MDO ISSX

Noordwijk aan Zee — The Netherlands

June 17-21, 2012

19th International Symposium on Microsomes and Drug Oxidations
12th European Regional ISSX Meeting

Meeting Proceedings and Abstracts

Dear Colleagues,

On behalf of the Meeting Organizing Committee, it is our pleasure to welcome you to the 19th Microsomes and Drug Oxidations (MDO) and 12th European International Society for the Study of Xenobiotics (ISSX) meeting.

Microsomes and Drug Oxidations (MDO, www.mdo.ki.se) constitutes the major series of international symposia in the field of drug metabolism and related areas. MDO has a long tradition of outstanding symposia since the first held in Bethesda, Maryland, USA in 1968. MDO meetings bring together scientists from both academia and industry and constitute a fruitful meeting place where synergistic interactions are established and where the knowledge regarding the latest developments in the field are discussed.

The International Society for the Study of Xenobiotics (ISSX, www.issx.org) is the premier international scientific organization for researchers interested in the disposition and safety of xenobiotics and in other related disciplines. ISSX was established in 1981 under the aegis of the Gordon Research Conferences on Drug Metabolism, which created an international society to promote the interaction of scientists dedicated to the study of xenobiotics in living systems. The mission of ISSX is to promote scientific development and to foster communication among researchers worldwide.

This meeting is the first joined meeting from both Societies and we think it provides an extremely valuable and truly unique opportunity for researchers to gather and exchange ideas and expertise. In addition to an outstanding scientific program, the meeting provides you with access to state-of-the-art exhibits and ample opportunities to present your work during our poster presentation sessions.

We have also made much effort to offer a social programme including a welcome reception among our exhibiting companies and posters, an optional excursion highlighting the scenic and cultural essence of Amsterdam, as well as fruitful social interactions at the banquet at the Breaker's Beach House in Noordwijk aan Zee.

The Grand Hotel Huis ter Duin also provides you with the possibility for relaxing activities on the North Sea, morning jogging on the beach and relatively immediate contact with the Dutch capital Amsterdam, and cities like Leiden, Haag and Rotterdam.

We do sincerely hope that you will consider the first joined 19th MDO / 12th European ISSX meeting both scientifically and socially very rewarding.

On behalf of the Meeting Organizing Committee, with the best personal regards,

Nico P.E. Vermeulen, Ph.D. and Magnus Ingelman-Sundberg, Ph.D.
Co-Chairs
19th International Symposium on Microsomes and Drug Oxidations (MDO) and 12th European Regional ISSX Meeting

19th MDO and 12th European Regional ISSX Meeting

June 17 – 21, 2012

Noordwijk aan Zee, the Netherlands

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Course Leader: Meindert Danhof, LACDR Leiden, Leiden, the Netherlands</td><td>09:00 – 12:15 | Pické III
Short Course 2. Genomics, Epigenomics, and Proteomics in Evaluation of Drug Effects
Course Leader: Bob van de Water, Leiden University, Leiden, the Netherlands</td></tr>
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Bill Smith, ISSX President</td></tr>
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Hubert G. Leufkens, Utrecht Institute for Pharmaceutical Sciences and the Dutch Medicines Evaluation Board, Utrecht, the Netherlands</td></tr>
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Matthias Schwab, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany</td></tr>
<tr><td colspan="2">12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer
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14:00 – 15:30 | Pické I + II **Parallel Session 1. Novel Enzyme Reactions in Drug Metabolism** *Co-Chairs: R. Scott Obach, Pfizer, Inc., Groton, CT, USA; F. Peter Guengerich, Vanderbilt University School of Medicine, Nashville, TN, USA*	**14:00 – 15:30 | Pické III** **Parallel Session 2. MicroRNAs as Biomarkers for Carcinogenicity and Toxicity** *Co-Chairs: Jan Hoeijmakers, Erasmus Medical Centre, Rotterdam, the Netherlands; Tsuyoshi Yokoi, Kanazawa University, Kanazawa, Japan*
15:30 – 16:00 Refreshment Break | Keizerzaal + Foyer **Refreshment Break, Visit Exhibitors, View Posters P1 – P100: Analytical to Enzyme Induction**	
16:00 – 17:30 | Pické I + II **Parallel Session 3. Adverse Drug Reactions: Focus on Mechanisms and Biomarkers** *Co-Chairs: Ann Daly, Newcastle University, Newcastle, UK; Gerry Kenna, AstraZeneca, Cheshire, UK*	**16:00 – 17:30 | Pické III** **Parallel Session 4. War Stories in Drug Discovery and Development** *Co-Chairs: R. Scott Obach, Pfizer, Inc., Groton, CT, USA; Kevin Beaumont, Pfizer Worldwide R&D, Cambridge, MA, USA*
Tuesday, June 19, 2012	
07:30 – 17:00 | Keizerzaal Foyer **Registration Open**	
07:20 – 08:20 | Pické III **Industry-Supported Symposium – Presented by Waters Corporation** *The Evolving Role of Exact Mass MS for Quantification in DMPK Studies*	
08:30 – 10:00 | Pické I + II **Plenary Session 3. Pharmacometrics and PK-PD** *Co-Chairs: Meindert Danhof, LACDR Leiden, Leiden, the Netherlands; Martijn Rooseboom, Merck Sharp & Dohme (MSD), Oss, the Netherlands*	
10:00 – 10:30 | Keizerzaal + Foyer **Refreshment Break, Visit Exhibitors, View Posters P101 – P205: Enzyme Inhibition/Inactivation to Metabolism**	
10:30 – 12:00 | Pické I + II **Parallel Session 5. Drug Transporters: Novel and Translational Aspects** *Co-Chairs: Mikko Niemi, University of Helsinki, Helsinki, Finland; Frans G.M. Russel, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands*	**10:30 – 12:00 | Pické III** **Parallel Session 6. Metabolomics for Discovery of Biomarkers for Drug Safety and Efficacy** *Co-Chairs: Frank J. Gonzalez, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; Thomas Hankemeier, Leiden University, Netherlands Metabolomics Centre, Leiden, the Netherlands*
12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer **Lunch Break, Poster Presentations P101 – P205 and Poster Awards Finalist Presentations A7 – A12, Visit Exhibitors**	
14:00 – 15:30 | Pické I + II **Parallel Session 7. Omics and Epigenomics in Drug Action and Toxicity** *Co-Chairs: Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden; Ivonne Rietjens, Wageningen University, Wageningen, the Netherlands*	**14:00 – 15:30 | Pické III** **Parallel Session 8. Translational Pharmakokinetics (Animal to Man, *in vitro* to *in vivo*, Healthy to Disease Patients, Patient Subpopulations)** *Co-Chairs: Brian Houston, University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, UK; Geoffrey Tucker, University of Sheffield, Sheffield, UK*
15:30 – 16:30 | Pické I + II **Plenary Session 4. ISSX Scientific Achievement Awards Session** *Bill Smith, Pfizer Inc., La Jolla, CA, USA*	
17:00 – 21:00 | Meet in Hotel Lobby **Excursion to Amsterdam – Motor coaches depart from the Grand Hotel Huis ter Duin promptly at 17:00.** **Bring your event ticket!** *Tickets may still be available. Inquire at the MDO-ISSX Meeting registration counter in the Keizerzaal Foyer.*	

Program-at-a-Glance

Wednesday, June 20, 2012

07:30 – 17:00 | Keizerzaal Foyer
Registration Open

07:20 – 08:20 | Pické III
Industry-Supported Symposium – Presented by Covance
Integrated Metabolism Investigations to Streamline Compound Development

08:30 – 10:00 | Pické I + II
Plenary Session 5. Immune Mediators in DILI
Co-Chairs: Dean Naisbitt, University of Liverpool, Liverpool, UK;
Dirk Wohlleber, Institute of Molecular Medicine, University of Bonn, Bonn, Germany

10:00 – 10:30 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P206 – P297: Non-P450 Phase I Enzymes to Transporters

10:30 – 12:00 | Pické I + II **Parallel Session 9. Structural Analyses of Transporters and Enzymes** *Co-Chairs: Eric F. Johnson, The Scripps Research Institute, La Jolla, CA, USA;* *Emily Scott, University of Kansas, Lawrence, KS, USA*	**10:30 – 12:00 | Pické III** **Parallel Session 10. Novel Aspects of Non-Coding RNA in Regulation of ADME Genes** *Co-Chairs: Xiaobo Zhong, University of Kansas Medical Center, Kansas City, KS, USA;* *Aiming Yu, University at Buffalo, SUNY, Buffalo, NY, USA*

12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer
Lunch Break, Poster Presentations P206 – P297, Visit Exhibitors

14:00 – 15:30 | Pické I + II **Parallel Session 11. Nrf2 Signaling in Disease and Chemical Exposure** *Co-Chairs: John Hayes, University of Dundee, Dundee, UK;* *B. Kevin Park, University of Liverpool, Liverpool, UK*	**14:00 – 15:30 | Pické III** **Parallel Session 12. Omics to Identify and Classify Functional Signaling Networks** *Co-Chairs: Ulrich Zanger, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany;* *Bob van de Water, Leiden University, Leiden, the Netherlands*

15:30 – 16:00 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P206 – P297: Non-P450 Phase I Enzymes to Transporters

16:00 – 17:30 | Pické I + II **Parallel Session 13. Abstract-Based Session** *Co-Chairs: Frans G.M. Russel, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands;* *Erik Eliasson, Karolinska University Hospital Huddinge, Stockholm, Sweden*	**16:00 – 17:30 | Pické III** **Parallel Session 14. Abstract-Based Session** *Co-Chairs: Ivonne Rietjens, Wageningen University, Wageningen, the Netherlands;* *Bob van de Water, Leiden University, Leiden, the Netherlands*

19:00 | Breakers Beach House
Banquet
Tickets may still be available. Inquire at the MDO-ISSX registration counter in the Keizerzaal Foyer.

Thursday, June 21, 2012

07:30 – 15:00 | Keizerzaal Foyer
Registration Open

07:20 – 08:20 | Pické III
Industry-Supported Symposium – Presented by Thermo Scientific
Tools to Accelerate Discovery DMPK – Better Answers, Faster.

08:30 – 10:00 | Pické I + II
Plenary Session 6. Imaging for Drug Efficacy and Disposition
Co-Chairs: Julia C. Stingl, University of Ulm, Ulm, Germany;
Christer Halldin, Karolinska Institutet, Stockholm, Sweden

10:00 – 10:30 | Keizerzaal Foyer
Refreshment Break, View Awards Finalist Posters

10:30 – 12:00 | Pické I + II **Parallel Session 15. Adverse Drug Reactions: Focus on Translational Aspects and *in vivo*** *Co-Chairs: Paul Watkins, The Hamner UNC - Institute for Drug Safety Sciences, Research Triangle Park, NC, USA;* *Ina Schuppe-Koistinen, AstraZeneca R&D, Södertälje, Sweden*	**10:30 – 12:00 | Pické III** **Parallel Session 16. Novel Aspects in Cytochrome P450 Catalysis and Dynamics** *Co-Chairs: James R. Halpert, Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, CA, USA;* *Paul R. Ortiz de Montellano, University of California, San Francisco, CA, USA*
12:00 – 13:00 | La Terrasse and Keizerzaal Foyer **Lunch Break, View Awards Finalist Posters**	
13:00 – 14:30 | Pické I + II **Parallel Session 17. Omics and Bioinformatics in Drug Efficacy and Safety** *Co-Chairs: Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden;* *Laura Suter-Dick, F. Hoffmann-La Roche, Ltd., Basel, Switzerland*	**13:00 – 14:30 | Pické III** **Parallel Session 18. Reactive Metabolites and Drug Safety: Predictive, Mechanistic, and Translational Aspects** *Co-Chairs: Nico P.E. Vermeulen, VU Amsterdam, LACDR, Amsterdam, the Netherlands;* *Thomas A. Baillie, University of Washington, Seattle, WA, USA*
14:30 – 14:45 | Pické I + II **Poster Awards Presentation and Closing Remarks**	

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VU University Amsterdam
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Karolinska Institutet Stockholm
Section of Pharmacogenetics
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Microsomes and Drug Oxidations (MDO) constitutes the major series of international symposia in the field of drug metabolism and related areas. MDO has a long tradition of outstanding symposia since the first held in Bethesda, Maryland, USA in 1968 and now occurring every two years. MDO has an International Advisory Board with distinguished scientists in the field. MDO meetings bring together scientists from both academia and industry and constitute a fruitful meeting place where synergistic interactions are established and where the knowledge regarding the latest developments in the field are discussed.

Save the Date to attend the
MDO Meeting 2014 in Stuttgart, Germany
May 18-22, 2014

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The International Society for the Study of Xenobiotics (ISSX) is the premier international scientific organization for researchers interested in the metabolism and disposition of xenobiotics and in other related disciplines. ISSX was formed in 1981 under the aegis of the Gordon Research Conferences on Drug Metabolism, which created an international society to promote the interaction of scientists dedicated to the study of xenobiotics in living systems. ISSX's mission is to promote scientific development and to foster communication among researchers worldwide.

Save the Date to attend the
18th North American Regional ISSX Meeting in Dallas, Texas, USA
October 14 – 18, 2012

ISSX International Society for the Study of Xenobiotics

Meeting Location

Grand Hotel Huis ter Duin
Koningin Astrid Boulevard 5
Postbus 85
2200 AB Noordwijk aan Zee
The Netherlands
Tel: +31 (0)71-361 9220

Welcome Reception

All meeting attendees and their registered guests are invited to attend the Welcome Reception on Sunday, June 17 from 19:00 – 21:00 in the Keizerzaal + Foyer.

Amsterdam Excursion and Dinner Cruise (Ticketed Event)

Attendees will meet in the hotel lobby and motor coaches will depart from the Grand Hotel Huis ter Duin promptly at 17:00 and transport participants to the city center where they will enjoy a cozy and exclusive cruise through the Amsterdam canals on a comfortable private boat. **Please bring your event ticket!**

A separate registration fee of $100 applies. Tickets may still be available. Inquire at the registration counter in the Keizerzaal Foyer.

Banquet (Ticketed Event)

Join your colleagues on Wednesday, June 20 at 19:00 to celebrate with acquaintances new and old at the banquet to be held in the new Breakers Beach House at the Grand Hotel Huis ter Duin.

A separate registration fee of $100 applies. Tickets may still be available. Inquire at the registration counter in the Keizerzaal Foyer.

Invited Speaker Information

An audio-visual technician will be available to assist speakers with loading their presentations. Speakers should bring their presentations to their assigned session room no less than 30 minutes prior to the start of their session.

Graduate/Predoctoral and Postdoctoral Poster Awards Competition

The Graduate/Predoctoral Poster Awards finalists will present posters on Monday, June 18 in the Keizerzaal Foyer from 13:00 – 14:00. Postdoctoral Poster Awards finalists will present posters on Tuesday, June 19 from 13:00 – 14:00 in the Keizerzaal Foyer. View the finalist posters throughout the meeting and plan to attend the Poster Awards presentation on Thursday, June 21 at 15:30 in Pické I + II.

Abstract Publication

Accepted abstracts are published online at **www.issx.org/onlineabstracts**.

Meeting Registration | Keizerzaal Foyer

Sunday, June 17	08:00 – 19:00
Monday, June 18	07:30 – 17:00
Tuesday, June 19	07:30 – 17:00
Wednesday, June 20	07:30 – 17:00
Thursday, June 21	07:30 – 15:00

Exhibit Schedule | Keizerzaal l·yŘ Foyer

Sunday, June 17 *Welcome Reception*	19:00 – 21:00
Monday, June 18	10:00 – 16:00
Tuesday, June 19	10:00 – 17:00
Wednesday, June 20	10:00 – 16:00

Poster Presentation Schedule Keizerzaal l·yŘ Foyer

Monday, June 18 *Posters P1 – P100*	12:00 – 13:00
Tuesday, June 19 *Posters P101 – P205*	12:00 – 13:00
Wednesday, June 20 *Posters P206 – P297*	12:00 – 13:00

Poster Awards Competition Keizerzaal Foyer

Monday, June 18 – Thursday, June 21 – Poster Viewing

Poster Awards Competition Presentations Keizerzaal Foyer

Monday, June 18 – Graduate/Predoctoral	13:00 – 14:00
Tuesday, June 19 – Postdoctoral	13:00 – 14:00

Scientific Program

Sunday, June 17

09:00 – 12:15 | Pické I
Short Course 1. Pharmacometric Methods and PK-PD Modeling
Course Leader: Meindert Danhof, LACDR Leiden, Leiden, the Netherlands

09:00 – 09:10
PHARMACOMETRIC METHODS AND THE PK-PD
Meindert Danhof, LACDR Leiden, Leiden, the Netherlands

09:10 – 09:45
SC1 - PK-PD MODELING OF CNS DRUGS: IMPLEMENTING RECEPTOR BINDING
Johannes Proost, University of Groningen, Groningen, the Netherlands

09:45 – 10:20
SC2 - PK-PD MODELING OF BIOLOGICS, ROLE OF TARGET MEDIATED DRUG DISPOSITION
Philip Lowe, Novartis Pharma AG, Basel, Switzerland

10:20 – 10:40 | Pické Foyer
Refreshment Break for Short Course Attendees

10:40 – 11:30
SC3 - TRANSLATIONAL PK-PD MODELING: TARGET ENGAGEMENT LINKING TO EFFICACY/SAFETY
Sandra Visser, AstraZeneca R&D, Södertälje, Sweden

11:30 – 12:15
SC4 - PK-PD MODELING OF DISEASE PROCESSES AND PROGRESSION: DISEASE SYSTEM ANALYSIS
Teun Post, Merck Sharp & Dohme (MSD), Eindhoven Area, the Netherlands

09:00 – 12:15 | Pické III
Short Course 2. Genomics, Epigenomics, and Proteomics in Evaluation of Drug Effects
Course Leader: Bob van de Water, Leiden University, Leiden, the Netherlands

09:00 – 09:40
SC5 - METABOLOMICS IN DRUG EFFICACY AND SAFETY EVALUATION
Johan Lindberg, AstraZeneca R&D, Södertälje, Sweden

09:40 – 10:20
SC6 - TRANSCRIPTOMICS AND EPIGENOMICS IN DRUG SAFETY
Remi Terranova, Novartis Institutes for Biomedical Research, Basel, Switzerland

10:20 – 10:40 | Pické Foyer
Refreshment Break for Short Course Attendees

10:40 – 11:30
SC7 - FROM OMICS-BASED SAFETY BIOMARKERS TO HIGH THROUGHPUT ASSAYS
Harry Vrieling, Leiden University Medical Centre, Leiden, the Netherlands

11:30 – 12:15
SC8 - RNA INTERFERENCE TO IDENTIFY FUNCTIONAL GENES UNDERLYING DRUG EFFICACY AND SAFETY
Bob van de Water, Leiden University, Leiden, the Netherlands

12:15 – 13:30 | La Terrasse
Short Course Attendee Lunch

13:30 – 16:45 | Pické I
Short Course 3. Human *in vitro* Systems for Predicting Drug Transport, Metabolism, and Adverse Effects
Course Leaders: Per Artursson, Uppsala University, Uppsala, Sweden;
Geny Groothuis, University of Groningen, Groningen, the Netherlands

13:30 – 14:15
SC9 - 2D CELL CULTURE AND SUBCELLULAR MODELS FROM INTESTINE AND LIVER FOR STUDIES OF DRUG TRANSPORT, DRUG-DRUG TRANSPORTER INTERACTIONS AND TRANSPORTER-MEDIATED SIDE EFFECTS
Per Artursson, Uppsala University, Uppsala, Sweden

14:15 – 15:00
SC10 - ROLE OF DIFFERENT CELL TYPES IN CO-CULTURES TO RECONSTRUCT HUMAN LIVER
Edward L. LeCluyse, Triangle Research Laboratories at The Hamner Institutes, Research Triangle Park, NC, USA

15:00 – 15:20 | Pické Foyer
Refreshment Break for Short Course Attendees

15:20 – 16:00
SC11 - HEPATIC 3D CELL CULTURES OF HUMAN LIVER IN DRUG DISCOVERY
Tommy B. Andersson, AstraZeneca R&D, Mölndal, Sweden

16:00 – 16:45
SC12 - *EX VIVO* PREPARATIONS OF HUMAN TISSUES FOR DRUG METABOLISM, TOXICITY, AND TRANSPORT
Geny Groothuis, University of Groningen, Groningen, the Netherlands

13:30 – 16:45 | Pické III
Short Course 4. Linking Pre-clinical Knowledge of PK Variability to PD Outcomes (Hands-On)
Sponsored by Simcyp, Ltd.
Course Leader: Geoffrey Tucker, University of Sheffield, Sheffield, UK

13:30 – 14:15
SC13 - INTRODUCTORY LECTURE - PROPAGATION OF PK VARIABILITY TO DRUG RESPONSE
Geoffrey Tucker, University of Sheffield, Sheffield, UK

14:15 – 15:00
SC14 - PK AND PD ANALYSIS OF EFAVIRENZ DOSE REDUCTION USING AN *IN VITRO - IN VIVO* EXTRAPOLATION MODEL
Marco Siccardi, University of Liverpool, Liverpool, UK

15:00 – 15:15 | Pické Foyer
Refreshment Break for Short Course Attendees

15:15 – 16:45
HANDS-ON WORKSHOP
Workshop Tutors:
Geoffrey Tucker, University of Sheffield, Sheffield, UK
Marco Siccardi, University of Liverpool, Liverpool, UK
Khaled Abduljalil, Simcyp Ltd, Groton, CT, USA
Zoe Barter, Simcyp Ltd, Groton, CT, USA
David Plowchalk, Pfizer, Groton, CT, USA
Karen Rowland-Yeo, Simcyp Ltd, Groton, CT, USA

18:00 – 18:15 | Pické I + II
Opening Session – Welcome Remarks

Nico P. E. Vermeulen and Magnus Ingelman-Sundberg, 19th MDO and 12th European ISSX Meeting Co-Chairs;
Bill Smith, ISSX President

18:15 – 19:00 | Pické I + II
Keynote Lecture
S1 - RISK MANAGEMENT IN THE NEW EU PHARMACOVIGILANCE LEGISLATION: GOOD SCIENCE IS MORE THAN EVER NEEDED
Hubert G. Leufkens, Utrecht Institute for Pharmaceutical Sciences and the Dutch Medicines Evaluation Board, Utrecht, the Netherlands

19:00 – 21:00 | Keizerzaal + Foyer
Opening Welcome Reception + Meet the Exhibitors

Monday, June 18

08:30 – 10:00 | Pické I + II
Plenary Session 1. Stem Cells in Drug Efficacy and Safety Assessment
Co-Chairs: David Hay, University of Edinburgh MRC CRM, Edinburgh, UK;
Stephen Strom, Karolinska Institutet and Hospital, Stockholm, Sweden

08:30 – 09:00
S2 - DERIVING METABOLICALLY ACTIVE AND PREDICTIVE HEPATOCYTES FROM PLURIPOTENT STEM CELLS
David Hay, University of Edinburgh MRC CRM, Edinburgh, UK

09:00 – 09:30
S3 - STEM CELL-DERIVED HEPATOCYTES AND HUMANIZED MICE, NEW TOOLS FOR RESEARCH
Stephen Strom, Karolinska Institutet and Hospital, Stockholm, Sweden

09:30 – 10:00
S4 - TOWARDS THE GENERATION OF A HEPATIC MICRO-BIOREACTOR FOR DRUG TOXICITY AND METABOLIZATION STUDIES
Catherine Verfaillie, Katholieke Universiteit Leuven, Leuven, Belgium

10:00 – 10:30 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P1 – P100: Analytical to Enzyme Induction

10:30 – 12:00 | Pické I + II
Plenary Session 2. Pharmacogenomic Biomarkers in Clinical Medicine (Including Regulatory Aspects)
Co-Chairs: Urs A. Meyer, University of Basel, Basel, Switzerland;
Matthias Schwab, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

10:30 – 11:00
S5 - CLINICAL PHARMACOGENOMICS, AN UPDATE
Matthias Schwab, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

11:00 – 11:30
S6 - REGULATORY PHARMACOGENOMICS
Felix Frueh, Medco Research Institute, LLC, Bethesda, MD, USA

11:30 – 12:00
S7 - THE USE OF GWAS IN ELUCIDATION OF THE GENETIC ARCHITECTURE OF COMMON DISEASES AND PHARMACOGENETICS
Colin N.A. Palmer, University of Dundee, Dundee, UK

12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer
Lunch Break, Poster Presentations P1 – P100 and Poster Awards Finalist Presentations A1 – A6, Visit Exhibitors

12:00 – 13:00 | Keizerzaal + Foyer
Poster Presentations P1 – P100: Analytical to Enzyme Induction, Visit Exhibitors

13:00 – 14:00 | Keizerzaal Foyer
Graduate/Predoctoral Poster Awards Finalist Presentations A1 – A6

12:00 | TBD
MDO International Scientific Advisory Committee Luncheon

14:00 – 15:30 | Pické I + II
Parallel Session 1. Novel Enzyme Reactions in Drug Metabolism
Co-Chairs: R. Scott Obach, Pfizer, Inc., Groton, CT, USA;
F. Peter Guengerich, Vanderbilt University School of Medicine, Nashville, TN, USA

14:00 – 14:30
S8 - THE NOVEL DRUG METABOLISM ENZYME MARC
Bernd Clement, Institute of Pharmacy, University of Kiel, Kiel, Germany

14:30 – 15:00
S9 - ALDEHYDE OXIDASE IN DRUG RESEARCH
R. Scott Obach, Pfizer, Inc., Groton, CT, USA

15:00 – 15:30
S10 - UNUSUAL REACTIONS CATALYZED BY CYTOCHROME P450 ENZYMES
F. Peter Guengerich, Vanderbilt University School of Medicine, Nashville, TN, USA

14:00 – 15:30 | Pické III
Parallel Session 2. MicroRNAs as Biomarkers for Carcinogenicity and Toxicity
Co-Chairs: Jan Hoeijmakers, Erasmus Medical Centre, Rotterdam, the Netherlands;
Tsuyoshi Yokoi, Kanazawa University, Kanazawa, Japan

14:00 – 14:30
S11 - MicroRNA REGULATION OF DRUG ACTION
Noam Shomron, Tel Aviv University, Tel Aviv, Israel

14:30 – 15:00
S12 - MicroRNAs, THE DNA DAMAGE RESPONSE, AND CANCER
Joris Pothof, Erasmus Medical Centre, Rotterdam, the Netherlands

15:00 – 15:30
S13 - TOXICOLOGICAL IMPLICATIONS OF MODULATION OF GENE EXPRESSION BY MicroRNAs
Tsuyoshi Yokoi, Kanazawa University, Kanazawa, Japan

15:30 – 16:00 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P1 – P100: Analytical to Enzyme Induction

16:00 – 17:30 | Pické I + II
Parallel Session 3. Adverse Drug Reactions: Focus on Mechanisms and Biomarkers
Co-Chairs: Ann Daly, Newcastle University, Newcastle, UK; Gerry Kenna, AstraZeneca, Cheshire, UK

16:00 – 16:30
S14 - DRUG-INDUCED LIVER INJURY: MECHANISMS, PREDICTION, AND AVOIDANCE
Gerry Kenna, AstraZeneca, Cheshire, UK

16:30 – 17:00
S15 - GENETIC PREDISPOSITION TOWARDS DRUG-INDUCED ARRHYTHMIA
Elijah R. Behr, St. George's University of London, London, UK

17:00 – 17:30
S16 - USING INBRED MICE TO IDENTIFY ADR MECHANISMS
Paul Watkins, The Hamner UNC – Institute for Drug Safety Sciences, Research Triangle Park, NC, USA

16:00 – 17:30 | Pické III
Parallel Session 4. War Stories in Drug Discovery and Development
Co-Chairs: R. Scott Obach, Pfizer, Inc., Groton, CT, USA; Kevin Beaumont, Pfizer Worldwide R&D, Cambridge, MA, USA

16:00 – 16:30
S17 - THE DISCOVERY OF NEUROKININ-2 ANTAGONISTS - A WAR OF ATTRITION
Kevin Beaumont, Pfizer, Inc., Pfizer Worldwide R&D, Cambridge, MA, USA

16:30 – 17:00
S18 - DRUG-INDUCED LIVER INJURY IN HUMANS: THE CASE OF XIMELAGATRAN
Tommy Andersson, AstraZeneca R&D, Mölndal, Sweden

17:00 – 17:30
S19 - IN THE TRENCHES WITH VISMODEGIB: AN APPROVED HEDGEHOG PATHWAY INHIBITOR WITH UNIQUE PHARMACOKINETIC AND METABOLIC PROPERTIES
Cornelis E.C.A. Hop, Genentech, Inc., South San Francisco, CA, USA

Tuesday, June 19

08:30 – 10:00 | Pické I + II
Plenary Session 3. Pharmacometrics and PK-PD
Co-Chairs: Meindert Danhof, LACDR Leiden, Leiden, the Netherlands;
Martijn Rooseboom, Merck Sharp & Dohme (MSD), Oss, the Netherlands

08:30 – 09:00
S20 - TRANSLATIONAL PK-PD: PREDICTING EFFICACY AND SAFETY IN MAN FROM NON-CLINICAL DATA
Piet van der Graaf, Pfizer, Kent, UK

09:00 – 09:30
S21 - MODELING OF MATURATION: PREDICTING PK AND PD IN PEDIATRICS
Catherijne Knibbe, St. Antonius Hospital, Nieuwegein, the Netherlands and LACDR/St. Antonius Hospital, Leiden, the Netherlands

09:30 – 10:00
S22 - DISEASE SYSTEMS ANALYSIS: PREDICTING DRUG EFFECT ON DISEASE PROGRESSION
Bart Ploeger, AstraZeneca, Södertälje, Sweden

10:00 – 10:30 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P101 – P205: Enzyme Inhibition/Inactivation to Metabolism

10:30 – 12:00 | Pické I + II
Parallel Session 5. Drug Transporters: Novel and Translational Aspects
Co-Chairs: Mikko Niemi, University of Helsinki, Helsinki, Finland;
Frans G.M. Russel, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

10:30 – 11:00
S23 - CLINICAL PHARMACOGENETICS OF DRUG TRANSPORTERS
Mikko Niemi, University of Helsinki, Helsinki, Finland

11:00 – 11:30
S24 - ROLE OF DRUG TRANSPORTERS IN DRUG EXPOSURE AND SAFETY
Frans G.M. Russel, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

11:30 – 12:00
S25 - MICRODOSING AND PET STUDIES IN DRUG TRANSPORTER RESEARCH
Yuichi Sugiyama, University of Tokyo, Tokyo, Japan

10:30 – 12:00 | Pické III
Parallel Session 6. Metabolomics for Discovery of Biomarkers for Drug Safety and Efficacy
Co-Chairs: Frank J. Gonzalez, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; Thomas Hankemeier, Leiden University, Netherlands Metabolomics Centre, Leiden, the Netherlands

10:30 – 11:00
S26 - DISCOVERY OF DISEASE AND TOXICITY BIOMARKERS AND THEIR CONTRIBUTION TO MECHANISMS
Frank Gonzalez, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

11:00 – 11:30
S27 - METABOLOMICS IN TRANSLATIONAL DRUG RESEARCH
Thomas Hankemeier, Leiden University, Netherlands Metabolomics Centre, Leiden, the Netherlands

11:30 – 12:00
S28 - PREDICTING DRUG RESPONSE FROM METABOLIC PROFILES
Hector Keun, Imperial College London, London, UK

12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer
Lunch Break, Poster Presentations P101 – P205 and Poster Awards Finalist Presentations A7 – A12, Visit Exhibitors

12:00 – 13:00 | Keizerzaal + Foyer
Poster Presentations P101 – P205: Enzyme Inhibition/Inactivation to Metabolism

13:00 – 14:00 | Keizerzaal Foyer
Postdoctoral Poster Awards Finalist Presentations A7 – A12

14:00 – 15:30 | Pické I + II
Parallel Session 7. Omics and Epigenomics in Drug Action and Toxicity
Co-Chairs: Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden; Ivonne Rietjens, Wageningen University, Wageningen, the Netherlands

14:00 – 14:30
S29 - EPIGENETICS IN TOXICOLOGY; INTRODUCTION TO THE FIELD
Ivonne Rietjens, Wageningen University, Wageningen, the Netherlands

14:30 – 15:00
S30 - INTERINDIVIDUAL DIFFERENCES IN THE HUMAN HEPATIC EPIGENOME: IMPLICATIONS FOR DRUG METABOLISM AND TRANSPORT
Marina Kacevska, Karolinska Institutet, Stockholm, Sweden

15:00 – 15:30
S31 - PHARMACOGENOMICS OF THE RNA WORLD: STRUCTURAL RNA POLYMORPHISMS IN DRUG THERAPY
Wolfgang Sadee, The Ohio State University, Columbus, OH, USA

14:00 – 15:30 | Pické III
Parallel Session 8. Translational Pharmakokinetics (Animal to Man, *in vitro* to *in vivo*, Healthy to Disease Patients, Patient Subpopulations)
Co-Chairs: Brian Houston, University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, UK;
Geoffrey Tucker, University of Sheffield, Sheffield, UK

14:00 – 14:30
S32 - TRANSLATIONAL PHARMACOKINETICS – HUMAN IN VITRO-IN VIVO EXTRAPOLATION
Brian Houston, University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, UK

14:30 – 15:00
S33 - THE VIRTUAL PATIENT - UNDERSTANDING COMPLEXITY AND VARIABILITY
Geoffrey Tucker, University of Sheffield, Sheffield, UK

15:00 – 15:30
S34 - TRANSLATIONAL PK - ANIMAL - HUMAN EXTRAPOLATION
Natilie Hosea, Pfizer Inc., San Diego, CA, USA

15:30 – 16:30 | Pické I + II
Plenary Session 4. Scientific Achievement Awards Session
Bill Smith, Pfizer Inc., La Jolla, CA, USA

17:00 – 21:00 | Meet in Hotel Lobby
Excursion to Amsterdam
(Ticketed Event)

Motor coaches depart from the Grand Hotel Huis ter Duin promptly at 17:00. Bring your event ticket!

Scientific Program

Wednesday, June 20

08:30 – 10:00 | Pické I + II
Plenary Session 5. Immune Mediators in DILI
Co-Chairs: Dean Naisbitt, University of Liverpool, Liverpool, UK;
Dirk Wohlleber, Institute of Molecular Medicine, University of Bonn, Bonn, Germany

08:30 – 09:00
S35 - IMMUNE MEDIATORS IN DILI: LESSONS TO BE LEARNED FROM STUDIES WITH HUMAN CELLS
Dean Naisbitt, University of Liverpool, Liverpool, UK

09:00 – 09:30
S36 - CHARACTERIZATION OF NOVEL PATHOPHYSIOLOGICAL MECHANISMS CAUSING DRUG-INDUCED IMMUNE MEDIATED LIVER INJURY
Dirk Wohlleber, Institute of Molecular Medicine, University of Bonn, Bonn, Germany

09:30 – 10:00
S37 - THE P-I CONCEPT: PHARMACOLOGICAL INTERACTION OF DRUGS WITH IMMUNE RECEPTORS
Werner Pichler, University of Bern, Bern, Switzerland

10:00 – 10:30 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors,
View Posters P206 – P297: Non-P450 Phase I Enzymes to Transporters

10:30 – 12:00 | Pické I + II
Parallel Session 9. Structural Analyses of Transporters and Enzymes
Co-Chairs: Eric F. Johnson, The Scripps Research Institute, La Jolla, CA, USA;
Emily Scott, University of Kansas, Lawrence, KS, USA

10:30 – 11:00
S38 - LIGAND BINDING TO CRYSTALLINE P450 2D6: AN EXPERIMENTAL PLATFORM FOR RAPID, STRUCTURAL CHARACTERIZATION OF SUBSTRATE AND INHIBITOR BINDING
Eric F. Johnson, The Scripps Research Institute, La Jolla, CA, USA

11:00 – 11:30
S39 - CYTOCHROME P450 STRUCTURES IN PREDICTION OF DRUG AND PROCARCINOGEN METABOLISM
Emily Scott, University of Kansas, Lawrence, KS, USA

11:30 – 12:00
S40 - STRUCTURAL ANALYSIS OF ATP-BINDING CASSETTE TRANSPORTERS USING ELECTRON- AND X-RAY CRYSTALLOGRAPHY
Robert Ford, The University of Manchester, Manchester, UK

10:30 – 12:00 | Pické III
Parallel Session 10. Novel Aspects of Non-Coding RNA in Regulation of ADME Genes
Co-Chairs: Xiaobo Zhong, University of Kansas Medical Center, Kansas City, KS, USA;
Aiming Yu, University at Buffalo, SUNY, Buffalo, NY, USA

10:30 – 11:00
S41 - COOPERATION BETWEEN MicroRNA AND ALTERNATIVE POLYADENYLATION IN REGULATION OF CYP3A4 EXPRESSION IN HEPATOCYTES IN RESPONSE TO DRUG INDUCTION
Xiaobo Zhong, University of Kansas Medical Center, Kansas City, KS, USA

11:00 – 11:30
S42 - MicroRNAs IN REGULATION OF DRUG TRANSPORTER GENES - CASES AND PERSPECTIVES
Aiming Yu, University at Buffalo, SUNY, Buffalo, NY, USA

11:30 – 12:00
S43 - MicroRNAs, LincRNAs AND EPIGENETIC FACTORS REGULATING HEPATIC CYPS - LESSONS FROM DEEP SEQUENCING
David J. Waxman, Boston University, Boston, MA, USA

12:00 – 14:00 | La Terrasse and Keizerzaal + Foyer
Lunch Break, Poster Presentations P206 – P297, Visit Exhibitors

12:00 – 13:00 | Keizerzaal + Foyer
Poster Presentations P206 – P297: Non-P450 Phase I Enzymes to Transporters

14:00 – 15:30 | Pické I + II
Parallel Session 11. Nrf2 Signaling in Disease and Chemical Exposure
Co-Chairs: John Hayes, University of Dundee, Dundee, UK;
B. Kevin Park, University of Liverpool, Liverpool, UK

14:00 – 14:30
S44 - NRF2 SIGNALING MECHANISMS IN RESPONSE TO CELLULAR STRESS
John Hayes, University of Dundee, Dundee, UK

14:30 – 15:00
S45 - THE NRF2-KEAP1-ARE PATHWAY AND THE DUAL ROLE OF NRF2 IN CANCER
Donna Zhang, University of Arizona, Tucson, AZ, USA

15:00 – 15:30
S46 - ROLE OF NRF2 IN PROTECTION AGAINST DRUG-INDUCED DISEASE
B. Kevin Park, University of Liverpool, Liverpool, UK

14:00 – 15:30 | Pické III
Parallel Session 12. Omics to Identify and Classify Functional Signaling Networks
Co-Chairs: Ulrich Zanger, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany; Bob van de Water, Leiden University, Leiden, the Netherlands

14:00 – 14:30
S47 - IMPACT OF TUMOR-DERIVED CYTOKINES ON XENOBIOTIC CLEARANCE PATHWAYS IN CANCER: LIVER PROTEOME AND TRANSCRIPTOME OF TUMOR-BEARING MICE
Graham Robertson, ANZAC Research Institute at Concord RG Hospital, Sydney, Australia

14:30 – 15:00
S48 - GENETICS/GENOMICS APPROACHES TO STUDY REGULATION OF HEPATIC P450 GENE EXPRESSION
Ulrich Zanger, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

15:00 – 15:30
S49 - RNA-INTERFERENCE-BASED FUNCTIONAL GENOMICS OF DRUG-INDUCED LIVER INJURY RESPONSES
Bob van de Water, Leiden University, Leiden, the Netherlands

15:30 – 16:00 | Keizerzaal + Foyer
Refreshment Break, Visit Exhibitors, View Posters P206 – P297: Non-P450 Phase I Enzymes to Transporters

16:00 – 17:30 | Pické I + II
Parallel Session 13. Abstract-Based Session
Co-Chairs: Frans G.M. Russel, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; Erik Eliasson, Karolinska University Hospital Huddinge, Stockholm, Sweden

P16 - INVESTIGATION OF HEPATIC CLEARANCE OF DRUGS IN CHIMERIC MICE WITH HUMANIZED LIVER
Françoise Brée, Xenoblis, Saint Grégoire, France

P93 - CLASSIFICATION OF 331 DRUGS INVOLVED IN SIGNIFICANT PHARMACOKINETIC DRUG-DRUG INTERACTIONS
Akihiro Hisaka, The University of Tokyo Hospital, Tokyo, Japan

P117 - HUMAN CYP2C8 IS POST-TRANSCRIPTIONALLY REGULATED BY MIRNAS 103 AND 107 IN HUMAN LIVER
Sailesh Surapureddi, National Institutes of Health, Research Triangle Park, NC, USA

P122 - REGULATION OF PXR FUNCTION BY POST-TRANSLATIONAL MODIFICATION
Junko Sugatani, University of Shizuoka, Shizuoka, Japan

P167 - ABACAVIR METABOLISM IN ANTIGEN PRESENTING CELLS GENERATES A FUNCTIONAL ANTIGEN FOR T-CELLS
Catherine C. Bell, University of Liverpool, Liverpool, UK

P202 - EZETIMIBE AS A BIOMARKER FOR LIVER DIRECTED UGT1A1 GENE THERAPY FOR CRIGLER- NAJJAR TYPE I
Nina Sneitz, University of Helsinki, Helsinki, Finland

16:00 – 17:30 | Pické III
Parallel Session 14. Abstract-Based Session
Co-Chairs: Ivonne Rietjens, Wageningen University, Wageningen, the Netherlands; Bob van de Water, Leiden University, Leiden, the Netherlands

P27 - PROTEIN S-GLUTATHIONYLATION: A ROLE FOR GLUTATHIONE S-TRANSFERASE PI IN MEDIATING CYTOPROTECTION?
David J. McGarry, University of Dundee, Dundee, UK

P52 - BIOACTIVATION OF CLOZAPINE - INVOLVEMENT OF HUMAN CYTOCHROME P450S
Sanja Dragovic, Vrije Universiteit, Amsterdam, the Netherlands

P40 - D2-DOPAMINERGIC RECEPTOR LINKED PATHWAYS: CRITICAL REGULATORS OF CYP3A, CYP2C AND CYP2D
Maria Konstandi, University of Ioannina, School of Medicine, Ioannina, Greece

P150 - IMAGING-BASED TECHNIQUES AS FUNCTIONAL READOUTS FOR DRUG TOXICITY
Bram Herpers, Leiden University, the Netherlands

P155 - TRANSFER OF A BILE TRANSPORT ANALYSIS METHOD FROM 2D TO A 3D MULTI-COMPARTMENT BIOREACTOR SYSTEM
Marc Lübberstedt, Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité University Medicine Berlin, Berlin, Germany

P206 - THE MITOCHONDRIAL AMIDOXIME REDUCTASE A NOVEL LIPOGENIC ENZYME SYSTEM?
Etienne PA. Neve, Karolinksa Institutet, Stockholm, Sweden

19:00 | Breakers Beach House
Banquet
(Ticketed Event)

Thursday, June 21

08:30 – 10:00 | Pické I + II
Plenary Session 6. Imaging for Drug Efficacy and Disposition
Co-Chairs: Julia Stingl, University of Ulm, Ulm, Germany; Christer Halldin, Karolinska Institutet, Stockholm, Sweden

08:30 – 09:00
S50 - VISUALIZING PHARMACOGENETICS IN THE BRAIN: MODULATION OF BRAIN PERFUSION BY PHARMACOGENETIC POLYMORPHISMS AND DRUG EFFECTS
Julia Stingl, University of Ulm, Ulm, Germany

09:00 – 9:30
S51 - PET STUDIES IN DRUG DEVELOPMENT
Christer Halldin, Karolinska Institutet, PET Centre, Stockholm, Sweden

09:30 – 10:00
S52 - PET IN DRUG DISCOVERY AND DEVELOPMENT
Antony Gee, King's College London, London, UK

10:00 – 10:30 | Keizerzaal Foyer
Refreshment Break, View Awards Finalist Posters

10:30 – 12:00 | Pické I + II
Parallel Session 15. Adverse Drug Reactions: Focus on Translational Aspects and *in vivo*
Co-Chairs: Paul Watkins, The Hamner UNC – Institute for Drug Safety Sciences, Research Triangle Park, NC, USA; Ina Schuppe-Koistinen, AstraZeneca R&D, Södertälje, Sweden

10:30 – 11:00
S53 - GENETIC PREDICTORS FOR DRUG-INDUCED LIVER INJURY
Ann Daly, Newcastle University, Newcastle, UK

11:00 – 11:30
S54 - CLINICAL BIOMARKER QUALIFICATION FOR DRUG-INDUCED INJURY TO KIDNEY, LIVER, AND THE VASCULAR SYSTEM WITHIN THE INNOVATIVE MEDICINES INITIATIVE (IMI) PROJECT SAFE-T
Ina Schuppe-Koistinen, AstraZeneca R&D, Södertälje, Sweden

11:30 – 12:00
S55 - CARDIOVASCULAR TOXICITY: A CHALLENGING OPPORTUNITY FOR TRANSLATIONAL BIOMARKERS
Philip Milliken, GlaxoSmithKline, Ware, UK

10:30 – 12:00 | Pické III
Parallel Session 16. Novel Aspects in Cytochrome P450 Catalysis and Dynamics
Co-Chairs: James Halpert, Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, CA, USA; Paul R. Ortiz de Montellano, University of California, San Francisco, CA, USA

10:30 – 11:00
S56 - STRUCTURE AND DYNAMICS OF CYTOCHROME P450 FROM MOLECULAR SIMULATION
Chris Oostenbrink, University of Natural Resources and Life Sciences, Vienna, Austria

11:00 – 11:30
S57 - UNDERSTANDING AND PREDICTION OF CYTOCHROME P450 MEDIATED METABOLISM
Lars Olsen, University of Copenhagen, Copenhagen, Denmark

11:30 – 12:00
S58 - LABORATORY EVOLUTION OF REGIO- AND STEREOSELECTIVE P450 ENZYMES
Manfred T. Reetz, Philipps-Universität Marburg, Marburg, Germany and Max-Planck-Institut für Kohlenforschung, Mülheim, Germany

12:00 – 13:00 | La Terrasse and Keizerzaal Foyer
Lunch Break, View Awards Finalist Posters

13:00 – 14:30 | Pické I + II
Parallel Session 17. Omics and Bioinformatics in Drug Efficacy and Safety
Co-Chairs: Magnus Ingelman-Sundberg, Karolinska Institutet, Stockholm, Sweden; Laura Suter-Dick, F. Hoffmann-La Roche, Ltd., Basel, Switzerland

13:00 – 13:30
S59 - INTRODUCTION AND INTEGRATED ANALYSIS OF OMICS (GENETICS, PROTEOMICS, METABOLOMICS)
Laura Suter-Dick, F. Hoffmann-La Roche Ltd., Basel, Switzerland

13:30 – 14:00
S60 - EXPANDING THE BOUNDARIES OF TOXICOGENOMICS
Ivan Rusyn, University of North Carolina, Chapel Hill, NC, USA

14:00 – 14:30
S61 - PRACTICAL APPLICATIONS OF GENOMICS AND NEXT GENERATION SEQUENCING IN DRUG AND CHEMICAL DEVELOPMENT
Tim Gant, University of Leicester, Leicester, UK

13:00 – 14:30 | Pické III
Parallel Session 18. Reactive Metabolites and Drug Safety: Predictive, Mechanistic, and Translational Aspects
Co-Chairs: Nico P.E. Vermeulen, VU Amsterdam, LACDR, Amsterdam, the Netherlands; Thomas A. Baillie, University of Washington, Seattle, WA, USA

13:00 – 13:30
S62 - METABOLIC ACTIVATION AND DRUG-INDUCED TOXICITY: NEW INSIGHTS FROM COVALENT DRUGS
Thomas A. Baillie, University of Washington, Seattle, WA, USA

13:30 – 14:00
S63 - WAYS TO ADDRESS CHEMICALLY REACTIVE METABOLITES DURING DRUG DEVELOPMENT
Ian Knemeyer, Merck Research Laboratories, Boston, MA, USA

14:00 – 14:30
S64 - DRUG BIOACTIVATION VIA REACTIVE METABOLITE FORMATION: IMPORTANCE OF SULFENIC ACIDS
Daniel Mansuy, Université Paris Descartes, Paris, France

14:30 – 14:45 | Pické I + II
Awards PresŜẏűl·ṁ2ẏ
JBC/Herb Taylor Award presented by F. Peter Guengerich and poster awards presentation moderated by Nico P.E. Vermeulan and Magnus Ingelman-Sundberg

Closing Words
Future MDO Meeting:
Uli Zanger, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany
Future ISSX Meetings: *Bill Smith, Pfizer Inc., La Jolla, CA, USA*

Poster Awards Abstract Presentation Schedule | Keizerzaal Foyer

Category	Set-up Time	Poster Viewing	Authors Present	Dismantle Time
GRADUATE/ PREDOCTORAL	Monday, June 18 07:30 – 08:30	Monday, June 18 – Thursday, June 21	Monday, June 18 13:00 – 14:00	Thursday, June 21 17:30 – 18:30
POSTDOCTORAL	Monday, June 18 07:30 – 08:30	Monday, June 18 – Thursday, June 21	Tuesday, June 19 13:00 – 14:00	Thursday, June 21 17:30 – 18:30

Poster Abstract Presentation Schedule | Keizerzaal + Foyer

Day	Set-up Time	Poster Viewing	Authors Present	Dismantle Time
Monday, June 18 **POSTER SESSION 1**	07:30 – 08:30	10:00 – 16:00	**Abstracts P1–P50** 12:00 – 12:30 **Abstracts P51–P100** 12:30 – 13:00	17:30 – 18:30
Tuesday, June 19 **POSTER SESSION 2**	07:30 – 08:30	10:00 – 17:00	**Abstracts P101–P153** 12:00 – 12:30 **Abstracts P154–P205** 12:30 – 13:00	17:30 – 18:30
Wednesday, June 20 **POSTER SESSION 3**	07:30 – 08:30	10:00 – 16:00	**Abstracts P206–P252** 12:00 – 12:30 **Abstracts P253–P297** 12:30 – 13:00	17:30 – 18:30

OUT OF COURTESY TO THE AUTHORS, PLEASE REFRAIN FROM TAKING PHOTOGRAPHS OR VIDEO RECORDING.

Please note: *Posters will be attended during designated presentation times. This is your opportunity to ask authors about their research. To obtain a copy of specific information that is being presented, contact the author directly or access the QR Code on the poster if one appears.*

Poster Details

GRADUATE/PREDOCTORAL POSTER FINALISTS (A1 – A6)

A1 - HLA-B*5701-RESTRICTED ACTIVATION OF FLUCLOXACILLIN-RESPONSIVE CD8+ T-CELLS
Manal Monshi, The University of Liverpool, Liverpool, United Kingdom

A2 - IMPACT OF CHRONIC RENAL FAILURE ON THE EXPRESSION AND ACTIVITY OF BRAIN CYTOCHROME P450 IN RATS: IMPLICATION OF PARATHYROID HORMONE
Judith Naud, Centre de recherche de l'Hopital Maisonneuve-Rosemont, Montreal, QC, Canada

A3 - ZEBRAFISH: AN IN VIVO HIGH-THROUGHPUT SCREENING MODEL FOR CYP3A4 INDUCTION AND INHIBITION
Hui Ting Chng, National University of Singapore, Singapore, Singapore

A4 - EPIGENETIC REGULATION IS A CRUCIAL FACTOR FOR DEFECTIVE EXPRESSION OF UGT1A1 IN HUMAN KIDNEY
Shingo Oda, Kanazawa University, Kanazawa, Japan

A5 - UDP-GLUCURONOSYLTRANSFERASE (UGT) 2B7 AND 1A9 SUPPRESS CYTOCHROME P450 3A4 FUNCTION: EVIDENCE FOR THE INVOLVEMENT OF THE CYTOSOLIC TAIL OF UGT IN THE SUPPRESSION
Yuu Miyauchi, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

A6 - UNEXPECTED SPECIES DIFFERENCES AND STRUCTURAL DETERMINANTS OF STATIN TRANSPORT BY SODIUM-TAUROCHOLATE CO-TRANSPORTING POLYPEPTIDE (NTCP/SLC10A1)
Marianne K. DeGorter, The University of Western Ontario, London, ON, Canada

POSTDOCTORAL POSTER FINALISTS (A7 – A12)

A7 - EVIDENCE FOR HNRNP L AS A SPECIFIC TRANSCRIPTION FACTOR OF THE CYP2C19*17 ALLELE
Isa Cavaco, CBME/IBB, University of Algarve, Faro, Portugal

A8 - MODULATION OF HEPATIC TRANSPORTER GENES BY CITROBACTER RODENTIUM INFECTION
Matthew D. Merrell, Emory University, Atlanta, GA, USA

A9 - THE TOXTRACKER ASSAY: NOVEL GFP REPORTER SYSTEMS THAT PROVIDE MECHANISTIC INSIGHT INTO THE GENOTOXIC PROPERTIES OF CHEMICALS
Giel Hendriks, Leiden University Medical Center, Leiden, Netherlands

A10 - CLINICAL RELEVANCE OF DRUG-DRUG INTERACTIONS WITH ORAL ANTIDIABETIC DRUGS OCCURRING AT THE LEVEL OF OATP1B1
Evita van de Steeg, TNO, Zeist, Netherlands

A11 - COMPARISONS OF CYP2A6 GENOTYPE AND ENZYME ACTIVITY BETWEEN SWEDES AND KOREANS
Natasa Djordjevic, Medical Faculty, University of Kragujevac, Kragujevac, Serbia

A12 - PHYSIOLOGICALLY BASED MODELING OF FIRST PASS METABOLISM IN THE GUT WALL – ESTABLISHMENT AND PRELIMINARY VALIDATION OF IN VITRO-IN VIVO SCALING FACTORS FOR INTESTINAL METABOLISM IN BEAGLE DOG
Aki T. Heikkinen, Hoffmann-La Roche AG, pRED, Pharma Research & Early Development, Basel, Switzerland

ANALYTICAL (P1 – P5)

P1 - METABOLITE PREDICTION IN THE IHUMITE WORKFLOW FOR FIRST-IN-MAN AND PRECLINICAL COVERAGE STUDIES
Peter L. Jacobs, MSam, Oss, Netherlands

P2 - ENSURING ANALYTICAL QUALITY IN A HT-ADME ENVIRONMENT: TIERED LC-MS/MS SUPPORT AND SUPPORTING INFORMATION SYSTEMS PLATFORMS
Graeme T. Clark, Cyprotex, Macclesfield, United Kingdom

P3 - AN LC-MS/MS ASSAY FOR THE DETERMINATION OF ZEYLENONE IN PLASMA AFTER STABILIZED BY THE PRESENCE OF ESTERASE INHIBITORS
Qi Chang, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

P4 - HIGH-THROUGHPUT ANALYSIS OF 1'-HYDROXYMIDAZOLAM IN PLASMA USING ULTRA-FAST SPE/MS/MS
Vaughn P. Miller, Agilent Technologies, Wakefield, MA, USA

P5 - DEVELOPMENT AND VALIDATION OF BIOASSAYS FOR THE DEVELOPMENT OF BIOTHERAPEUTICS
Massimo Breda, Accelera Srl, Nerviano - Milano, Italy

BIOAVAILABILITY (P6 – P10)

P6 - PRECLINICAL PHARMACOKINETICS OF DECURSINOL
Jin Sook Song, Korea Research Institute of Chemical Technology, Dae jeon, South Korea

P7 - PHARMACOKINETICS AND BIOAVAILABILITIES OF TWO DAMMARANE SAPOGENINS IN RATS
Qi Chang, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

P8 - HUMAN SMALL INTESTINAL AND COLONIC TISSUE MOUNTED IN THE USSING CHAMBER AS A TOOL FOR CHARACTERIZING THE INTESTINAL ABSORPTION OF DRUGS
Veronika Rozehnal, Daiichi-Sankyo Europe, München, Germany

P9 - EC FP7 HEALTH - 2007- GRANT PREDICTIV: THE STRATEGY APPLIED TO MEASURE BIOKINETIC PARAMETERS AND THEIR CRUCIAL ROLE IN IN VITRO TESTING
Lysiane Richert, KaLy-Cell, Strasbourg, France

P10 - EC FP7 HEALTH - 2007- GRANT PREDICTIV: PROFILING THE TOXICITY OF NEW DRUGS: A NON-ANIMAL-BASED APPROACH INTEGRATING TOXICODYNAMICS AND BIOKINETICS – EVALUATION OF THE EXPOSURE OF PRIMARY RAT AND HUMAN HEPATOCYTES TO IBUPROFEN AFTER SINGLE OR REPEATED TREA
Lysiane Richert, KaLy-Cell, Strasbourg, France

CLEARANCE PREDICTION (P11 – P16)

P11 - PREDICTION OF HUMAN PHARMACOKINETICS BY ALLOMETRIC SCALING USING CHIMERIC MICE WITH HUMANIZED LIVER
Seigo Sanoh, Hiroshima University, Hiroshima, Japan

P12 - RELATIONSHIP OF METABOLIC STABILITIES IN CARBOXYLESTERASE AND STRUCTURES OF ACETAMINOPHEN CARBOXYLIC ESTERS
Zhaoming Liu, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

P13 - BINDING OF BOVINE SERUM ALBUMIN TO FATTY ACIDS: IMPROVED THE CLEARANCE PREDICTION FOR CYP1A2 SUBSTRATE
Nitsupa Wattanachai, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

P14 - USING RECOMBINANTLY EXPRESSED HUMAN URIDINE 5'-DIPHOSPHO-GLUCURONOSYLTRANSFERASE (UGT) ENZYMES TO PREDICT TOTAL BODY CLEARANCE FOR SIX MERCK COMPOUNDS
Christopher Ray Gibson, Merck & Co Inc, West Point, PA, USA

P15 - TEMPORAL DEGRADATION OF ENZYME ACTIVITY IN HUMAN CRYOPRESERVED HEPATOCYTE SUSPENSIONS AND PLATED CULTURES
Julie A. Price, LifeTechnologies, Paisley, United Kingdom

P16 - INVESTIGATION OF HEPATIC CLEARANCE OF DRUGS IN CHIMERIC MICE WITH HUMANIZED LIVER
F. Bree, Xenoblis, Saint Grégoire, France

CONJUGATION REACTIONS AND ENZYMES

(P17 – P29, P211)

P17 - ALTERATION IN THE FUNCTION OF THE UDP-GLUCURONOSYLTRANSFERASE 1A SUBFAMILY BY CYTOCHROME P450 3A4: DIFFERENT SUSCEPTIBILITY OF UGT ISOFORMS AND UGT1A7 VARIANTS
Yuji Ishii, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

P18 - EFFECTS OF UGT2B SUBSTRATES AND INHIBITORS ON R- AND S- LORAZEPAM GLUCURONIDATION BY HUMAN LIVER MICROSOMES
Verawan Uchaipichat, Faculty of Pharmaceutical Sciences, Khon Kaen, Thailand

P19 - EFFECTS OF THE DISRUPTION OF *GLUTATHIONE S-TRANSFERASE MU 1* GENE AND GLUTATHIONE DEPLETION IN 1,2-DICHLORO-4-NITROBENZENE-INDUCED TOXICITY IN MICE
Shingo Arakawa, Daiichi Sankyo Co., Ltd., Fukuroi, Shizuoka, Japan

P20 - EFFECT OF PHENOBARBITAL TREATMENT ON UDP-GLUCURONOSYLTRANSFERASE 1A SUBFAMILY IN RAT BRAIN
Yukiko Sakakibara, Meijo University, Nagoya, Japan

P21 - THE ACTIVITIES AND EXPRESSION OF HEPATIC GLUTATHIONE S-TRANSFERASES AND CARBONYL REDUCTASE 1 IN YOUNG AND OLD MALE RATS
Lenka Skalova, Charles University, Faculty of Pharmacy, Hradec Kralove, Czech Republic

P22 - SYNTHETIC STILBENOIDS: NOVEL SUBSTRATES FOR HUMAN HEPATIC, RENAL, AND INTESTINAL UDP-GLUCURONOSYLTRANSFERASES
Anna Radominska-Pandya, University of Arkansas for Medical Science, Little Rock, AR, USA

P23 - THE EFFECT OF C-4 SUBSTITUTIONS ON ESCULETIN METABOLISM: DIFFERENCES IN METABOLIC PROFILE, UGT ISOFORM SELECTIVITY AND METABOLIC RATE
Ling Yang, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

P24 - THE "ALBUMIN EFFECT" IN IN VITRO STUDIES OF UGTS 1A7, 1A8, AND 1A10
Nenad Manevski, Faculty of Pharmacy, Helsinki University, Helsinki, Finland

P25 - SELECTIVE CONJUGATION OF 7-HYDROXYCOUMARIN BY RECOMBINANT HUMAN URIDINE 5'-DIPHOSPHO-GLUCURONOSYLTRANSFERASE (UGT)
Roberto Tolando, Celsis In Vitro Technologies GmbH, Neuss, Germany

P26 - UGT2B7 IS RESPONSIBLE FOR HEPATIC GLUCURONIDATION OF DIETHYLSTILBESTROL (DES) AND DES IN TURN POTENTLY INHIBITS CATALYTIC ACTIVITIES OF MANY UGT ENZYMES
Liangliang Zhu, Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

P27 - PROTEIN S-GLUTATHIONYLATION: A ROLE FOR GLUTATHIONE S-TRANSFERASE PI IN MEDIATING CYTOPROTECTION?
David J. McGarry, University of Dundee, Dundee, United Kingdom

P28 – ABSTRACT WITHDRAWN

P29 - SOME PHASE II METABOLITES OF LU AA21004 (1-[2-(2,4-DIMETHYL-PHENYLSULFANYL)-PHENYL]-PIPERAZINE) AND THEIR FORMATION
Lars Dalgaard, H Lundbeck A/S, Copenhaven-V, Denmark

P211 - A PROTEIN TYROSINE KINASE INHIBITOR, GEFITINIB, INHIBITS UDP-GLUCURONOSYLTRANSFERASE 1A9 (UGT1A9) BY BINDING TO THE ALLOSTERIC SITE SHARED WITH ADENINE NUCLEOTIDES
Yuji Ishii, Labratory of Molecular Life Sciences, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

CYTOCHROME P450 (P30 – P38, P40 – P67, P213)

P30 - EVALUATION OF THE MODULATORY EFFECT OF LABISIA PUMILA EXTRACTS ON CYTOCHROME P450 2C8 IN VITRO
Yan Pan, International Medical University, Kuala Lumpur, Malaysia

P31 - MOLECULAR STUDIES ON INHIBITORY EFFECTS OF EPILOBIUM HIRSUTUM L. ON DRUG METABOLIZING CYP2B1, CPY2C6, CYP2D2 AND CYP3A4 ENZYMES IN RAT LIVER MICROSOMES
Serdar Karakurt, METU, Ankara, Turkey

P32 - FORMATION OF DIHYDROXYLATED AND GLUTATHIONE CONJUGATE METABOLITES DERIVED FROM THALIDOMIDE AND 5-HYDROXYTHALIDOMIDE IN HUMANIZED TK-NOG MICE
Hiroshi Yamazaki, Showa Pharmaceutical Univ, Machida, Japan

Poster Details

P33 - CHARACTERIZATION OF CYNOMOLGUS P450 ENZYMES IN THE INTESTINE AND LIVER USING TYPICAL SUBSTRATES AND INHIBITORS FOR HUMAN P450 ENZYMES
Noriaki Yoda, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

P34 - LOW POTENTIAL FOR DRUG INTERACTION OF CAPPARIS OVATA WATER EXTRACT
Alaattin Sen, Pamukkale University, Kinikli, Turkey

P35 - PROBING THE MECHANISM OF NICOTINE DEGRADATION BY MAMMALIAN CYTOCHROME P450 MONOOXYGENASES
Marcel Delaforge, CNRS, Gif sur Yvette, France

P36 - DOWN-REGULATION OF HUMAN CYTOCHROME P450 1A1 AND 1B1 BY SINGLE-WALLED CARBON NANOTUBE
Miki Katoh, Meijo University, Nagoya, Japan

P37 - ENHANCED EXPRESSION OF CYTOCHROME P450 GENES BY HEPATOCYTE NUCLEAR FACTOR-6 IN HEPATOCYTE-LIKE CELLS DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS
Shogo Takahashi, Tohoku Pharmaceutical University, Sendai, Japan

P38 - DRUG INTERACTION AND CARCINOGEN ACTIVATING POTENTIAL OF O-COUMARIC ACID
Sevki Arslan, Pamukkale University, Denizli, Turkey

P40 - D2-DOPAMINERGIC RECEPTOR LINKED PATHWAYS: CRITICAL REGULATORS OF CYP3A, CYP2C AND CYP2D
Maria Konstandi, University of Ioannina, School of Medicine, Ioannina, Greece

P41 - PHENOTYPIC DIFFERENCES OF HUH7 CELLS INDUCED DURING CONFLUENCE: BASIS FOR PXR MEDIATED INDUCTION OF CYP3A4 EXPRESSION
Louise Sivertsson, Karolinska Institutet, Stockholm, Sweden

P42 - SIMULTANEOUS EVALUATION OF INFLUENCE OF SODIUM TANSHINON IIASILATE ON CYP450 ISOFORMS BY COCKTAIL PROBE DRUGS
Jianjie Jiao, Tianjin Medical University, Tianjin, China

P43 - CLONING AND CHARACTERISATION OF THE SECOND TAMMAR WALLABY (MARCOPUS EUGENII) CYP4B SUBFAMILY MEMBER, CYP4B3
Natalie L. Milic, Charles Darwin University, Darwin, Australia

P44 - CHARACTERIZATION OF DRUG METABOLIZING CYP102A1 MUTANTS GENERATED BY DOMAIN-EXCHANGE AND DIRECTED EVOLUTION
Ji-Yeon Kang, Chonnam National University, Gwang-Ju, South Korea

P45 - PEROXIDE-DEPENDENT OXIDATION OF VARIOUS SUBSTRATES CATALYZED BY CYP191A1 FROM MYCOBACTERIUM SMEGMATIS
Hye Yeong Jo, Chonnam National University, Gwangju, South Korea

P46 - GENERATION OF HUMAN METABOLITES OF ATORVASTATIN AND FLUVASTATIN BY BACTERIAL CYP102A1 MUTANTS
GunSu Cha, Chonnam National University, Gwang-Ju, South Korea

P47 - GENERATION OF 5'-HYDROXYOMEPRAZOLE BY CYP102A1 MUTANTS FROM BACILLUS MEGATERIUM
Hyeong In Im, Chonnam National University, Gwangju, South Korea

P48 - FLAVIN CONTAINING REDUCTASE DOMAIN OF CYTOCHROME P450 BM3 AS A SURROGATE FOR MAMMALIAN NADPH-P450 REDUCTASE
Bo-Yeon Park, Chonnam national university, Gwangju, South Korea

P49 - IDENTIFICATION OF NOVEL CYTOCHROME P450S IN THE ACARI
Kirsty Graham, Northumbria University, Newcastle-Upon-Tyne, United Kingdom

P50 - ROSUVASTATIN IN HIGH CHOLESTEROL DIET SUPRESSES CYP2C11 AND CYP2C6 IN RATS
Alice Zacharova, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

P51 - MOLECULAR MECHANISMS OF CYTOCHROME P450 CYP2S1 REGULATION BY CHEMICALS, ULTRAVIOLET RADIATION (UVR) AND OXIDATIVE STRESS
Gillian Smith, University of Dundee, Dundee, United Kingdom

P52 - BIOACTIVATION OF CLOZAPINE - INVOLVEMENT OF HUMAN CYTOCHROME P450S
Sanja Dragovic, Vrije Universiteit, Amsterdam, Netherlands

P53 - PHOTO-CYTOCHROME B5 – A NEW MEMBRANE CROSS-LINKING TOOL
Petr Hodek, Charles University in Prague, Prague, Czech Republic

P54 - EFFICIENT SCREENING OF P450 BM3 MUTANTS TO METABOLIZE DRUG-LIKE MOLECULES
Jelle Reinen, Vrije Univ, Amsterdam, Netherlands

P55 - 4-HYDROXY TRANS-RETINOIC ACID: ONE OF MAJOR AND UNIQUE METABOLITES FROM T-RA IN THE LIVER OF FETAL CYP3A TRANSCHROMOSOMIC MICE
Yasuko Tsukazaki, Mitsubishi Chemical Medience Corporation, Tsukuba, Japan

P56 - FUNCTIONAL CHARACTERIZATION OF EIGHT HUMAN CYP1A2 VARIANTS: THE ROLE OF CYTOCHROME B_5
Michel Kranendonk, Universidade Nova de Lisboa, Fac Medical Sciences, Lisbon, Portugal

P57 - CYTOCHROME P450 ACTIVE SITE ACCESS AND EGRESS PATHS OPENINGS DIFFER IN THEIR MEMBRANE POSITIONS
Michal Otyepka, Palacky University Olomouc, Olomouc, Czech Republic

P58 - WHAT IS THE CRUCIAL BINDING FEATURE FOR METABOLIC SPECIFICITY OF BIOSYNTHETIC MONOOXYGENASES CYTOCHROME P450 17?
Chun-zhi Ai, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

P59 - INTERACTIONS OF INHIBITOR MOLECULES WITH THE HUMAN CYP2E1 ENZYME ACTIVE SITE
Laura E. Martikainen, University of Eastern Finland, Kuopio, Finland

P60 - IN VIVO EFFECT OF EPILOBIUM HIRSUTUM L. ON PROTEIN AND MRNA EXPRESSIONS OF RAT LIVER VITAMIN D3 METABOLIZING CYP27B1 AND CYP24A1 ENZYMES
Serdar Karakurt, METU, Ankara, Turkey

P61 - A COMPARISON OF INDUCTION OF CYP2E1 IN THE RABBIT LIVER AND KIDNEY BY ACRYLAMIDE
Emel Arinç, Middle East Technical University, Ankara, Turkey

P62 - CYTOCHROME P450 1A1 STRUCTURE AND UTILITY IN PREDICTING DRUG AND XENOBIOTIC METABOLISM
Grazyna D. Szklarz, West Virginia University, Morgantown, WV, USA

P63 - THE MAMMALIAN CYP51: FROM MOUSE KNOCKOUT MODELS TO GENOTYPING IN HUMANS
Damjana Rozman, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

P64 - EFFORTS TO IDENTIFY ELECTRON DONORS TO ER LUMEN ORIENTED CYP2W1: A PILOT STUDY
Jia Guo, Karolinska Institutet, Stockholm, Sweden

P65 - IDENTIFICATION AND FUNCTIONAL STUDIES OF CYTOCHROME P450S IN HUMAN PLATELETS
Su-Jun Lee, Inje University College of Medicine, Busan, South Korea

P66 - COMPARISON OF CYP3A4 AND CYP3A5: THE EFFECTS OF CYTOCHROME B5 AND NADPH-CYTOCHROME P450 REDUCTASE ON TESTOSTERONE HYDROXYLATION ACTIVITIES
Su-Jun Lee, Inje University College of Medicine, Busan, South Korea

P67 - EFFECT OF AZOLE DRUGS ON ACTIVITY OF OXYSTEROL-7ALPHA-HYDROXYLASE, CYP7B1
Andrei A. Gilep, Institute of Bioorganic Chemistry NASB, Minsk, Belarus

P213 - STRUCTURAL RATIONALIZATION OF REGIO- AND STEREO-SELECTIVE HYDROXYLATION BY DRUG-METABOLIZING CYTOCHROME P450 BM3 MUTANTS
Daan P. Geerke, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

DIFFERENCES IN METABOLISM (SPECIES, GENDER, AGE, DISEASES) (P68 – P79, P253)

P68 - METABOLIC INVESTIGATION OF URINE AND PLASMA PROFILES OBTAINED FROM ALCOHOL-DOSED MICE USING ACCURATE MASS LC/MS/MS
Stephanie N. Harden, Waters AG, 5405 Baden-Dättwil, Switzerland

P69 - SELECTING THE MOST RELEVANT NON-RODENT SPECIES FOR NON-CLINICAL ASSESSMENT: A DECISION TOOL COMPARING MINIPIG, DOG AND MONKEY TO MAN
Lars Dalgaard, LD ADME Consult, Roslev, Denmark

P70 - HYPERVARIABLE CYTOCHROME P450 ACTIVITY IN CHILDREN WITH SEVERE HEPATIC DYSFUNCTION
Lies De Bock, Ghent University, Gent, Belgium

P71 - NOVEL CYP 3A KNOCKOUT CHIMERIC MOUSE WITH HUMANIZED LIVER AND METABOLIC PROFILING OF NEFAZODONE FOR ACCURATE PRE-CLINICAL HUMAN PREDICTION
Chise Tateno, PhoenixBio Co., Ltd., Higashihiroshima, Japan

P72 - SEX DIFFERENCES IN PROPOFOL BIOTRANSFORMATION, CLINICAL IMPLICATIONS
Eva Choong, Karolinska Institutet, Stockholm, Sweden

P73 - THE INABILITY OF BARBER'S POLE WORM (HAEMONCHUS CONTORTUS) TO METABOLIZE IVERMECTIN
Barbora Szotakova, Charles University, Faculty of Pharmacy, Hradec Kralove, Czech Republic

P74 - DETERMINANTS OF CYP3A4 EXPRESSION AND METABOLIC ACTIVITY IN THE HUH7 HUMAN HEPATOMA CELL MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE
Sarah J. Woolsey, The University of Western Ontario, London, ON, Canada

P75 - COMPARISON OF GLIBENCLAMIDE METABOLISM IN HUMAN AND MOUSE LIVER MICROSOMES
Selvan Ravindran, Sai Advantium Pharma Limited, Pune, India

P76 - COMPARATIVE METABOLISM OF AILDENAFIL IN MOUSE, RAT, DOG AND HUMAN LIVER MICROSOMES
Duan Yun Si, Tianjin Institute of Pharmaceutical Research, Tianjin, China

P77 - DISPOSITION OF FLUOXETINE IN NEWBORN LAMBS UP TO 1 YEAR OF AGE
Timothy Chow, University of British Columbia, Vancouver, BC, Canada

P78 - SPECIES-SPECIFIC IN VITRO GLUCURONIDATION OF THE MYCOTOXIN DEOXYNIVALENOL
Christiane K. Fæste, Norwegian Veterinary Institute, Oslo, Norway

P79 - VARIATIONS IN HUMAN DRUG AND STEROID METABOLISM CAUSED BY MUTATIONS IN NADPH P450 OXIDOREDUCTASE
Amit V. Pandey, University of Bern, Bern, Switzerland

P253 - USE OF HUMANIZED MOUSE MODELS IN DRUG-DRUG INTERACTION, PHARMACOKINETIC AND SAFETY TESTING OF COMPOUNDS
Nico Scheer, TaconicArtemis, Köln, Germany

DISPOSITION (P80)

P80 - ORAL ABSORPTION AND EXCRETION OF ICARITIN, AN AGLYCONE AND ALSO ACTIVE METABOLITE OF PRENYLFLAVONOIDS FROM THE CHINESE MEDICINE HERBA EPIMEDII, IN RATS
Qi Chang, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

DRUG DISCOVERY AND DEVELOPMENT (P81 – P84)

P81 - RAPID QUANTITATIVE METHOD DEVELOPMENT FOR DRUG METABOLITES (DMS) IN HUMAN PLASMA USING ISOTOPE DILUTION
Jim Vrbanac, MPI Research, Mattawan, MI, USA

P82 - SELECTION OF DRUG CANDIDATES USING RAPID PK SCREENING
Mira Wenker, WIL Research, Hertogenbosch, Netherlands

P83 - A NOVEL APPROACH TO THE STORAGE AND RETRIEVAL OF INFORMATION IN XENOBIOTIC METABOLISM STUDIES TO IMPROVE DECISION MAKING AND KNOWLEDGE SHARING BETWEEN SCIENTISTS, FACILITIES AND LOCATIONS
Mark D. Wrona, Waters Corporation, Milford, MA, USA

P84 - THE INFLUENCE OF DACARBAZINE ON HEMATOLOGICAL AND HISTOLOGICAL CHANGES IN HAMSTERS WITH FIBROSARCOMA
Boris Milijasevic, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia, Novi Sad, Serbia

DRUG INTERACTION (P85 – P93)

P85 - ANALYSIS OF THE REPAGLINIDE CONCENTRATION INCREASE BY GEMFIBROZIL AND ITRACONAZOLE BASED ON THE INHIBITION OF HEPATIC UPTAKE TRANSPORTER AND METABOLIC ENZYMES
Kiyomi Ito, Musashino University, Tokyo, Japan

P86 - VALIDATION OF AN IN VIVO PHENOTYPING COCKTAIL FOR THE EARLY DETECTION OF THE INDUCTIVE OR INHIBITORY POTENTIAL OF NEW DRUGS
Nicoline Treijtel, Kinesis-Pharma, Breda, Netherlands

P87 - A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING APPROACH FOR REPAGLINIDE CLEARANCE AND DRUG-DRUG INTERACTIONS: IMPACT OF IN VITRO SYSTEM AND ROLE OF TRANSPORTER-METABOLISM INTERPLAY
Carolina Säll, Centre for Applied Pharmacokinetic Research, University of Manchester, Manchester, United Kingdom

P88 - VALIDATION OF ENDOGENOUSLY EXPRESSED OCT2 IN HUMAN RENAL PROXIMAL TUBULAR CELL MODEL AS A TOOL FOR COMPOUND SCREENING
Martijn J. Wilmer, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

P89 - ANTHOCYANIDIN PELARGONIDIN DOES NOT INHIBIT HUMAN LIVER CYTOCHROMES P450 AT PHYSIOLOGICALLY RELEVANT CONCENTRATIONS
Eva Anzenbacherova, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic

P90 - APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING AND SIMULATION FOR WAIVER OF ORTERONEL DRUG-DRUG INTERACTION TRIALS
Chuang Lu, Millennium Pharmaceuticals, Inc., Cambrdge, MA, USA

P91 - MANAGING DIGOXIN DRUG INTERACTION POTENTIAL BY RECEIVER OPERATING CHARACTERISTICS ANALYSIS
H. Wortelboer, TNO, Zeist, Netherlands

P92 - WHAT IS THE CONTRIBUTION OF CYP2B6 TO BUPROPION METABOLIC CLEARANCE? IMPLICATIONS FOR THE PREDICTION OF CYP2B6 MEDIATED DRUG-DRUG INTERACTIONS
Z. E. Barter, Simcyp Ltd, Sheffield, United Kingdom

P93 - CLASSIFICATION OF 331 DRUGS INVOLVED IN SIGNIFICANT PHARMACOKINETIC DRUG-DRUG INTERACTIONS
Akihiro Hisaka, The University of Tokyo Hospital, Tokyo, Japan

ENZYME INDUCTION (P94 – P100)

P94 - REACTIVE OXYGEN SPECIES SCAVENGING AND DETOXIFICATION POTENTIALS OF BLIGHIA SAPIDA POLYPHENOLIC EXTRACT IN RAT MICROSOMES
Taofeek O. Ajiboye, Fountain University, Osogbo, Nigeria

P95 - COMPLEX EFFECTS OF DIETARY PHYTOCHEMICAL SULFORAPHANE ON CYP3A4 GENE EXPRESSION
Tomas Smutny, Charles University in Prague, Hradec Kralove, Czech Republic

P96 - PLATEABLE CRYOPRESERVED HUMAN HEPATOCYTES FOR THE ASSESSMENT OF CYTOCHROME P450 INDUCIBILITY: EXPERIMENTAL CONDITION-RELATED VARIABLES AFFECTING THEIR RESPONSE TO INDUCERS
Lysiane Richert, KaLy-Cell, Strasbourg, France

P97 - A TRANSGENIC MOUSE MODEL TO STUDY EXPRESSION OF THE HUMAN GLUTATHIONE TRANSFERASE P GENE
Colin Henderson, University of Dundee, Dundee, United Kingdom

P98 - EVALUATION OF CYP3A4 INDUCTION BIOMARKERS: A CASE STUDY OF A CANDIDATE DRUG
Jan Snoeys, Janssen Research and Development, Beerse, Belgium

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Bo Feng, Department of Pharmacokinetics, Dynamics & Metabolism, Pfizer Inc., Groton, CT, USA

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Tuesday, June 19, 2012

7:20 – 8:20 | Pické III
Presented by Waters Corporation
The Evolving Role of Exact Mass MS for Quantification in DMPK Studies
Mark Wrona, Senior Applications Specialist, Pharmaceutical & Life Sciences, Waters Corporation

The objective of this symposium is to provide the attendees with an understanding of the strength and weakness of the different mass spectrometry systems for DMPK studies and how to best design the experiments to elucidate the information desired. The information disseminated will allow the attendees to perform their studies more efficiently and gain a greater understanding of the fate of the molecules being tested.

Wednesday, June 20, 2012

7:20 – 8:20 | Pické III
Presented by Covance
Integrated Metabolism Investigations to Streamline Compound Development
1: Radiolabelled ADME package
2: Robust early metabolite investigation
Dr. David Lankester
Ms. Caroline Anderson, Covance

The objective of the two presentations in this session is to highlight the advantages of integrating Metabolism investigations to reduce timelines whilst generating data to support compound development and in line with current regulatory guidance. The first part of the presentation will focus on defining and executing radiolabelling strategies to ensure successful and timely completion of metabolism investigations. The scope of the radiolabelled ADME studies (non-clinical & clinical) required to answer compound specific questions will be included. The second part of the presentation will focus on iterative approaches to metabolite investigations using samples from in vitro, non-clinical and clinical studies. Consideration of the ICH M3 and FDA Safety Testing of Metabolites guidance will be included. Using specific examples, illustrations of compound specific questions and how they can be answered will be presented.

Thursday, June 21, 2012

7:20 – 8:20 | Pické III
Presented by Thermo Scientific
Tools to Accelerate Discovery DMPK – Better Answers, Faster.
Tim Stratton, Thermo Scientific

This seminar will educate the meeting attendees regarding the latest analytical LCMS workflow applications for DMPK, including metabolite identification, structural elucidation and quantitation, and how the newly available tools will help researchers improve and accelerate their Xenobiotics studies.

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Cypex

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Booth 17

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SafeSciMET
Table-top in Foyer

SafeSciMET is a pan-European education and training network, which solves a shortfall in current drug safety education and training in Europe where an integrative and translational approach is lacking. The consortium consists of top academic institutions for drug safety education and research and Industrial pharmaceutical companies, all members of EFPIA. The network is supported by the Innovative Medicines Initiative Joint Undertaking (IMI-JU).

Exhibitor Directory

Simulations Plus, Inc.
Booth 41

Modeling and simulation software programs and consulting services - GastroPlus, DDDPlus, ADMET Predictor, MedChem Studio, and MedChem Designer - are used across discovery and development for data mining and drug design, ADME-Tox property prediction, simulation of in vitro dissolution experiments, and prediction of absorption/PBPK/PD/DDI for humans and animals through various administration routes.

SOLVO Biotechnology
Booth 43

SOLVO Biotechnology has outstanding knowledge on transporters, the widest drug-transporter assay portfolio and the largest R&D Department among its competitors. SOLVO continuously expands its product and service portfolio (with 10-15 new launches annually), based on the latest results in transporter science, the needs of the pharmaceutical industry and the various regulatory agencies such as FDA and EMA. SOLVO's mainstream products and services are in vitro and in vivo assay systems to detect the interaction of test compounds with a wide array of ABC and SLC Transporter proteins for ADME/Tox research. All major pharma and biotech companies use SOLVO's assays, either in-house or as contract research.

tebu-bio
Booth 27

Since 1998, tebu-bio has been helping ADME scientists to build an accurate picture of ADME properties, drug-drug interactions, transporter assessment and pharmacokinetics prediction throughout their studies. We select the best brands from world renowned manufacturers, guaranteeing the highest quality of service all over Europe. (www.tebu-bio.com)

Thermo Scientific
Booth 33

Thermo Fisher Scientific is the world leader in serving science, enabling our customers to make the world healthier, cleaner and safer by providing analytical instruments, equipment, reagents & consumables, software & services. We serve over 350,000 customers worldwide including pharmaceutical and biotechnology, hospitals, clinical diagnostic labs, universities, research institutes and government agencies.

TNO Triskelion
Booth 22

TNO Triskelion's Analytical Research Centre focuses on high-end expertise and advanced facilities in analytical chemistry (bioanalysis, immunogenicity, metabolite ID, LC-MS and ligand binding assays). We support our pharma partners with specific knowledge and expertise to meet their scientific objectives, budgets and timelines.

Exhibitor Directory

Triangle Research Labs
Booth 23

Triangle Research Labs focuses on in vitro and in vivo ADME, Toxicology, and Metabolic Disorders. TRL offers an array of services focused on drug metabolism, drug-drug interactions, in vivo pharmacokinetics and toxicology, drug characterization, drug transporters, diabetes research, and hepatotoxicity.

Unilabs York Bioanalytical Solutions Ltd.
Booth 3

Unilabs York Bioanalytical Solutions is a specialist provider of bioanalytical, metabolism and biomarker services to pharmaceutical and biotech companies, supporting development of small and large molecules, from discovery through to post-marketing. •Bioanalytical specialists •Experienced scientists use the latest technology to provide high quality data and reports in the timelines that you require •Our in-depth understanding of the technical and regulatory issues allows us to advise our clients, adding value to their projects •We deliver both qualitative and quantitative data providing key information to impact chemical design in early discovery and accelerate compound progression throughout drug development

Waters European Headquarters
Booth 45

For over 15 years, Waters has worked alongside the world's largest pharmaceutical companies, listening to their needs and creating innovative solutions specific to those needs. By partnering with those who lead the industry, Waters excellence in Metabolite Identification has been and will be the standard for others to follow. Learn more at www.waters.com/metID.

WIL Research Company
Booth 6

WIL Research is the global CRO which has listening down to a science. We custom design toxicological, bioanalytical, and formulation services for pharmaceutical, biotechnology, chemical, agrochemical, and food companies. With over 900 dedicated scientific, technical, and support personnel WIL Research offers technological expertise, flexible study design, and quality outcomes.

Xenotech, LLC.
Booth 26

XenoTech began as a pioneer in P450 research and has grown into an innovator of preclinical in vitro studies covering a variety of enzymes implicated in drug-drug interactions. XenoTech is dedicated to providing the highest quality in vitro drug metabolism and drug-drug interaction studies to pharmaceutical and chemical companies worldwide.

SC1 - PKPD MODELING OF CNS DRUGS, IMPLEMENTING RECEPTOR BINDING

Johannes H. Proost
Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, Netherlands

Pharmacokinetic-pharmacodynamic (PKPD) modeling tools are extensively used to characterize quantitatively the processes between drug administration and its effects. In the last decades, PKPD models with a mechanistic and physiological basis are increasingly used. These models explicitly express and separate the drug- and system- specific components contributing to the PKPD properties and drug effects, and provide an opportunity to scale model parameters and model structure between species. Such models have the potential ability to predict human PKPD properties using prior information from in vitro and preclinical studies. This will be elaborated in the lecture, using an example in the field of antipsychotic drugs. In schizophrenia drug therapy and research, dopamine D2 receptor occupancy (D2RO) is often used as a target biomarker to quantify the relationship between dose, efficacy and side effects. Target occupancy is important both in early drug discovery, where accurate knowledge of the level of occupancy could help to determine the suitability of a drug candidate for further development, and later in the drug development process, when target site occupancy measurements can guide dose selection. First, using plasma concentration, brain concentration and D2RO data from preclinical studies in rats, a mechanism- and physiologically-based PKPD model was developed to describe the time course of D2RO of several antipsychotics, with emphasis on the modeling of blood-brain-barrier transport and receptor association/dissociation kinetics. In the second stage, this model was utilized to translate the in vitro and preclinical information to D2RO in human striatum. The rat pharmacokinetic and brain physiology parameters were substituted with human population pharmacokinetic parameters and human physiological information. To predict the passive transport across the human blood brain barrier, apparent permeability values were scaled based on rat and human brain endothelial surface area. Active efflux clearance in brain was scaled from rat to human using transporter gene expression information. Binding constants at the D2 receptor were corrected based on the differences between in vitro and in vivosystems of the same species. The predictive power of this physiology-based approach was determined by comparing the D2RO predictions with the observed human D2RO of six antipsychotics at clinically relevant doses. The predicted human D2RO was in good agreement with the clinically observed D2RO for five antipsychotics. Human D2RO predictions improved when in vitro and in vivoinformation was integrated in the model. However, D2RO was underpredicted for haloperidol. This example demonstrates that a PKPD model structure developed from rat data, integrated with in vitro and preclinical in vivo model parameters and human pharmacokinetic and physiological information, constitutes a scientific basis to predict the time course of a relevant biomarker in man. Applications of this predictive tool are not limited to predicting D2RO in early drug discovery, but may also include selecting appropriate first in human doses. It is not anticipated that such predictive tools will completely replace the need for in vivo experiments, but it is plausible that this tool can help to design more informative and more efficient clinical studies.

SC2 - PKPD MODELING OF BIOLOGICS: ROLE OF TARGET MEDIATED DRUG DISPOSITION

Philip Lowe
Modeling and Simulation, Novartis Pharma AG, Basel, Switzerland

The goal of this talk is to give some examples of how drug-target binding can be quantitated based upon either assays of captured target and/or nonlinear pharmacokinetics. This drug-target binding is then used within the context of preclinical and clinical drug development. The majority of the talk will focus on pharmacokinetic-pharmacodynamic model based analyses, as there are serious interpretation issues if classical non-compartmental analyses are blindly applied to the nonlinear behaviours common with biopharmaceuticals. By incorporating aspects of mechanism into PKPD models, one gains a deeper understanding of the basis of drug disposition and pharmacology, greater biological realism and thereby better extrapolatability, or prediction, of patient responses. For biopharmaceuticals especially, mechanistic aspects of physiology and biochemistry can be linked by explicitly describing drug distribution and elimination together with drug target production (expression) and elimination, i.e. target turnover. Drug-target binding is modelled enabling target capture or occupancy to be quantitated and correlated with clinical responses. Examples will include:

- Omalizumab capturing IgE thereby alleviating the signs and symptoms of allergic asthma.
- Non-clinical to clinical translation of a monoclonal antibody with highly nonlinear pharmacokinetics characteristic of target mediated drug disposition (TMDD).

- Canakinumab capturing interleukin-1β thereby alleviating the pain and inflammation experienced by patients with cryopyrin associated periodic syndromes.

The overall objective of a PKPD modelling and simulation process could be realised, to deliver physiologically and biochemically reasonable predictions of the effectiveness of the drugs. Through simulation from the models, suitable posologies – doses and regimens – for specific groups of patients were suggested.

SC3 - TRANSLATIONAL PK-PD MODELING: TARGET ENGAGEMENT LINKING TO EFFICACY/SAFETY

Sandra A.G. Visser
Global Network Leader Non-Clinical Modeling & Simulation, Global DMPK, AstraZeneca R&D Södertälje, Södertälje, Sweden

One of the major challenges for the pharmaceutical industry is to improve phase II success. To improve this success rate, there is a large focus on developing translational biomarkers and in vivo models. To do this, we must derive a solid quantitative pharmacokinetic-pharmacodynamic (PKPD) understanding of the drug action at the target and the relationship between target engagement and the disease (efficacy) and/or safety in pre-clinical models. This will in turn provide increased confidence in interspecies translation and facilitate decision making in early Development. In this lecture, it will be discussed how quantitative pharmacology concepts can be used for translation from in vivo results to the clinical phase. We will address the issues and challenges you face in translating in vitro and in vivo data to humans with examples of PKPD models and modeling approaches in the Pain, Alzheimer and Safety research areas.

SC4 - PK-PD MODELING OF DISEASE PROCESSES AND PROGRESSION: DISEASE SYSTEM ANALYSIS

Teun M. Post
Merck Sharp & Dohme (MSD), Oss, Netherlands

An invariable status or baseline is generally assumed when investigating physiological systems. This is often an unrealistic description of a biological system, because functions may change naturally or deteriorate over time, also during the treatment period. The addition of a pertinent time-variant component adds a distinct dimension to pharmacodynamics models, turning them into disease progression models. For progressive diseases, such as osteoporosis, diabetes mellitus, Alzheimer's and Parkinson's disease, the factor time forms an important addition in establishing and differentiating treatment effects based on their mechanism of action. In this short course, various concepts and aspects of modeling disease processes and disease progression will be discussed. A separation between the disease process and the progression of the disease status is important when drugs specifically modify the time course of the disease rather than having a symptomatic effect. The identification and separation of these components within a population approach is referred to as Disease System Analysis. Knowledge about the underlying (patho)physiology then becomes an important factor in establishing the actual qualitative treatment effect on the disease progression. There are distinct levels at which the disease processes and pharmacodynamics effects can be assessed and each of these levels has its own time scale, time course and mechanistic complexity. Biomarkers and eventually clinical outcome determine the time scale and mechanistic complexity that can be captured within the analysis. Descriptive disease models separate the natural progression of the disease from the treatment effects, but typically lack a description of the underlying disease processes. In contrast, mechanism-based disease progression models make a clear separation between the disease process(es), the disease progression and the treatment effects. The mechanism-based approach takes the middle between purely empirical models and systems biology models because it takes into account the physiological pathways and their time-scales, but it also considers the ability to identify the model structure and to estimate the corresponding parameters. Overall, the participants will be provided with a basic understanding of the concepts and aspects of disease progression modeling.

SC5 - METABOLOMICS IN DRUG EFFICACY AND SAFETY EVALUATION

Johan Lindberg
Molecular profiling, Safety Assessment, AstraZeneca R&D Södertälje, Södertälje, Sweden

Within the field of metabolomics you aim for measuring a complete profile of all endogenous metabolites in a cell or organism. The obtained profile function as metabolic fingerprint for any physiological state or process of a cell or

organism and has proven value in areas such as biomarker discovery, disease state characterization and problem solving within toxicology. This lecture will give a general background on the technology. The wide diversity of the chemical structure among the endogenous metabolites and the large variation in their quantities motivate the use of several different bio-analytical platforms. Pros and cons for various analytical technologies e.g. NMR, LC (GC)-MS (/MS) and MS imaging will be discussed including their use as targeted or untargeted platforms. A major difficulty within metabolomics is the fact that many detected analytes are unidentified and structural elucidation methods including the use of databases are crucial in order to do the biological interpretation. Furthermore the profiling approach sets special demands on the data analysis. Sample running often progress of several days or even weeks and quality control, instrument and biological normalization are important steps. **Fibrodysplasia** is the most significant toxicological issue associated with the matrix metalloproteinase inhibition in the pre-clinical species. The metabolomic workflow and results will be shown using an investigational dog study where several metabolite and protein platform was used, including their integration. **Drug Induced Liver Injury (DILI)** is a major cause of attrition within safety and there is a need for new and more sensitive biomarkers. Bile acids, synthesized in the liver, are candidate biomarkers. An untargeted platform for profiling bile acids and their conjugates will be presented. Altogether more than 100 different bile acids have measured across different species and sample matrixes. Results will be shown from pre-clinical studies that show bile duct hyperplasia and from patients with DILI. MS imaging is a promising new technology which makes it possible to detect low molecular analytes directly on the tissue. Examples will be shown where it is used for problem solving of pre-clinical studies showing **crystal nephropathy** as well as a biomarker discovery tool.

SC6 - TRANSCRIPTOMICS AND EPIGENOMICS IN DRUG SAFETY

Remi Terranova[1], Harri Lempiäinen[1], Raphaelle Luisier[1], Arne Mueller[1], Philippe Couttet[1], Federico Bolognani[1], Valerie Dubost[1], Elif Unterberger[2], John Thomson[3], Albert Braeuning[2], Jay Goodman[4], Olivier Grenet[1], Pierre Moulin[1], Michael Schwarz[2], Richard Meehan[3] and Jonathan Moggs[1]

[1]Novartis Institutes for Biomedical Research, Basel, Switzerland, [2]Dept. of Toxicology, University of Tübingen, Tübingen, Germany, [3]IGMM, Western General Hospital, MRC Human Genetics Unit, Edinburgh, United Kingdom, [4]Center for Integrative Toxicology, Michigan State University, East Lansing, MI

Epigenetic mechanisms have an important role in human disease, and may also be involved in the pharmacodynamic and adverse effects associated with many drugs and toxicants. The development of powerful epigenomic profiling technologies and the construction of relevant bioinformatic frameworks for epigenome analysis and integration provide unique opportunities for identifying mechanisms and biomarkers of drug-mediated toxicity during both the preclinical and clinical phases of drug development.

Non-genotoxic carcinogenesis (NGC) is a common drug-induced toxicity finding in rodent models for which no well-validated short-term assays exist. Here we have used genome-wide epigenomic and transcriptomic profiling, coupled to molecular histopathology, to investigate the temporal sequence of events and identify early-mechanisms and biomarkers of NGC in vivo in a well-characterized rodent model for liver tumor promotion. Exposure of mice to a tumor-promoting dose of phenobarbital (PB) led to transcriptional perturbations including the progressive increase in hepatic expression of non-coding RNAs from an epigenetically imprinted locus. Molecular histopathology and genetic analyses revealed the tissue-specificity and dependence to cancer-relevant pathways of the non-coding RNAs up-regulation. Finally, whole-genome methylome profiling identified precise perturbations of liver 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) patterns over the promoter regions of PB target genes, highlighting a potentially important role for 5hmC in early mechanisms of NGC that may ultimately enhance the identification of novel early biomarkers for cancer risk assessment. Our data highlight novel potential early mechanisms and identify novel candidate early biomarkers for liver tumor promotion, providing new opportunities for assessing the carcinogenic potential of environmental cues including novel therapeutics. Continued progress towards the mapping of epigenomes from humans and other toxicologically-relevant species will enhance our ability to interpret the biological significance of xenobiotic-induced epigenetic perturbations and may also provide novel biomarkers for susceptibility to adverse events.

SC7 - FROM OMICS-BASED SAFETY BIOMARKERS TO HIGH THROUGHPUT ASSAYS

Harry Vrieling[1], Giel Hendriks[1] and Bob van de Water[2]
[1]Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands, [2]Toxicology, Leiden University, Leiden, Netherlands

People are exposed to an ever-increasing number of chemical compounds that are developed by industry for a wide range of applications. These compounds may react with different cellular components and depending on the type of damage inflicted specific defense mechanisms are activated that provide protection against the toxic, mutagenic and possibly oncogenic consequences of exposure. Legislation demands rigorously testing of novel chemical compounds for potential harmful properties to prevent exposures that may jeopardize human health. We postulated that monitoring of the activation of specific cellular signaling pathways upon exposure will not only allow reliable assessment of potential (geno)toxic properties of chemicals, but also provide insight into their primary mode of toxicity. To this end, we performed extensive transcriptional profiling in mouse embryonic stem (mES) cells to identify biomarker genes that are predictive for specific classes of (geno)toxic compounds. Expression of selected biomarker genes was either driven by fusion of promoter regions to a DsRed fluorescent reporter gene or by construction of C-terminal GFP-tagged fusion proteins through bacterial artificial chromosome (BAC) transgenomics. Following transfection stably integrated reporter cell lines were isolated in mES cells. Developed mES reporter cell lines have differential selectivity towards either genotoxic compounds or agents that induce oxidative stress. For some reporters, the upstream signaling route was investigated in more detail using DNA replication inhibiting drugs, DNA damage kinase inhibitors and siRNA mediated knockdown of potential upstream regulatory genes. The reporters allow rapid and reliable (geno)toxicity testing and assessment of the primary reactive properties of known and unknown chemicals. In addition, they provide a powerful system for mechanistic studies into the cellular signaling pathways that are activated upon exposure to these agents.

SC8 - RNA INTERFERENCE TO IDENTIFY FUNCTIONAL GENES UNDERLYING DRUG EFFICACY AND SAFETY

Bob van de Water
Division of Toxicology, Leiden Amsterdam Center for Drug Research, Leiden University, Leiden, Netherlands

Multi-omics analysis of biological responses through either transcriptomics, proteomics or metabolimics, in relation to drug safety evaluation allow for a systems toxicology-based analysis of biological perturbations at either the cellular, organ or organism level. Such omics approaches allow the identification of the biological programs and signalling networks that are significantly affected in relation to the adverse effects. However, the functional role and biological relevance of each of the individual molecular components of the affected networks can only be speculated. Recent advances in RNA-interference technologies now allow for the genome-wide analysis of the functional relevance of individual genes in the adverse cellular response to xenobiotic exposure. The methodologies involve either synthetic siRNA- or (viral) construct-based shRNA-mediated knock down of individual genes followed by the evaluation of the biological response by for example simple measurements such as cell survival or gene-reporter assays. Alternatively, advanced (live cell) high-content imaging-based approaches followed by multi-parameter quantitative image analysis allows the extraction of more detailed biological relevant information. In this presentation the RNA-interference technologies will be explained and the application in the understanding of the molecular mechanisms of adverse drug reactions and/or the evaluation of drug efficacy will be exemplified.

SC9 - 2D CELL CULTURE AND SUBCELLULAR MODELS FROM INTESTINE AND LIVER FOR STUDIES OF DRUG TRANSPORT, DRUG-DRUG TRANSPORTER INTERACTIONS AND TRANSPORTER MEDIATED SIDE EFFECTS

Per Artursson
Department of Pharmacy, Uppsala University, Uppsala, Sweden

In vivo, drugs are transported into and out of cells by both passive and active transport mechanisms. Further, drugs that are substrates of transport proteins often interact with more than one transporter and may also interact with drug metabolizing enzymes. This complexity makes in vivo observations of potential transport mechanisms and DDIs difficult to interpret and, as a result of this, most transport mechanisms and DDIs with drug transporting proteins have been identified using controlled conditions in vitro. Cell culture models for studies of drug transport range from demanding primary cultures, over multifunctional organotypic cell lines derived from intestinal or liver backgrounds, to simpler cells lines without tissue resemblance, and even subcellular membrane vesicles. The established cell models are useful tools that give good qualitative estimates of drug transport mechanisms and DDIs. More

quantitative cell culture models are currently developed e.g. based on new, more reliable data on the expression of ADMET proteins, considerations of free intracellular concentrations and improved dynamic PBPK modeling. This presentation will first provide a brief overview of 2D cell culture methodologies and their application. Then, recent attempts to make these models more quantitative will be discussed.

SC10 - ROLE OF DIFFERENT CELL TYPES IN CO-CULTURES TO RECONSTRUCT HUMAN LIVER

Edward LeCluyse
Triangle Research Laboratories at the Hamner Institutes for Health Sciences, Research Triangle Park, NC

The liver is comprised of cells that are broadly divided into two categories: parenchymal cells (hepatocytes) and non-parenchymal cells (NPC). The non-parenchymal fraction predominantly includes liver sinusoidal endothelial cells (LSEC), hepatic stellate cells (HSC), and Kupffer cells (KC). While often relegated to the status of the "other" cell types of the liver when discussing hepatocytes, NPC are important contributors to basic hepatocyte health and function. Direct and indirect interactions and communications between the different cell types of the liver play a much greater role than originally appreciated in the maintenance of normal liver function and the causes of drug-induced hepatotoxicity both in vivo and in vitro. These functions include the secretion of growth factors and other mediators of cellular function, including transport and metabolism. Chemical-induced hepatotoxicity often occurs in specific regions of the liver and is due, in part, to the natural configuration and relationship of the different cell types in the zonal microenvironments. NPC can serve as the primary targets of certain hepatotoxins, or can mediate the physiological or pathological response to other cells. Therefore, assessing drug- or chemical-induced effects on liver function or adaptive responses in vitro can be enhanced by the ability to control the presence or absence of NPC in order to determine their relative role for specific biological responses of the liver. In this presentation, the roles of the different cell types in co-culture systems and examples of their benefits for different applications will be described.

SC11 - HEPATIC 3D CELL CULTURES OF HUMAN LIVER IN DRUG DISCOVERY

Tommy B. Andersson
AstraZeneca R&D Mölndal, DMPK Innovative Medicines, Mölndal, Sweden

Within the scope of developing an in vitro culture model for pharmacological research on human liver functions, a three-dimensional multicompartment hollow fiber bioreactor proven to function as a clinical extracorporeal liver support system was scaled down from 800mL to a size suitable for preclinical drug testing. Primary human liver cells cultured over 14 days in 800, 8, or 2mL bioreactors exhibited comparable time course profiles for most of the metabolic parameters in the different bioreactor size variants. Major drug metabolizing cytochrome P450 activities analyzed in the 2mL bioreactor were preserved over up to 23 days. Immunohistochemical studies revealed tissue-like structures of parenchymal and nonparenchymal cells in the miniaturized bioreactor, indicating physiological reorganization of the cells. Moreover, the canalicular transporters multidrug-resistance-associated protein 2, multidrug-resistance protein 1 (P-glycoprotein), and breast cancer resistance protein showed a similar distribution pattern to that found in human liver tissue. In addition the cell line HepaRG inoculated into the bioreactor exhibits promising properties such as expression and function of drug-metabolizing enzymes and transporter proteins, which resemble those found in freshly isolated. The model P450 substrates, which were introduced to the bioreactor system mimicking in vivo bolus doses, showed stable metabolism over the entire experimental period of several weeks with the exception of bupropion hydroxylase, which increased over time. Ketoconazole treatment decreased the CYP3A4 activity and rifampicin induced the CYP3A4- and CYP2B6-dependent activity which predicts well the vivo situation. Moreover, polarity of transporter expression and formation of tissue-like structures including bile canaliculi were demonstrated by immune histochemistry in HepaRG cells. In conclusion, the down-scaled multicompartment hollow fiber technology allows stable maintenance of primary human liver cells and HepaRG cells provides an innovative tool for pharmacological and kinetic studies of hepatic functions with small cell numbers.

SC12 - EX VIVO PREPARATIONS OF HUMAN TISSUES FOR DRUG METABOLISM, TOXICITY AND TRANSPORT

Geny M.M. Groothuis
Division Pharmacokinetics, Toxicology and Targeting, Department of Pharmacy, University of Groningen, Groningen, Netherlands

Before new drugs are allowed on the market, their safety and metabolite profile should be extensively tested, as often reactive metabolites are the ultimate toxicant. The exposure of the target cell to the drug and its metabolites is determined by the expression levels of the transporters and the metabolic enzymes in the target cells. Thus to predict drug-induced toxicity not only the intrinsic biological effects of the drug but also the exposure to the ultimate toxicant needs to be predicted. In the past decades, in vitro techniques were increasingly used to predict drug-induced toxicity, not only to reduce the use of experimental animals and to obtain insight in the mechanism, but also to be able to obtain human-specific information by using human cells. Although cellular and subcellular models have been proven to be very useful, they do not fully represent the complex intact tissues, and very often the tissue-specific differentiation is lost in cell lines. In the liver the presence of the Kupffer, stellate, biliary epithelial and endothelial cells are of importance for the toxic effects of drugs. In the intestine the situation is even more complex as the expression levels of the relevant transporters and metabolic enzymes show significant gradients along the length of the intestine. Therefore ex vivo tissue models have proven to be useful to fill the gap between in vitro cell models and the in vivo situation. In this lecture the use of ex vivo models of liver and intestinal tissue are presented. In precision-cut tissue slices, prepared from liver or intestine, all the cell types are present in their original conformation and cell-cell and cell-matrix interactions are intact. Their viability is maintained for up to several days and metabolism and toxic effects can be studied using innovative analytical methods including omics technologies. The Ussing Chamber technique allows to study the vectorial transport of drugs from the lumen to the serosal side and vice versa in addition to their metabolism and toxicity. These techniques can be applied to animal and human tissue and allow to study interspecies differences under identical experimental circumstances. Recent improvements in the technology and applications in prediction of toxicity will be discussed.

SC13 - PROPAGATION OF PHARMACOKINETIC VARIABILITY TO DRUG RESPONSE

G. T. Tucker
University of Sheffield and Simcyp Ltd, Sheffield, United Kingdom

With the availability of comprehensive physiologically-based pharmacokinetic (PBPK) models that capture population variability in demographics (including age and ethnicity), physiology, metabolism/transport, pharmaceutical formulation, genetics, disease and drug-drug interactions, it is now possible to link such models through to the investigation of the impact of such variability on pharmacodynamic (PD) outcome (PBPK-PD modelling and simulation). After review of the basic principles of quantitative pharmacology, applications of PBPK-PD modelling will be discussed with regard to a hierarchy of PKPD models from those where plasma or organ exposure is linked to empirical Hill functions, through the empirical PKPD link (k_{e0}) model to more mechanistic representations based on receptor binding, stimulus-response-transduction and indirect response.

SC14 - PK AND PD ANALYSIS OF EFAVIRENZ DOSE REDUCTION USING AN IN VITRO - IN VIVO EXTRAPOLATION MODEL

Marco Siccardi[1], Lisa M. Almond[2], Alessandro Schipani[1], Chantal Csajka[3], Catia M. Marzolini[4], Christoph Wyen[5], Norbert H. Brockmeyer[6], Marta Boffito[7], Andrew Owen[1] and David Back[1]
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Background: Efavirenz (EFV) is one of the most common components of anti HIV therapy and its pharmacokinetics (PK) has been correlated with efficacy and toxicity in several clinical studies. The PK of EFV is characterized by large inter-patient variability. CYP2B6 is the main enzyme responsible for EFV metabolism and CYP2B6 polymorphisms can markedly affect EFV exposure, with 516G>T considered to be the main genetic variant. In vitro-in vivo extrapolation (IVIVE) can be used to predict drug absorption, distribution, clearance, and metabolic drug-drug interactions from in

vitro data. The aim of this study was to develop and assess an IVIVE model for EFV PK and pharmacodynamic (PD). The IVIVE model was compared to a population PK approach and subsequently the effect of dose reduction on PK and PD was investigated in virtual subjects with different CYP2B6 genotypes. **Methods**: In vitro data describing the physiochemical properties, absorption and metabolism of EFV and the effect of CYP2B6 516 genotype on CYP2B6 protein expression in liver tissue were obtained from published literature or obtained with standard methods. PK/PD was characterised using data from Csajka et al. and Marzolini et al. and a significant correlation between $C_{8\text{-}16hr,}$ viral suppression and CNS toxicity was identified. These data were used to simulate EFV PK and PD in a virtual population of 500 patients using Simcyp Population-based Simulator at standard regimen (600 mg once daily) and following dose reduction to 400 mg and 200mg. **Results:** The mean population estimates of the IVIVE model for CL/F in subjects who were 516GG, 516GT and 516TT were 15.3 L/h, 11.4 L/h and 7.2 L/h, respectively, were comparable to the population PK model (GG = 13.3 L/h, 9.7 L/h and 2.6 L/h). At standard dose the simulated probability of viral suppression was 80% and for CNS toxicity was 28%. Following a dose reduction to 400mg, the probability of viral suppression was reduced to 69%, 75% and 82% and the probability of CNS toxicity was 21%, 24% and 29% for 516GG, 516GT and 516TT, respectively. With reduction to 200mg, the probability of viral suppression decreased to 54%, 62% and 72% and probability of CNS toxicity to 13%, 18% and 20% for 516GG, 516GT and 516TT, respectively. **Discussion** The developed IVIVE model predicted the PK and PD of EFV in individuals with different CYP2B6 genotypes. The main PK variables for different dosing strategies have been simulated and the effect of CYP2B6 516 G>T on EFV clearance has been characterised. Inclusion of other genetic variants and a more detailed description of absorption, metabolism and induction of enzyme expression could improve the accuracy of IVIVE models. This simulation approach can be used to investigate clinically relevant 'what-if' questions, such as whether genotype-based dose reduction strategies are feasible to manage inter individual differences in exposure. This information can now be used to design prospective clinical studies.

S1 - RISK MANAGEMENT IN THE NEW EU PHARMACOVIGILANCE LEGISLATION:

Hubert G. Leufkens
Utrecht Institute for Pharmaceutical Sciences (UIPS) and the Dutch Medicines Evaluation Board (MEB), Utrecht, Netherlands

Decision making around medicines is a constant trade-off of wanted (efficacy, effecttiveness) and adverse effects (harm, costs). In the interest of the patient and public health, it is important that the benefits of drug treatment outweigh the risks. Finding the right balance is an integral part of the product-life cycle of medicine, and this balance may change over time. In the advent in the new EU Legislation on pharmacovigilance, expected to come into force in a few days from now, risk management of medicinal products will enter a complete new era in terms of legal framework, procedures, responsibilities of stakeholders, but also scientific challenges. Trust building, increased transparency and better science are aimed to support the prompt and robust management of possible drug induced safety concerns in an informed and public health driven fashion. This pharmacovigilance model intends to produce a shift from a reactive, passive to a more pro-active, planned and science based mode of risk management. A key player in this new environment will be the Pharmacovigilance Risk Assessment Committee (PRAC) based at the EMA in London. When looking at the current agenda of drug safety issues, ranging from concerns about antidiabetic induced cancer risk to rare and serious events seen in users of biologicals (i.e. PML, severe infections), it becomes clear that no single method can be ruled out or can do all the work. This philosophy requires the availability of extensive and longitudinal data on drug usage, relevant outcomes, advanced medical record linkage systems and an integrative knowledge base of toxicology, clinical medicine and epidemiology. The system of Risk Management Plans is an important vehicle to link possible and identified pre-approval safety issues to concrete post-approval studies. The 'real world' of pharmacotherapy with conditions not reflected by the controlled clinical trial environment and the erratic nature of all the different market forces, fuel the need for a wide area of study scenarios. This presentation will address various available methods for risk assessment and aims to provide a broader scope on how create an innovative and sustainable system of medicines' safety evaluation.

S2 - DERIVING METABOLICALLY ACTIVE AND PREDICTIVE HEPATOCYTES FROM PLURIPOTENT STEM CELLS

David C. Hay
MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom

Maintaining stable and differentiated somatic cell function in cell culture is essential to a range of biological endeavours. However, current technologies, for example, employing primary human hepatocytes are limited by

supply, expense, and functional instability on biological cell culture substrata. As such, defined and biologically active substrates have the potential to improve cell culture-based assay applications. Adopting an unbiased approach we screened polymer microarrays and identified a polyurethane matrix which promoted hepatocyte viability, gene expression, drug-inducible metabolism. Most recently, our inter-disciplinary approach has led to the construction of an in vitro system which is sensitive (IC50 values compare to freshly isolated primary human hepatocytes) and predictive of human compound toxicity in situations where accepted rodent models do not. These data and other new findings will be discussed at the meeting.

S3 - STEM CELL-DERIVED HEPATOCYTES AND HUMANIZED MICE, NEW TOOLS FOR RESEARCH

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Our group has reported the generation of mice with "humanized" livers. In collaboration with Markus Grompe, we reported that when transplanted with human hepatocytes, the liver of special mutant mice hereafter called FRG mice are efficiently repopulated. The FRG(-/-) mouse is a model of Hereditary Tyrosinemia Type 1 (HT1). When Fah, is knocked out, native (mouse) hepatocytes are continually damaged by high levels of tyrosine and oxidative damage. After transplantation with FAH proficient human hepatocytes, mouse hepatocytes die and are rapidly replaced by human hepatocytes. Importantly, every human donor tested has engrafted. We have experience with more than 25 donors ranging in age from 0.5 -64. Human hepatocytes from one repopulated mouse can be serially transplanted to create dozens more "humanized" mice, which also maintains useful cells past the lifetime of a single animal. Engraftment efficiency improves with serial passage; 2^{o} or 3^{o} recipients frequently display 75-98% repopulation with human hepatocytes. Twenty-two normal human liver genes were examined and were expressed in humanized mice at levels within the range observed for normal human liver.

S4 - TOWARDS THE GENERATION OF A HEPATIC MICRO-BIOREACTOR FOR DRUG TOXICITY AND METABOLIZATION STUDIES

Catherine Verfaillie
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Abstract not available.

S5 - CLINICAL PHARMAGENOMICS: AN UPDATE

Matthias Schwab
Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

Variation in drug disposition and response among patients is a major concern associated with many therapeutical agents used in all disciplines of medicine. The clinical relevance of variability is most evident with drugs that have a narrow therapeutic window (i.e., the dose used is close to the dose probably resulting in drug-related toxicity in most individuals). With increasingly information available from the Human Genome Project, the HapMap Project, the Cancer Genome initiative, etc. pharmacogenomics aims to elucidate the genomic determinants of drug efficacy and toxicity. For instance, variants in genes that are relevant for ADME processes such as drug metabolizing enzymes, drug transporters and nuclear receptors but also other drug targets have profound effects on patient outcome. Recent clinically important examples are pharmacogenomics of warfarin, clopidogrel, abacavir, imatinib, erlotinib, cetuximab, peginterferon, etc. However, it is unlikely that one single gene will affect exclusively disease or treatment outcome, and therefore a more comprehensive approach will be to consider genetic polymorphisms in entire biological/ pharmacological pathways. Recently developed '-omics' appoaches (e.g. genomics, transcriptomics, proteomics, metabolomics) will be helpful to identify further putative targets for better prediction of drug response and will complement each other. Array technologies (e.g. cDNA arrays, GWA), next generation sequencing and metabolomics have shown to be helpful for identifying novel genes, redefining disease diagnosis and predicting therapy response to specific drugs. Finally, non-genetic factors as well as epigenetics (e.g. methylation, miRNA) have to be considered more intensively in the future. Experimental as well as computational approaches are required to obtain holistic, mechanistic information on disease networks and drug response. Thus, only systems pharmacology allows the integration of the systems-level understanding of drug response with genome medicine to promote the idea of personalized medicine and clinical pharmacogenomics.

S6 - REGULATORY PHARMACOGENOMICS

Felix Frueh
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Abstract not available.

S7 - THE USE OF GWAS IN ELUCIDATION OF THE GENETIC ARCHITECTURE OF COMMON DISEASES AND PHARMACOGENETICS

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The advent of high content genotyping arrays has revolutionised the field of human genetics. During the 1980s family based linkage analysis provided great insights into monogenic diseases, but this technique proved unable to provide meaningful insight into common disease. The development of hybridisation microarrays that simultaneously assessed over 100,000 variants has led to an explosion in genetic studies with genes signals for over 237 traits being described by the middle of 2011. These traits included common diseases such as type 2 diabetes, and rheumatoid arthritis, and quantitative traits such as serum cholesterol levels and even coffee consumption. The use of GWAS in pharmacogenetics has now started to emerge with major findings in drug induced liver injury and statin-induced myopathy. GWAS has largely confirmed the genetic architecture of response to warfarin and clopidogrel. GWAS studies of pharmacogenetics has been based on clinical trials and collections of samples from adverse events registers, but increasingly large population based studies have been utilised where drug exposure and outcome have been defined using the Electronic Medical Record. The GoDARTS study in Tayside is such a study, where we have recruited 18000 individuals with and without type 2 diabetes. Large scale genotyping in this population allows for many hypothesis to be tested due to the complete record linkage of clinical data including drug prescribing history. Genetic studies of disease outcomes including cardiovascular disease and retinopathy will be discussed, as will findings regarding the genetics of LDLc lowering effectiveness of statins and in the variability in HbA1c reduction by metformin.

S8 - THE NOVEL DRUG METABOLISM ENZYME MARC

Bernd Clement
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The investigation of oxygen-insensitive nitrogen reductive drug metabolism in mitochondria resulted in the discovery of a completely unknown mammalian molybdenum enzyme in our laboratory[1]. Upon reconstitution with the electron transport proteins cytochrome b5 and its reductase this molybdenum enzyme is capable of reducing N-hydroxylated compounds. Thus it was named “mitochondrial amidoxime reducing component” (mARC), because initially the N-reduction of amidoxime structures was studied with this isolated enzyme[2]. In continuation of our drug metabolism studies a suitable in-vitro assay using the recombinant human enzymes was developed. Since mammalian genomes encode for two homologues mARC enzymes (mARC1/mARC2), both mARC enzymes were tested and are able to reduce N-hydroxylated compounds together with cytochrome b5 and its reductase in vitro[3]. By RNA interference and knockout studies the involvement of mitochondrial cytochrome b5 and at least one mARC protein in the N-reductive pathway was confirmed [2, 4]. Using in-vitro assays of the recombinant enzymes or isolated mitochondria as well as cellular systems we clearly demonstrate that the mARC-containing enzyme system plays a major role in drug metabolism. For example we show that this molybdenum enzyme system is able to reduce N-hydroxylated derivatives of amidines, guanidines, oximes, and sulphonamides as well as N-oxides. Furthermore, it is involved in the detoxification of mutagenic N-hydroxylated DNA bases and hydroxylamines[3, 5; 6]. Unusual for xenobiotic metabolizing enzymes all tested extrahepatic tissues show N-reductive activity. The highest conversion rates are observed in liver, kidney, thyroid and pancreas. This corresponds to the expression patterns of both mARC forms. Although both mARC proteins can be detected in mitochondria, only one mARC is present in the outer membrane. Furthermore additional evidence of a previously reported peroxisomal localization[7; 8] of mARC is provided.

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S9 - ALDEHYDE OXIDASE IN DRUG RESEARCH

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Aldehyde oxidase (AOX) is a molybdenum cofactor containing enzyme that is capable of oxidizing aldehydes, imines, and aromatic azaheterocyclic compounds. Some basic aspects of its biochemistry will be reviewed. It is different from aldehyde dehydrogenase, a family of enzymes that metabolizes aldehydes. AOX has generally not been a focus in drug research, however, an increase in the prevalence in the use of aromatic azaheterocyclics as substituents in drug design has caused an increase in the importance of AO. But when left unexamined in drug design, an impact of AO on the clearance of a new chemical entity can result in an unexpectedly low exposure in humans. Research we have done in order to improve our understanding of this enzyme and its role in drug disposition will be described. We have developed an in vitro approach whereby human clearance due to AO catalyzed metabolism can be estimated by comparison to known compounds. Additionally, we have recently developed a method wherein the relative contribution of AO to total metabolism can be estimated using human hepatocytes. Finally, strategies to reduce or remove AO mediated clearance will be discussed.

S10 - UNUSUAL REACTIONS CATALYZED BY CYTOCHROME P450 ENZYMES

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Cytochrome P450 (P450) catalyzes a wide variety of reactions, most of which are oxidations but including reactions and even some non-redox reactions. Most of the oxidations can be rationalized in the context of FeO3+ (ÒCompound IÓ) chemistry, although some may involve FeOOH reactions. Rearrangements may occur with reaction intermediates, or reaction products may rearrange to yield unusual products. Oxidations of conjugates are known (e.g. glucuronides, lysolecithins). Sequential reactions are also common (i.e. 2- or 3-step oxidations), and P450s vary regarding the processivity of these multi-step sequences. Another unusual group of oxidative reactions involves coupling of ring structures, commonly seen in plant metabolism and also other P450s. Many of the P450 rearrangement products are important in that they are involved in electrophilic addition to tissue nucleophiles and to cell toxicity. (Supported in part by USPHS grant R37 CA0090426.)

S11 - MICRORNA REGULATION OF DRUG ACTION

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Pharmacogenomics aims to predict which drugs will be most effective and safe for a particular individual based on their genome sequence or expression profile, thereby allowing personalized treatment. The bulk of pharmacogenomic research has focused on the role of single nucleotide polymorphisms (SNPs), copy number variations (CNVs) or differences in gene expression levels of drug metabolizing or transporting genes and drug targets. We focus instead on microRNAs which are small non-coding RNAs that negatively regulate gene expression in many cellular processes. I will discuss how microRNAs, by regulating the expression of pharmacogenomic-related genes, can play a pivotal role in drug efficacy and toxicity. I will present our comprehensive analysis of all possible

gene-microRNA interactions related to drug efficacy. Altogether our work maps the potential clinical implications of microRNAs on pharmacogenomic-related genes for personalized medicine.

S12 - MICRORNAS, THE DNA DAMAGE RESPONSE AND CANCER

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Many carcinogenic agents such as ultra-violet light from the sun and various natural and man-made chemicals act by damaging the DNA. In addition, DNA is also damaged by several endogenous sources such as oxidative stress and metabolic byproducts. When not repaired properly, these DNA lesions will be fixed in the genome as mutations, deletions or chromosomal rearrangements that can drive carcinogenesis. When not repaired at all, DNA damage accumulation during life is a causal factor of aging. To deal with these potentially detrimental effects of DNA damage, cells induce a complex DNA damage response (DDR) that includes DNA repair, cell cycle checkpoints, damage tolerance systems and, when damage is beyond repair, apoptosis. The DDR is a potent barrier against carcinogenesis and defects within this response are observed in many, if not all, human tumors. Within tumors, DDR defects not only fuel the evolution of precancerous cells to malignant tumors, but can also induce sensitivity to DNA damaging agents in cancer cells, which can be therapeutically exploited by the use of DNA damaging treatment modalities. Regulation of and coordination between sub-pathways within the DDR is very important for maintaining genome stability and counteract the adverse effects of DNA damage as seen in carcinogenesis or aging. Although regulation of the DDR has been extensively studied at the transcriptional and post-translational level, less is known about post-transcriptional gene regulation by microRNAs. We will show that DNA damage induces specific microRNA expression changes. There is a remarkable overlap between these DNA damage responsive microRNAs and differentially regulated microRNAs in aged mouse organs. On the other hand, we found several DNA damage responsive microRNAs deregulated in human breast and/or lung cancer. Together these observations are implying an important role for DNA damage responsive microRNAs in tumorigenesis and aging. Indeed, many DNA damage responsive microRNAs that are induced in aging or deregulated in cancer protect cells against genotoxic stress. As an example, we will demonstrate the intricate role of PTEN silencing by microRNAs in genotoxic stress resistance. In summary, we demonstrate that microRNA action is an integral part of the DNA damage response important for tumorigenesis and aging.

S13 - TOXICOLOGICAL IMPLICATIONS OF MODULATION OF GENE EXPRESSION BY MICRORNAS

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MicroRNAs (miRNAs) are a large family of non-coding RNAs that are evolutionarily conserved, endogenous, and 21-23 nucleotides in length. MiRNAs regulate gene expression by targeting messenger RNAs (mRNAs) by binding to complementary regions of transcripts to repress their translation or mRNA degradation. MiRNAs are encoded by the genome, and approximately 1500 human miRNAs have been identified so far. MiRNAs are predicted to target ~60% of human mRNAs and are expressed in all animal cells. Research on miRNA is growing exponentially, and is becoming clear that miRNAs have fundamental roles in cellular responses to xenobiotic stresses, which affect a large range of physiological processes such as development, immune responses, metabolism, tumor formation, disease development as well as toxicological outcomes. Recently, many studies concerning miRNAs related to cancer have been published, however, the miRNA research in the metabolism of xenobiotics and endobiotics and in toxicology has only recently been established. In this symposium, miRNAs with reference to target prediction, potential modulation of toxicology-related changes of miRNA expression, role of miRNA in immune-mediated drug-induced liver injury, miRNA in plasma as potential toxicological biomarkers and relevance, and miRNA-related genetic polymorphisms are discussed.

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S14 - DRUG INDUCED LIVER INJURY: MECHANISMS, PREDICTION AND AVOIDANCE

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Drug induced liver injury (DILI) remains a frequent cause of delayed or terminated drug development, restricted drug usage and precautionary regulatory action. A key challenge for the pharmaceutical industry is to develop novel approaches which enable early identification and deselection of compounds with high propensity to cause DILI in humans. This has proved very challenging, especially since many drugs cause DILI only infrequently in certain susceptible individuals. Although the mechanisms which underlie such idiosyncratic DILI remain poorly defined, it is considered that they comprise a combination of compound related properties and patient related properties. In principle, it may be possible to minimise or eliminate undesirable compound related properties which can result in DILI via use of a tiered test cascade, which addresses key chemical and cellular processes and includes in silico tools, in vitro assays, in vivo models and translational biomarkers. However, whereas many promising assays, models and candidate DILI biomarkers have been described, currently there is no consensus across pharmas or within the scientific community on which approaches should be used or how they should be integrated together. Recently this topic has been prioritised by the EU/EFPIA Innovative Medicines Initiative for pre-competitive research funding. A consortium has been formed (Mechanism Based Integrated Systems for the Prediction of Drug Induced Liver Injury: acronym MIP-DILI) which comprises 11 pharmas, 9 academic research groups and 6 SME partners and will tackle the problem from 2012-2017. The primary goal is to identify and validate a panel of in vitro “best practice assays” for prediction of DILI in humans. Enhanced understanding of the relationship between in vitro assay signals and DILI in vivo, in animals and humans, is an important supportive goal, as is development of novel modelling approaches that integrate multiple preclinical data types to improve DILI prediction. An additional benefit will be improved understanding amongst academia, pharmas and regulators of the value and limitations of new and existing approaches for DILI hazard identification and risk assessment.

S15 - GENETIC PREDISPOSITION TOWARDS DRUG-INDUCED ARRHYTHMIA

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Drug-induced arrhythmia is a rare, unpredictable and life-threatening serious adverse event. It can be caused by both cardiac and non-cardiac drugs and has become a major issue in novel drug development and for the regulatory authorities. The majority are due to Torsades de Pointes an arrhythmia characteristic of the ‘congenital’ form of the long QT syndrome, a genetic disorder of prolonged repolarisation associated with the risk of sudden death. An ‘acquired’ form may be induced by exposure to drugs that impair cardiac repolarisation, typically by blocking the rapid rectifier current IKr. It has also become apparent that these may in part be due to an underlying genetic predisposition to the congenital condition, a ‘forme fruste’. This lecture will describe the problem, recognised clinical risk factors and the underlying rare and common genetic variation predisposing to drug-induced Torsades de Pointes. The future potential for pharmacogenomic guided and personalized prescription to prevent this adverse event will also be discussed and placed into clinical context.

S16 - USING INBRED MICE TO IDENTIFY ADR MECHANISMS

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Several recent studies have indicated a role of genetic variation in the predisposition to serious adverse drug reactions (ADRs) and the introduction of low cost genotyping and sequencing technologies has provided an unprecedented opportunity to define the genetic basis for ADR susceptibility in the clinic. However, the small cohort sizes that are often available for genomic association studies may preclude detection of true associations with rare allelic variants. The ideal model to study these effects would not only identify target genes and pathways for interrogation, but also improve detection of ADR potential during preclinical testing. One approach is to use a panel of genetically defined inbred mouse strains (mouse diversity panel; MDP) as a toxicity screening strategy to mimic the genetic diversity of the human population. We have previously shown the utility of the model to predict quantitative trait loci that underlie susceptibility to acetaminophen-induced liver injury in te MDP and in human populations treated with this drug. Recently, we conducted a study using novel anti-trypanosomal drug DB289, for which clinical development was halted due to severe kidney injury necessitating prolong dialysis in one clinical trial subject. Using the MDP approach, we were able to detect the potential for kidney proximal tubular epithelial injury using sensitive renal biomarker KIM-1. Genomic and metabolomic analysis across the strains indicate that a potential mechanism for DB289 renal toxicity is the disruption of lipogenic factors. Together, these data indicate the potential for using the MDP to predict the potential for an ADR, to generate hypotheses regarding the toxicity mechanisms, and to determine the genetic basis of ADR susceptibility in man.

S17 - THE DISCOVERY OF NEUROKININ-2 ANTAGONISTS - A WAR OF ATTRITION

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The Neurokinin-2 (NK_2) receptor is a G-protein coupled receptor (GPCR) with the 21 amino acid peptide as the endogenous ligand. Antagonism of the NK_2 receptor has potential for the treatment of bladder disorders, such as urge urinary incontinence (UUI). As part of a Discovery effort to identify potent antagonists of the NK_2receptor, a series of compounds with a basic dichlorophenyl lactam pharmacophore were identified. The core structure provided a scaffold for NK_2antagonism, with substituents providing H-bonding potential and lipophilicity required for appropriate potency. However, this scaffold also produced a basic centre, molecular weight of 320 and significant lipophilicity. Consequently, following appropriate substitution to achieve highly potent antagonists, physicochemical parameters of candidate molecules were at the extremes of those required for appropriate pharmacokinetics. This is not unusual for antagonists of peptidic GPCR targets. Subsequently, several candidates were identified and taken forward for further evaluation. The first clinical candidate failed in FIH studies for poor oral absorption. Attempts to improve oral absorption led to a candidate with significant lipophilicity that failed for preclinical toxicity. Further compounds could not balance QTc liabilities and one final attempt led to a clinical compound led to a compound with poor oral bioavailability due to extensive hepatic first-pass metabolism. Overall, the balance of requisite potency against the target could not be combined with appropriate ADME characteristics in this series.

S18 - DRUG INDUCED LIVER INJURY IN HUMANS: THE CASE OF XIMELAGATRAN

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Ximelagatran was the first orally available direct thrombin inhibitor under clinical development that also reached the market. Ximelagatran was tested in an extensive clinical programme. Short-term use (<12 days) in humans including the phase III clinical trials did not indicate any hepatotoxic potential. Increased hepatic enzyme levels were first observed at a higher frequency when evaluating the long-term (>35 days) use of ximelagatran (incidence of>3_ upper limit of normal (ULN) plasma ALT was 7.9%). The frequency of elevated total bilirubin levels was similar in the ximelagatran and the comparator groups. However, the combination of ALT>3_ULN and total bilirubin>2_ULN was 0.5% among patients treated with ximelagatran and 0.1% among patients in the comparator group. Symptoms such as fever and rash potentially indicating hypersensitivity (immunologic type of reaction) were low and did not differ between ximelagatran and the comparators. The withdrawal of ximelagatran from the market and termination of the ximelagatran development program was triggered by safety data from a 35-day study, indicating that severe hepatic injury in a patient could develop after exposure to the drug has been completed and that regular liver

function monitoring may not mitigate the possible risk of severe hepatic injury. As for many drugs causing liverinjury, the standard preclinical toxicological studies provided no indication that ximelagatran affected hepatic functions. In addition, extensive investigations using human-based in vitro models have not been able to define mechanisms explaining the pattern of hepatic injury observed in long-term clinical trials. A pharmacogenomic study provided evidence that the ALT increases were associated with major histocompatibility complex (MHC) alleles DRB1'07 and DQA1*02 suggesting a possible immunogenic pathogenesis. This example provides important clues to the mechanism of idiosyncratic drug-induced liver toxicity.

S19 - IN THE TRENCHES WITH VISMODEGIB, AN APPROVED HEDGEHOG PATHWAY INHIBITOR WITH UNIQUE PHARMACOKINETIC AND METABOLIC PROPERTIES

Cornelis E.C.A. Hop[1], Richard A. Graham[2], Harvey Wong[1], Mark J. Dresser[2], Stephen E. Gould[3], S. Cyrus Khojasteh[1], Young G. Shin[1] and Qin Yue[1]

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Vismodegib (Erivedge®) is a first-in-class small molecule hedgehog pathway inhibitor that has recently been approved by the FDA for the treatment of advanced basal cell carcinoma (BCC). Vismodegib treatment provided significant clinical benefit in both locally advanced and metastatic BCC in a pivotal phase II study. Vismodegib has a unique pharmacokinetic profile characterized by a long terminal half-life of 12 days, and a distinct non-linear increase in exposure with repeat dosing. There appeared to be multiple factors contributing to the unique pharmacokinetics: non-linear absorption, saturable high affinity binding to alpha-1-acid glycoprotein (AAG) and extremely low clearance. In this presentation details of preclinical and clinical studies will be presented to explain the unique PK features of vismodegib. **Experiments and Results:** Integrated PK/PD/efficacy studies were performed to characterize the exposure – pathway modulation - tumor growth inhibition relationship and the pharmacokinetics in preclinical species. From an absorption and clearance point of view the dog was an appropriate model for the human pharmacokinetics. The clearance in dogs was low, 0.3 ml/min/kg, and the absorption was non-linear due to non-sink permeation associated with the extremely low solubility, 0.0001 mg/ml at pH 6.5-7.4. The very long half-life in humans prevented a traditional mass balance study. Thus, extensive studies were conducted in healthy volunteers with trace amounts of ^{14}C labeled vismodegib to determine the iv and oral pharmacokinetics and mass balance. Detection involved accelerator mass spectrometry which allowed quantification of radioactivity in plasma and excreta through the 56 days study duration. A single-dose PK study was performed with 150 mg oral vismodegib and a tracer dose via iv. The iv clearance in humans was extremely low, 0.01 ml/min/kg, and the absolute bioavailability was determined to be 32%. Because of the non-linear pharmacokinetics, this study was also performed at steady-state following pretreatment with 6 oral doses of 150 mg vismodegib. The iv clearance increased to 0.02 ml/min/kg and the absolute bioavailability decreased to 7% under steady-state conditions. The mass balance study with oral administration of 150 mg vismodegib and a trace of ^{14}C labeled vismodegib indicated that on average 82% of the dose was recovered in the feces and 4% in the urine, which represents excellent recovery for a compound with such a long half life. The circulating radioactivity was almost exclusively unchanged vismodegib, but metabolites were detected in feces and urine despite the extremely low clearance. The main metabolic pathway is via oxidation. Unique metabolites involving opening of the pyridine ring were observed as well and their formation will be described. The saturation of binding to AAG was systematically studied both preclinically and clinically. It was determined that there is a strong relation between the AAG and vismodegib concentrations in plasma along with an increased free fraction and total clearance after multiple dosing. **Summary:** Collectively these data fully explain the unique single dose and steady state pharmacokinetics of vismodegib in humans.

S20 - PKPD: PREDICTING EFFICACY AND SAFETY MAN FROM NON-CLINICAL DATA

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In a recent commentary, Sperling et al. (Science Translational Medicine 3: 1-5, 2011) suggest that 3 hypotheses can explain the disappointing clinical results with novel pharmacological interventions for the treatment of Alzheimer's Disease (AD): (1) We are targeting the wrong pathophysiological mechanisms; (2) Drugs do not engage with the intended target (see also Morgan et al, Drug Discovery Today 17: 419-424, 2012); (3) Drug intervention is started at the wrong stage of the disease. It is our proposal that quantitative, model-based drug discovery and development approaches, including translational pharmacokinetics/pharmacodynamics (PKPD) and Quantitative Systems

Pharmacology (QSP), can address each of these 3 issues and therefore could be provide a key tool for a more rational, efficient and effective paradigm not only in AD (Van Der Graaf, NIA/NIH Alzheimer's Disease Research Summit, Bethesda 14th May 2012; http://videocast.nih.gov/summary.asp?Live=11196) but in all pharmaceutical R&D areas (Van Der Graaf & Benson, Pharmaceutical Research 28: 1460-1464, 2011). QSP (the merger of systems biology and pharmacology) draws on existing ideas and established concepts from traditional pharmacology, physiology and target-based drug discovery and will therefore serve as a link between pharmacology/ physiology and new systems-level and "omics" approaches. This approach is breaking decisively with a "one-gene, one-receptor, one-mechanism" approach in favour of a network-centric view that relies on mathematical models to achieve the necessary integration of data and hypotheses. This will involve cross-disciplinary collaborations beyond the boundaries of currently existing networks and infrastructure. Significant gains can be made by establishing new research partnerships and improving communication and alignment between existing skills, expertise and capabilities which are currently unconnected. A general framework for the development and implementation of a QSP approach has recently been provided by the NIH QSP Workshop Group (http://www.nigms.nih.gov/News/Reports/201110-syspharma.htm), which recommended that interdisciplinary research programs should focus initially on eight specific research challenges, multi-faceted training activities and private-public partnerships aimed at engaging investigators from industry, regulatory agencies and academics in the biological, mathematical, engineering and medical communities. Because of the acute need for trainees with strong skills in quantitative reasoning, network biology, and animal and human pharmacology, industry should be engaged in education as well as research. To address these challenges, multi-faceted research programs should be established that combine experimental and computational studies and span biochemical, genetic, animal and clinical approaches. These new research programs should involve research centers that bring cross-cutting expertise to the creation of multi-disciplinary computational, experimental, training and outreach programs. Recent success in the establishment of centers of excellence in systems biology and of biomedical computing (in NIGMS, NCI and the Common Fund) provides a template for similar large-scale efforts in QSP. In addition, established programs in pharmacology or systems biology should be encouraged to add elements (and/or collaborators) from other disciplines explicitly to cut across scientific and technological barriers to advance the study of therapeutic drugs at multiple levels of biological complexity. An integration of computational and experimental approaches is required.

S21 - MODELING OF MATURATION: PREDICTING PK AND PD IN PEDIATRICS

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Children differ from adults in their response to drugs. These differences may be caused by changes in the pharmacokinetics (PK) and/or pharmacodynamics (PD) between children and adults and may also vary between children of different ages. The maturation rates of these developmental changes vary however between the pathways and receptors and often do not correlate solely with the increase in bodyweight of the child. As a result, instead of an empiric dosing regimen that is based on bodyweight in a linear function, paediatric dosing regimens should be based on an understanding of the PK-PD relationship of the drug in children. Clinical trials in children should therefore consider age-related variability in both PK and PD simultaneously in order to be able to develop rational dosing schemes. In practice, this age-related variability in PK and/or PD must be considered in the context of all other sources of intra- and inter-individual variability resulting from genetic-, environmental- and disease related factors and drug interactions. If population PK-PD models need to be applied to every single drug in paediatrics, large costs and significant time will be needed to develop evidence-based dosing schedules for each drug. An important question is, to what extent population PK-PD models developed for specific drugs constitute a basis for the development of dosing guidelines for drugs other than those that have actually been studied. In this respect, the kinetics of age-related changes in renal function, the functionality of drug metabolizing enzymes, drug transporters, as well as the expression function of pharmacological receptors are patient specific or biological system-specific properties. These system-specific properties, derived from one 'model' drug, could in principle serve as a basis for the prediction of age related changes in the PK and PD of other drugs (so called extrapolation). Using simulations for drugs other than those used to generate biological system specific information may significantly reduce the time and costs needed to develop drug dosing guidelines for individual drugs. During the presentation, results will be shown of different PhD projects in paediatric PK-PD modelling which include maturation in in vivo UGT glucuronidation and CYP3A oxidation. Results of meta PK-PD analyses with novel paradigms for individualized dosing in children are discussed together with validation approaches specific for paediatric datasets. Furthermore, results of proof-of-principle studies are presented on the extrapolation of the system-specific parts of the models to other drugs sharing

the same pathway. It is concluded that future research should focus on the development of validated physiological PK-PD models for specific elimination routes and targets that have predictive and extrapolation potential, making them of use in designing algorithms to derive first-time-in-child doses and to derive individualized dosing guidelines in paediatrics. This methodology will improve the efficacy/safety balance of dosing guidelines which will be of benefit to the individual child.

S22 - DISEASE SYSTEMS ANALYSIS: PREDICTING DRUG EFFECT ON DISEASE PROGRESSION

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In conventional PK-PD analyses, the model parameters for the status of a biological system in absence of a drug are kept invariable with time. In addition, physiology is generally considered constant at baseline. However, this assumption is not valid for progressive and chronic diseases, as biological functions will change over time. In a disease progression analysis, time-dependent changes in the dynamics of the disease are accounted for to distinguish the time course of the disease from that of the drug effect(s) (1). Hence, it allows, on the basis of adequately designed clinical trials, separating symptomatic effects with a short onset, which will disappear after treatment cessation, from disease-modifying effects, which are considered slow and persistent. When biomarker data are available that provide specific insight into the nature of the disease and mechanism of action of the drug, a clear distinction can be made between disease process (i.e. the processes that reflect the underlying mechanism of the disease) and disease status. This approach might ultimately allow for predicting long-term drug effects on the basis of short-term biomarker responses. In mechanism-based approaches, a disease is considered as a disturbance of the steady-state situation (i.e. homeostasis) in (a part of) the organism. Hence, the observed disease status results from time-dependent changes in the underlying biological processes. This disease system analysis approach allows distinguishing between disease status and disease process, which can be utilized to analyse the progress of chronic and degenerative diseases, and the mechanisms of drug action. In this presentation, the different implications of a descriptive approach versus a disease systems analysis are illustrated by applying both approaches to quantify treatment effects in slowly progressive diseases such as Parkinson's disease (2), diabetes (3) and osteoporosis (4).

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S23 - CLINICAL PHARMACOGENETICS OF DRUG TRANSPORTERS

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Abstract not available.

S24 - ROLE OF DRUG TRANSPORTERS IN DRUG EXPOSURE AND SAFETY

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There is an increasing appreciation of the role that transport proteins play in the systemic and tissue exposure of a wide variety of drugs in clinical use. Membrane transporters can be the rate-determining step in the uptake and/or excretion of a compound or metabolite. These transporters are classified into efflux transporters belonging to the ATP-binding cassette (ABC) family and solute carrier (SLC) family members that mediate the influx or bidirectional movement of drugs across the cell membrane. Their coordinated expression and activities at the basolateral and apical side of transporting epithelia are significant determinants of drug exposure and toxicity. Adverse transporter-

mediated drug reactions can result from increased plasma exposure of drug or endogenous compound (e.g. bile acids or uric acid) due to drug-inhibited uptake and/or excretion in clearance organs such as liver and kidney. In toxicological target organs like liver, kidney and brain enhanced uptake or reduced efflux of the drug can give rise to harmful intracellular exposure levels. Since there exists a considerable functional redundancy in transporters, clinically relevant drug–drug interactions and variability in exposure are difficult to predict from in vitro experiments. An important step is to elucidate the relative contribution of the target transporter to the overall membrane transport of a specific drug. This lecture will summarize recent advances in understanding and studying the role of clinically relevant drug transporters expressed in liver, kidney and blood brain barrier, with special reference to recent examples from our research on hepatic uptake transporters and BBB efflux transporters.

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S25 - MICRODOSING AND PET STUDIES IN DRUG TRANSPORTER RESEARCH

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Microdose clinical trials can be utilized for the purpose to gain pharmacokinetic information on a tentative drug candidate in human using labelled compound(s) and AMS or imaging technology using PET, or non-labelled compound(s) and LC/MS/MS, and to select drug candidate by ensuring adequate properties in early stage. In 2008, the NEDO (New Energy and Industrial Technology Development Organization) project entitled " Establishment of Evolutional Drug development with the Use of Microdosing Clinical Trial" was adopted. In this project, the quantitative prediction method on drug absorption, distribution, metabolism and excretion(ADME) using modeling and simulation will be applied to human to validate this methodology. That is, based on the in vitro data on metabolism, transport and binding with animal and human tissues, the drug concentration-time profiles in the plasma and target tissues such as brain, tumor, liver and kidney will be predicted and the validity of the predictions will be investigated by using clinical studies under its microdose and therapeutic dose conditions. PET is a promising approach to determine the functional change of transporters associated with genetic polymorphisms or drug-drug interactions. Labeled PET probes are being developed for specific transporters in this NEDO project. For example, the effects of changes in uptake and efflux transporters' activity on systemic and hepatic exposure of statins were simulated and the validity of model prediction will be shown by clinical studies with PET. Here in this presentation, I will share will you our recent progress in the use of the analysis of plasma clearance of drugs and PET imaging to evaluate the transporter function in vivo including PET probes for hepatic uptake transporters (OATP1B1, OATP1B3) and biliary excretion transporters (MRP2) and those in other tissues.

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S26 - DISCOVERY OF DISEASE AND TOXICITY BIOMARKERS AND THEIR CONTRIBUTION TO MECHANISMS

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Metabolomics is being used to identify biomarkers for diseases such as diabetes and diabetic complications, alcoholic-induced liver disease, and cancer, using mouse and monkey models and samples taken from normal and diseased human patients. This approach has provided novel, relevant, and translatable perspectives of these diseases and given unprecedented insights into their mechanisms of pathogenesis and progression. Using ultraperformance liquid chromatography electrospray ionization-quadrupole mass spectrometry (UPLC-ESI-QTOFMS) and gas chromatography coupled to mass spectrometry (GCMS) platforms, cells and tissue extracts, serum, and urine can be probed to discover and develop chemical fingerprints left from various biological processes. Biomarkers can be identified in easily obtainable biofluids such as urine or serum for cancer diagnosis with the promise of developing early-stage prognostic and diagnostic indicators. Biomarkers can be used to monitor cancer therapy to determine drug efficacy and distinguish responders from non-responders. In many cases, biomarkers identified by metabolomics can provide mechanistic insight into disease pathogenesis, where an enrichment or depletion of a certain metabolite or metabolites indicates perturbed metabolic processes and therefore point to alterations at the genomic, epigenomic, transcriptomic, and/or proteomic levels as revealed by a complete systems biology approach. Indeed, the metabolome might be the most accurate indicator of cellular physiology. Metabolomics can be used to study the metabolism of drugs and identify the full profile of metabolites produced in humans and determine which metabolite leads to efficacy and which leads to toxicity. Metabolites that are identified as biomarkers for diseases can also lead to clues for unraveling novel signal transduction pathways. Evidence will be presented on discovery of biomarkers for hepatotoxicities and their underlying mechanisms.

S27 - METABOLOMICS IN TRANSLATIONAL DRUG RESEARCH

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Metabolic processes are the core of physiology, and consequently, metabolomics is in principally ideally positioned to distinguish between health and disease, and to predict the efficiency and adverse effects of interventions. Therefore we aim at a metabolomics-based systems biology approach for personalized medicine. For this, several metabolomics platforms have been developed to follow the intervention of drugs such as global profiling of lipids, central metabolism and targeted platforms such as for oxylipins. These platforms can be used to for human, animal and cell-based studies in drug research. As metabolites are essential the same with similar function in human, animal and at cell level, metabolomics is ideally suited for translational studies. Several examples will be discussed how drug response profiles were measured in human or animal models in order to understand, and better predict, the efficacy and adverse side effects of drugs. Patient stratification requires the identification of sub-groups in patients, and examples of identifying such sub-groups will be discussed for cardiovascular diseases. A better understanding why patients react different to drugs will not only help in clinical decisions, but will also allow to better understand individually variation in drug response and may be allow new routes to drug development. An example to study toxicity from a systems perspective will be shown. It will be discussed how results from clinical studies can be translated to animal or cell models, and vice versa. The possible future role of metabolomics in translational drug research as well as in personalized medicine will be discussed.

S28 - PREDICTING DRUG RESPONSE FROM METABOLIC PROFILES

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Abstract not available.

S29 - EPIGENETICS IN TOXICOLOGY; INTRODUCTION TO THE FIELD

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Epigenetics may present one of the most exciting developments in modern biology and life sciences and will have its impact in toxicology as well. It refers to the study of heritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence, explaining why the field is called epi- (Greek: above, over, on top of) genetics. The molecular basis of epigenetics includes 1) DNA modifications such as DNA methylation at the 5-position of cytosine residues, 2) histone modifications affecting chromatin folding and packaging of DNA around nucleosomes via covalent histone modification such as acetylation, methylation, phosphorylation and other protein modifications, and 3) RNA interference (RNAi). This presentation will provide an introduction to the field presenting an overview of these basic mechanisms of epigenetics also focussing on the possible role of epigenetics in the biological effects of chemicals on human health. It will illustrate that epigenetics will have an important impact in the field of toxicology and the understanding of the mode of action of drugs and other xenobiotics. This also raises issues as to what test strategies and models systems can be employed, if effects observed can be displayed in dose-response curves, whether effects are beneficial or adverse and how these effects can be taken into account in safety assessment. Some examples of how chemicals affect the epigenome and the resulting biological outcome will be presented. All together the lecture aims at presenting an introduction to a field expected to be of utmost importance for toxicology in the 21st century.

S30 - INTERINDIVIDUAL DIFFERENCES IN THE HUMAN HEPATIC EPIGENOME: IMPLICATIONS FOR DRUG METABOLISM AND TRANSPORT

Marina Kacevska[1], Maxim Ivanov[1], Anders Rane[2], Silva Kasela[3], Mart Kals[3], Andres Metspalu[3], Lili Milani[3] and Magnus Ingelman-Sundberg[1]
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Adverse drug reactions (ADRs) are a major problem in drug therapy and drug development. Many ADRs are highly dependent on the variability in enzymes responsible for drug absorption, distribution, metabolism, and excretion (ADME). It is becoming increasingly evident that epigenetic programming generates interindividual differences in phenotype beyond genetic variability that may determine susceptibility to ADRs. There is a growing body of evidence that such epigenetic regulation can affect the expression of several ADME genes (1). Therefore, fully understanding epigenetic regulation of ADME encoding genes may provide an additional dimension of interindividual differences important in the variable response to drug treatment as well as ADRs. Our group is investigating the epigenome as well as the genome, transcriptome and proteome in 95 human adult and 14 fetal livers. Epigenetic regulation, involving DNA methylation is examined using various approaches; a novel targeted bisulfite next-generation sequencing method selective for approximately 200 different ADME genes and their flanking regions; whole genome DNA methylation using the Illumina Infinium Human Methylation 450K BeadChip assay; and focused bisulfite sequencing to examine DNA methylation states on the CYP3A4 promoter. The newly discovered 5-hydroxymethylation (5hmC) in mammals is also investigated for its functional role in human livers by selective capture of 5hmC-containing fragments using the recently developed bio-ortogonal 5hmC labeling method (2). These studies aim to reveal DNA elements subject to transcriptional control by DNA methylation/hydroxymethylation that may explain differences between fetal and adult as well as interindividual variation in ADME gene expression. In this presentation some of our recent findings of the ontogeny of epigenetic regulation of ADME genes and the possible implications on therapeutic outcome will be discussed.

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S31 - PHARMACOGENOMICS OF THE RNA WORLD: STRUCTURAL RNA POLYMORPHISMS IN DRUG THERAPY

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Pharmacogenomic biomarkers increasingly serve in drug therapy to reduce adverse effects and enhance efficacy. Yet the genetic contributions to treatment outcomes often remain uncertain. Clinical utility of genetic biomarkers can be diminished with use of surrogate markers, rather than causative polymorphisms that alter the biological processes. In addition to polymorphisms that change protein coding sequence (cSNPs), variants affecting mRNA and protein expression are prevalent but their discovery has proven difficult. Regulatory variants come in two flavors, those affecting transcription (rSNPs), and a mechanistically distinct class that acts specifically on RNA functions – termed structural RNA SNPs (srSNPs) (1). Processes frequently affected by srSNPs include RNA splicing, folding, turnover, and translation. I will discuss the role of srSNPs in drug metabolism, transport, and response, to highlight their abundance and clinical relevance. Tapping into numerous molecular mechanisms, srSNPs require distinct experimental approaches for discovery and characterization. I will present examples where srSNPs have promise as pharmacogenomic biomarkers predictive of treatment outcomes. Supported by U01 GM092655.

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S32 - TRANSLATIONAL PHARMACOKINETICS – HUMAN IN VITRO-IN VIVO EXTRAPOLATION

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In recent years the translation of in vitro-derived parameters valuable for the early prediction of drug pharmacokinetics has become more demanding requiring mechanistic approaches with sound methodologies. Our ongoing appreciation of the intricacies of hepatic drug disposition indicates that the use of more than one in vitro system is essential (to cover metabolism via various phase 1 and 2 enzyme systems, action of uptake and effux transporters and other intracellular interactions) which presents challenges for high throughput generation of data and a holistic interpretation is required. That said in vitro techniques offer many opportunities for generating reliable kinetic parameters for both transporter and metabolic activities and for delineating the interrelationship between these processes and other cellular events. It has become clear that the use of pure scaling factors (based on either enzyme or transporter protein recovery) for in vitro to in vivo extrapolation is not adequate and empirical factors are also required to compensate for the quantitative inadequacies of in vitro systems and allow accurate translation of these parameters. This situation is confounded by drug and system dependences which have yet to be resolved. An up-to-date appraisal of the utility of human hepatocytes will be provided including demonstration of the need for pragmatism and justification of incorporating empirical considerations with accepted scaling procedures. The value of using both hepatic microsomal and hepatocellular data will be stressed. By taking advantage of our sound basis for scaling of metabolic clearance, there is great promise for the evolution of analogous transporter scaling strategies. While the complexities associated with accommodating influx and efflux processes, together with P450 and conjugation reactions are evident, the challenges are tractable through the use of physiologically based pharmacokinetic (PBPK) modelling approaches. While acknowledging the species differences in expression of both enzymes and transporter proteins, rat hepatocytes continue to be a very valuable model system for exploring general principles and establishing elusive relationships. The role of passive permeability, rate limiting steps and calculation of Kp (hepatocyte to plasma or medium concentration ratios) and fucell (unbound fraction of drug concentration within the hepatocyte) will be discussed. The potential utility of particular in vitro approaches for the simultaneous measurement of several of the above processes (dual incubations and sandwich culture) will also be addressed.

S33 - THE VIRTUAL PATIENT - UNDERSTANDING COMPLEXITY AND VARIABILITY

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The application of physiologically-based pharmacokinetic (PBPK) modelling is coming of age in drug development and regulation, reflecting significant advances over the past 10 years in the predictability of key pharmacokinetic parameters from human in vitro data and in the availability of dedicated software platforms and associated data bases. With respect to understanding co-variates and variability, focus in applying PBPK has been on anticipating the

quantitative impact of drug-drug interactions, age, genetics, racial differences, food effects and pharmaceutical formulation. In principle, it is also possible to incorporate pathological features in PBPK models to predict PK in specific disease states. However, although there is a large and diverse body of literature documenting changes in relevant parameters in other diseases, systematic attempts to assimilate the information into PBPK models are only now beginning. As a start, some progress has been made in predicting the effects of liver cirrhosis and morbid obesity (including bariatric surgery) based on prior knowledge of physiological and relevant biochemical changes. The consequences of impaired drug metabolism as an accompaniment to progressive deterioration in renal function have also been incorporated into prediction of the impact of renal disease. Inflammatory disease is an obvious target for mechanistic PBPK modelling, to incorporate known changes in the levels of enzymes, transporters and plasma binding proteins. These extensions of PBPK modelling, along with the incorporation of the PK of biologicals and moves towards linking PBPK to pharmacodynamic (PD) outcome, are clearly of benefit in understanding extremes of risk in different patient populations as part of the process of drug development. Indeed, mechanistic PBPK modelling is the only efficient methodology that can anticipate the combined effects of many patient variables acting simultaneously. The alternative, 'top down', approach based on population pharmacokinetics (POPPK) is limited in this respect inasmuch as its power to detect significant co-variates is highly dependent on both the size of the signal and the frequency of the sub-group within the cohort. A way forward is to incorporate the generic framework of PBPK models into more mechanistic POPPK and POPPKPD modelling. Apart from its use in drug development, PBPK also has potential application in the health care arena as an educational tool and for the provision of computerised, 'point of care' advice on personalised drug dosage. Multi-drug treatment of the complex patient (e.g. an elderly, obese lady with cardiac failure, rheumatoid arthritis, renal impairment, Alzheimer's disease, and a 'poor metaboliser' to boot) is a considerable clinical challenge. One day, when sufficient information is available in the patient, clinicians may be able to link that person to his or her virtual twin within a PBPK-PD model on an iPad to provide safe, effective, individualised dosage, and to avoid undesired drug-drug interactions. If the physician considers this too complex, the friendly clinical pharmacist will be looking over his/her shoulder to provide further guidance. The challenge of complexity in drug treatment is already with us and can only increase.

S34 - TRANSLATIONAL PK- ANIMAL TO HUMAN EXTRAPOLATION

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Quantitative human pharmacokinetic (PK) predictions play a critical role in assessing the quality of potential oral drug candidates where clearance, volume of distribution, bioavailability and the plasma-concentration-time profiles are the desired endpoints. Many methods for conducting predictions have been reported and span from utilizing in silico data to in vivo data, and employ scaling algorithms to complex computation approaches. Despite the multitude of methods, extrapolation of animal PK information to human remains beneficial for evaluating compound qualities with respect to total body clearance and disposition. More specifically, mechanisms of clearance may be observed in vivo which may not be reliably tested or scaled from in vitro studies. Two case studies will be discussed which demonstrate the utility & predictability of animal scaling to human. Ultimately, the predicted human PK parameter estimates from animal extrapolation can be combined with computational approaches to define a human plasma-concentration time profile and enable prediction of total human body exposure under various dosing regimens.

S35 - IMMUNE MEDIATORS IN DILI: LESSONS TO BE LEARNED FROM STUDIES WITH HUMAN CELLS

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Immunologically-mediated reactions are one of the most feared as they are difficult to predict, they show no simple dose-response relationship and they affect almost every organ. The presence of drug-responsive T-lymphocytes in blood and skin of patients with cutaneous adverse reactions provides a robust case for their involvement in the disease pathogenesis. In contrast, the role of T-cells in reactions targeting the liver is less well defined. Flucloxacillin is a common cause of drug-induced liver injury (DILI). Delay in the reaction onset and identification of HLA-B*5701 as a susceptibility factor are indicative of an immune pathogenesis. Despite this, drug-responsive T-cells have not been detected and as such the role of the HLA risk allele in the disease is not known. We have recently characterized flucloxacillin-responsive CD4+ and CD8+ T-cells from patients with DILI and shown that naive T-cells from volunteers expressing HLA-B*5701 are activated with flucloxacillin when dendritic cells present the drug antigen. T-cell clones secreted IFN-γ, Th2 cytokines, perforin, granzyme B and FasL following drug stimulation. It is well-established that an

obligatory step in β-lactam reactions is the formation of covalent bonds with lysine residues on protein. Using mass spectrometric methods, we identified albumin as the major protein modified with β-lactam antibiotics, defined the profile of drug protein conjugation at specific lysine residues and characterized for the first time the minimum levels of modification associated with the stimulation of a clinically relevant drug-specific T-cell response. Flucloxacillin-derived haptens were found to bind to albumin in a time-dependent manner and the level of binding correlated directly with the stimulation of clones. Activation of CD8+ clones with flucloxacillin was processing-dependent and restricted by HLA-B*5701 and the closely related HLA-B*5801. Clones displayed additional reactivity against β-lactam antibiotics, but not abacavir. Collectively, these studies define the immune basis for flucloxacillin-induced liver injury and link the genetic association to the iatrogenic disease.

S36 - CHARACTERIZATION OF NOVEL PATHOPHYSIOLOGICAL MECHANISMS CAUSING DRUG INDUCED IMMUNE MEDIATED LIVER INJURY

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We identified recently a novel CD8 T cell (CTL) effector function against viral infections in the liver. This effector function relies on the secretion of TNF by activated CTL after antigen recognition. Secreted TNF kills specifically infected hepatocytes whereas uninfected hepatocytes remain healthy. The ligation of the TNF receptor on hepatocytes can trigger both, pro-survival pathways via NFkB signaling, and apoptotic pathways involving generation of ROS and caspase activation. What discriminates between these two pathways remains still largely unknown. We identified viral infection to fatefully influence this decision towards cell death. We found that viral infection overcomes different checkpoints regulating TNF apoptosis signaling. Viral infection interfered with TNF signaling at early steps, triggering an activation of the initiator caspase 8 that was not observed in uninfected cells. In infected hepatocytes the subsequent cleavage of Bid and the activation of caspase 9 culminated in the activation of effector caspase 3 and thus induced the execution of apoptosis. Thereby the mitochondrial apoptotic pathway was essentially involved, as an inhibition of caspase 8 or 9 prevented liver damage. More importantly, viral infection contributed to sensitization of hepatocytes towards apoptosis by augmenting mitochondrial vulnerability. We observed increased mitochondrial outer membrane permeability in infected hepatocytes leading to cytosolic release of IMS proteins such as, cytochrome C, HTRA2/Omi and Smac/DIABLO. Those mitochondria showed augmented sensitivity towards caspase 8 mediated degradation, leading to massive cytochrome c release. We used our experimental system of viral infection and TNF exposure to test different drugs for hepatotoxicity. We found that some drug metabolism pathways are altered during viral infection and change the outcome of viral hepatitis. The interaction between inflammation in the liver and drug metabolism may allow liver toxicity to surface that has remained clinically silent in absence of the other condition.

S37 - THE P-I CONCEPT: PHARMACOLOGICAL INTERACTION OF DRUGS WITH IMMUNE RECEPTORS

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Drug hypersensitivity reactions lead to a great variety of sometimes puzzling diseases with massive immune stimulations. It is hotly depated how drugs stimulate the immune system: on one hand it is an immunological dogma that small molecules per se do not stimulate the immune system, and that an immune response to drugs can only arise if the drug acts as a hapten and covalently binds to a carrier molecule. However, we have recently established an additional model how T cells are stimulated by drugs in drug hypersensitivity: this concept is called the p-i concept (=pharmacological interaction with immune receptors). It postulates that some drugs, which lack hapten characteristics, can bind directly and reversibly (non-covalently) to immune receptors and thereby stimulate the cells. Thereby, two forms of p-i interactions can be distinguished: a) p-i (TCR): a certain drug may bind to a particular T-cell receptor (TCR), and this drug binding suffices - together with TCR interactions with the HLA - to stimulate the T-cell to secrete cytokines, to proliferate and to exert cytotoxicity. B) p-i (HLA): the drug binds directly to a certain HLA-molecule, which would explain the striking HLA associations of some drug hypersensitivity reactions, as the drug can only bind sufficiently affine to a certain allele (e.g. HLA-B*57:01). The p-i concept is based on extensive investigations of T cell clones from patients with drug hypersensitivity diseases, which react with the drug. It has been well documented by various groups for severe drug hypersensitivity reactions elicited by carbamazepine,

abacavir and sulfamethoxazole. Stimulation of drug specific T-cells occurs within seconds to minutes as revealed by a rapid Ca++ influx after drug addition to drug specific T-cell clones, thus before metabolism and processing can occur. In the abacavir model, no evidence of metabolism or processing could be found in peripheral blood cells. The use of hybridoma cells transfected with the drug specifc alpha/beta-TCR for the drug confirmed the need of specific TCR; modelling of drug interactions with specific TCR-CDR3 or -CDR2 sites highlighted possible interaction site and their fine specificity. As the immune system can only react in an immunological way, the symptoms arising after drug stimulation of immune receptors imitate an immune response following recognition of a peptide antigen. Of note, it is not the result of an own, drug specific immune response but actually a pharmacological ("off target") stimulation of some T cells via their alpha/beta-TCRs. The peptide specificity of the drug stimulated T cells is still unknown. For the risk assessment of drugs in preclinical development, both the hapten and p-i concept should be addressed. It may be easier to test the hapten features, which is however, in my opinion, far rarer causing systemic drug allergies than drugs directly interacting with immune receptors.

S38 - LIGAND BINDING TO CRYSTALLINE P450 2D6: AN EXPERIMENTAL PLATFORM FOR RAPID, STRUCTURAL CHARACTERIZATION OF SUBSTRATE AND INHIBITOR BINDING

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Cytochrome P450 2D6 contributes extensively to human drug clearance, and there is a significant potential for metabolic drug-drug interactions with P450 2D6. Crystal structures P450 2D6 provide information that can aid in drug design to reduce the likelihood of drug-drug interactions. Currently, two crystal structures of P450 2D6 are available in the Protein Database, one with the inhibitor prinomastat bound in the active site (3QM4) and another crystallized without a ligand (2F9Q). The active site architectures of the two structures differ significantly due to conformational differences for the N-terminal and C-terminal loops, beta-sheet 1, the helix F-G loop and the helix B-C loop that form the distal outer surface of the substrate binding cavity. Additional structural information would provide a more complete understanding of structural adaptations that occur when substrates bind. With this in mind, P450 2D6 was crystallized with thioridazine. The crystallized complex exhibits an open conformation with one molecule of thioridazine bound in the active site and another molecule bound in an entrance channel. Examination of the crystal lattice identified extensive lattice interactions around the edges of P450 2D6 molecule with the distal face of the molecule exposed to a solvent channel. Conditions were identified that enabled ligand exchanges for the thioridazine bound in the enzyme active site of the crystallized complex. As a result of sparse lattice contacts with the distal surface, the enzyme displays adaptations for binding alternative ligands. These results characterize ionic, hydrogen bonding and hydrophobic interactions between P450 2D6 and quinidine, quinine, dextromethorphan and ajmalicine. Additionally, this crystal form of P450 2D6 provides an experimental platform for rapid determination of P450 2D6 structures with new chemical entities. (Supported by NIH Grant GM031001)

S39 - CYTOCHROME P450 STRUCTURES IN PREDICTION OF DRUG AND PROCARCINOGEN METABOLISM

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Cytochromes P450 1A1, 2A6, and 2A13 play distinct key roles in human metabolism of various components of tobacco smoke. While hepatic CYP2A6 appears to play a primary role in the metabolism of nicotine, CYP1A1 and CYP2A13 are well known for their involvement in the bioactivation of the procarcinogens benzo[a]pyrene and the nicotine-derived nitrosaminoketone known as NNK, respectively. New structures of each enzyme in concert with docking approaches suggest structural features that mediate the entry, binding, and perhaps metabolism of these physiologically important compounds in tobacco-induced lung carcinogenesis. A comparison of docking predictions with experimental results suggests both the strengths and weaknesses of in silico approaches based on currently available structural information about these cytochrome P450 enzymes.

S40 - STRUCTURAL ANALYSIS OF ATP-BINDING CASSETTE TRANSPORTERS USING ELECTRON- AND X-RAY CRYSTALLOGRAPHY

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ATP-binding cassette (ABC) transporters are a large family of membrane proteins which are generally composed of two transmembrane domains and two cytoplasmic nucleotide-binding domains. They are active transporters, utilising ATP to drive the passage of allocrites (ie transported substrates) across a membrane, usually against a concentration gradient. Several members of this family are involved in drug efflux from cells, including at least three human ABC transporters that are also associated with the acquisition of drug resistance in cancer cells. In addition there are many ABC transporters that actively efflux drugs / antibiotics from bacterial cells, hence there is considerable interest in their structure/function relationships. Interestingly, this family has yielded several structures for intact ABC transporters via X-ray crystallography. This includes a eukaryotic (murine) multidrug transporter, ABCB1. In addition there are multiple structures for the more tractable cytoplasmic domains of this family. Despite the relative glut of structural data, it is only recently that we have begun to understand the relationship between structure and function in these transporters. Moreover, the contribution of lower resolution data obtained from electron microscopy and electron crystallography to this recent understanding cannot be overlooked. A general model of active transporters suggests that energy, usually in the form of ATP binding or hydrolysis, generates a conformational change in the transporter such that a binding site for the allocrite becomes exposed on the opposite side of the membrane. Hence two conformations are predicted, outward- and inward-facing, with ATP driving the shift from one to the other (dependent on whether the transporter is an importer or exporter). Structural data has begun to confirm the applicability of this model to ABC transporters, although with some important caveats. For example, unregulated ATP binding and hydrolysis would be wasteful in the absence of allocrite, and indeed experimental data for well-characterised ABC transporters show a clear stimulation of ATPase activity by addition of allocrite. Hence there must be structural cross-talk between allocrite binding site (assumed to lie in the transmembrane region) and ATP hydrolysis site (unambiguously in the cytoplasmic domains). That ATP binding in the cytoplasmic domains drives the conformation change via dimerisation of these domains has been widely accepted, and this dates from some of the earliest structural data for ABC transporters. However the accumulating data from X-ray crystallography and electron crystallography are beginning to be somewhat inconsistent with this simple hypothesis for the mechanism of action of ABC transporters. In this presentation I will review data obtained by X-ray crystallography and electron microscopy/crystallography for ABC transporters and attempt to draw conclusions about the subtle structure/function relationships that these data reveal.

S41 - COOPERATION BETWEEN MICRORNA AND ALTERNATIVE POLYADENYLATION IN REGULATION OF CYP3A4 EXPRESSION IN HEPATOCYTES IN RESPONSE TO DRUG INDUCTION

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Abstract not available.

S42 - MICRORNAS IN REGULATION OF DRUG TRANSPORTER GENES

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There are considerable variations in drug absorption, distribution, metabolism and excretion (ADME) in humans, which may lead to unwanted adverse drug effects during pharmacotherapy. Some of the mechanistic causes are well documented, e.g., genetic polymorphism, inhibition and transcriptional regulation of ADME genes. However, little is known how ADME is regulated at the posttranscriptional level. MicroRNAs (miRNAs) are a large group of short, noncoding RNAs that control posttranscriptional expression of target genes. Currently, more than 1,000 miRNAs have been identified in human genome, which may regulate thousands of protein-coding genes. Some miRNAs have been shown to directly or indirectly control the expression of ABC transporters. Herein we present our findings on noncoding miRNAs in regulation of ABC transporter BCRP/ABCG2 and MRP1/ABCC1. Consequently, intervention of miRNA signaling may modulate target gene expression, alter the capacity of cellular drug disposition, and influence the sensitivity of cells to xenobiotic drugs. In addition, the expression of some ADME regulatory miRNAs is significantly changed in cells following the exposure to xenobiotics. Therefore, miRNA-mediated epigenetic

regulation of ABC transporters may represent a new mechanism underlying interindividual variations in ADME as well as multidrug resistance.

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S43 - MICRORNAS, LINCRNAS AND EPIGENETIC FACTORS REGULATING HEPATIC CYPS: LESSONS FROM DEEP SEQUENCING

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Advances in high-throughput RNA sequencing (RNA-seq) and computational analysis have stimulated the discovery of novel transcripts and facilitated quantification of gene expression on a genome-wide scale. Presently, we employed RNA-seq to comprehensively capture the transcriptomes and gene expression patterns in male and female mouse liver, with the ultimate goal of identifying regulatory networks that control the clinically significant sex differences in drug and steroid metabolism, hepatophysiology, and liver diseases found in humans. We utilized RNA-seq data assembled from ~68 million small RNA sequence reads (15-35 bp) and ~83 million polyA+ mRNA reads (>200 bp) to discover novel RNAs underpinning the endocrine regulation of liver cytochromes P450 (CYPs) and other key hepatic enzymes. We identified 12 male-biased and 32 female-biased **microRNAs** (male-female difference in expression >2, $p<0.0005$), several of which are either oncomirs or tumor suppressors whose sex-dependent expression patterns may help explain the greater susceptibility of males to hepatocellular carcinoma. Further investigation of two of these sex-biased microRNAs confirmed their strong sex-dependent expression and revealed tight regulation by **growth hormone (GH)**, a major endocrine regulator of sex-differences in the liver. In addition to microRNAs, **long intergenic non-coding RNAs (lincRNAs)** represent an important class of regulatory RNAs increasingly recognized for their effects on the epigenetic landscape and chromatin states. To test the hypothesis that GH-regulated and sex dependent lincRNAs contribute to sex specific chromatin states via interaction with chromatin modifying enzymes, we performed **strand-specific RNA-seq** using liver nuclear poly-A RNA. This rich dataset included 48 novel male-specific and 18 novel female-specific lincRNAs that are intergenic, unidirectionally transcribed and showed strong nuclear localization; many are also regulated by GH. Moreover, several of these novel sex-dependent lincRNAs are tightly bound to chromatin and are physically associated with polycomb repressive complex-2 (PRC2), suggesting they have a role in laying down PRC2-dependent repressive chromatin marks. Our findings also illustrate how RNA-seq can be used to distinguish closely related genes and transcripts in complex liver drug metabolizing enzyme families, providing information critical for understanding the regulation of closely related genes. Examples include members of the Cyp2c and Gstp gene subfamilies, which we were previously unable to distinguish by microarray and qPCR but are now able to quantify at the individual gene and transcript level with high precision. Thus, we have leveraged the power of RNA-seq technology to obtain high resolution maps of the liver transcriptome, identify key microRNAs, lincRNAs and other epigenetic factors that may contribute to sex-biased expression of hepatic CYPs, and lay a strong foundation for further functional studies elucidating the complex regulatory networks controlling liver metabolic processes with a major impact on human health. Supported in part by NIH grant DK33765 (to D.J.W.).

S44 - NRF2 SIGNALING MECHANISMS IN RESPONSE TO CELLULAR STRESS

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Electrophilic chemicals, pro-oxidants, heavy metals and cancer chemopreventive agents increase the expression of a range of cytoprotective proteins. Genes induced by these stressors include those encoding antioxidant enzymes, drug-metabolizing enzymes, drug-efflux pumps and 26S proteasomal subunits. These genes are co-regulated through an antioxidant response element (ARE, 5'-$^{A}/_{G}$TGACnnnGC$^{A}/_{G}$-3') present in their promoter regions. Induction of ARE-driven genes by electrophiles and oxidants is mediated by the Nrf2 cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factor. Activation of Nrf2 in response to various environmental and physiological stimuli represents an important mechanism by which cells adapt to a wide spectrum of stressors. Under normal homeostatic conditions Nrf2 has a relatively short half-life, but during stress it becomes relatively stable, and this is sufficient to allow induction of its target genes. The stress-dependent stabilization of Nrf2 is due to antagonism of the E3 ubiquitin ligase substrate adaptors Keap1 and beta-TrCP, which allow Cul3 and Cul1 to ubiquitylate the CNC-bZIP factor and target it for proteasomal degradation. The Neh2 domain of Nrf2 contains DLG and ETGE motifs to which Keap1 binds, and the Neh6 domain of Nrf2 contains DSGIS and DSAPGS motifs to which beta-TrCP binds. The ability of beta-TrCP to bind Nrf2 is influenced by glycogen synthase kinase-3 (GSK3) activity, thereby providing a mechanism by which the PI3K-PKB/Akt signal transduction pathway influences the stability of Nrf2 and the expression of cytoprotective genes. The stimuli that antagonise repression of Nrf2 by Keap1 and beta-TrCP appear to be distinct, with the former being inactivated by redox stressors, electrophiles and certain metals, and the latter being blocked by agents that activate PI3K-PKB/Akt thereby inhibiting GSK3.

S45 - THE NRF2-KEAP1-ARE PATHWAY AND THE DUAL ROLE OF NRF2 IN CANCER

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Nrf2 (NF-E2-related factor-2) is a transcription factor that is ubiquitously expressed, but at low levels in all human organs. It regulates an adaptive defense response by inducing the expression of genes bearing an antioxidant response element (ARE) in their promoters. Under oxidative stress, Nrf2 induces the transcription of cellular protective genes to neutralize harmful reactive intermediates. Since oxidative stress is implicated in the initiation and progression of cancer, activation of the Nrf2 pathway is important in chemoprevention and many chemopreventive compounds have been identified as Nrf2 activators. However, the “dark” side of Nrf2 has recently been revealed. High constitutive levels of Nrf2 are present in many types of tumors and cancer cell lines. Moreover, overexpression of Nrf2 in cancer cells protects them from the cytotoxic effects of anti-cancer therapies, resulting in chemoresistance. In this presentation, I will be discussing the mechanism of Nrf2 regulation, its dual roles in cancer, and the feasibility of using our recently discovered Nrf2 inhibitor, brusatol, to combat chemoresistance.

S46 - ROLE OF NRF2 IN PROTECTION AGAINST DRUG-INDUCED DISEASE

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Chemical stress is a potentially deleterious state induced by exposure of cells to non-physiological levels of drugs and metabolites that can play a role in drug and chemical induced disease. Target organs include the liver, kidney and the lung. Chemical stress may result in both acute changes, such as alteration of the oxidised/reduced glutathione ratio (GSSG/GSH), and longer term effects, such as altered protein function through covalent modification. There is overwhelming evidence supporting a major role for the transcription factor Nrf2 in the sensing and response to chemical stress to model liver, kidney and lung toxins (such as acetaminophen, cis-platin and bleomycin) largely through work in in vitro systems and rodent models. Whilst sensing of chemical stress and induction of Nrf2 is clearly critical in adaptation, basal Nrf2 activity is also important to the phenotype with regards to the immediate response to chemical stress; Nrf2 has a key role not only in the detoxication of xenobiotics but also the metabolism of endobiotics. Nrf2 is not the sole transcriptional pathway that is involved in adaptation to chemical exposure, and its basal and inducible activity may ultimately form part of a larger transcriptional defence network. It is also important to note that Nrf2 plays a role in the regulation of other systems (e.g. the immune system, lipid homeostasis) that

have a potential role in adverse drug reactions. From a pharmacological perspective it is important to know 1) how the Nrf2 system may modulate the toxicity of a particular drug and 2) how the status of an individual's Nrf2 system may determine susceptibility to DILI. For this purpose we need to develop biomarkers that can be used in physiologically relevant in vitro models, animal models and in human studies.

S47 - IMPACT OF TUMOR-DERIVED CYTOKINES ON XENOBIOTIC CLEARANCE PATHWAYS IN CANCER: LIVER PROTEOME AND TRANSCRIPTOME OF TUMOR BEARING MICE

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Abstract not available.

S48 - GENETICS/GENOMICS APPROACHES TO STUDY REGULATION OF HEPATIC P450 GENE EXPRESSION

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Drug metabolizing cytochromes P450 and other genes involved in drug absorption, distribution, metabolism and exretion (ADME) are regulated by numerous complex signaling pathways including in particular xenobiotic-induced nuclear receptor networks but also networks related to endogenous and pathophysiological processes. The recent omics revolution enables novel approaches to study gene regulation, as will be shown by several examples. One promising approach is the unbiased identification of genetic loci associated with gene expression (expression quantitative trait loci, eQTL) by genome-wide association, which can reveal not only cis-acting gene polymorphisms but also trans-acting factors involved in regulation. Several eQTL studies were recently carried out on liver tissue and thousands of novel eQTLs were identified (Schadt et al., PLoS Biology 2008; Innocenti et al., PLoS Genetics 2011; Schröder et al., Pharmacogenomics J 2011). However, due to limited power only very few trans-eQTLs with relevance for P450 regulation were discovered this way. An alternative approach is illustrated by our pathway-guided analysis in which we tested association of gene polymorphisms in relevant transcriptional and other regulatory pathways with mRNA, protein and enzymatic function of CYP3A4. A total of 334 SNPs in 40 genes including transcription factors (HNFs, C/EBP, NFkB, USF1) and nuclear receptors (PXR, CAR, PPAR, FXR, VDR, GR), cytokines (IL1b, IL6), and drug transporters (MDR1, SLCO) were analyzed. Statistical analysis identified SNPs in several regulatory proteins (ARNT, GR, PGRMC2, PPARA) to be consistently and significantly associated with CYP3A4 phenotype (Klein et al., CPT 2012). Moreover, PPARA genotype was also shown to be associated with PPARA protein expression in liver. In yet another approach a systems biology-based computational analysis workflow was applied to genome-wide, time-resolved RNA expression changes in primary human hepatocytes challenged with atorvastatin, in order to reconstruct transcription factor-based regulatory mechanisms. Interestingly, this approach also identified PPARA as a novel potential regulator of CYP3A4 expression (Schröder et al., Bioinformatics 2011). Thus, diverse omics tools can be used to gain novel insight into gene regulation. However, functional validation of novel findings is indispensable to establish causality. Using gene knock-down by shRNA-encoding lentivirus, reporter gene assays, chromatin-immunoprecipitation, and EMSA, we confirmed PPARA as novel transcriptional regulator of CYP3A4 and other ADME genes. Finally, genome-wide transcriptomics was used to compare the PPARA "regulome" with those of the traditional xenosensors CAR and PXR, showing considerable overlap with respect to ADME gene regulation. These findings establish PPARA as a previously underestimated transcriptional regulator of CYP/ADME genes and they provide a novel link between lipid homeostasis, inflammation, and regulation with implications for drug treatment and pharmacogenomics. Supported by the network program Virtual Liver of the German Federal Ministry of Education and Science, and by the Robert-Bosch Foundation, Stuttgart, Germany.

S49 - RNA-INTERFERENCE-BASED FUNCTIONAL GENOMICS OF DRUG-INDUCED LIVER INJURY RESPONSES

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Drug-induced liver injury (DILI) is an important clinical problem which involves cytokine-mediated signaling. While numerous studies have unraveled DILI-related stress response pathways in vitro and in vivo models using genome-wide transcriptomics analysis, the functional relevance of such pathways in the hepatoxicity response is largely unclear. High throughput RNAinterference-based knock down of large sets of individual genes allows the disclosure of their functional importance in cytotoxicity. We have applied siRNA-based gene knock down in combination with

high content imaging to identify the critical cell signaling components that drive drug-induced liver cell death. In hepatocytes tumor necrosis factor (TNF) synergizes with various hepatotoxic drugs (e.g. diclofenac, carbamazepine, ketoconazole, amiodarone and nefazodone) that all cause idiosyncratic liver failure in the clinic. Gene expression profiling identified Nrf2 signaling, endoplasmic reticulum (ER) stress responses and death receptor signaling as key pathways of the cellular stress response caused by hepatotoxic drugs. We used individual knock down of alternatively expressed genes that define these pathways to uncover the critical role of individual Nrf2, ER stress and death receptor signaling components in the TNF/hepatotoxicant interaction. In addition, we have applied genome-wide kinase and ubiquitinase siRNA screens (in total ~1600 genes) to define novel signaling adverse outcome pathway components that regulate the pro-apoptotic TNF signaling during hepatotoxicity. Our studies successfully exemplify the power of siRNA technologies to discover in detail the new functional relevant molecular programs that govern drug-induced liver cell toxicities.

S50 - VISUALIZING PHARMACOGENETICS IN THE BRAIN: MODULATION OF BRAIN PERFUSION BY PHARMACOGENETIC POLYMORPHISMS AND DRUG EFFECTS

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Recent data showing that drug metabolizing enzymes (DME) are expressed and active not only in the liver, but also in the brain. This raises the question of what influence brain DMEs may have on brain metabolism of drugs, xenobiotics, and endogenous substrates. Relevant in this respect is evidence suggesting that genetic polymorphisms in DMEs may cause differences in brain function and homeostasis. The visualization of the modulation of brain function or local brain drug metabolism by genetic polymorphic DMEs remains technically challenging in humans. Therefore, complementary research strategies have focused on the identification of intermediate phenotypes assessing the genetic modulation of the involved enzymes in the context of brain function. This is the approach followed by genetic neuroimaging, where the brain correlates of genetic polymorphisms are investigated within clinically relevant neural circuitry-behavior models. A neuroimaging modality that has proven to be particularly interesting in this respect is perfusion imaging. In the past, quantitative methods such as perfusion imaging have been the domain of PET techniques. Arterial spin labelling (ASL) is an MRI technique that is cost-effective and safe, offers high reproducibility over long time periods, and has much higher spatial resolution than electroencephalography. Perfusion imaging is especially important in pharmacological imaging, because pharmacological agents often change activation at rest, which cannot be assessed with BOLD imaging. Due to its close association with measures of neural metabolism, brain perfusion at rest constitutes a basic index of baseline brain function that can be investigated under the action of diverse pharmacological agent. Therefore, perfusion imaging allows monitoring in vivo progressive changes in rest perfusion induced by psychotropic drugs over the course of weeks or months. In addition to general pharmacological effects, individual differences in the effect of medication including those associated with genetic polymorphisms can be assessed. The study of CYP2D6 and other xenobiotic metabolizing enzymes and transporters in the brain in combination with drug effects will produce a new type of studies that may be characterized as pharmacogenetic neuroimaging. The aim of pharmacogenetic imaging is the development of translational approaches for quantitative phenotypes that index individual differences (for example, based on genetic differences or vulnerability factors) as well as variation in the response to psychopharmacological medication. A recent genetic neuroimaging study identified a candidate endophenotype of pharmacogenetic polymorphisms in the brain by reporting an effect of CYP2D6 genetic polymorphism on resting brain function. These results were confirmed by a second independent study using a working memory and face recognition task. Genetic and pharmacological neuroimaging studies may uncover the brain functions through which associations between polymorphic DMEs and clinical phenotypes operate. This prospects the future relevance of pharmacogenetic polymorphisms for genetic and pharmacological neuroimaging.

S51 - PET STUDIES IN DRUG DEVELOPMENT

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The human genome contains over 23.000 genes determining structural and/or functional proteins. According to recent estimates, ~4.000 of them can be targeted at ~12.000 various target sites. Using advanced molecular imaging techniques and molecular imaging biomarkers, molecular targets including disease biomarkers and/or disease

modifiers can be visualized in the living organism. Complemented with animal disease models, with special regard to transgenic models, this approach has significant benefits in the field of both diagnostic biomarker and therapeutic drug development. Due to the lack of data on the functional significance of these sites, pharmacologists are now challenged to find the physiological roles of these receptors and identify selective agents and possible therapeutic indications. PET provides a new way to image the function of a target from rodents to human and by elevating the mass, to pharmacologically modify the function of the target. The main applications of radioligands in brain research concern human neuropsychopharmacology and the discovery and development of novel drugs to be used in the therapy of psychiatric and neurological disorders. A basic problem in PET brain receptor studies is the lack of useful radioligands with ideal binding characteristics. During the past decade various ^{11}C- and ^{18}F-labeled radioligands have been developed for labeling some of the major central neuroreceptor systems. There is still a need to develop pure selective PET tracers for all the targets of the human brain. This presentation will review recent examples in neuroreceptor radioligand development, PET in drug development and the translational potential from rodents to human of in vivo imaging of neurotransmitter systems. A basic problem in the discovery and development of novel drugs to be used in for example the therapy of neurological and psychiatric disorders is the absence of relevant in vitro or in vivo animal models that can yield results to be extrapolated to man. Drug research now benefits from the fast development of functional imaging techniques such as translational PET-imaging.

S52 - PET IN DRUG DISCOVERY AND DEVELOPMENT

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Abstract not available.

S53 - GENETIC PREDICTORS FOR DRUG-INDUCED LIVER INJURY

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Genome-wide association studies and genome sequencing have both facilitated the identification of genes that predict susceptibility to hepatotoxicity reactions linked to particular drugs. Data from at least six different genome-wide association studies concerned with different drugs associated with liver injury are now available. Each shows an important contribution to susceptibility by various HLA alleles. In some cases, reactions to drugs that are apparently unrelated chemically are associated with the same HLA class II allele; for example the HLA haplotype which includes HLA-DRB1*15:01 is a risk factor for drug-induced liver injury related to the nonsteroidal antiinflammatory drug lumiracoxib and to the antimicrobial co-amoxiclav. There is increasing evidence that hepatotoxicity reactions that are HLA-associated involve a T-cell response to either the parent drug or a metabolite directly or with conjugate formation. A recent genome-wide association suggests that HLA genotype is not a major predictor for all hepatotoxicity reactions and has provided some evidence for roles for genes that encode proteins relevant to drug disposition and the innate immune response. Some of these findings are consistent with previous candidate gene studies. Additional genetic factors, including rare variants that cannot be detected by genome-wide association studies, may contribute to both HLA-associated and non-HLA associated drug induced liver injury reactions. Exome sequencing of cases of liver injury due to co-amoxiclav has yielded some interesting new associations. Further genetic studies require larger sample collections relating to particular drugs and current efforts are directed towards achieving this.

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S54 - CLINICAL BIOMARKER QUALIFICATION FOR DRUG-INDUCED INJURY TO KIDNEY, LIVER AND THE VASCULAR SYSTEM WITHIN THE INNOVATIVE MEDICINES INITIATIVE (IMI) PROJECT SAFE-T

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The SAFE-T consortium was one of the first projects established under the EU's Innovative Medicines Initiative (IMI), starting on June 15, 2009. SAFE-T is working to address the current lack of sensitive and specific clinical tests to diagnose and monitor drug-induced injury to the kidney, liver and vascular systems in man, which is a major hurdle

in drug development. The consortium is a public private partnership of 25 organisations from pharmaceutical industry, small to medium sized enterprises, academic institutions and clinical units of excellence with representatives from health authorities as external observers and advisors. Key objective of the five-year project is qualification of biomarkers for drug-induced kidney, liver and vascular injury in translational studies and obtaining regulatory acceptance in translational and clinical contexts. The qualification program is split into an exploratory phase, assessing variability of candidate markers across different populations and obtaining initial performance estimates, and a confirmatory phase, evaluating diagnostic and predictive value of the most promising candidates in more depth. The study program consists of a set of prospective trials specifically designed for purposes of the consortium, and sampling from clinical or epidemiological studies performed by the consortium partners. Current biomarker lists for qualification include 22 candidates for Drug-Induced Kidney Injury (DIKI), 19 for Drug-Induced Liver Injury (DILI), and 34 for Drug-Induced Vascular Injury (DILI). The talk will give an overview on the consortium, the qualification program, and the biomarker candidates. Preliminary data from the exploratory qualification phase will be presented for some of the selected biomarkers.

S55 - CARDIOVASCULAR TOXICITY: A CHALLENGING OPPORTUNITY FOR TRANSLATIONAL BIOMARKERS

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The efficiency of pharmaceutical R&D is heavily dependent upon the rates of attrition of therapeutic agents progressing through the pipeline and improved safety is a key component of the drive for differentiated, reimbursable medicines. It is now accepted that cardiovascular toxicity is a primary cause of compound attrition during preclinical and clinical development and hence failure of drugs to reach patients (Valentin & Hammond, 2008). Furthermore, and of more concern to patients, prescribers, regulatory agencies and pharmaceutical companies alike is that cardiovascular toxicity has been the reason for labelling restrictions on drug use and withdrawal of medicines from the marketplace. In addition to the reported patient safety consequences there is a significant and poorly quantified economic burden of cardiovascular toxicity on state health care systems. The recognition of the extent and severity of cardiovascular toxicity has led to the publication of Regulatory guidance documents that specifically describe testing requirements for preclinical and clinical cardiovascular safety assessment of new medicines. Within pharmaceutical companies the impact can be seen in terms of loss of investment at the point of compound termination, often long before development costs are recouped, as well as potential for serious reputational consequences. It is clear that the consequences of cardiovascular toxicities are serious and that a compound showing cardiovascular liability may require additional studies to demonstrate an acceptable benefit:risk balance. Our fundamental understanding of the underlying causes of certain cardiovascular toxicities, such as abnormal changes in cardiovascular electrophysiology are relatively well advanced, with effective strategies optimised for hazard identification and risk assessment in place. Other causes of cardiovascular attrition such as damage to the heart tissue, blood vessels or heart valves tend to emerge during repeat dosing and consequently can be difficult to predict early in discovery. The presentation will focus on the preclinical response within one pharmaceutical company to the challenge posed by cardiovascular toxicity comprising both functional and morphological variants including examples of established and emerging translational biomarkers. Case studies are used to exemplify the approaches now available to prosecute cardiovascular toxicity. A view to emerging technologies and future challenges is also included.

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S56 - STRUCTURE AND DYNAMICS OF CYTOCHROMES P450 FROM MOLECULAR SIMULATION

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As the most important phase I drug metabolizing enzymes, the human Cytochromes P450 display an enormous versatility in the molecular structures of possible substrates. Individual isoforms may preferentially bind specific classes of molecules, but also within these classes, some isoforms show remarkable levels of promiscuity. In our work, we try to link this promiscuity to the versatility and malleability of the active site. Mainly focusing on the flexibility of protein structures and the presence or absence of water molecules, we establish molecular reasons for

observed promiscuity, determine the relevant factors to take into account when predicting binding poses and rationalize the role of individual interactions in the process of ligand binding [1,2]. A high level of active site flexibility does not only allow for the binding of a large variety of substrates and inhibitors, but also appears to be important to facilitate ligand binding and unbinding. We show that dynamic aspects need to be taken into account quantitatively calculating the free energy of binding of inhibitors and substrates and subsequently understand the molecular reasons for e.g. stereospecificity or cooperativity [3].

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S57 - UNDERSTANDING AND PREDICTION OF CYTOCHROME P450 MEDIATED METABOLISM

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The cytochrome P450 enzymes are involved in the metabolism of the majority of all drug compounds. They are a family of promiscuous enzymes, metabolizing many different compounds leading to multiple products for each drug compound. Prediction of what metabolites that the enzymes generate is still a major challenge. In principle, the binding free energy and activation energy need to be estimated accurately enough, which require advanced and time-consuming calculations. In this talk, we will discuss how precomputed activation energies on fragments found in common drug compounds can be used to predict of the site of metabolism. We will also discuss how simple extensions of the activation-energy-based models can be developed to predict the site of metabolism for some of the most important cytochrome P450 isoforms. Moreover, how to consider whether at all a compound is likely to be recognized by the enzymes will be discussed.

S58 - LABORATORY EVOLUTION OF REGIO- AND STEREOSELECTIVE P450 ENZYMES

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Since its conception in 1997,[1] the idea of directed evolution of stereoselective enzymes as a new approach to asymmetric catalysis has been generalized to include essentially all of the known enzyme types, including hydrolases, reductases, oxygenases, transferases, and C-C bond forming enzymes such as aldolases and oxynitrilases.[2] It involves repeating cycles of gene mutagenesis and screening, which builds up "evolutionary pressure". It is the Darwinian character of this evolutionary machine for generating highly enantio- and/or regioselective biocatalysts that the experimenter relies on. Since the screening step is the bottleneck of laboratory evolution, the real challenge is to obtain mutant libraries of highest quality requiring a minimum of screening effort. In this endeavor the so-called iterative saturation mutagenesis (ISM) has proven to be an extremely valuable tool.[2] The most recent example of ISM concerns the control of regio- and stereoselectivity of P450-catalyzed oxidative hydroxylation of steroids, which involves CH-activation. Mutants selective for the 2-position or 15-position were evolved on an optional basis with complete diastereoselectivity.[3] Small molecules can also be regio- and stereoselectively hydroxylated using P450 mutants produced by directed evolution, such oxidative CH-activation being of considerable synthetic interest.[4] P450 enzymes can also be tuned by utilizing additives such as per-fluoro fatty acids which are chemically inert, but which upon entering the large binding pocket activate the enzyme by "turning on" the high spin active state of Fe-heme.[5] In combination with directed evolution, interesting effects regarding selectivity can be achieved.[6] The lecture will focus on current methodology developments as applied to P450 enzymes.

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S59 - INTEGRATED ANALYSIS OF OMICS

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Safety assessment is striving to include new technologies able to provide better outcome, in terms of sensitivity, predictivity and mechanistic understanding of toxicity. Also, these technologies should aid identifying biomarkers to monitor adverse effects in the preclinical and clinical setting. Holistic approaches such as the now widespread toxicogenomics, metabonomics and proteomics, are ideally suited to generate the data to address these needs. However, a set of data is not sufficient to generate biological meaningful knowledge, like collection of words is not a book. Thus, technologies are evolving together with bioinformatics tools, pathway analysis and a profound understanding of cell biology to allow the interpretation of the data in the context of safety assessment. In the integrated EU Framework 6 project (PredTox), a project partly funded by the EU, was tasked with the goal of systematically using 'omics technologies to better understand the molecular mechanisms underlying liver and kidney injuries. Liver hypertrophy (LH), a recurrent preclinical finding, was studied by analysing the effects of six selected compounds administered to rats for up to 14 days. The integrated analysis of transcriptomic data across studies and the inclusion of additional 'omics markers provided information for the generation of mechanistic models that explained the underlying pathology. Some of the administered drugs caused a marked increase in the expression of xenobiotic metabolizing enzymes (XME) accompanied by the accumulation of newly-synthesized proteins within the smooth endoplasmic reticulum (SER). Another group of compounds, including troglitazone, caused mainly marked peroxisomal proliferation. Both molecular mechanisms acted by activating different nuclear receptors that led to a similar phenotypic outcome. Molecular analysis based mainly on gene expression and pathway analysis was used to better understand the differences. Similarly, the results obtained in the PredTox study suggested putative biomarkers of nephrotoxicity and bile duct necrosis. In particular for biomarker identification, the combination of conventional endpoints with transcriptomics and metabolomics has proven very useful. Specific kidney biomarkers have been identified and qualified in the past. In our laboratory, using a subset of compounds known to cause DILI and in particular cholestasis, biomarker performance was greatly improved when combining clinical chemistry endpoints with metabonomics (in serum) and gene expression in the liver tissue. In addition, the combination of metabolomics and transcriptomics has also been of use to elucidate drug-induced phospholipidosis. Besides of the quasi-traditional 'omics technologies, new markers are widely studied in the field of omics. Among the most prominent ones are miRNAs: small, single stranded RNAs able to regulate hundreds of transcripts, generally by suppressing translation. miRNAs are believed to be tissue specific and have been shown to be stable in serum after release into the circulation upon tissue damage. This makes them ideal candidates as biomarkers for disease and safety assessment. Bioinformatic tools are currently evolving in order to assess the tissue specificity and the target genes for each miRNA, in order to understand the biological effects related with their release and/or upregulation after toxic insult.

S60 - EXPANDING THE BOUNDARIES OF TOXICOGENOMICS: UNDERSTANDING THE DOSE-RESPONSE AT THE PATHWAY LEVEL AND DELINEATING GENETIC BACKGROUND-DEPENDENT AND –INDEPENDENT MECHANISMS OF TOXICITY

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Toxicogenomics is a mature field which provides invaluable information on the molecular events preceding or accompanying toxicity; however, most traditional use of gene expression and other –omic data in toxicology is limited to the mode-of-action analysis, classification/prediction, and biomarker discovery. Elucidation of (i) the shape of dose-response relationships at the pathway level, and (ii) the impact that inter-individual differences in gene expression patterns may have on both efficacy and safety outcomes, represent some of the novel frontiers in toxicogenomics. The number of toxicogenomic studies which incorporate dose-response and population-based designs is on the rise, and the applicability of such data to hazard assessment is also increasing. First, we will

describe a user-friendly computational approach for dose-response analysis of gene expression data at the pathway level. A fast dose-response curve fitting procedure is applied to the expression data, the pathways enriched for dose-responsive genes are detected using a resampling-based test of categorical significance, and pathway-based dose-response profiles are generated, which enables estimation of a pathway-based EC_{50} value while accounting for variability and uncertainty. Second, we posit that the challenge of elucidation of the genetic determinants for inter-individual differences in toxicity may be met through a combined analysis of the toxicity phenotypes, genetic and gene expression data from the population-based experimental in vivo and in vitro model systems. Using data obtained with several toxicants, we will demonstrate that the intricate interplay between genetic polymorphisms and gene expression requires careful consideration of the genetic background-dependent and –independent perturbed pathways.

S61 - PRACTICAL APPLICATIONS OF GENOMICS AND NEXT GENERATION SEQUENCING IN DRUG AND CHEMICAL DEVELOPMENT

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The genomics 'revolution' started in the mid 1990s with the advent of capillary sequencing. Capillary sequencing allowed the complete sequencing of mRNA libraries. The consequence of this was the rapid development of collections of cDNA clones that could be easily accessed by researchers and replaced the hitherto small collection of clones held, and exchanged, by individual laboratories. This drove the need for a platform to make use of this new resource with a greater capacity for parallel gene hybridisation than could be achieved with Northern's and Dot blots. The answer was the development of the microarray platform first used for measuring multiple transcriptome abundance but which now has developed multiple applications including epigenetic and structural genome analysis. These technologies immediately found an application in understanding transcriptome responses in cells that could be used to discern chemical mechanisms of toxicity, as well as having potential in redefining limits such as NOAELs. Foresight was demonstrated early in the technology development both in the US (GEO omnibus) and Europe (EBI ArrayExpress) in collecting these data in databases that could provide a resource for others involved in biological, chemical and drug research. Now holding a substantial amount of data these are a rich, and free, resource for utilisation. Consequentially applications have developed to use these data for example in connectivity mapping; a method that can be used to discern novel properties in new or existing chemical entities. Development of the sequencing technology that led originally to microarrays did not stop at capillary sequencing. Recent methods of sequencing fragments attached to a solid substrate have greatly increased the capacity and meant that it is now quite possible for one person to sequence one or more mammalian genomes in a few days. Consolidation of these machines in sequencing centres multiplies this productivity still further. In addition bioinformatic applications have developed so it is no longer arduous to analyse the data generated. Sequencing technology is now at a point where reasonable prediction is being made that our genome will be part of our medical record within a decade. For both drug and chemical development this presents opportunities and challenges. The opportunities are in the application of these technologies to development and improve products. The challenge is in assessing the impact of toxicity in an era where quite possibly genome based individual, or at least sub population, risk assessments will need to be made.

S62 - METABOLIC ACTIVATION AND DRUG INDUCED TOXICITY: NEW INSIGHTS FROM COVALENT DRUGS

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The metabolism of xenobiotics to chemically reactive intermediates that covalently modify cellular macromolecules has been implicated as one general mechanism of drug-induced toxicity, although it is recognized that some reactive electrophiles appear to be benign while others are not. The factors that determine the toxicological potential of a given reactive species are poorly understood, and this limitation in our knowledge has hampered the development of predictive models of drug toxicity. Recently, a number of pharmaceutical companies have identified multiple risk factors for drug-induced liver injury, which typically include evidence of metabolic activation (as measured by covalent binding to protein, GSH adduct formation, etc) along with projected clinical dose (ie "body burden" of reactive metabolites). Additional insight has been provided in the form of a growing database on "structural alerts" for toxicity, as well as a consideration of those therapeutic agents whose pharmacological activity depends on covalent modification of their respective biological targets. Indeed, there has been renewed interest in recent years

in targeted covalent inhibitors as drug candidates, notably in the field of oncology, which has further stimulated discussion on the nature of xenobiotic-protein modifications that are safe relative to those that result in toxicity. This presentation will focus on those new developments that are advancing our understanding of the molecular basis of drug toxicity resulting from metabolic activation events.

S63 - WAYS TO ADDRESS CHEMICALLY REACTIVE METABOLITES DURING DRUG DEVELOPMENT

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Human idiosyncratic toxicity cannot always be predicted by preclinical toxicology models. Although idiosyncratic toxicity is defined by its low incidence, it can be life-threatening. Due to its low incidence, human idiosyncratic toxicity often only becomes apparent during late-stage trials or even later during post-marketing and can result in development termination or post-marketing withdrawal. Since patients' safety could potentially be at risk, efforts to reduce the likelihood of human idiosyncratic toxicity are warranted. The issue is, as stated at the outset, that human idiosyncratic toxicity is not consistently predicted by preclinical toxicology models and thus, the pharmaceutical industry as a whole is at a loss for how to best address these concerns. A generally accepted hypothesis is based on literature reports suggesting that drugs manifesting human idiosyncratic toxicity can either be experimentally-demonstrated or theoretically-expected to generate reactive metabolites in humans. Further, it is hypothesized that those reactive metabolites either form conjugates with endogenous protein via covalent binding, thus perturbing their function or, by forming the conjugate, generate a new hapten in the body that triggers an immune reaction. Although the link between reactive metabolite formation and human idiosyncratic toxicity is only hypothesized, it is still generally accepted that a complex link likely exists and it would be prudent to limit the generation of reactive metabolites. The pharmaceutical industry has taken various approaches to form an effective strategy to address chemically reactive metabolites that includes identifying and removing structurally alarming features in the drug or metabolites, running of covalent protein binding experiments, evaluating ability to chemically-trap metabolites, assessing quasi- and fully-irreversible inhibition of metabolizing enzymes, and assessing cellular toxicity during following drug incubation in metabolically-competent in vitro systems. These approaches and other novel approaches will be discussed including their benefits and limitations.

S64 - DRUG BIOACTIVATION VIA REACTIVE METABOLITES: IMPORTANCE OF SULFENIC ACIDS

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Sulfenic acid intermediates are formed during the oxidation of cysteine residues of proteins and play key roles in enzyme catalysis, redox homeostasis and regulation of cell signalling. However few data are presently available on the formation and fate of sulfenic acids as reactive intermediates during the metabolism of xenobiotics. Recent data will be presented about the formation of such intermediates during the metabolic activation of anti-thrombotic drugs of the tetrahydro-thienopyridine series, ticlopidine, clopidogrel and prasugrel. These data allowed us to explain the origin of a recent controversy about the nature of the enzyme responsible for the in vivo transformation of clopidogrel into pharmacologically active metabolites that act as inhibitors of platelet aggregation. They confirmed that two cytochrome P450-dependent oxidation steps are involved in clopidogrel bioactivation, and showed that paraoxonase-1 only catalyzes the formation of a minor metabolite which is an isomer of the pharmacologically active metabolite. Thus P450 2C19, but not paraoxonase-1, is a major determinant of clopidogrel efficacy. Other drugs such as omeprazole and disulfiram become pharmacologically active after transformation into sulfenic acid reactive metabolites. In a more general manner, the different mechanisms by which a sulfur-containing xenobiotic can be metabolized with the intermediate formation of a sulfenic acid, the different possible fates of these sulfenic acids, and the possible implications of their formation in pharmacology and toxicology will be discussed.

A1 - HLA-B*5701-RESTRICTED ACTIVATION OF FLUCLOXACILLIN-RESPONSIVE CD8+ T-CELLS

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Flucloxacillin is a common cause of drug-induced liver injury (DILI). Delay in the reaction onset and identification of HLA-B*5701 as a susceptibility factor are indicative of an immune pathogenesis[1]. Despite this, drug-responsive T-cells have not been detected and as such the role of the HLA risk allele in the disease is not known. We test the following hypothesis: drug-responsive T-cells circulate in patients with flucloxacillin-induced liver injury and the drug antigen is presented to T-cells by HLA-B*5701. PBMC were isolated from 6 patients with a history of flucloxacillin-induced liver injury (5 of the patients express HLA-B*5701) and tolerant controls. PBMC responses to flucloxacillin were characterized using enzyme-linked immunosorbent spot (ELISpot). We also utilized our recently established *in vitro* T-cell priming assay that recapitulates key elements of events that occur *in vivo* during elicitation of an immunological drug reaction by combining naive CD3+ T-cells from 3 HLA-B*5701 positive volunteers with flucloxacillin and dendritic cells in culture[2]. Drug-responsive T-cells were expanded for 8 days prior to analysis of IFN-γ secreting cells. T-cells from patients and volunteers were cloned and flucloxacillin-specific proliferation and cytokine secretion measured. The mechanism of flucloxacillin presentation to T-cells and the basis for the association with HLA-B*5701 was investigated. Flucloxacillin-responsive CD4+ and CD8+ T-cells were characterized from patients with DILI. Furthermore, naive CD8+ T-cells from volunteers expressing HLA-B*5701 were activated with flucloxacillin when dendritic cells present the drug antigen. T-cell clones secreted IFN-γ, Th2 cytokines, perforin, granzyme B and FasL following drug stimulation. Flucloxacillin-derived haptens bound to selective lysine residues on albumin in a time-dependent manner and the level of binding correlated directly with the stimulation of clones. Activation of CD8+ clones with flucloxacillin was processing-dependent and restricted by HLA-B*5701 and the closely related HLA-B*5801. Clones displayed additional reactivity against β-lactam antibiotics, but not abacavir. These studies define the immune basis for flucloxacillin-induced liver injury and link the genetic association to the iatrogenic disease.

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A2 - IMPACT OF CHRONIC RENAL FAILURE ON THE EXPRESSION AND ACTIVITY OF BRAIN CYTOCHROME P450 IN RATS: IMPLICATION OF PARATHYROID HORMONE

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Background: Chronic renal failure (CRF) impedes not only renal excretion of drugs but also their metabolism by reducing liver and intestine cytochrome P450 (CYP450), especially the CYP3A family. We have shown that uremic serum contains factors that decrease liver CYP450 protein expression and metabolic activity. We have identified parathyroid hormone (PTH) as one of those factors. The present study aimed to investigate *(1)* the repercussions of CRF on rat brain CYP450, *(2)* the role of PTH in the downregulation of brain CYP450 in rats with CRF, and *(3)* the effects of PTH on CYP450 in astrocytes. For this purpose, CYP450 isoenzymes mRNA and protein expression in whole brain as well as different brain regions (cortex, cerebellum, hippocampus, and rest of brain parenchyma) have been studied in order to determine the effects of CRF on cerebral drug metabolism by CYP450. Also, the effect of parathyroidectomy (PTX) on whole brain CYP450 was evaluated in rats with CRF, and, finally, the effects of PTH on CYP450 in astrocytes were determined. **Methods:** The brain of CRF rats (induced by 5/6th nephrectomy) and control rats (sham laparotomy) was harvested and microsomal fractions of either whole brain or dissected brain regions were prepared. Whole brain microsomal fractions from control or CRF rats that underwent PTX one week prior to nephrectomy were also prepared. Protein and mRNA expression of Cyp1a, Cyp2c11, Cyp2e1 and Cyp3a were

assessed by Western Blot assay and Real Time PCR, respectively. Cyp3a activity was assessed in brain microsomal preparation. For the *in vitro* study, primary cultured astrocytes were incubated either with serum from CTL, CTL-PTX, CRF or CRF-PTX rats or in normal serum with or without PTH, and Cyp3a protein expression was assessed. **Results:** We found downregulations (at least 40%) of Cyp1a, Cyp2c11 and Cyp3a in whole brain and brain regions of CRF rats. These downregulations were not associated with decreases in mRNA expression. A significant decrease in Cyp3a activity was also observed. We found that PTX prevents the decrease in Cyp3a expression in whole brain microsomes from CRF rats. Finally, serum form CRF rats decreased Cyp3a expression in rat astrocytes while serum from CRF-PTX rats had no effect on Cyp3a in astrocytes. **Conclusion:** CRF is associated with a decrease in some major brain drug-metabolizing enzymes, which could explain an increase in bioavailability of drugs in the brain. PTH is a major mediator implicated in these downregulations.

A3 - ZEBRAFISH: AN IN VIVO HIGH-THROUGHPUT SCREENING MODEL FOR CYP3A4 INDUCTION AND INHIBITION

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High-throughput *in vitro* assays are widely employed during early drug discovery to predict xenobiotic metabolism. While *in vitro* drug metabolism models such as recombinant enzymes, liver microsomes and hepatocytes are well established for drug metabolism research, they do not mimic the *in vivo* physiological conditions completely. For example, binding of drug to plasma proteins and blood cells, and extra-hepatic metabolism are not modeled using such *in vitro* systems. Human CYP3A4 is the single most important drug-metabolizing enzyme. It accounts for roughly 40% of the total cytochrome P450 in human liver microsomes and metabolizes more than 50% of the clinically used drugs. In this study, a novel *in vivo* high-throughput zebrafish model was explored for the screening of CYP3A4 induction and inhibition activities. Each experiment utilizing 5-day post fertilization wild-type zebrafish larvae was conducted via five replicates in a 96-well format, with each replicate pooled from three larvae. For induction studies, zebrafish larvae were incubated for 48 hours with varying concentrations of nine FDA-recommended human CYP3A-inducers (carbamazepine, dexamethasone, efavirenz, nafcillin, phenytoin, pioglitazone, prednisone, rifampicin and rufinamide) with subsequent measurement of CYP3A activity using the CYP3A4-specific chemiluminescing probe substrate, Luciferin-IPA. Gene expression levels of zebrafish CYP3A65 (homologous to human CYP3A4) were determined for dexamethasone and rifampicin via real time-reverse transcriptase polymerase chain reaction. For inhibition studies, four FDA-recommended human CYP3A-inhibitors (cimetidine, erythromycin, ketoconazole and verapamil) were co-incubated with Luciferin-IPA for 1 hour followed by measurement of CYP3A4 activity. Our results demonstrated that five out of the nine human CYP3A4-inducers exhibited statistically significant ($P<0.05$) enzyme fold-induction (FI) at a concentration level of 100 μM (carbamazepine, 1.8 ± 0.3 FI; dexamethasone, 1.9 ± 0.3 FI; nafcillin, 1.4 ± 0.2 FI; phenytoin, 1.6 ± 0.2 FI; pioglitazone, 2.4 ± 0.1 FI). Furthermore, pioglitazone and carbamazepine dosed at 0.1, 1, 10 and 100 μM exhibited a concentration-dependent enzyme induction. CYP3A65 gene was expressed 2.6-fold and 1.7-fold higher in each of the respective 100 μM dexamethasone- and rifampicin-treated samples. Unlike dexamethasone, rifampicin treatment induced CYP3A65 at the mRNA level but not functional enzyme induction. Of the four human CYP3A4-inhibitors tested, ketoconazole showed concentration-dependent enzyme inhibition with an IC_{50} value of 5.61 μM. Collectively, our proof-of-principle study established the novel potential of zebrafish as an *in vivo* high-throughput model for screening CYP3A4 induction and inhibition functional activities. While human CYP3A4 induction and inhibition phenotypes are only partially modeled using the wild-type zebrafish, the development of transgenic zebrafish model that closely mirrors human liver metabolism would facilitate its future application in the screening of xenobiotic metabolism.

* H.T.C. and R.F.S.L. contributed equally to the project.

A4 - EPIGENETIC REGULATION IS A CRUCIAL FACTOR FOR DEFECTIVE EXPRESSION OF UGT1A1 IN HUMAN KIDNEY

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Human UGT1A1 is predominantly expressed in liver and intestine, but not in kidney. It has been reported that hepatocyte nuclear factor (HNF) 1α is involved in transactivation of UGT1A1. Considering the expression of HNF1α in various tissues including liver and kidney, the mechanism of defective expression of UGT1A1 in kidney is open to question. The purpose of this study was to uncover the mechanism of defective expression of UGT1A1 in kidney,

focusing on the epigenetic regulation. *In silico* analysis predicted a putative CpG rich region near the transcription start site (TSS) of UGT1A1. Bisulfite sequence analysis revealed that the CpG rich region near the TSS was hypermethylated in the kidney, whereas was hypomethylated in the liver, suggesting that DNA methylation would contribute to the differences in the UGT1A1 expression. Chromatin immunoprecipitation assay demonstrated that histone H3 around UGT1A1 promoter region was highly acetylated in liver, but not in kidney. In addition, recruitment of HNF1α to the promoter was observed in liver, but not in kidney. These observations suggest that DNA hypermethylation in kidney is linked with a defect of histone H3 acetylation and binding of HNF1α. Next, we investigated the effects of inhibition of DNA methylation and histone deacetylation on UGT1A1 expression using cell lines. Liver-derived HuH7 and kidney-derived HK2 cells were transfected with HNF1α and treated with 5-aza-2'-deoxycytidine (5-aza-dC) and trichostatin A (TSA), which inhibit DNA methylation and histone deacetylation, respectively, and then the expression level of UGT1A1 mRNA was determined by real-time RT-PCR. UGT1A1 mRNA was substantially expressed in HuH7 cells, whereas it was marginally expressed in HK2 cells. UGT1A1 promoter was found to be hypomethylated in HuH7 cells, whereas it was hypermethylated in HK2 cells, suggesting the importance of DNA methylation in basal UGT1A1 expression. Treatment of HK2 cells with 5-aza-dC but not TSA resulted in an increase of UGT1A1 mRNA expression, and treatment with 5-aza-dC and TSA resulted in a synergistic increase of UGT1A1 mRNA. Furthermore, overexpression of HNF1α in HK2 cells increased the UGT1A1 mRNA only in the presence of 5-aza-dC. In contrast, neither inhibition of DNA methylation nor overexpression of HNF1α caused an increase of UGT1A1 expression in HuH7 cells. Taken together, we found that the DNA hypermethylation along with hypoacetylation of histone interferes the binding of HNF1α, resulting in the defective expression of UGT1A1 in the human kidney. Thus, epigenetic regulation is a crucial factor for defective expression of UGT1A1 in human kidney.

A5 - UDP-GLUCURONOSYLTRANSFERASE (UGT) 2B7 AND 1A9 SUPPRESS CYTOCHROME P450 3A4 FUNCTION: EVIDENCE FOR THE INVOLVEMENT OF THE CYTOSOLIC TAIL OF UGT IN THE SUPPRESSION

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Cytochrome P450 (P450, CYP) 3A4 is thought to contribute to the metabolism of about 50% of drugs. There are large inter-individual differences (~40-fold) in the expression level of hepatic CYP3A4. However, variance in the *in vivo* clearance of drugs metabolized by this P450 is much less (8~10-fold). Because of this discrepancy, it would be reasonable to suppose that variations in CYP3A4 function are caused by other distinct mechanisms, in addition to changes in expression levels. Regarding this issue, we focused on protein-protein interactions between CYP3A4 and UDP-glucuronosyltransferases (UGTs). Our previous studies have confirmed the association of P450 and UGTs by several methods including co-immunoprecipitation and overlay assay [1]. We have also shown that CYP3A4 alters the regio-selectivity of UGT2B7-catalyzed morphine glucuronidation [2]. However, the reverse effect whether UGT can modulate the activity of CYP3A4 has not yet been elucidated. To address this issue, we established an insect cell (Sf9) system co-expressing CYP3A4, NADPH-P450 reductase, and the major human UGT isoforms, UGT2B7/1A9, and CYP3A4 activity was compared between Sf9 microsomes with and without UGT co-expression. CYP3A4 activity was not affected by co-expressing calnexin, which has the same topology as UGT in the endoplasmic reticulum. In contrast, CYP3A4 activity was significantly suppressed by simultaneous expression with UGT2B7 and 1A9. These results suggest that the CYP3A4-UGT interaction reduces CYP3A4 function. Although both UGT2B7 and 1A9 exerted positive effects on CYP3A4 activity, subtle differences in kinetics were observed. For example, while UGT2B7 significantly reduced the Vmax of CYP3A4, UGT1A9 increased the S_{50} without affecting Vmax. Hence, UGT seems to suppress CYP3A4 catalysis in an isoform-specific manner. To identify the UGT domain that interacts with CYP3A4, a series of C-terminal-truncated UGT2B7 mutants were generated. Although a mutant lacking 11 amino acid residues (^{519}Phe - ^{529}Asp) of the C-terminus exhibited a similar suppressive effect on CYP3A4 activity as the wild-type enzyme, another construct lacking a longer sequence (^{511}Cys - ^{529}Asp) lost the suppressive effect. This, therefore, suggests that the region from ^{511}Cys to ^{518}Lys of the cytosolic tail of UGT2B7 plays a crucial role in the interaction with CYP3A4. This study showed, for the first time, an alteration of CYP3A4 function by UGT. This protein-protein interaction between UGT and CYP3A4 may be one of the reasons why there is a discrepancy between CYP3A4 levels and CYP3A4-mediated drug clearance.

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A6 - UNEXPECTED SPECIES DIFFERENCES AND STRUCTURAL DETERMINANTS OF STATIN TRANSPORT BY SODIUM-TAUROCHOLATE CO-TRANSPORTING POLYPEPTIDE (NTCP/SLC10A1)

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Sodium-taurocholate co-transporting polypeptide (NTCP/*SLC10A1*) is a well-studied hepatic bile acid uptake transporter that has been more recently recognized for its potential role as a drug transporter. Notably, statins are transported by human NTCP, but not rat Ntcp. We aimed to identify the region of NTCP involved in statin transport, and to further investigate species-related differences in statin transport by NTCP. We employed homologous recombination of linear plasmid DNA by *E. coli* to create a library of human NTCP and rat Ntcp chimera with randomly distributed junctions. The uptake of [^{3}H]-atorvastatin and [^{3}H]-rosuvastatin by the chimeric transporters, as well as human, mouse and rat NTCP, was assessed in HeLa cells. We observed mouse Ntcp and human NTCP were capable of atorvastatin and rosuvastatin transport, but rat Ntcp was not. By screening the NTCP chimera, we identified a region, spanning from Met160 to Val217, that when replaced in rat Ntcp with the corresponding sequence of human NTCP, conferred atorvastatin transport to rat Ntcp. The resulting rat Ntcp, when site-directed mutagenesis was carried out match the human NTCP sequence for 14 amino acids in this region, exhibited 98% of human NTCP atorvastatin transport, compared with negligible transport (<5%) exhibited by wildtype rat Ntcp ($p < 0.001$). This region is predicted to form intracellular loop 3 and the adjacent transmembrane helices 5 and 6. In conclusion, unlike rat Ntcp, mouse Ntcp was capable of statin transport, similar to human NTCP. In addition, we identified a key region of human NTCP which accounts for the observed difference in statin transport. These findings further confirm the importance of NTCP as not only a bile acid transporter, but also for hepatic drug uptake and that in some cases, caution is warranted in extrapolating between rodent models (mouse vs rat) and interpreting species-dependent effects during preclinical transporter studies.

A7 - EVIDENCE FOR HNRNP L AS A SPECIFIC TRANSCRIPTION FACTOR OF THE CYP2C19*17 ALLELE

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CYP2C19 plays an important role in the metabolism of several drugs including protein pump inhibitors such as omeprazole, the antidepressant escitalopram, the platelet aggregation inhibitor clopidogrel and the antimalarial proguanil. Inter-individual differences in the expression of the *CYP2C19* gene are associated with major effects in drug treatment efficacy. Thus, inter-individual differences in CYP2C19 activity can explain higher risk of adverse drug reactions or therapeutic failures. An important factor explaining these differences is the presence of genetic polymorphism and today than 30 known *CYP2C19* alleles have been described (http://www.cypalleles.ki.se/cyp2c19.htm), where the common *CYP2C19*17* allele (20 % frequency) causes a significantly higher expression of the gene. The increased expression of the *CYP2C19*17* gene was initially proposed to be linked to the recruitment of different transcription factor(s) to the canonical mutated -806 site which did not occur in the wt *CYP2C19*1* promoter. This suggests the presence of a different set of co-regulators that bind to the DNA for each allele and a study elucidating the basis for different modes of transcriptional control was initiated. Thus, we performed screening of the proteins binding to -806 region in the *CYP2C19* gene using nuclear extracts and oligonucleotide-affinity chromatography followed by LC/MS. The results obtained showed a specific binding to the biotinylated -806T oligonucleotide of some members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family. Antibodies toward hnRNP A3 and L caused a supershift in EMSA analysis. An oligonucleotide-affinity

chromatography followed by Western blot analysis showed, when using MCF7 and HepG2 and also human liver nuclear extracts, that there is a specific binding of hnRNP L to the element carrying -806T. Thus, hnRNP L is a strong candidate as being a major regulator of *CYP2C19*17* but not *CYP2C19*1* gene expression.

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A8 - MODULATION OF HEPATIC TRANSPORTER GENES BY CITROBACTER RODENTIUM INFECTION

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As the primary organ of drug and toxicant metabolism, the liver plays a key role in the detoxication and elimination of many drugs and xenobiotics. The capacity of the liver to clear toxicants from the body is impacted by disease and infection. For example, in the classical model of sterile infection, administration of bacterial lipopolysaccharide has been shown to alter hepatic drug metabolizing enzyme (DME) and transporter expression. Additionally, lipopolysaccharide treatment and experimental sepsis have been shown to induce cholestasis through altered enzyme and transporter expression. Previous work in our laboratory has detailed the modulation by live *Citrobacter rodentium* infection (a model of enteropathogenic *Escherichia coli*) of the expression of hepatic DMEs, and investigated the role of specific cytokines in that modulation. Since many of the processes and factors which regulate DME expression have also been shown to control transporter expression, we hypothesized that infection with live *C. rodentium* will modulate hepatic drug transporter expression as well as bile acid related enzymes and transporters. In order to investigate this hypothesis, mice lacking *Toll-like receptor 4* (*TLR4*) (in C3H background), *Interleukin-6* (*IL-6*), or *Interferon-gamma* (*IFNγ*) (in C57BL/6 background) as well as appropriate wild type animals were orally infected with live *C. rodentium* and sacrificed 7 days later. Hepatic mRNA expression of drug transport and other bile acid-related genes was determined using quantitative RT-PCR. In wild type mice, drug transporter mRNA expression was significantly decreased by infection for *Slc22a4*, *Slco1a1*, *Slco1a4*, *Slco2b1*, *Abcc2*, *Abcc3*, *Abcc4*, and *Abcc6*. In contrast, mRNA expression of *Slco3a1* and *Abcb1b* was increased in infected animals, while *Abcg2* was unresponsive to the infection. Interestingly, significant strain differences in the response to live infection were observed with two transport genes, *Slc22a5* and *Slco1b2,* with significant increases in infected C3H animals, and significant decreases in infected C57BL/6. Genes involved in bile acid synthesis and transport were also significantly impacted during C. rodentium infection. Expression of *Abcb11*, *Slc10a1*, and *Cyp7a1*, the two major hepatic bile acid transporters and the rate-limiting enzyme of bile acid synthesis, was significantly decreased in infected animals. The effect of infection on several of the genes analyzed appeared to be cytokine-dependent. Thus, the downregulation of *Slc22a4*, *Slco1a4* and *Cyp7a1* was absent in *IFNγ*-null mice. Similarly, the downregulation of *Slco1a1* was blocked in *IL-6*-null mice. The mRNA regulation of all genes in *TLR4*-null mice (HeJ) was comparable to those in wild type animals (HeOuJ). These data indicate that *C. rodentium* infection modulates hepatic drug processing through alteration of transporter expression as well as DMEs. Furthermore, this live infection downregulates important genes of bile acid synthesis and transport and may increase the risk for cholestasis. This work was supported by NIH grants R01 DK072372 and T32ES012870.

A9 - THE TOXTRACKER ASSAY: NOVEL GFP REPORTER SYSTEMS THAT PROVIDE MECHANISTIC INSIGHT INTO THE GENOTOXIC PROPERTIES OF CHEMICALS

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People are exposed to an ever-increasing number of chemical compounds that are developed by industry for a wide range of applications. These compounds may harmfully react with different cellular components and activate specific defense mechanisms that provide protection against the toxic, mutagenic and possibly oncogenic consequences of exposure. Monitoring the activation of specific cellular signaling pathways upon exposure may therefore allow reliable and mechanism-based assessment of potential (geno)toxic properties of chemicals, while providing insight into their primary mode of toxicity. By whole-genome transcription profiling of mouse embryonic stem (mES) cells we identified genes that were transcriptionally activated upon exposure to either genotoxic compounds or pro-oxidants. For selected biomarker genes we constructed reporters encoding C--terminal GFP-tagged fusion proteins. GFP reporter genes were located on bacterial artificial chromosomes (BACs) thereby enabling transcriptional

regulation of the reporters by their own physiological promoter. The Bscl2-GFP reporter is selectively activated after exposure to genotoxic agents and its induction is associated with inhibition of DNA replication and activation of the ATR-depedent DNA damage signaling pathway. The Srxn1-GFP reporter is preferentially induced upon oxidative stress and is part of the Nrf2 antioxidant response pathway. The Btg2-GFP reporter is controlled by the p53 tumor suppressor and is activated upon exposure to different classes of genotoxic and cytotoxic chemicals. These different mES reporter cell lines are combined in a novel, highly sensitive and selective (geno)toxicity assay (ToxTracker) that utilize the differential responsiveness of various reporter cell lines enables prediction of the primary reactive properties of known and unknown chemicals. Validation of the ToxTracker assay using an ECVAM-recommended compound library consisting of 50 genotoxic and non-genotoxic chemicals confirmed the sensitivity and reliability of the assay with nearly no false-positive test results. The ToxTracker assay provides a powerful tool for carcinogenic risk assessment of novel chemicals and can help to identify the various cellular signaling pathways that are activated upon exposure to these agents.

A10 - CLINICAL RELEVANCE OF DRUG-DRUG INTERACTIONS WITH ORAL ANTIDIABETIC DRUGS OCCURRING AT THE LEVEL OF OATP1B1

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Organic anion-transporting polypeptide 1B1 (OATP1B1) is an important uptake transporter expressed at the basolateral membrane of hepatocytes. Several polymorphic variants of OATP1B1 have been described, of which OATP1B1*15 (Asn130Asp and Val174Ala) has been associated with decreased transport activity, increased plasma exposure and increased toxicity of selected drugs, including many statins. Since statins are often concomitantly prescribed with oral antidiabetic drugs in the clinic, the aim of this study was to investigate possible drug-drug interactions between these drugs at the level of OATP1B1, and to study the effect of the *15 polymorphic variant in this. We therefore generated and characterized HEK293 cells stably overexpressing OATP1B1 or OATP1B1*15. Absolute expression levels of OATP1B1 and OATP1B1*15 in cellular membranes were similar between the two cell lines, as measured by LC-MS/MS. As expected, the V_{max} value for transport of $E_2$17β-G by OATP1B1 was decreased by more than 60% in HEK-OATP1B1*15 cells, whereas K_m values were comparable between OATP1B1 and OATP1B1*15. The uptake of rosuvastatin by HEK-OATP1B1 (K_m 13.1 ± 0.43 µM and V_{max} 202 ± 2.1 pmol/mg protein/min) was nearly absent in HEK-OATP1B1*15 cells. Interestingly, several oral antidiabetic drugs (glyburide, glimepiride, troglitazone, pioglitazone, and glipizide) were identified as significant inhibitors of the OATP1B1-mediated transport of both substrates, whereas gliclazide, tolbutamide and metformin did not affect the function of OATP1B1 (IC_{50} >250 µM). The IC_{50} values for inhibition of $E_2$17β-G uptake were comparable between HEK-OATP1B1 and HEK-OATP1B1*15 cells. To assess whether the established IC_{50} values are of clinical relevance, we compared them with reported C_{max} values of the studied oral antidiabetics after the maximum dose in patients. Importantly, the reported maximum plasma levels are within the same concentration range as some of the observed IC_{50} values in this study. However, as these oral antidiabetic drugs show a relatively high plasma protein binding *in vivo* (>98%), it remains to be established whether the studied oral antidiabetics affect the clinical pharmacokinetic profile of rosuvastatin.

A11 - COMPARISONS OF CYP2A6 GENOTYPE AND ENZYME ACTIVITY BETWEEN SWEDES AND KOREANS

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Cytochrome P450 2A6 (CYP2A6), involved in biotransformation of several endogenous and exogenous compounds, is well-known by its wide inter-individual and inter-ethnic variations. The aim of this study was to compare CYP2A6 genotype and enzyme activity between two distinct populations, namely Swedes and Koreans, and to investigate the

influence of genotype, sex, age, cigarette smoking and oral contraceptive (OC) use on enzyme activity. The study involved 190 Swedish and 144 Korean unrelated healthy volunteers, which were genotyped for CYP2A6*1B, *1x2, *4, *5, *7, *8, *9 and *10 variant alleles. In addition, using caffeine as a probe, in vivo CYP2A6 activity was estimated by the 17U/17X urinary ratio. The results revealed that, except for the 1436G>T polymorphism that was absent in both populations, the distribution of CYP2A6 variant alleles and genotypes was significantly different between Swedes and Koreans (P=0.002). The distribution of genotype groups, appointed according to the presence of functional (*1A, *1x2, or *1B1) or less functional/non-functional CYP2A6 alleles (*4, *7, *8, *9 and *10), also significantly differed between the populations (P<0.0001), indicating rapid and slow genotype groups as the most prevalent among Swedes and Koreans, respectively. CYP2A6 genotype significantly affected enzyme activity in both populations (P=0.004), while no effect of sex (P=0.14), age (P=0.32), cigarette smoking (P=0.39) or OC use (P=0.59) was observed. The 17U/17X ratios in Swedes (n=190) ranged from 0.00 to 2.81 (median: 0.50), while in Koreans (n=144) were between 0.00 and 1.96, with the median value of 0.22. The probit transformation indicated common antimode at 0.01, with 3.16% of Swedes and 18.75% of Koreans having phenotype of slow metabolizer (P<0.0001). CYP2A6 enzyme activity was significantly higher in Swedes compared to Koreans (P=0.0001). The observed differences between the two populations remained significant when controlling for the genotype effect, i.e. within rapid (P=0.0007) and intermediate (P=0.04) genotype groups. In conclusion, we report major differences in CYP2A6 genotype and enzyme activity between Swedes and Koreans. Swedes display significantly higher CYP2A6 activity compared to Koreans, and the observed difference is mainly, but not entirely due to significantly lower frequency of defective CYP2A6 variant alleles in former compared to latter. In addition to ethnicity, CYP2A6 genotype, but not sex, age, cigarette smoking and OC use, significantly affect CYP2A6 enzyme activity.

A12 - PHYSIOLOGICALLY BASED MODELING OF FIRST PASS METABOLISM IN THE GUT WALL – ESTABLISHMENT AND PRELIMINARY VALIDATION OF IN VITRO-IN VIVO SCALING FACTORS FOR INTESTINAL METABOLISM IN BEAGLE DOG

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Beagle dog is a widely used *in vivo* model to guide clinical formulation development and to explore the potential for food effects. However, screening in dog provides low throughput and results are often not directly translatable to human. Consequently, a physiologically based modeling approach has been proposed with the aim of improving human absorption predictions, by using the dog as a validation step to verify the model assumptions [1]. One current weakness in this strategy is related to intestinal metabolism, which can be a significant determinant of oral bioavailability. However, validated tools to incorporate intestinal metabolism into physiological models of the dog are lacking. Consequently, the aims of this work were to characterize the expression and activity of major metabolizing enzymes in the dog intestine and to establish and preliminarily validate *in vitro – in vivo* scaling factors for intestinal metabolism in the dog. Microsomes from different intestinal segments were prepared from eluted enterocytes of four beagle dogs. The amount of microsomal protein per unit tissue was determined using testosterone-6β-hydroxylation and 7-hydroxycoumarin glucuronidation activities, as the microsomal markers to correct for the losses during cell fractionation. Abundance of CYP2B11 and CYP3A12 in the microsomes were quantified using a mass spectrometry based assay [2] and was supplemented with estimates based on diazepam-N-demethylation and diazepam-3-hydroxylation activities for CYP2B11 and CYP3A12, respectively, for the samples from distal intestine with low abundance. Typical total tissue concentrations of CYP2B11 and CYP3A12 were estimated to be 70 and 130 pmol/cm intestine (post mortem length) in the proximal small intestine and 10 and 20 pmol/cm in the colon, respectively. Enzyme abundance based scaling factors for intestinal metabolism were implemented into a physiologically based intestinal absorption model in GastroPlus™ software. The fraction of absorbed dose escaping the gut wall metabolism (F_g) of 5 reference compounds (domperidone, felodipine, nitrendipine, quinidine and sildenafil) in beagle dog were estimated from intravenous and oral pharmacokinetic literature data. V_{max} and K_m for CYP2B11 and CYP3A12 mediated metabolism were determined *in vitro* in recombinant enzymes and were used as inputs for GastroPlus simulations. The predicted F_g of 4 out of 5 compounds were within a 1.5 fold range from the value estimated from *in vivo* data at the single dose levels given in the reference *in vivo* studies. This preliminary validation of intestinal metabolism scaling factors supports the utility of

this approach for prediction of gut wall extraction in the beagle dog. However, further studies are warranted for evaluation of simulations of dose dependence and impact of absorption site on gut wall extraction.

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Poster Abstracts

P1 - METABOLITE PREDICTION IN THE IHUMITE WORKFLOW FOR FIRST-IN-MAN AND PRECLINICAL COVERAGE STUDIES

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Introduction: A workflow is developed for the identification of major human plasma metabolites (iHumite®) during first-in-man studies. It includes drug metabolite profiling tools for both targeted identification on the basis of in silico metabolite prediction, and a non-targeted approach by comparing peaks detected in dosed plasma with a control sample (placebo dosed or pre-dose). The identification of major and human specific metabolites can be used to initiate early nonclinical characterization. False negative results for major human metabolite(s) in the cold study would delay such a decision until results of the 14C human ADME study become available (clinical phase 3). Metabolite prediction was evaluated for 14 compounds of which plasma data were recently published in 14C-ADME studies.Methods: Metabolite prediction is a key step in the iHumite workflow developed for the identification of human metabolites. Potential drug metabolites are generated on the basis of biotransformation rules derived from metabolic reactions observed in man. Predictions were made according to three subsequent reaction steps starting from the 2D chemical structures. The resulting list of metabolites and their calculated monoisotopic masses are used to perform a systematic and very sensitive (targeted) screen for the presence of potential metabolites in pooled human plasma and their coverage in preclinical species. In our workflow, detected peaks in LC-MS data of in vitro or in vivo metabolism studies, are compared against a control sample using MsXelerator processing software and judged on a number of characteristics, including chromatographic peak shape, isotope signature match, product ion and neutral loss ion scanning relative to the parent drug. The sequential approach of multiple filters ensures high confidence that detected peaks are real metabolites. Results and Conclusions: To estimate the current coverage of our targeted approach, we evaluated the predictions based on 14C-ADME studies published by 7 different companies in the period 2008-2012. This dataset included 14 compounds with different modes of action. For 11 compounds in this set a total of 17 major metabolites in plasma were reported. For 3 additional 14C-labeled compounds the most abundant metabolite in plasma was also included in our selection. Although not major metabolites according to the FDA/ICH guidelines, one of these 3 additional metabolites was disproportionately circulating in humans compared with animal species. Of this set of 20 selected metabolites 17 (85%, including all of the eleven 1-step metabolites) would have been covered in the experimental set of mass chromatograms derived from elemental formula of predicted metabolites as done in our workflow. Also 15 (75%) of the reported metabolite structures were in the predicted set (including all of the 1-step metabolite structures). MsXelerator software can read the metabolite prediction file format and is able to combine results from both targeted and non-targeted data analysis. By doing so, both expected and unexpected metabolites can be found in one single run. Where possible, incorporation of new biotransformations in the prediction rules will further increase coverage and consequently the efficiency of our iHumite workflow by reducing the time spent for the non-targeted approach.

P2 - ENSURING ANALYTICAL QUALITY IN A HT-ADME ENVIRONMENT: TIERED LC-MS/MS SUPPORT AND SUPPORTING INFORMATION SYSTEMS PLATFORMS

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Ensuring Analytical Quality in a HT-ADME Environment: Tiered LC-MS/MS Support and Supporting Information Systems Platforms G.T. Clark, J. Cartmell, T. Potter, P. Butler, C. Dilworth LC-MS/MS endpoint determination of HT-ADME assays has been the gold standard for over 10 years in the pharmaceutical industry. Increasing volume of samples observed in these early phase discovery assays has in recent years, tipped the balance in favour of speed over quality, with a large number of HT-ADME labs working under the assumption that generic HPLC conditions are able to provide a 'fit-for-purpose' platform capable of supporting a diverse chemical space. Whilst in vivo bioanalysis always considers the matrix of interest when designing an analytical assay, HT-ADME analysis invariably makes little difference between subcellular based matrix based assays (e.g. microsomal clearance) and more complex matrix based assays (e.g. plasma protein binding). The results of which are that, whilst generic LC-MS/MS approaches may provide adequate data for a buffer based assay, analysis of more complex matrices may provide poor analytical data (and vice versa). In order to obtain the best quality analytical data for HT-ADME assays, Cyprotex laboratories have employed commercially available compound optimization software in conjunction with a tiered LC-MS/MS approach.

After optimization the tiered approach determines the most appropriate system to support the compound's analysis, depending on its retention time on chromatography systems that have been characterized for areas of known matrix effects (ion suppression and enhancement). To ensure that the correct optimization and HPLC methods are utilized, supporting bespoke information systems have been developed in house. These information systems allow not only automated optimization and method selection, but also dynamic scripting of liquid handling robotics and subsequent automated analysis of LC/MS/MS data in bespoke assay result spreadsheets. The analytical and information system workflows are presented as examples of ensuring that high quality analytical data can be collected in support of diverse HT-ADME assays.

P3 - AN LC-MS/MS ASSAY FOR THE DETERMINATION OF ZEYLENONE IN PLASMA AFTER STABILIZED BY THE PRESENCE OF ESTERASE INHIBITORS

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Zeylenone is a naturally occurring cytotoxic agent that contains two benzoate groups. It is extremely labile in plasma and sensitive to esterase hydrolysis, leading to the formation of its inactive metabolite. This instability results in difficulty in developing and validating bio-analytical method for pharmacokinetic studies. In order to permit an accurate determination of zeylenone**,** blood and tissue samples need to be rapidly stabilized at the time of collection. As a result, six esterase inhibitors, namely EDTA·2Na^{+}, NaF, phenylmethanesulfonyl fluoride, dichlorvos, bis-nitrophenyl phosphate (BNPP) and thenoyltrifluoroacetone, and the binary mixtures of these inhibitors were screened for the stabilization of zeylenone in rat plasma. The results showed that the combination of NaF and BNPP exhibited the most effective stabilizing effect with the degraded content of zeylenone decreased from more than 60% (in the absence of inhibitors) to <5%. Following the stabilization, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method for the quantitation of zeylenone in rat plasma has been developed and validated using the mixture of NaF and BNPP as the esterase inhibitor to prevent zeylenone from converting to its inactive metabolite. The analyte and internal standards (IS), emodin, in rat plasma containing NaF (5 mM) and BNPP (5 mM) were acidified by formic acid and extracted into ethyl acetate at 0°C and the liquid–liquid extraction was free of matrix effect caused by esterase inhibitors. The chromatographic separation was achieved on a Phenomenex® Luna C18 column with a run time of 6 min. Detection was performed on a 3200 Q-Trap with positive ion electrospray mode, monitoring the ion transition m/z 383.2→105.0. The method was validated over the range from 2 to 1300 ng/mL with inter- and intra-run precision for the quality control samples being less than 6.8%. The assay accuracy was within100 ±7.0% of the nominal values. The mixture of NaF and BNPP effectively stabilized zeylenone during blood collection and storage. The validated method was applied to a pharmacokinetic study in rats after the administration of zeylenone solutions via intravenous route. The results demonstrated that the assay was adequately sensitive, stable, rapid and specific for the analysis of plasma samples in preclinical pharmacokinetic studies. [This work was supported by NSFC (No: 81172997) and MOST (International S&T Collaboration project No: 1108)].

P4 - HIGH-THROUGHPUT ANALYSIS OF 1'-HYDROXYMIDAZOLAM IN PLASMA USING ULTRA-FAST SPE/MS/MS

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There is an increasing demand for greater throughputs and efficiencies of mass spectrometry-based bioanalysis by DMPK researchers. We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze small molecule analytes in a plasma based matrix with much faster sample cycle times and similar analytical results compared to LC/MS/MS methods. Critical bioanalytical parameters were systematically investigated using the small molecule analyte 1'-hydroxymidazolam spiked into plasma. 1'-Hydroxymidazolam is the primary CYP3A4 metabolite of the benzodiazepine midazolam and is often used for clinical drug-drug interaction studies. Comparable accuracy, precision, and linearity were achieved at rates 10-30 fold faster than traditional LC/MS/MS methods. Hydroxymidazolam calibration curves and quality control standards were prepared in a wide dynamic range by spiking blank matrix with 1'-hydroxymidazolam. The samples were then precipitated with acetonitrile containing an isotopically labeled internal standard, followed by dilution. Sample analysis was performed at a rate of <10 seconds per sample using an Agilent RapidFire ultra-fast autosampler/online SPE system (reverse phase) coupled to an Agilent 6460 QqQ mass spectrometer. SPE methods were optimized for the analyte and the SPE/MS/MS results were

compared to LC/MS/MS analysis. Data analysis was performed using RapidFire Integrator software. Hydroxymidazolam had excellent linearity within the measured range of 10-1000 ng/ml with an R^2 value greater than 0.995. Intra- and interday accuracies and precision for all concentrations were within 10%. A robustness test comprising of 1500 sequential injections of the same hydroxymidazolam concentration in protein precipitated plasma revealed a CV of less than 3% with no changes to peak signal or SPE cartridge pressure. Ionization suppression was present, but it could be effectively managed by using the stable isotope labeled internal standard (IS). Carryover was found to be < 1% of the low standard. A very strong correlation of SPE/MS/MS results from the same samples analyzed by traditional LC/MS/MS was seen (correlation coefficient = 0.999). The ultra-fast SPE/MS/MS system had comparable accuracy, precision and linearity results to LC/MS/MS for 1'hydroxymidazolam in plasma. This methodology is capable of throughputs >400 samples per hour. The SPE/MS/MS system may be useful for the efficient bioanalysis of 1'-hydroxymidazolam clinical drug-drug interaction studies and for analysis of similar small molecules in plasma from animal and human research studies.

P5 - DEVELOPMENT AND VALIDATION OF BIOASSAYS FOR THE DEVELOPMENT OF BIOTHERAPEUTICS

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Biotherapeutics are biologically active molecules, in most cases recombinant DNA technology-derived biopolymers, used in the treatment or prevention of human diseases. They can be constructed to be as much as possible similar to body's key effector/signaling agents but also to be different, so to increase their potency, improve their pharmacokinetic properties or reduce their potential immunogenicity. The measurement of the concentration of a biotherapeutic in biological matrices is a crucial aspect during the process of its pre-clinical and clinical development. Toxico-kinetic data are in fact mandatory to support safety assessment, dose-regimen choice and, later on, for evaluating the safety versus efficacy ratio. Ligand-binding assays are the mainstream quantitative bioanalytical tools used for this purpose and, among them, ELISAs are the ones most often chosen. This because ELISAs are generally relatively easy to perform, they do not need sophisticated instruments, do not require steps of extraction or partial purification of the analyte from the matrix and are sufficiently flexible in terms of format and readout to satisfy many assay variables. Most of all, if appropriately developed, they often results endowed with the necessary sensitivity and, in particular, with the sufficient accuracy and precision to allow assay validation. The purpose of this presentation is to review the development, optimization and validation of ELISA methods during the development of a novel biotherapeutic agent. The advantages and possible limitations of the use of ELISAs for routine sample analysis are also discussed.

P6 - PRECLINICAL PHARMACOKINETICS OF DECURSINOL

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Decursinol, a major coumarin from the roots of Angelica gigas Nakai, have been known to possess numerous pharmacological against inflammation, angiogenesis, nociceptive pain, and alzheimer¡¯s disease in various animal models. Recently, it marketed as a functional food in USA and Europe for minor pain relief. In vitro and in vivo studies were conducted to characterize the metabolism and pharmacokinetics of decursinol. Decursinol exhibited high stability (> 80% for 2hrs) to NADPH and UDPGA dependent metabolism in human and rat liver microsomes. In Caco-2 cell monolayers, decursinol showed high permeability (>14 ¡¿ 10-6 cm/s) at all tested concentrations in the absorptive direction, which saturated at 100 ¥ìM. Secretion increased in a concentration-dependent manner, with an efflux ratio above 2 at 50 ¥ìM, indicating the participation of an active efflux transporters. Following intravenous and oral administration in rats, decursinol was eliminated dose-dependently, showing smaller clearance and longer half lives in higher dosages. Consequently, oral bioavailability of decursinol was significantly increased according to the doses. It showed that the saturation of absorption occurred in 10 mg/kg and Tmax of 20mg/kg was increased significantly than lower doses after oral administration of decursinol in rats.

P7 - PHARMACOKINETICS AND BIOAVAILABILITIES OF TWO DAMMARANE SAPOGENINS IN RATS

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Background Ginsenosides, the active components of well-known Chinese medicine Ginseng, had been found a wide range of biological activities. Many studies indicated that ginsenosides can hydrolyzed in the gastrointestinal tract to form their metabolites, which are more easily absorbed into the body and displayed more potential activities than original saponins(Hasegawa, 2004). Our research group uses proprietary method to hydrolyze ginsenosides to produce an active fraction, namely Dammarane Sapogenins (DS-1226). DS-1226, mainly composed of 33% protopanaxatriol (PPT) and 16% protopanoxadiol (PPD), displayed significant activities in decreasing chemotherapy-induced myelosuppression and improving learning and memory of mice in our previous studies(Yang et al.,2010). The present study was preformed to investigate the pharmacokinetic (PK) properties of PPD and PPT after being given with DS-1226 to rats for better understanding DS-1226 pharmacological activities. **Methods** Male SD rats received intravenous and oral administration of DS-1226 at 30 and 75 mg/kg, respectively. The blood samples were collected at time intervals after dosing and the plasma PPD and PPT concentrations were measured by a LC-MS method using selective ion monitoring at m/z: 495.5 (PPD) and 511.6 (PPT). The PK parameters were estimated by the plasma concentration-time profile of each rat. **Results** After intravenous administration, PPT were found more rapidly eliminated from the body than PPD, with average $t_{1/2,\lambda z}$ value of 6.49, 0.80 h, and CL of 0.95, 4.04 L/h/kg for PPD and PPT. The parameters Vd and $AUC_{0\text{-}inf}$ were 9.15, 4.95 L/kg and 5.13, 2.67 μg·h/mL for PPD and PPT, respectively. After oral administration, both PPD and PPT could be absorbed into the body, but their systemic exposures were quite different. PPT had a lower average value of $AUC_{0\text{-}inf}$ (0.26 μg·h/mL) than that of PPD (6.02 μg·h/mL), even its dosage was relative high. The C_{max}, t_{max}, $t_{1/2,\lambda z}$, CL/F, Vd/F of PPD were estimated as 1042.4 ng/mL, 2.13 h, 3.92 h, 2.23 L/h/kg and 11.7 L/kg, respectively. For PPT, the oral PK parameters could not be estimated because its plasma concentration was very low and failed to give an integrated time curve. The absolute bioavailabilities of PPD and PPT were 46.92%, 3.95%, respectively. **Conclusion** PPD and PPT have very similar chemical structures, but their PK properties were quite different. The reasons for lower bioavailability and fast elimination of PPT than those of PPD are deserve to further study. (Supported by the International S&T Collaboration Projects (2011DFA32730 and 1108) from MOST of People's Republic of China)

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P8 - HUMAN SMALL INTESTINAL AND COLONIC TISSUE MOUNTED IN THE USSING CHAMBER AS A TOOL FOR CHARACTERIZING THE INTESTINAL ABSORPTION OF DRUGS

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The purpose of this study was to validate human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for predicting the oral drug absorption in humans with the main focus on moderately and poorly permeable compounds. The obtained apparent permeability coefficient (P_{app}) of eleven test compounds was compared to their fraction absorbed (Fa) in humans taken from the literature. Beside the conventional P_{app} a new parameter, the apparent permeability coefficient total ($P_{app,total}$), involving both the apical-to-basolateral permeability and the time-dependent compound accumulation in the tissue was established. The permeability of lucifer yellow (LY), a fluorescent marker of the paracellular pathway and the test compounds showed no obvious differences between small intestine and colon. Furthermore, small intestinal and colonic tissue from a single donor showed similar permeability of both LY and a transcellularly transported compound metoprolol. All test compounds including low molecular weight hydrophilic compounds such as metformin, atenolol, sulpiride and famotidine showed adequate

permeability reflecting human Fa values (R^2 = 0.87). The P_{app} values of digoxin, a P-glycoprotein (P-gp) substrate, were not significantly affected by the addition of verapamil, a P-gp inhibitor. In contrast, the $P_{app,total}$ values of digoxin increased approximately three-fold in the presence of verapamil. In conclusion, both small intestinal and colonic tissue mounted in the Ussing chamber provide a good opportunity to predict the oral drug absorption rate in humans even for moderately and poorly absorbed compounds. The novel calculation of $P_{app,total}$ allows the study of the carrier-mediated drug-drug interactions in human intestine.

P9 - EC FP7 HEALTH - 2007- GRANT PREDICTIV: THE STRATEGY APPLIED TO MEASURE BIOKINETIC PARAMETERS AND THEIR CRUCIAL ROLE IN IN VITRO TESTING

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In vivo the kinetics of chemicals providing information on the actual internal dose reaching the target is the most relevant parameter in evaluating human and experimental animal exposure and in the quantitative risk assessment. At variance, only very few studies have addressed the issue of in vitro biokinetics, although kinetics is considered the crucial body of information for the design and performance of toxicological tests and interpretation of toxicity data (Adler et al, 2011). In in vitro models the nominal applied concentration rather than the actual level of cell exposure is quite often associated to an observed effect. And indeed, the difficulty in translating an in vitro concentration into an in vivo dose (in vitro–in vivo extrapolation) and in vitro/in vivo differences, often attributed to kinetics, are the major limitations of non-animal testing. The actual intracellular concentration may be affected due to altered bioavailability (interactions with medium/plate, abiotic processes) or to physiological cellular processes (transport across the membranes, biotransformation, bioaccumulation) after acute and even more after repeated treatments. Inclusion of biokinetic measurements in the in vitro test protocols is essential also to know whether cells are exposed to the parent compound and/or its metabolites. The aim of WP3 within the PredictIV project is the development of a strategy for measuring/estimating the real exposure of cells to drugs and/or their metabolites in the in vitro test systems, to contribute to the derivation of actual NOEC values in cellular model systems representative of in vivo target organs, from which it would be possible to extrapolate the corresponding in vivo dose as input for kinetic (PBTK) models to make possible the extrapolation to the corresponding in vivo dose. The application of the strategy to renal (RPTEC/TERT1 cells) and hepatic model (rat and human hepatocytes and HepaRG), treated with cyclosporine, ibuprofen and chlorpromazine (selected as model compounds) for 14 days at two doses (TC_{10} and 1/10 of TC_{10}) is described. Some relevant examples on kinetic profile at D0 and D13 will be described as examples: in the renal model treated with cyclosporine, the intracellular concentration was not linearly related to the nominal concentration used for treatment (a ratio of 10 vs the expected 3) and the comparison of D0 and D13 kinetics (a <4-fold higher differences) suggests a high potential for bioaccumulation and lack of metabolism in the model. In the hepatic model, the decrease in ibuprofen content in supernatant not counterbalanced by an equal increase in intracellular concentration, resulting in a low mass balance (only 2-20% after 24 h) was attributed to metabolism of the parent compound. A similar situation was obtained with chlorpromazine in rat hepatocytes. However, while chlorpromazine accumulated inside the cells over the 14-day exposure, no potential for bioaccumulation nor induction of ibuprofen metabolism was observed in rat hepatocytes, with relevant differences when compared to the human model.

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P10 - EC FP7 HEALTH - 2007- GRANT PREDICTIV: PROFILING THE TOXICITY OF NEW DRUGS: A NON-ANIMAL-BASED APPROACH INTEGRATING TOXICODYNAMICS AND BIOKINETICS – EVALUATION OF THE EXPOSURE OF PRIMARY RAT AND HUMAN HEPATOCYTES TO IBUPROFEN AFTER SINGLE OR REPEATED TREA

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The overall aim of PredictIV is to develop strategies to improve the safety assessment of therapeutic candidates in

the early stage of development and late discovery phase, making use of optimized existing in vitro models, integrating kinetic and dynamic data. Being the liver the organ most frequently affected by drugs in repeated dose toxicity studies and clinical drug development, primary rat (PRH) and human (PHH) hepatocytes (from 3 donors) cultured in a sandwich of Matrigel/collagen configuration were used to assess the real cell exposure after single or repeated treatment to a given test compound and/or its metabolites, to be considered in the evaluation of its hepatotoxicity potential. Cells were treated daily for 14 days (D0-D13) with Ibuprofen (IBU) at two concentrations: IC10 (high: 100 in PRH and 1000 µM in PHH) and 1/10 of IC10 (low:10 in PRH and 100 µM in PHH). IBU was quantified with a HPLC method in cell lysate/medium samples at five different time points (2 min-24 hours), to determine its kinetic profile at D0 and D13. IBU nominal concentration (measured in the medium at time0) and bioavailability were not affected by "abiotic" processes (i.e. adsorption to the plastic, stability in the medium, evaporation and sequestration by Matrigel and/or collagen), except for sequestration by collagen at D13 (~30%, used as correction factor). The profile of IBU decrease in the medium vs time was similar in the two models in any experimental conditions; however, some differences were observed when the time-courses of IBU intracellular content were compared. In PRH intracellular uptake was rapid and proportional to the added IBU amount: it increased up to 3 hours, then drastically dropped at 24 hours at both concentrations. The kinetic profile was similar at D0 and D13 suggesting that no bioaccumulation, saturation/induction phenomena occurred. The disappearance in the medium was not quantitatively counterbalanced by the intracellular content, giving rise to a progressively lower mass balance, attributable to CYP-dependent IBU metabolism. In PHH, although treated with 10-fold higher IBU concentrations, the intracellular content was much lower than in RPH, not because of a lower uptake, but very likely due to a much more efficient CYP-dependent metabolism. Indeed, starting from 2 min the mass balance indicated a time-dependent IBU 'loss', with a constant intracellular content up to 3 hours, suggesting that a steady-state was reached, attributable to an equilibrium between IBU entering the cells and readily metabolized. This could explain the 10-fold difference in the IC10 concentrations in the two models. At the low concentration, after 24 hours the metabolism was almost complete (with <10% IBU recovery). At the high IBU concentration at D13, higher mass balance values were calculated, suggesting the possible intervention of saturation processes. Although with the same kinetic profile, high variability among the 3 donors was obtained, corresponding to differences in their CYP2C9 activities. Kinetic results, providing the actual exposure metrics, are an essential requisite to give the correct interpretation to toxicity data.

This work was supported by the Seventh Framework Programme – PredictIV, grant # 202222.

P11 - PREDICTION OF HUMAN PHARMACOKINETICS BY ALLOMETRIC SCALING USING CHIMERIC MICE WITH HUMANIZED LIVER

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<Purpose> Prediction of human pharmacokinetics (PK) from animal data is difficult for species differences. We used chimeric mice with humanized liver (PXB mice®, PhoenixBio, Co., Ltd, Hiroshisma, Japan) which have been generated from urokinase-type plasminogen activator / severe combined immunodeficiency mice repopulated with approximately 80% human hepatocytes to evaluate the predictability for human PK. Previously, we reported that the predictability of human hepatic intrinsic clearance (CL_{int}) calculated by the well-stirred model, comparing in vitro CL_{int} from PXB mouse fresh hepatocytes and in vivo CL_{int} from PXB mice after i.v. administration of 13 clinical drugs which are metabolized in liver (Sanoh et al., 2012). Additionally, not only total clearance (CL) but also distribution volume (V_{dss}) predictions were conducted from in vivo data by the single species allometric scaling (SSS) of PXB mice in this study. The purpose is to evaluate the accuracy of predictability for human PK, comparing these in vitro and in vivo approaches including SSS methods. **<Methods>** (1) In vitro CL_{int} of PXB mice; Fresh hepatocytes were isolated from PXB mice by in situ collagenase perfusion method. In vitro CL_{int} values were calculated from time course of disappearance of test drugs during incubation with PXB mouse hepatocytes. (2) In vivo CL_{int} of PXB mice; PXB mice were i.v. administrated of drugs at 0.3-5mg/kg body weight (B.W.). In vivo CL_{int} values were calculated using the analysis based on the well-stirred model. (3) SSS of CL and V_{dss}; CL and V_{dss} predictions were estimated by SSS

methods (eq.1 and eq.2). $CL_{human} = CL_{PXB\ mice} \times (B.W._{human}/B.W._{PXB\ mice})^{e}$ (eq.1), $V_{dss\ human} = V_{dss\ PXB\ mice} \times (B.W._{human}/B.W._{PXB\ mice})^{e}$ (eq.2). Both exponential values of allometric equation were calculated from CL and V_{dss} of each drug in PXB mice and humans, respectively. (4) Human PK data; CL and V_{dss} values in human were applied from literature values. **<Result>** (1) For 2 of 13 (15%) compounds, in vivo CL_{int} in humans was predicted within a 3-fold error from in vitro CL_{int} of PXB mouse hepatocytes. (2) For 4 of 13 (31%) compounds, in vivo CL_{int} in humans was predicted within a 3-fold error from in vivo CL_{int} of PXB mice. (3) Each exponential value of eq.1 and eq.2 was determined to 0.84 and 0.95, respectively. The SSS of PXB mice predicted human CL within a 3-fold of actual values for 85%, although the direct comparison of CL between PXB mice and humans resulted in 23%. In the prediction for human V_{dss} within a 3-fold error, SSS of PXB mice resulted in 100%. **<Discussion and conclusion>** SSS approach using PXB mice, which showed accurate prediction for CL and V_{dss}, prove to be more effective. These observations demonstrated that in vivo experiments with PXB mice should be useful for quantitative prediction of human PK characteristics of drug candidates.

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P12 - RELATIONSHIP OF METABOLIC STABILITIES IN CARBOXYLESTERASE AND STRUCTURES OF ACETAMINOPHEN CARBOXYLIC ESTERS

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Relationship of metabolic stabilities in carboxylesterase and structures of acetaminophen carboxylic esters Purpose: To evaluate the metabolic stabilities of 13 acetaminophen carboxylic esters (11 of them were synthetized in our lab), and relationships of metabolic stabilities and structures were probed. Methods: The in vitro metabolic stabilities of 13 acetaminophen carboxylic esters were examined in recombinant human carboxylesterases, hCES1b and hCES2. The apparent half-lives were determined based on the disappearance of esters using reverse-phase HPLC with UV detector. Results: The 13 carboxylic esters were all recognized by hCES1b and hCES2. Differences between half-lives were obvious. Diphenylacetyl acetaminophen showed the longest half-life in hCES1b system, and 2-phenylpropionyl acetaminophen showed the longest half-life in hCES2 system, while ester of flurbiprofen and acetaminophen showed the shortest half-life in both systems. Conclusions: 2-phenylpropionyl substituent group on hydroxyl was more helpful to increase the metabolic stability in carboxylesterase. More long the carbon chains of carboxylic substituent groups, less metabolic stability of esters. Conjugated structure and steric hindrance contributed to increase the metabolic stability.

P13 - BINDING OF BOVINE SERUM ALBUMIN TO FATTY ACIDS: IMPROVED THE CLEARANCE PREDICTION FOR CYP1A2 SUBSTRATE

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Improved predictions of in vivo hepatic clearance (CL_H) from in vitro intrinsic clearance (CL_{int}) by bovine serum albumin (BSA) have been reported for drugs that were metabolized by certain cytochrome P450 (CYP) isoforms (i.e. CYP2C8 and CYP2C9) as well as certain UDP-glucuronosyltransferase (UGT) isoforms (i.e. UGT1A9, UGT2B4, and UGT2B7). BSA increases the activity of these enzymes by sequestering polyunsaturated fatty acids (PUFAs) that are released from membranes, especially when using human liver microsomes (HLM) as the enzyme source. The aims of this study were to characterize the effect of BSA on the kinetics of CYP1A2-mediated phenacetin O-deethylation and determine whether addition of BSA to incubation improves the prediction of in vivo hepatic clearance of phenacetin. The kinetics of phenacetin O-deethylation by HLM and E.coli-expressed CYP1A2 (rCYP1A2) were performed with and without BSA (2% w/v) supplementation. BSA increased CL_{int} for phenacetin O-deethylation, due to a reduction in K_m value for high affinity component of human liver microsomal phenacetin O-deethylation. The CL_{int} for phenacetin O-deethylation by rCYP1A2 was increased to a similar extent. A fatty acid mixture, comprising 3 µM each of oleic acid and linoleic acid plus 1.5 µM arachidonic acid, decreased phenacetin O-deethylation activity in rCYP1A2 due to an increase in K_m. The addition of BSA reversed the inhibition of fatty acid mixture. Mean values of predicted CL_H of phenacetin O-deethylation was in good agreement with reported CL_H from clinical study when in vitro data obtained

in the presence of BSA were used for clearance prediction. In conclusion, this study has demonstrated that supplementation of incubations of HLM and rCYP1A2 with BSA enhances the activity of CYP1A2-mediated phenacetin O-deethylation, thereby improving the accuracy of in vivo clearance prediction. This effect results from BSA sequestration of inhibitory PUFAs that are released from membranes of the enzyme sources during course of in vitro incubations.

P14 - USING RECOMBINANTLY EXPRESSED HUMAN URIDINE 5'-DIPHOSPHO-GLUCURONOSYLTRANSFERASE (UGT) ENZYMES TO PREDICT TOTAL BODY CLEARANCE FOR SIX MERCK COMPOUNDS

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The increasing number of compounds in discovery/development that are eliminated by UGT-mediated biotransformation has led to the need for robust and accurate in vitro models for prediction of UGT-mediated intrinsic clearance (CLint). Based on internal experience, there are issues with scaling from hepatocytes and liver microsomal CLint data to whole liver UGT-mediated clearances in humans with respect to both robustness and reliability of the predictions. To allow quantitative extrapolation of data from recombinantly expressed human UGT (rhUGT) systems, liver and kidney relative activity factors (RAF) were generated for recombinantly expressed human UGT1A1, 1A4, 1A6, 1A9 and 2B7 to enable their use as a quantitative tool for human prediction. Estradiol, serotonin, trifluroperazine, propofol and AZT were used as in vitro marker substrates for UGT1A1, 1A6, 1A4, 1A9 and 2B7, respectively. Liver RAFs for UGT1A1, 1A6, 1A4, 1A9 and 2B7 were 1.42, 4.25, 6.07, 0.34 and 1.3, respectively. Likewise the kidney RAFs for UGT1A6, 1A9 and 2B7 were 4.54, 0.55 and 1.70, respectively. The predictive accuracy of the in vitro in vivo extrapolation (IVIVE) approach using the tissue scalars in conjunction with CLint data generated from recombinant UGTs was evaluated in a retrospective manner for six UGT-substrates from the Merck pipeline. Intravenous pharmacokinetic data were available for all of these compounds which were expected based on preclinical and human in vivo and in vitro studies to be eliminated exclusively by UGT metabolism. The results are promising with all predicted clearance values being within 40% of the observed values, most (4 of 6) were within 20%. These data suggest the relative contribution of the kidney to the elimination of these compounds was minimal (~10% of the total clearance). Overall, the results are supportive of further research using rhUGT in vitro systems as models for human clearance and may also have utility in the estimating the fraction metabolized by individual UGT enzymes; a pre-requisite for accurate drug-drug interaction and pharmacogenetic assessment.

P15 - TEMPORAL DEGRADATION OF ENZYME ACTIVITY IN HUMAN CRYOPRESERVED HEPATOCYTE SUSPENSIONS AND PLATED CULTURES

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Temporal Degradation of Enzyme Activity in Human Cryopreserved Hepatocyte Suspensions and Plated Cultures Cornelia M. Smith, Christina K Nolan, Gillian C. Wallace, Julie Price and Stephen S. Ferguson Clearance is a major pharmacokinetic parameter used to assess the safety and efficacy of candidate drugs. Cryopreserved hepatocytes are utilized to make in vivo Clearance predictions. Accordingly, the hepatic model chosen can influence the predicted clearance. The objective of this study was to assess enzymatic activity in suspensions and plated cultures of cryopreserved human hepatocytes as a function of time. Suspensions of cryopreserved human hepatocytes were held in a humidified chamber at 37°C at 0, 0.5, 1, 2, 4, 6, 8 and 10 h and activities CYP1A2, CYP2C9, CYP2D6, CYP3A, UGT and SULT determined. Plated cultures of cryopreserved human hepatocytes were held under the same conditions as the suspensions for 0, 4, 6, 8, 12, 24, 48 and 72 h and activities CYP1A2 and CYP3A determined. Probe substrates used to determine enzymatic activity were phenacetin (CYP1A2), diclofenac (CYP2C9), dextromethorphan (CYP2D6), testosterone (CYP3A) and 7-hydroxycoumarin (UGT and SULT). Pharmacokinetic modeling revealed enzyme inactivation time (IT50) values in suspension of 2.49 h ± 0.53, 4.54 h ± 0.44, 2.92 h ± 0.97, 1.63 h ± 0.08 and 1.38 h ± 0.39 for CYP1A2, CYP2C9, CYP2D6, CYP3A and UGT, respectively. The IT50 values increased to 21.3 h ± 2.1 and 28.8 h ± 20.4 for CYP1A2 and CYP3A respectively when examined in plated cultures. These studies support enzyme stabilization when hepatocytes are cultured over a period of days as opposed to a few hours. The use of

hepatocytes in suspension are limited to ~4 hours as our data shows a fast degradation of enzymatic activity especially prevalent for CYP1A2 and CYP3A. The plated hepatocytes model is especially applicable to assessing clearance for low-turnover compounds in discovery.

P16 - INVESTIGATION OF HEPATIC CLEARANCE OF DRUGS IN CHIMERIC MICE WITH HUMANIZED LIVER

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Extrapolation of the metabolic, pharmacokinetic and toxicological data obtained from animals to humans is not always straightforward. The remarkable species difference in metabolism is mainly due to the differences in drug-metabolizing enzymes among species. Thus, humanized transgenic mouse models, in which the human drug-metabolizing enzymes are expressed in the humanized liver of the model, has been developed to address such challenges.
Since the liver is the key organ involved in the pharmacokinetics of drugs, human liver is essential for the prediction of human drug metabolism and pharmacokinetics. Thus, chimeric mice with humanized livers could have great advantages in pharmacokinetic studies. At Xenoblis we evaluated the clearance rate of drugs using a novel chimeric mouse model with a humanized liver, the Yecuris™ **FRG**™ knockout (KO) mouse. The transgenic mouse contains three mutations (Azuma: 2007): **F**AH knockout inducing liver disease/**R**ag2 (recombinant activating gene 2) knockout inducing T and B cell deficiency/ Il2r**g** (Interleukin 2 subunit γ-chain) knockout inducing NK cell deficiency. The model was developed originally on the C57Bl/6 strain and has recently been established on the non-obese diabetic (NOD) background for inclusion of immune components in studies. The presence of human drug-metabolizing enzymes and transporters in the humanized Yecuris™ **FRG**™ **KO** mouse was previously demonstrated (Azuma: 2007, S. Strom: 2010, C Chesne: 2010). The aims of this work were to evaluate the Yecuris™ **FRG**™ **KO** mice with at least 80% human hepatocyte replacement index, to predict human in vivo pharmacokinetics and metabolism, assess the suitability of the model for use in drug-drug interaction (DDI) studies and provide mass spectrometry response factors for relative quantification of human metabolites. Propafenone was selected for investigation in the **FRG**™ **KO** mouse model, a compound known to have specific CYP2D6 species metabolism (Bateman: 2009). Previous attempts to observe human specific metabolites in vivo in other models have been unsuccessful (Kamimura: 2010). Rag2-/-/Il2rg-/-/C57Bl6 mice were used as controls to idenitify the mouse metabolites. Blood samples were collected at selected time points following IV dosing with PK profiles generated using 10 µL aliquots of plasma for LC-MS/MS analysis. Following propafenone administration, preferential formation of the human metabolite, 5-hydroxypropafenone, is only observed in animals with humanized livers. Humanized **FRG**™ **KO** mice are a valuable model for understanding human-specific metabolism in vivo and are a novel tool for improved understanding of the role of human drug-metabolizing enzymes in drug clearance, pharmacokinetics, and in the prediction of potential drug-drug interactions.

P17 - ALTERATION IN THE FUNCTION OF THE UDP-GLUCURONOSYLTRANSFERASE 1A SUBFAMILY BY CYTOCHROME P450 3A4: DIFFERENT SUSCEPTIBILITY OF UGT ISOFORMS AND UGT1A7 VARIANTS

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Cytochromes P450 (P450s, CYPs) are very important drug-metabolizing enzymes, and are involved in the metabolism of a huge number of drugs. Of P450s, CYP3A4 is the most abundantly-expressed isoform in the liver. We have demonstrated that the regioselectivity of UDP-glucuronosyltransferase 2B7 (UGT2B7)-catalyzed morphine glucuronidation is altered by protein-protein interaction with CYP3A4 [1]. A subsequent study has suggested that the J-helix or the surrounding domains of CYP3A4 contribute to the interaction with UGT2B7 [2]. However, to the best of our knowledge, it remains unknown whether CYP3A4 modifies the functions of the UGT1A subfamily. Therefore, functional protein-protein interactions between UGT1A isoforms and CYP3A4 were studied. To this end, UGT1A-catalyzed glucuronidation was assayed in Sf-9 cells which simultaneously expressed UGT and CYP3A4. When CYP3A4 was co-expressed either with UGT1A1 or 1A7, the Vmax for the glucuronidation of irinotecan metabolite (SN-38) was significantly increased compared with UGT single expression. The Km was little affected by simultaneous expression of CYP3A4. In the case of UGT1A6-catalyzed glucuronidation of serotonin, both Km and Vmax were increased by

CYP3A4. We also examined the effects of UGT1A7 allelic variants on UGT function and interaction with CYP3A4. Kinetic analyses were performed using three substrates, 4-methylumbelliferone, SN-38 and 4-hydroxybiphenyl. While simultaneous expression of CYP3A4 reduced the Vmax of UGT1A7*3 (R129K, R131K and W208A), those of *1 (wild-type) and *2 (R129K and R131K) were increased. The Kms for all substrates were unaffected by CYP3A4. In the kinetics involving different concentrations of UDP-glucuronic acid (UDP-GlcUA), the Km for UDP-GlcUA was significantly higher in *2 and *3 than in *1. The Km for UDP-GlcUA was increased by CYP3A4 in UGT1A7*1 and *3, whereas *2 did not exhibit any such change. The results obtained suggest that 1) CYP3A4 changes the catalytic function of the UGT1A subfamily in a UGT isoform-specific manner and 2) non-synonymous mutations of amino acids 129, 131 and 208 in UGT1A7 reduce not only the ability of UGT to utilize UDP-GlcUA, but also CYP3A4-mediated enhancement of catalytic activity. [References] 1. Takeda S et al., (2005) Modulation of UDP-glucuronosyltransferase function by cytochrome P450: evidence for the alteration of UGT2B7-catalyzed glucuronidation of morphine by CYP3A4, Mol Pharmacol, 67: 665-672. 2. Takeda S et al., (2009) Interaction of cytochrome P450 3A4 and UDP-glucuronosyltransferase 2B7: evidence for protein-protein association and possible involvement of CYP3A4 J-helix in the interaction. Mol. Pharmacol., 75: 956-964.

P18 - EFFECTS OF UGT2B SUBSTRATES AND INHIBITORS ON R- AND S- LORAZEPAM GLUCURONIDATION BY HUMAN LIVER MICROSOMES

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Recent in vitro data has shown that UGT 2B4, 2B7 and 2B15 are likely to be the major enzymes responsible for human liver microsomal R- and S-lorazepam glucuronidation (1). Data regarding metabolic drug-drug interactions arising from inhibition of UGT2B catalyzed lorazepam glucuronidation is limited. Thus, this study aimed to: (i) investigate effects of the known UGT2B substrates and/or inhibitors codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, valproic acid and zidovudine on R- and S-lorazepam glucuronidation using HLM as the enzyme source, and (ii) predict the magnitude of selected interactions arising from inhibition of R- and S-lorazepam glucuronidation in vivo. IC_{50} values were determined using four concentrations of each putative inhibitor at an R,S-lorazepam concentration of 100 µM, which corresponds to a concentration of each enantiomer of 50 µM. This concentration is close to the K_m for R- and S- lorazepam glucuronide formation by HLM (1). The magnitude of an inhibitory drug-drug interaction in vivo, expressed as the ratio of the area under the plasma drug concentration-time curve (AUC) in the presence and absence of an inhibitor, was predicted from the ratio of the inhibitor concentration in vivo and the inhibition constant (K_i) determined in vitro. Weak inhibition of R- and S-lorazepam glucuronide formation was observed for codeine, fluconazole, morphine, valproic acid and zidovudine, with the IC_{50} values ranging from 809 to 12800 µM. Ketamine and methadone showed moderate inhibition of R- and S-lorazepam glucuronidation, with IC_{50} values ranging from 118 to 205 µM. However, reasonably potent inhibition of R- and S-lorazepam glucuronidation was observed for ketoconazole, with the respective IC_{50} values of 22 and 18 µM. The mechanism of inhibition and K_i values for ketoconazole and valproic acid inhibition on R- and S-lorazepam glucuronidation were further investigated. Ketoconazole inhibition of R- and S- lorazepam glucuronide formation was noncompetitive and competitive, respectively.Valproic acid inhibited both R- and S-lorazepam glucuronidation in a non-competitive manner. K_i values for inhibition of R- and S- lorazepam glucuronide formation by ketoconazole were 15 ± 0.1 µM and 4 ± 0.1 µM, respectively, whereas the respective K_i values for valproic acid inhibition were 3997 ± 0.1 µM and 3286 ± 0.1 µM. Based on total inhibitor concentration in plasma at a dose of 200 mg once daily for ketoconazole and 500 mg twice a day for valproic acid, predicted AUC increases for R- and S-lorazepam when coadministered with ketoconazole were 18% and 64%, respectively. Only a minor interaction was predicted with valproic acid; predicted AUC increases for R- and S-lorazepam were in the range 15-18%, which is consistent with the reported interaction in humans. These data suggest a potential metabolic drug-drug interaction arising from ketoconazole inhibition of lorazepam glucuronidation.

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P19 - EFFECTS OF THE DISRUPTION OF GLUTATHIONE S-TRANSFERASE MU 1 GENE AND GLUTATHIONE DEPLETION IN 1,2-DICHLORO-4-NITROBENZENE-INDUCED TOXICITY IN MICE

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We investigated the involvement of the enzyme (glutathione S-transferase Mu 1, GSTM1) and the coenzyme (glutathione, GSH) in 1,2-dichloro-4-nitrobenzene (DCNB)-induced toxicity using Gstm1-null mice and L-buthionine (S,R)-sulfoximine (BSO), an inhibitor of GSH synthesis. After a single oral dose of DCNB, methemoglobinemia was more marked in Gstm1-null mice compared with wild-type mice, but no apparent effect of BSO on methemoglobinemia was observed. Therefore, the enzyme GSTM1 seems to be an important factor in DCNB-induced methemoglobinemia. Additionally, hepatocyte necrosis/degeneration in the presence of BSO was observed in wild-type mice, but not in Gstm1-null mice. Therefore, the coenzyme GSH seems to be an important factor in DCNB-induced hepatotoxicity in wild-type mice, and Gstm1-null mice were resistant to DCNB-induced hepatotoxicity under the condition of GSH depletion. Plasma DCNB concentration, which was measured 2 hours after DCNB administration in females, was higher in Gstm1-null mice than in wild-type mice, regardless of BSO treatment, suggesting that exposure to DCNB was related to the methemoglobinemia, but not to the hepatotoxicity. In the presence of BSO, a marked increase in phosphorylated c-jun N-terminal kinase (JNK) was observed in wild-type mice 2, 4, and 8 hours after DCNB administration, but the increase was suppressed in Gstm1-null mice. Therefore, the suppressed phosphorylation of JNK may be a mechanism of the resistance to DCNB-induced hepatotoxicity in Gstm1-null mice under the condition of GSH depletion. In conclusion, GSTM1 as a conjugation enzyme is an important factor in DCNB-induced methemoglobinemia, while GSH as a coenzyme is an important factor in DCNB-induced hepatotoxicity.

P20 - EFFECT OF PHENOBARBITAL TREATMENT ON UDP-GLUCURONOSYLTRANSFERASE 1A SUBFAMILY IN RAT BRAIN

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UDP-glucuronosyltransferase (UGT) catalyzes a major phase II reaction in the drug-metabolizing enzyme system. The UGT1A subfamily is expressed in both the liver and the brain. Therefore, it is possible that the UGT1A subfamily is involved in the metabolism of several drugs that show pharmacological effects in the brain and is responsible for drug interactions within the brain. Although there are numerous reports concerning the induction of UGT1A expression and enzyme activity in the liver, the induction in the brain remains unclear. The purpose of the present study was to clarify the effect of phenobarbital, one of the major inducers of drug-metabolizing enzymes, on Ugt1a mRNA expression and enzyme activities in the rat brain. Eight-week-old male Sprague-Dawley rats were treated intraperitoneally with phenobarbital (80 mg/kg), once daily for 7 days. The rats were decapitated 1 day after the last treatment and their brains were divided into 9 regions (cerebellum, frontal cortex, parietal cortex, piriform cortex, hippocampus, medulla oblongata, olfactory bulb, striatum, and thalamus). Each region was pooled (mRNA, n = 5; enzyme activity, n = 10). Expressions of Ugt1a1, Ugt1a6, and Ugt1a7 mRNAs were measured by real-time polymerase chain reaction with each specific primer. 7-Ethyl-10-hydroxy-camptothecin (SN-38) and acetaminophen glucuronidation, which are catalyzed mainly by Ugt1a1 and Ugt1a6/Ugt1a7, respectively were determined using high performance liquid chromatography or liquid chromatography-tandem mass spectrometry. Phenobarbital treatment induced Ugt1a mRNA expression and enzyme activities in some regions, and the degree of induction differed among the 9 regions. Ugt1a1 mRNA expression and SN-38 glucuronidation were increased by 4.0- and 1.4-fold, respectively in the striatum. Ugt1a6 and Ugt1a7 mRNA expression was induced, particularly in the striatum and thalamus (Ugt1a6: 3.0- and 2.9-fold, respectively; Ugt1a7: 2.6- and 2.6-fold, respectively). In those regions, acetaminophen glucuronidation was increased 1.8- and 1.2-fold, respectively, by phenobarbital treatment. However, a correlation between changes in mRNA expression and enzyme activities was not observed. In conclusion, we clarified that phenobarbital treatment induced Ugt1a1, Ugt1a6, and Ugt1a7 mRNA expression and enzyme activities in some regions of the rat brain, suggesting that the induction of the drug-metabolizing enzyme could be involved in drug interactions in the brain.

P21 - THE ACTIVITIES AND EXPRESSION OF HEPATIC GLUTATHIONE S-TRANSFERASES AND CARBONYL REDUCTASE 1 IN YOUNG AND OLD MALE RATS

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Age-dependent differences in drug metabolism have been recognized for a long time. In old animals and human, a marked fall in cytochromes P450 activities and drugs oxidative biotransformation has been repeatedly described. However, information about age- differences in activities of drug-metabolizing reductases and conjugation enzymes has been limited. The aim of presented study was to compare the activities and expression of carbonyl reductase 1 (CBR1) and glutathione S-transferases (GSTs) in young and old rats. For this purpose, twelve male Wistar rats at the age of 6 weeks (young) and 20 months (old) were used. All animals were from the same breed and kept under the same conditions. Subcellular fractions of liver homogenates were prepared. GST specific activity was assessed in the cytosolic fraction by standard colorimetric method using substrate 1-chloro-2,4-dinitrobenzene, specific activity of CBR1 was determined by spectrofluorimetric method using menadione as a substrate. GSTA and CBR1 protein level was monitored by immunoblotting with specific antibody and normalized to the amount of β-actin. The results showed that activities and protein levels of CBR1 as well as GSTs were significantly elevated in old animals comparing to young ones. The more pronounced increase was observed in GST where specific activity was about 6-fold higher and corresponding protein was significantly increased in 20-month-old rats than in young animals. The elevation of these enzymes in old animals may lie in regulation of their expression via increased oxidative stress. Elevated activities of GST and CBR1 may protect old animals against xenobiotic as well as eobiotic electrophiles and reactive carbonyls, but it may alter metabolism of drugs which are CBR1 and especially GSTs substrates.

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P22 - SYNTHETIC STILBENOIDS: NOVEL SUBSTRATES FOR HUMAN HEPATIC, RENAL, AND INTESTINAL UDP-GLUCURONOSYLTRANSFERASES

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UGTs are membrane enzymes which are responsible for rapid clearance of resveratrol (Res) from the plasma. Three synthetic stilbenoids: (E)-3-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylic acid (NI-12a), (E)-2,4-dimethoxy-6-(4-methoxystyryl)benzaldehyde oxime (NI-ST-05), and (E)-4-(3,5-dimethoxystyryl)-2,6-dinitrophenol (DNR-1) have been designed and synthesized in our laboratory. Each of these novel compounds was based on the structure of Res with the majority of the hydroxyl groups capped by O-methylation and other functional moieties added. The activities of 12 major recombinant human UGTs, including UGT1A and 2B isoforms, were examined for their capacity to metabolize these compounds. The formation of glucuronides was identified using HPLC and β-glucuronidase hydrolysis. The structures of these metabolites were elucidated by LC-MS/MS. NI-12a was glucuronidated at both the -COOH and -OH functionalities, NI-ST-05 formed a novel N-O-glucuronide, and no glucuronidated product was observed for DNR-1. UGT1A1, 1A3, 1A7, 1A8, 1A9, 1A10 and 2B4 are involved in glucuronidation of NI-12a and NI-ST-05. NI-12a is primarily metabolized by the hepatic and renal isoform, UGT1A9, whereas NI-ST-05 is primarily metabolized by an extrahepatic isoform, UGT1A10, with apparent K_m values of 240 and 6.2 μM, respectively. The involvement of hepatic and intestinal metabolism of both compounds was further confirmed from studies with a panel of human liver and intestinal microsomal preparations. High individual variation was demonstrated between individual donors. In summary, these studies clearly establish that modified Res-based stilbenoids may be preferable alternatives to Res itself due to increased bioavailability via altered conjugation. Similar compounds have been shown to bind to cannabinoid receptors and/or exhibit anticancer properties in vitro. Based on this data, we hypothesize that modified analogs of Res likely represent a useful scaffold for the design of highly selective and efficacious analogs which could potentially be developed as therapeutics. [Funded by GM075893 and DoD (Award number X81XWH-11-1-0795) USAMRMC (ARP), and the Arkansas Research Alliance (PAC)].

P23 - THE EFFECT OF C-4 SUBSTITUTIONS ON ESCULETIN METABOLISM: DIFFERENCES IN METABOLIC PROFILE, UGT ISOFORM SELECTIVITY AND METABOLIC RATE

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Abstract: Esculetin and its derivatives have multiple pharmacological activities, such as anti-inflammatory and antioxidant et al [1,2]. However, the poor metabolic stability of these compounds strongly influences their in vivo pharmacological activities and clinical applications. UDP-glucuronosyltransferases (UGTs) play a major role in the metabolic elimination of these compounds, but the effect of structural modification on the UGTs selectivity and the metabolic rate remained unclear. This study investigated the in vitro metabolism of esculetin, 4-methyl esculetin and 4-phenyl esculetin, based on their metabolic profiles, UGTs selectivity, as well as metabolic rates. All of these compounds can be biotransformed to a major metabolite (7-O-glucuronide), while a minor metabolite could also be detected for 4-methyl and 4-phenyl esculetin. UGT1A6 and UGT1A9 were the major isoforms involved in the metabolism of esculetin and its 4-methyl derivative, while UGT1A9 played a significant role in the metabolism of 4-phenyl esculetin. The Km values of these compounds in HLM were 264 uM, 73.5 uM and 7.7 uM for the formation of corresponding 7-O-glucuronide, respectively, suggesting that the Km values decreased with the increasing of the hydrophobicity of C-4 substitutions. Moreover, the C-4 substitutions also led an alteration of kinetics model, since esculetin and 4-methyl esculetin obeyed Michaelis-Menten model but 4-phenyl esculetin displayed a substrate inhibition model. In summary, C-4 hydrophobic substitutions of 6,7-dihydroxycoumarin can improve the affinity to UGTs, which is helpful for the design of new esculetin derivatives with proper metabolic stability.
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Keywords: Esculetin; 4-Methyl esculetin; 4-Phenyl esculetin; UGT; Metabolic stability.

P24 - THE "ALBUMIN EFFECT" IN IN VITRO STUDIES OF UGTS 1A7, 1A8, AND 1A10

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Human UDP-glucuronosyltransferases (UGTs) are phase II metabolic enzymes responsible for elimination of numerous endo- and xenobiotics from the body. The ability to accurately estimate the glucuronidation activity in vivo by performing cost-effective in vitro experiments is crucial for modern drug development. Recently we reported that inclusion of bovine serum albumin (BSA) greatly enhances the in vitro activity of UGT1A9 by both K_m decrease and V_{max} increase (Manevski et al., 2011). Here we expanded our study of albumin effect to a group of highly homologous enzymes: UGTs 1A7, 1A8, and 1A10. Entacapone, 4-methylumbelliferone, 1-naphthol, and beta-estradiol were used as probe substrates in detailed enzyme kinetics experiments in the presence and absence of BSA. The substrates' nonspecific binding to BSA was measured by rapid equilibrium dialysis. Our experiments show that this group of UGT enzymes is highly amenable to albumin effect in vitro. Inclusion of BSA led to significant activity enhancement and profound changes in all basic enzyme kinetic parameters: K_m, V_{max}, and enzyme kinetic model. The results set new standards for the accuracy of in vitro UGT assays and are expected to facilitate the in vitro – in vivo correlation, the important goal of drug metabolism studies.

P25 - SELECTIVE CONJUGATION OF 7-HYDROXYCOUMARIN BY RECOMBINANT HUMAN URIDINE 5'-DIPHOSPHO-GLUCURONOSYLTRANSFERASE (UGT)

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7-Hydroxycoumarin (7-HC) has been used as a probe substrate for phase II enzymes uridine 5'-diphospho-glucuronosyltransferase (UGT) and sulfotransferase (ST) in both subcellular fractions and in cellular systems. It may

be used directly as a substrate to measure phase II activities or may be the intermediate metabolite from the phase I metabolism of the parent substrates 7-ethoxycoumarin or couarmin. In humans, 7-HC glucuronide is the predominant phase II metabolite. However, the specificity of 7-HC as a phase II substrate for the UGT isozymes has not been reported. To investigate 7-HC specificity as a substrate for UGT isozymes, we used a panel of recombinant human enzymes (UGT1A1, 1A3, 1A4, 1A6, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17). Rank order of the activity showed a stratification amongst the UGTs tested: (1A6, 1A9) > (1A1, 1A10, 2B7, 2B15) > (1A3, 2B4, 2B17). These groups spanned a wide range of activities from UGT1A6 at 3808 pmol/min/mg, to UGT1A1 at 289 pmol/min/mg to UGT1A3 at 31 pmol/min/mg with UGT1A4 not showing any significant activity. Km and Vmax values ranged from 200 µM to 1620 µM and 108 pmol/min/mg to 6945 pmol/min/mg, respectively. The results showed that 7-HC is not metabolized equally between the UGT isozymes but is a substrate for 9 of the 10 rUGTs tested. This data may aid in interpreting metabolism differences between tissues or donors due to differential distribution of UGT isozymes.

P26 - UGT2B7 IS RESPONSIBLE FOR HEPATIC GLUCURONIDATION OF DIETHYLSTILBESTROL (DES) AND DES IN TURN POTENTLY INHIBITS CATALYTIC ACTIVITIES OF MANY UGT ENZYMES

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Abstract: Diethylstilbestrol (DES) is a synthetic estrogen famous for its severe carcinogenic effects. It was once widely prescribed to millions of pregnant women in false hopes of preventing miscarriage and other pregnancy complications, and also found widespread application as a growth promoter in feeding cattle, sheep, and poultry. After widespread and extensive exposures to DES, numerous serious health problems were raised, such as increasing cancer risks and malformations of genital tracts of men and women who were exposed to DES before birth. DES can undergo extensive glucuronidation in human in vivo that is also considered to be a sole detoxification pathway. However, DES glucuronidation is very poorly understood in human. This study was designed to characterize uridine 5-diphosphate glucuronosyltransferases (UGTs) responsible for DES glucuronidation, and further to identify UGTs strongly inhibited by DES. Results indicate that UGT1A1, 1A3, 1A8 and 2B7 can catalyze DES glucuronidation, while other UGTs display no DES glucuronidation activity. Kinetic researches, chemical inhibition studies and correlation analysis all demonstrate that UGT2B7 is a major enzyme responsible for DES glucuronidation in human liver microsomes (HLM). In addition, this study reveals that DES acts as a potent inhibitor of UGT1A1, 1A3, 1A9, 2B4 and 2B7. In vivo inhibition could likely occur in the case of high oral dosage or intravenous drip infusion of DES. In summary, this study indicates that UGT2B7 plays an important role in DES glucuronidation, and DES can in turn strongly inhibit many UGTs, implying DES may act as a toxicity enhancer increasing toxicities of other toxicants via suppressing the essential glucuronidation metabolism.
Key words: Diethylstilbestrol; UGT2B7; Inhibition.

P27 - PROTEIN S-GLUTATHIONYLATION: A ROLE FOR GLUTATHIONE S-TRANSFERASE PI IN MEDIATING CYTOPROTECTION?

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The Pi class Glutathione S-transferase (GSTP) is the most abundant extra-hepatic isoform in humans, increased expression of this enzyme in human tumours, and its contribution to anti-cancer drug resistance, is well documented. In addition to xenobiotic metabolism, GSTP is a modulator of stress response pathways acting as an inhibitor of c-Jun NH_2-terminal kinase (JNK)[1] and TNF receptor-associated factor (TRAF2)[2] independently of its catalytic function. Recently, Gstp has been implicated in mediating protein S-glutathionylation[3], a reversible post-translational modification involved in sulfhydryl homeostasis. The aim of this study was to examine the mechanism underlying Gstp-mediated S-glutathionylation and the role of Gstp in glutathione homeostasis in vivo. In contrast to previously published data, silencing of Gstp expression in the HCT116 colon carcinoma cell line using siRNA significantly increased glutathionylation of a large number of proteins and increased cellular oxidised glutathione content which correlated with increased resistance to the thiol blocking agent N-ethylmaleimide. Examination of subcellular fractions from Gstp-silenced HCT116 cells found increased S-glutathionylation of a number of mitochondrial proteins while dichlorodihydrofluorescein diacetate staining demonstrated no change in reactive oxygen species (ROS). In order to address the functional role of Gstp in glutathione homeostasis in vivo, our group and others have identified a tyrosine to phenylalanine mutation at codon 7 which results in an enzyme devoid of

catalytic activity, yet able to bind glutathione and other cellular substrates. We have designed a transgenic mouse model in which this point mutation has been constitutively knocked into the Gstp1 gene yielding a non-catalytic Gstp protein ($Gstp^{Y7F}$). Compared to wild-type mice ($Gstp^{WT}$), $Gstp^{Y7F}$ mice and mice nulled for Gstp ($Gstp1/2^{-/-}$) demonstrate increased resistance to a single oral dose of acetaminophen (APAP, 300mg/kg), the toxicities of which are associated with depletion of hepatic glutathione. At 24h following APAP treatment, ALT levels increased significantly in $Gstp^{WT}$ mice (>5000 U/l) but remained unchanged in $Gstp^{Y7F}$ and $Gstp1/2^{-/-}$ mice. Increased ALT levels in $Gstp^{WT}$ mice correlated with centrilobular necrosis which was absent in $Gstp^{Y7F}$ and $Gstp1/2^{-/-}$ mice. However, APAP treatment did elicit oxidative stress in all genotypes as evident from immunohistochemical staining of heme-oxygenase 1 (HO-1) which localised in centrilobular regions in $Gstp^{Y7F}$ and $Gstp1/2^{-/-}$ mice and around the centrilobular 'necrotic rings' in $Gstp^{WT}$ mice. Our data shows that the catalytic activity of Gstp promotes APAP hepatic toxicity; we currently hypothesise that this occurs because of an inability to regenerate hepatic glutathione levels, as preliminary data suggests glutathione regeneration is not perturbed in $Gstp^{Y7F}$ and $Gstp1/2^{-/-}$ mice. Taken together, these results demonstrate a potentially novel catalytic function of Gstp in regulating glutathione homeostasis and protein S-glutathionylation.

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P28 – ABSTRACT WITHDRAWN

P29 - SOME PHASE II METABOLITES OF LU AA21004 (1-[2-(2,4-DIMETHYL-PHENYLSULFANYL)-PHENYL]-PIPERAZINE) AND THEIR FORMATION

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Lu AA21004 (1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is currently in late stage clinical development for the treatment of major depressive disorder. In the present study, the biotransformation of Lu AA21004 was investigated in vitro with regards to the formation of phase I metabolites and some phase II metabolites (glucuronides) and compared with the proposed in vivo biotransformation (scheme 1). This includes M12, a new class of metabolite, a piperazine N-oxide/N-glucuronide: 4-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-1-b-D-glucuronic acid-piperazine 1-oxide (1) that were found in samples together with the glucuronides M3 and M4(b)**.** In vitro, CYP2C9 and CYP2C19 are the primary enzymes responsible for the formation of N-hydroxylated Lu AA21004 (2) that possibly could lead to M11 and M12. Indeed, conjugation with glucuronic acid in the presence of UDPGA in human liver microsome incubations leads to the formation of the two glucuronides (M11 and M12) that can be cleaved with beta-glucuronidase. The other glucuronides were not studied in detail in vitro, but were identified from their mass spectra. The UDP-glucuronosyltransferases (UGT's) likely involved in the glucuronidations will be discussed. In conclusion, the metabolism of Lu AA21004 has been studied in detail and found to follow expected biotransformation pathways, except for the formation of the piperazine N-oxide/N-glucuronide, which is probably a more frequent type of glucuronide than previously anticipated in drug metabolism.

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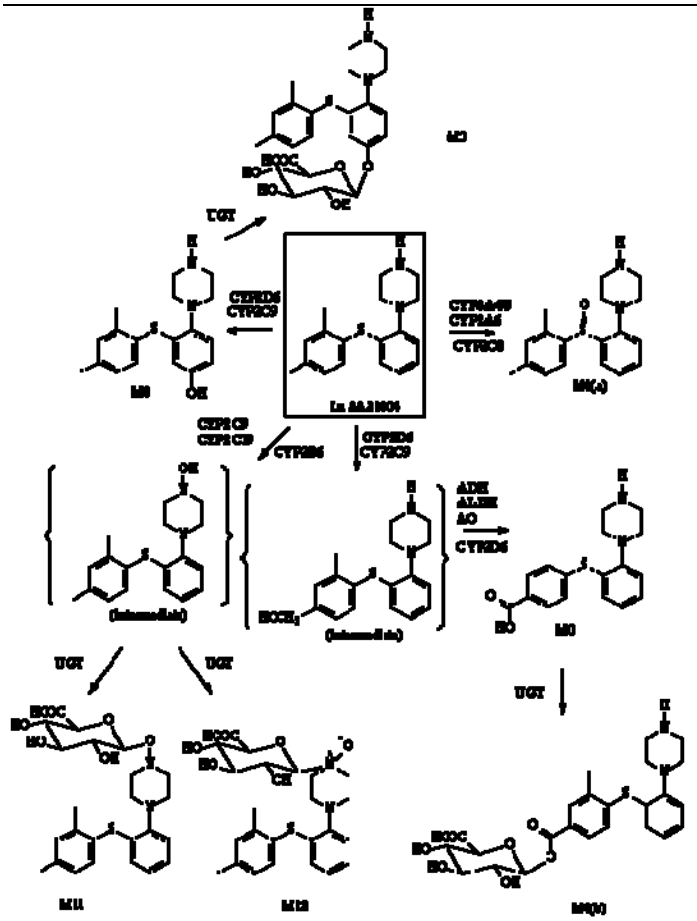

Scheme 1

P30 - EVALUATION OF THE MODULATORY EFFECT OF LABISIA PUMILA EXTRACTS ON CYTOCHROME P450 2C8 IN VITRO

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Cytochrome P450 (CYP) was found to be largely involved in the metabolism of various endogenous (steroids, bile acids, fatty acids, prostaglandins) and foreign compounds (drugs, alcohols, anti-oxidants, organic solvents, anaesthetic agents, dyes, environmental pollutants and chemicals). CYP2C8 , contributing to 5-8 % of CYP-mediated metabolism, is involved in the metabolism of more than 60 drugs, such as antimalarials (amodiaquine), antidiabetics (pioglitazone, repaglinide and rosiglitazone), statins (cerivastatin and fluvastatin) and anticancer agents (paclitaxel). Co-administration of inhibitor with certain CYP2C8 substrate may lead to accumulation of this substrate, which has a potential to cause adverse drug reaction. Herbal products contain a wide spectrum of constituents, of which the potentials of drug-herb interactions are always overlooked by public. Labisia pumila Blume (Myrsinacea family), or known as Kacip Fatimah in Malaysia, has been used traditionally for dysentery, flatulence, dysmenorrhea and gonorrhea. Thus far investigations of this herb mainly focus on the phytochemical properties and biological activities including antimicrobial, antioxidant, antistress activities as well as treatment of metabolic disorders associated with polycystic ovary syndrome. This study was performed to investigate the modulatory effects of various Labisia pumila extracts (aqueous, dichloromethane, ehtanol, hexane) on CYP2C8 activities in vitro. Dibenzylflourescein (DBF) was used as the substrate probe for CYP2C8 to establish a fluorescence-based assay. Our results indicate that ethanol, hexane and dichloromethane extracts significantly inhibited CYP2C8 activity with K_i values of 26.6 µg/ml, 33.9 µg/ml 0.70 µg/ml respectively. Thus, cautions need to be taken when Labisia pumila products are consumed with CYP2C8 substrates if these results can be confirmed with in vivo models.

P31 - MOLECULAR STUDIES ON INHIBITORY EFFECTS OF EPILOBIUM HIRSUTUM L. ON DRUG METABOLIZING CYP2B1, CPY2C6, CYP2D2 AND CYP3A4 ENZYMES IN RAT LIVER MICROSOMES

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Epilobium hirsutum L. (E.H) known as its high number of phenolic and flavonoid contents is distributed in worldwide. As a medicinal herb it has been used as an anticoagulant, anti-inflammatory and rectal and menstrual bleeding preventer. CYP2B1, CYP2C6, CYP2D6 and CYP3A1 are the important isoforms of cytochrome P450 enzymes with their private role on drug metabolism. Identification of phenolic compounds of E.H was done by using liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS). 18.75 mg extract/kg body weight/day was intraperitoneally (i.p.) injected to rats for 9 days to investigate in vivo effects of E.H on liver drug metabolizing CYP2B1, CYP2C6, CYP2D2 AND CYP3A1 mRNA and protein expressions as well as enzyme activities. Cytotoxic effect of E.H was tested by measuring lactate dehydrogenase activity. Although E.H. treatment caused 53 % inhibition of the benzphetamine N-demethylase (CYP2B1) and erythromycin N-demethylase (CYP3A1) enzyme activities ($p<0.0001$), no significant effect was observed on aminopyrine N-demethylase (CYP2C6). QRT-PCR analysis of mRNA expression showed that CYP2B1 (6.7 fold), CYP2D2 (7.9 fold) and CYP3A1 (12.7 fold) were significantly inhibited ($p<0.0001$), whereas CYP2C6 (1.6 fold) mRNA expression slightly inhibited ($p<0.05$). Western blot protein expression analysis also demonstrated that CYP2B1, CYP2D2 and CYP3A1 protein expressions were decreased significantly 55% ($p<0.0001$), 32% ($p=0.0043$) and 83% ($p<0.0001$) in comparison to control group. These results indicate that E.H. is a potent inhibitor of CYP2B1, CYP2D2 and CYP3A1; hence i.p. administration of Epilobium hirsutum L. may modulate pharmacological action of drugs and toxicants metabolized by those enzymes.

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P32 - FORMATION OF DIHYDROXYLATED AND GLUTATHIONE CONJUGATE METABOLITES DERIVED FROM THALIDOMIDE AND 5-HYDROXYTHALIDOMIDE IN HUMANIZED TK-NOG MICE

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Thalidomide was previously withdrawn because of its teratogenic effects in humans but has been approved for the treatment of refractory multiple myeloma. We reported that human cytochrome P450 3A enzymes in liver mediated thalidomide 5-hydroxylation and further oxidation, leading to non-enzymatic GSH conjugation, which may be relevant to the pharmacological and toxicological actions. However, it is not known if these two-step aromatic oxidations of thalidomide and the trapping of reactive metabolites by GSH occur in human in vivo situations, especially secondary catechol formation and other oxidation. The formation of dihydroxythalidomide and glutathione (GSH) conjugate(s) of 5-hydroxythalidomide was investigated in chimeric mice modified with "humanized" liver, i.e. novel humanized TK-NOG mice were prepared by introduction of thymidine kinase, followed by induction with ganciclovir, and human liver cells were transplanted. Following oral administration of racemic thalidomide (100 mg/kg), plasma concentrations of 5-hydroxy- and dihydroxythalidomide were higher in humanized mice than in controls. After administration of 5-hydroxythalidomide (10 mg/kg), higher concentrations of dihydroxythalidomide were detected. These results indicate that livers of humanized mice mediate thalidomide oxidation, leading to possibly catechol(s) and/or the GSH conjugate in vivo and suggest that thalidomide activation occurs.

P33 - CHARACTERIZATION OF CYNOMOLGUS P450 ENZYMES IN THE INTESTINE AND LIVER USING TYPICAL SUBSTRATES AND INHIBITORS FOR HUMAN P450 ENZYMES

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Cynomolgus monkeys (Macaca fascicularis) are widely used to predict human pharmacokinetic and/or toxic profiles in the drug developmental stage because the monkeys are closer to humans than other experimental animals in terms of their sequence identity. Some studies have revealed the properties of monkey cytochrome P450s (P450s, CYPs) such as the expression profiles and enzymatic functions; however, these are not enough to understand the species difference. Therefore, characterization of the substrate specificity and inhibitor selectivity of cynomolgus P450s was conducted using typical substrates and inhibitors for human P450s to clarify the species difference between monkeys and humans. Kinetic parameters were determined in pooled monkey intestinal and liver microsomes (MIM and MLM, respectively) using nine maker reactions for human P450s. Some kinetic parameters were unable to be determined because of the low activities. Similar to human P450 reactions, testosterone 6β-hydroxylation in MIM and MLM showed sigmoidal rate-substrate concentration profiles known as the CYP3A-specific reaction pattern, where Hill coefficient were 1.40 and 1.54, respectively. Intrinsic clearance (CL_{int}) for coumarin 7-hydroxylation and bufuralol 1'-hydroxylation were higher than those of other reactions in MLM, with the values of 238 and 158 μL/min/mg, respectively. On the other hand, paclitaxel 6α-hydroxylation, diclofenac 4'-hydroxylation, and S-mephenytoin 4'-hydroxylation showed low activities in MIM and MLM with CL_{int} values of less than 0.125 and 5.65 μL/min/mg, respectively. IC_{50} values of selective inhibitors for human P450s were determined in MIM and MLM. Ketoconazole strongly inhibited intestinal and hepatic testosterone 6β-hydroxylation with IC_{50} values of less than 0.0124 μM. Tranylcypromine showed inhibition on hepatic chlorzoxazone 6-hydroxylation but did not show on intestinal chlorzoxazone 6-hydroxylation. On the other hand, the inhibitory effect of ketoconazole on intestinal chlorzoxazone 6-hydroxylation was more potent than that on hepatic chlorzoxazone 6-hydroxylation. Furafylline and sulfaphenazole showed little inhibition in monkey microsomes on phenacetin O-deethylation and diclofenac 4'-hydroxylation, respectively. Because furafylline is also known as a mechanism-based inhibitor for human CYP1A2, time-dependent inhibition assay was carried out with MLM. The inhibitory effect of furafylline on hepatic phenacetin O-deethylation was weak even when furafylline was preincubated with MLM in the presence of NADPH/NADH (43.8% inhibition at 100 μM). Our studies provide comprehensive enzymatic profiles of cynomolgus P450s in the intestine and liver. These contribute to a better understanding of the species difference in substrate specificity and inhibitor selectivity between cynomolgus monkeys and humans.

P34 - LOW POTENTIAL FOR DRUG INTERACTION OF CAPPARIS OVATA WATER EXTRACT

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The aim of the present study was to evaluate the in vivo effects of the lyophilised water extract from combination of flowering buds, flowers and fruit of Capparis ovata on the expression of drug metabolizing enzymes in mice. Caper parts were supplied by Asci Murat Capers, Ice Cream, Dessert and Pickle Manufacturing & Export Co., Ltd. Caper has been widely used by the traditional medicine for healing effects in certain pathological conditions. The extract contains many constituents, in particular some flavonoids (rutin and quercetin derivatives) and hydrocinammic acids with several known biological effects such as the anti-inflammatory and the antioxidant. Recently, we have demonstrated that this extract expressed protective and healing effects on experimental animal model of multiple sclerosis (EAE) with our R & D Innovation project of KOSGEB (unpublished data, ongoing patent application process) In this study, we have assayed the in vivo effect of this extract on C57BL/6 mice drug metabolizing enzymes, namely CYP1A1, 1A2, 2A4, 2B9, 2C37, 2C39, 2D9, 2E1, 2F2, 2J5, 3A11, for its possible drug interaction potential. C57BL/6 mice, about 3 months old and weighing 18-22 g (n=16), received 500 mg extract per kg body weight intragastrically for 28 days while control subjects received only water. Animals were killed by decapitation and liver were removed. Total RNA was isolated using TRIzol (Invitrogen, UK) in accordance with the manufacturer's standard protocol. Primers for above mentioned mouse CYP450s were taken from literature and binding to target sequence was confirmed by BLAST (National Institute for Health, USA). RT-PCR was performed using the First-Strand cDNA

Synthesis System (Applied Biological Materials, Canada) following the manufacturer's protocol. We have observed the same levels of expressions for all the cytochrome P450s studied in both Capparis ovata extract fed- and control-mice. Capparis ovata extract did not significantly elicit the any major CYP450 enzyme expression. Therefore, our results suggest that the drug interaction potential of Capparis ovata water extract is unlikely or very low.

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P35 - PROBING THE MECHANISM OF NICOTINE DEGRADATION BY MAMMALIAN CYTOCHROME P450 MONOOXYGENASES

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In order to enhance the lifetime of drugs active against neurotoxic and neurodegenerative disorders, an approach towards the design of cytochrome P450 monooxygenase (CYPs) transition state analogues related to nicotine-based derivatives exhibiting therapeutic activities is being pursued. So as to study in detail the metabolism of nicotine by various mammalian CYPs potentially involved in its degradation – thus to identify reaction intermediates and to design transition-state analogues – three approaches are combined: experimentally-determined kinetic isotope effects (KIE); molecular dynamics (MD); theoretical calculation of KIEs. CYPs can metabolize nicotine either by oxidation at the methyl group to N-hydroxymethyl-nornicotine and demethylation to nornicotine, or by oxidation at the 5’ position to 5’-hydroxynicotine, with subsequent oxidation to cotinine. In human metabolism, cotinine is the dominant product. The aim of this project is to understand the factors that favour the formation of one or the other product. Biotransformation by rat microsomes of nicotine species specifically deuterated in either the methyl or the 5’,5’ position was studied in vitro. Two iminium ion intermediates were postulated by LC-MS analysis of the reaction products. Significant KIEs for cotinine formation were obtained. In contrast, no KIE was found for the consumption of nicotine to nornicotine. Calculations of the active site volume of several CYPs revealed a bell curve relationship of volume with the catalytic activity toward nicotine degradation. Interestingly, secondary cavities in the CYP structural channels with stable nicotine positions were identified using molecular docking simulations. During unconstrained MD simulations, hydrophobic spontaneous channelling of nicotine towards the final position close to the heme was observed.

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P36 - DOWN-REGULATION OF HUMAN CYTOCHROME P450 1A1 AND 1B1 BY SINGLE-WALLED CARBON NANOTUBE

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Nanotechnology has drastically emerged over the past 10 years, and a large variety of nanomaterials, which have a wide range of applications in nanoscience, medicine, and engineering, have been created. The application of single-walled carbon nanotube (SWCNT) as a drug delivery agent has been proposed owing to its distinct structural properties that enable the loading of molecules such as antibodies or drugs along the length of the nanotube sidewall. Although trans-pulmonary delivery has been actively investigated, drug-metabolizing enzymes are expressed in the lung and play important roles in lung homeostasis and drug detoxification. Clarifying the effect of SWCNT on these drug-metabolizing enzymes is of importance for its application as a drug carrier. The effects of SWCNT on the expression of 86 drug-metabolizing enzymes were investigated in normal human bronchial epithelial cells by real-time polymerase chain reaction, and we focused on the significantly down-regulated cytochrome P450 1A1 (CYP1A1) and CYP1B1 genes, which are involved in the metabolic activation of procarcinogens in the lung. To clarify the down-regulation mechanism, CYP1A1- and CYP1B1-expressing cells, such as human hepatocarcinoma HepG2 and breast carcinoma MCF-7 cells, were exposed to SWCNT at a concentration of 0.1 mg/ml for 24 h. The effect of SWCNT on the basal and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced mRNA expression and enzymatic activities of CYP1A1 and CYP1B1 were analyzed. Chromatin immunoprecipitation assay was also performed to quantitate the binding of aryl hydrocarbon receptor (AHR) protein to the enhancer region of these

genes. SWCNT down-regulated the basal and TCDD-induced mRNA expression of CYP1A1 and CYP1B1, and the TCDD-induced CYP1A1 and CYP1B1 enzymatic activities were inhibited in HepG2 and MCF-7 cells. In addition, the binding of AHR to the enhancer regions of CYP1A1 and CYP1B1 was inhibited significantly. These results indicate that inhibition of the binding of activated AHR to the enhancer regions of these genes is at least one of the mechanisms in the down-regulation of CYP1A1 and CYP1B1 mRNAs and their enzymatic activities. In conclusion, down-regulation of CYP1A1 and CYP1B1 genes by SWCNT may affect the lung defense mechanisms by reducing procarcinogen bioactivation and drug metabolism in the human lung.

P37 - ENHANCED EXPRESSION OF CYTOCHROME P450 GENES BY HEPATOCYTE NUCLEAR FACTOR-6 IN HEPATOCYTE-LIKE CELLS DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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[Purpose] The expression of drug-metabolizing enzymes changes significantly during liver development. Liver development is regulated by a dynamic network of liver-enriched transcription factors (LETFs), such as hepatocyte nuclear factor (HNF)-1, HNF-3, HNF-4, HNF-6, and CCAAT/enhancer binding proteinα. Although many of LETFs have been reported to modulate the regulation of transporter and drug-metabolizing enzyme genes, regulation of cytochrome P450 (P450) gene expression by HNF-6 has not been established. In the present study, we investigated whether HNF-6 could modulate P450 gene expression in hepatocyte-like cells derived from induced pluripotent stem cells (iPSHCs). **[Methods]** We constructed a human HNF-6-expressing adenovirus (AdhHNF-6). HepG2 and human fetal liver cells (HFLs) were infected with the AdhHNF-6. The human iPS cells (iPSCs) were provided by Dr. Akihiro Umezawa, National Institute for Child Health and Development. These cells were induced to differentiate into fetal hepatocyte-like cells using a 3-stage method (sequential addition of activin A, dimethylsulfoxide, and 3 cytokines, total 25 days culture). This iPSHCs were infected with AdhHNF-6 on day 15, day 19, and day 22. To evaluate the effect of HNF-6 overexpression on gene expression in HepG2, HFLs, and iPSHCs, the expression levels of hepatic marker, transcription factor, and P450 genes were measured using real-time PCR. **[Results and Discussion]** HepG2 and HFLs, which were infected with AdhHNF-6 showed a MOI-dependent upregulation of P450 genes compared with non-infected cells. In iPSHCs, AdhHNF-6 infection induced a 1,000-fold increase in the expression of CYP3A4 mRNA, but did not affect the expression of albumin (ALB) mRNA. These results suggested that HNF-6 could modulate the expression of the P450 genes. However, the expression levels of CYP3A4 and ALB mRNA were lower in the iPSHCs than in the human hepatocytes. Therefore, further studies are needed to improve the cell differentiation method for attaining the final maturation stage.

P38 - DRUG INTERACTION AND CARCINOGEN ACTIVATING POTENTIAL OF O-COUMARIC ACID

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Coumaric acids are hydroxy derivatives of cinnamic acid. There are three isomers, o-coumaric acid, m-coumaric acid, and p-coumaric acid that differ by the position of the hydroxy substitution of the phenyl group. o-Coumaric acid (trans-2-Hydroxycinnamic acid) is found in vegetables, fruits, coffee, tea and nuts and it is one of the common phenolic antioxidant in the human diet. Although humans are exposed widely to o-Coumaric acid (o-CA), its drug interaction and carcinogen activating potential remains to be elucidated. In this respect, the aim of the present study is investigate the effects o-CA acid on various cytochrome P450 dependent drug metabolizing enzymes, namely CYP1A1, CYP1A2, CYP2E1, CYP2C9 and CYP3A4 in human hepatocarcinoma cells (HepG2). For this purpose, the cytotoxicity of o-CA in HepG2 cells was investigated by crystal violet staining assay. It was found that 7.95 mM o-CA was 50% cytotoxic dose of HepG2 cells. This dose was selected for further studies. o-CA was caused 1.30- and 1.60-fold increases in CYP1A1 and CYP1A2 mRNA levels ($p<0.05$). Similarly, it was caused 5.65-fold increase in CYP2E1 mRNA level ($p<0.001$). Moreover 2.30-fold increase was observed in CYP2C9 mRNA level as a result of o-CA treatment ($p<0.05$). Unlike these, CYP3A4 mRNA level was decreased 2.50 fold with respect to untreated cells ($p<0.05$). Based on the observed effects on CYP450s, we have shown that o-CA may stimulate the metabolic breakdown of toxic chemicals metabolized not only by CYP2E1 but also by CYP1A1/1A2 through increased expression of these isozymes, which consequently may result in increased amounts of reactive metabolite

formation. This may in turn further potentiate the risk of toxicity, carcinogenesis, mutagenesis and malignant transformation. Moreover, it would be expected to change the disposition of essential medications. Therefore, until further clinical drug interaction studies are completed, the use of o-CA along with drugs should be avoided.

P39 - EFFECTS OF MEDICINAL PLANT VISCUM ALBUM L. ON RAT LIVER FLAVIN-CONTAINING MONOOXYGENASE ACTIVITY AND EXPRESSION

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Viscum album L., a species of mistletoe, contains lectins, polypeptides, mucilage, sugar alcohols, flavonoids, lignans, triterpenes, and phenylallyl alcohols[1]. The leaves and twigs of Viscum album L., taken as tea, have been traditionally used for hypertension, stomachache, diarrhea, dysuria and also as analgesic and cardiotonic agent in Anatolia, Turkey[2]. In addition, in Europe, sterile extracts of Viscum album L. are among the most common herbal extracts applied in cancer treatment and have been used as prescription drugs, while in US, considered as dietary supplement[3]. Flavin-containing monooxygenases (FMOs, EC 1.14.13.8) are FAD-containing phase I enzymes and responsible for the oxidation of wide-range of nucleophilic nitrogen, sulfur, phosphorus, and selenium heteroatom-containing drugs such as tamoxifen, methimazole and imipramine, pesticides, neurotoxins, and other chemicals by using NADPH as cofactor[4]. The aim of this study was to determine the in vivo effects of Viscum album L. on FMO activity, mRNA and protein expression in rat liver. The water extract of Viscum album was injected intraperitonally (i.p) into 15 wistar albino rats as 10 mg/kg of body weight for 9 consecutive days, while 11 rats were used as control. Then, following the decapitation, the livers were removed and microsomal fractions were prepared. FMO activity using methimazole as substrate, mRNA expression by quantitative Real-Time PCR, and protein expression by Western Blot were determined. The results showed that extract of Viscum album L. decreased mRNA, and protein expressions as well as enzyme activity of FMO with respect to controls. Liver microsomal FMO activity was decreased from 5.1±0.9 to 4.1±1.1 nmol/min/mg ($p<0.05$). mRNA expression of FMO in extract injected animals was found to be 2.6 fold lower compared to controls ($p<0.0001$). In addition, the plant extract decreased the FMO protein level 28% with respect to controls ($p<0.05$). In conclusion, the metabolism of xenobiotics by FMO-catalyzed reactions may be altered due to the changes in FMO expression and activity by Viscum album L. extract.

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P40 - D2-DOPAMINERGIC RECEPTOR LINKED PATHWAYS: CRITICAL REGULATORS OF CYP3A, CYP2C AND CYP2D

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D2-dopaminergic receptor linked pathways: critical regulators of CYP3A, CYP2C and CYP2D Evangelos P. Daskalopoulos1, Matti A. Lang2,3, Marios Marselos1, Foteini Malliou1 and Maria Konstandi1* 1Department of Pharmacology, School of Medicine, University of Ioannina, Ioannina GR-45110, Greece 2University of Queensland, National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains QLD 4108, Australia 3University of Uppsala, Department of Biochemistry, Husargatan 3, Uppsala, Sweden ABSTRACT Introduction/Aim: It is well established that various hormonal and monoaminergic systems hold determinant roles in the regulation of several hepatic drug-metabolizing CYPs. Growth hormone (GH), prolactin (PRL) and insulin are involved in CYP regulation and their release is under dopaminergic control. This study has focused on the role of D2-dopaminergic systems in the regulation of the major drug-metabolizing CYPs, the CYP3A, CYP2C and CYP2D in the liver of male rats.

Results/Discussion: The data showed that blockade of D2-dopaminergic receptors with sulpiride (SULP) markedly down-regulated CYP3A1/2, CYP2C11 and CYP2D1 expression in the rat liver. This suppressive effect appears to be mediated by the insulin/PI3K/Akt/FOXO1 signaling pathway. Furthermore, inactivation of the GH/STAT5b signaling pathway seems also to play a role in the SULP-mediated down-regulating effect on these CYPs. SULP suppressed plasma GH levels, followed by reduced activation of STAT5b, the major GH pulse-activated transcription factor which has an up-regulating effect on various CYPs in hepatic tissue. PRL, which possesses a down-regulating control on several CYPs, was increased by SULP, and may thus also contribute in the SULP-mediated suppression of the CYPs. Finally, it appears that the SULP-induced inactivation of the cAMP/PKA/CREB signaling pathway, which is a critical regulator of PXR and HNF1á, as well as inactivation of JNK, contribute in the SULP-induced down-regulation of the above mentioned CYPs. These mechanisms could therefore also contribute to the down-regulation. Conclusion: Taken together the present data provide evidence that drugs acting as D2-antagonists, could interfere with several major signaling pathways involved in the regulation of CYP3A, CYP2C and CYP2D, critical enzymes in drug-metabolism, thus affecting the effectiveness of the majority of prescribed drugs, as well as the toxicity and carcinogenic potency of a plethora of toxicants and carcinogens.

P41 - PHENOTYPIC DIFFERENCES OF HUH7 CELLS INDUCED DURING CONFLUENCE: BASIS FOR PXR MEDIATED INDUCTION OF CYP3A4 EXPRESSION

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Human primary hepatocytes (HPH) are widely used in drug discovery as an in vitro cell model for drug metabolism and toxicity studies. However, there are several limitations with the use of HPH; the often low availability of fresh liver samples and inter-individual donor variability. In addition, hepatocytes in two-dimensional cultures de-differentiate, resulting in a rapid reduction in the expression levels of liver-specific and drug metabolizing genes. As an alternative, cell lines can be used with advantages, such as their availability and stable phenotype. Many display, however, reduced hepatocyte-like functions and metabolic capabilities, thereby limiting their use in drug metabolism and toxicity. CYP3A4 is the most important drug metabolizing enzyme. It has a wide substrate spectrum and is involved in the metabolism of approximately 60% of the marketed drugs. Previously we have shown that when the human hepatoma cell line Huh7 is grown confluent, CYP3A4 mRNA and protein levels are up-regulated and the CYP3A4 activity is significantly increased. A whole genome gene array analysis indicated that the confluent cells acquired a phenotype more similar to HPH. Thus, several KEGG-pathways, related to liver functions, were significantly up-regulated in the cells grown confluent compared to control cells. By contrast, pathways related to proliferation and apoptosis were down-regulated in the confluent cells. Three DNA elements known to be important for CYP3A4 regulation were cloned and reporter gene expression of these CYP3A4 promoter constructs revealed that the CYP3A4 gene is transcriptionally activated during confluence. Using western blot and immunocytochemistry we show that the increase in CYP3A4 expression in confluent cells was accompanied by a nuclear translocation of the pregnane X receptor (PXR) in the absence of added ligands. The endogenous PXR levels were expressed at significant higher levels in the nucleus of confluent cells compared to control cells. In confluent cells the PXR ligand rifampicin further increased the transcription of CYP3A4, and siRNA knock-down of PXR resulted in significantly decreased CYP3A4 expression levels. After trypsination and re-plating of the confluent cells, the levels of CYP3A4 together with the nuclear levels of PXR were drastically decreased, suggesting that proper cell-cell contacts are important for CYP3A4 expression. In conclusion, our results show that Huh7 cells grown confluent are a CYP3A4-competent cell line, due to activation and nuclear translocation of endogenously expressed PXR. We hypothesize that PXR in turn, is regulated by decreased proliferation as a result of cell-cell contact inhibition.

P42 - SIMULTANEOUS EVALUATION OF INFLUENCE OF SODIUM TANSHINON IIASILATE ON CYP450 ISOFORMS BY COCKTAIL PROBE DRUGS

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BACKGROUNDS: Sodium Tanshinon Ⅱ Asilate (STA) is a sulfonate of Tanshinon ⅡA which is extracted from the traditional Chinese drug salvia miltiorrhza. It is widely used in clinic to treat cardiovascular disorders with other drugs, so it is very important to evaluate its influence on CYP isoforms to predict the drug-drug interactions.

OBJECTIVES: To evaluate the effect of STA on the activities of cytochrome P450 (CYP450) isoforms CYP1A2, CYP 2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 with a six-probe cocktail in rats. METHODS: The rats were randomly divided into four groups, the control group, the low dose STA group, the middle dose STA group and the high dose STA group. The rats in each group were given intraperitoneally normal saline 5ml/kg, STA 10mg/kg, STA 20mg/kg, STA 40mg/kg, respectively, once a day for seven days. On the eighth day, the six-probe cocktail including caffeine (2.5mg/kg), tolbutamide (2.5mg/kg), omeprazole (10mg/kg), metoprolol (10mg/kg), chlorzoxazone (5mg/kg) and midazolam (5mg/kg) was intravenously injected to all the rats. Before and after injection, the blood samples were collected at a series of time-points and the concentrations of six probe drugs in plasma were determined by a HPLC method with UV detection in a single run. The main pharmacokinetic parameters were calculated by the DAS 2.0 software (Drug And Statistics 2.0 edition, provided by Chinese Pharmacological Society). RESULTS: The AUCs and t1/2s of caffeine were significantly lower in high dose, middle dose and lower dose STA group (8.01 ± 2.04 mg/L•h, 9.56 ± 2.03 mg/L•h, 9.16 ± 3.01 mg/L•h; 1.71 ± 0.51 h , 1.67 ± 0.44 h, 1.69 ± 0.32 h, respectively) than those in control group (21.11 ± 5.14 mg/L•h, 2.21±1.01 h, $P<0.05$). The CLs of caffeine were 0.252 ± 0.041 L/h/kg, 0.256±0.034 L/h/kg and 0.307 ± 0.071 L/h/kg, in high dose, middle dose and lower dose STA group which were significantly higher than that in control group (0.119 ± 0.034 L/h/kg, $P<0.05$). Meanwhile, the AUCs of chlorzoxazone were significantly lower in lower, middle and high dose STA group than those in control group (14.34 ± 7.25 mg/L•h, 13.54 ± 5.11 mg/L•h, 8.54 ± 1.97 mg/L•h vs 30.01 ± 9.26 mg/L•h, $P<0.05$) and CLs of chlorzoxazone were significantly higher (0.494 ± 0.131 L/h/kg, 0.501 ± 0.165 L/h/kg, 0.621 ± 0.227 L/h/kg vs 0.179 ± 0.057 L/h/kg; $P<0.05$). Compared with control group, the main pharmacokinetic parameters of tolbutamide, omeprazole, metoprolol and midazolam have no significant differences in three dose STA groups ($P>0.05$). CONCLUSION: STA can inhibit the activity of CYP1A2 and CYP2E1. STA has no influence on the activities of CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The results suggest that when STA is co-administered with the drugs metabolized by CYP1A2 and CYP2E1, the dosage of both drugs should be adjusted. This work was supported by a grant from Tianjin Natural Science Foundation key project of China, No. 09JCZDJC21500.

P43 - CLONING AND CHARACTERISATION OF THE SECOND TAMMAR WALLABY (MARCOPUS EUGENII) CYP4B SUBFAMILY MEMBER, CYP4B3

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The cytochrome P450 4B subfamily (CYP4B) exhibits bioactivation of aromatic amines and species- and tissue-specific regulation. For many years, CYP4B1 was the only gene represented within this family, often referred to as a lung-specific enzyme. A second member, CYP4B2, was subsequently isolated from goat lung1. Hypothesis: This study aimed to investigate the CYP4B subfamily in the Tammar wallaby (Macropus eugenii). Furthermore, we examined the microsomal bioactivation of 2-aminofluorene (2-AF) (a rabbit CYP4B substrate) in Australian marsupial tissues. Methods: The Tammar wallaby cDNA clone was amplified by reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) methodology. Activation of 2-AF was determined in Tamar wallaby, Eastern gray kangaroo and koala lung, liver and kidney microsomes using the Ames Salmonella mutagenicity assay. Results: A 1580 base pair Tammar wallaby full-length lung cDNA clone was isolated and designated CYP4B3. The clone displayed 77% nucleotide and 75% amino acid similarity to Tammar wallaby CYP4B1, and encoded a protein of 510 amino acids. The size of the deduced protein was consistent with other reported CYP4B enzymes, and contained several common cytochrome P450 conserved regions, plus the CYP4 family specific region. 2-AF activation in the kidneys of both male and female wallabies was found to be more than 40-fold higher compared to rabbit ($p<0.05$). Tammar wallaby liver was also found to have higher activity compared to rabbit liver ($p<0.05$). Cell expression of the Tammar wallaby and rabbit CYP4B cDNAs resulted in activation of 2-AF with the rabbit CYP4B1 cDNA-expressed proteins, while negligible activity was observed with the Tammar wallaby CYP4B cDNA-expressed proteins. Conclusion: A second CYP4B subfamily member from Tammar wallaby lung, CYP4B3, was cloned, and found to exhibit a high degree of similarity to the previously cloned eutherian CYP4B isoforms. This molecular data enables evolutionary analysis of the CYP4B subfamily in Australian marsupials. 1. Carr BA, Ramakanth S, Dannan GA, Yost GS. Characterisation of pulmonary CYP4B2, specific catalyst of methyl oxidation of 3-methylindole, Molecular Pharmacology, 63(5), 1137-1147, 2003.

P44 - CHARACTERIZATION OF DRUG METABOLIZING CYP102A1 MUTANTS GENERATED BY DOMAIN-EXCHANGE AND DIRECTED EVOLUTION

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Cytochrome P450 enzymes are remarkably diverse oxygenation catalysts found throughout all classes of life. Recently, it was reported that CYP102A1 can be developed as a potentially versatile biocatalyst for the generation of human P450 drug metabolites. Therefore, it was suggested that CYP102A1 can be used to produce large quantities of the metabolites of human P450-catalyzed reactions. In this study, extremely highly active chimeric fusion proteins were obtained by exchanging reductase domains of CYP102A1 natural variants and mutants at key catalytic residues of the heme domains and random mutagenesis was used to improve the activity of these chimeras. To determine whether the chimeras have enhanced catalytic activity and, more importantly, whether they acquired activities of biotechnological importance, we measured the oxidation activities of the chimeras on human P450 substrates, mainly drugs. some chimeric proteins showed dramatically activity towards typical human P450 drug substrates. This study shows that chimeric proteins can be used as a template to develop industrial enzymes for the production of fine chemicals including drugs and their metabolites.

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P45 - PEROXIDE-DEPENDENT OXIDATION OF VARIOUS SUBSTRATES CATALYZED BY CYP191A1 FROM MYCOBACTERIUM SMEGMATIS

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Bacterial cytochrome P450 enzymes are capable of catalyzing various substrates via "peroxide shunt pathway" in the presence of peroxide, including hydrogen peroxide (H_2O_2), t-butylhydroperoxide (t-BHP), in place of the reductase system, oxygen and the costly cofactor such as NAD(P)H. Moreover, a continuous assay using peroxide allows a simple measurement of CYP191A1 activity. Genome analysis of Mycobacterium smegmatis suggests that it contains 40 putative P450 enzymes, including CYP51 and CYP164A2. In this study, The CYP191A1 from M. smegmatis was expressed in Escherichia coli, and purified. CYP 191A1 gene is 1,215 bp in length and encodes a protein a 416 amino acids and molecular mass of 46 kDa. It showed a typical reduced CO-difference spectrum with a maximum at 450 nm. Spectral analysis showed that the ferric form of the enzyme was in the low spin state with a Soret band at 416 nm, while the ferrous protein had a broad absorption peak around 420 nm. The CYP191A1 was also measured the catalytic activity for various substrates, including fatty acids, steroids, chromogenic and fluorogenic substrates, and several human substrates, in the presence of 6 types of electron transfer system, such as rat NADPH-cytochrome P450 reductase (CPR), Candida CPR (CaNPR), ferredoxin/ferredoxin reductase (FDR/Fdx), puridaredoxin/puridaredoxin reductase (PDR/Pdx), H_2O_2, and t-BHP. Reactions contained the hydroxylation and O-dealkylations and formation of the metabolites was confirmed by high-performance liquid chromatography, liquid chromatography-mass spectroscopy, and gas chromatography. CYP191A1 showed a preference for catalyzing the peroxide dependent oxidation of a variety of substrates. We expect that these "peroxygenase activity" of CYP191A1 offer effective biocatalytic tools to synthesize metabolites of drug or drug candidates cost-effectively and simply.

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P46 - GENERATION OF HUMAN METABOLITES OF ATORVASTATIN AND FLUVASTATIN BY BACTERIAL CYP102A1 MUTANTS

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Cytochrome P450s catalyze many different reactions involved in the biosynthetic pathways of a large variety of primary and secondary metabolites. Recently, it was reported that the various xenobiotic substrates including pharmaceuticals of human P450 enzymes was oxidized by wild-type and mutant forms of CYP102A1 from Bacillus megaterium. Atorvastatin and fluvastatin is known to be used to treat hypercholesterolemia and to prevent cardiovascular disease. Atorvastatin is oxidized by human CYP3A4 enzyme to produce metabolites including 2-OH atorvastatin and 4-OH atorvastatin, and fluvastatin is oxidized by human CYP2D6 enzyme to produce 5-OH fluvastatin and 6-OH fluvastatin. The aim of present study was to show that oxidation of atorvastatin and fluvastatin was catalyzed by wild-type and its mutants of CYP102A1 which were generated by site-directed and random mutagenesis. Metabolites formation was confirmed with high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectroscopy (LCMS). The results with CYP102A1 mutants show that in atorvastatin, major hydroxylated product (4-OH atorvastatin) and minor hydroxylated product (2-OH atorvastatin) were produced and in fluvastatin, two hydroxylated product (5-OH fluvastatin and 6-OH fluvastatin) which was shown to regioselectivity product were produced. To synthesize metabolite of atorvastatin and fluvastatin have not been reported by chemical methods. These results demonstrate that CYP102A1 mutants can be used to produce human metabolites of atorvastatin and fluvastatin.

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P47 - GENERATION OF 5'-HYDROXYOMEPRAZOLE BY CYP102A1 MUTANTS FROM BACILLUS MEGATERIUM

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Recently, wild-type and mutant forms of bacterial P450 BM3 (CYP102A1) from Bacillus megaterium have been found to metabolize various drugs through reactions similar to those catalyzed by human P450 enzymes. In this study, it has been shown that the hydroxylation reaction of human CYP3A4 and CYP2C19 substrate (omeprazole) are catalyzed by wild-type CYP102A1 and its mutants. Omeprazole, a proton pump inhibitor (PPI), has been widely used for many years as an acid inhibitory agent for the treatment of gastric acid hypersecretion disorders. Omeprazole is hydroxylated by the wild-type and a set of CYP102A1 mutants to produce one metabolite, hydroxyomeprazole. The activity of CYP102A1 mutant #10 (R47L/F87V/L188Q) is highest than wild-type CYP102A1 and other mutants. In this study, the structure of omeprazole metabolite was characterized by LC-MS and NMR spectroscopy. The metabolite is 5'-hydroxyomeprazole but not 3'-hydroxyomeprazole. These results suggest that there is a common mechanism for the hydroxylation reaction catalyzed by both the bacterial CYP102A1 and human P450 enzymes. In vivo effect of 5'-hydroxyomeprazole was also confirmed using ulcer animal model. The effect of 5'-hydroxyomeprazole was to treat ulcer animal model comparable to treat of omeprazole.

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P48 - FLAVIN CONTAINING REDUCTASE DOMAIN OF CYTOCHROME P450 BM3 AS A SURROGATE FOR MAMMALIAN NADPH-P450 REDUCTASE

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Cytochrome P450 BM3 (CYP102A1) from Bacillus megaterium is a self-sufficient monooxygenase which consists of heme domain and FAD/FMN-containing reductase domain. The reductase domain (BMR) of CYP102A1 has been intensively studied by numerous groups to utilize BMR as a surrogate reductase for the microsomal P450 complex. In this report, the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) by BMR was measured as a method for monitoring BMR activity. The electron transfer proceeds from NADPH to BMR and then to the substrates of BMR, MTT and CTC. MTT and CTC are monotetrazolium salts, which form formazans upon reduction. The reduction of MTT and CTC followed classical Michaelis-Menten kinetics (k_{cat} = 4120 min^{-1}, K_m = 77 mM for MTT and k_{cat} = 6580 min^{-1}, K_m = 51 mM, for CTC). A continuous assay using MTT and CTC allows a simple measurement of BMR activity in short analysis time. The ability of BMR to metabolize the substrates of mammalian NADPH-cytochrome P450 reductase (CPR) was examined and the results showed that BMR was able to metabolize mitomycin C and doxorubicin which are anticancer drug substrates for CPR, and produce the same metabolites as those produced by CPR. Moreover, to examine whether BMR can interact with human P450 enzymes and mediate electron transfer, activity assay for 7-ethoxycoumarin and phenacetin with CYP1A2 and BMR were performed. BMR was able to interact with CYP1A2 and transfer the electrons to promote the hydroxylation reaction by CYP1A2 and CYP2E1. The results of this study suggest the possibility of the utilization of BMR as a surrogate for mammalian CPR.

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P49 - IDENTIFICATION OF NOVEL CYTOCHROME P450S IN THE ACARI

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Cytochromes P450 are the major phase I drug metabolising enzymes found in most species including arthropods. Much of the work within the area of xenobiotic metabolism in this phylum has centred round mosquito species e.g. Anopheles gambiae and Culex quinquefasciatus due to their role as vectors of plasmodium falciparum. Current research on these mosquito species has identified members of the CYP6 and CYP9 families as playing an important role in the detoxification of pesticides and development of resistance, most notably, CYP6Z1 and CYP6P3 [1]. In addition to mosquitos this phylum contains many other medically/veterinary important ectoparasites many of which are members of Acari subclass (ticks and mites). The work presented here focuses on the identification of xenobiotic metabolising P450s from the cattle tick Rhipicephalus (Boophilus) microplus, an ectoparasite of cattle and a vector to numerous diseases most notably babesiosis, that costs beef producing countries millions of dollars each year. To date only 3 CYP genes have been identified within this species, none of which belong to the key CYP3 clade or have been characterised functionally [2, 3]. The research in acari, is currently greatly hindered by the lack of genome data available however, transcriptomic data is now starting to become available [4, 5]. Using transcriptomic data, 115 putative CYP sequences from R. microplus were put through detailed bioinformatic analysis and following the

construction of phylogenic trees, these sequences have been grouped into families and putative CYP6 and CYP9 homologues identified. From these partial DNA sequences gene specific primers (GSP) have been designed and used in 5'/3' RACE reactions to isolate full length cDNA sequences. cDNA was generated from RNA obtained from tick cell cultures (BME/CTVM2)[6]. The isolation of these novel CYP cDNAs will facilitate detailed enzymatic characterisation and investigation into their role in pesticide resistance. Finally this data will ultimately assist in designing better/specific and hopefully more environmentally friendly compounds for the control of these ectoparasites and additionally provide a base that could be extended to other acari members. 1. Chiu, T.L., et al., Comparative molecular modeling of Anopheles gambiae CYP6Z1, a mosquito P450 capable of metabolizing DDT. Proceedings of the National Academy of Sciences of the United States of America, 2008. 105(26): p. 8855-8860. 2. Crampton, A.L., C.D. Baxter, and S.C. Barker, A new family of cytochrome P450 genes (CYP41) from the cattle tick, Boophilus microplus. Insect Biochemistry and Molecular Biology, 1999. 29(9): p. 829-834. 3. He, H., et al., Molecular cloning and nucleotide sequence of a new P450 gene, CYP319A1, from the cattle tick, Boophilus microplus. Insect Biochemistry and Molecular Biology, 2002. 32(3): p. 303-309. 4. Bellgard, M.I., et al., CattleTickBase: An integrated Internet-based bioinformatics resource for Rhipicephalus (Boophilus) microplus. Int J Parasitol. 42(2): p. 161-9. 5. Guerrero, F.D., et al., Sequencing a new target genome: The Boophilus microplus (Acari : Ixodidae) genome project. Journal of Medical Entomology, 2006. 43(1): p. 9-16. 6. Bell-Sakyi, L., et al., Tick cell lines: tools for tick and tick-borne disease research. Trends in Parasitology, 2007. 23(9): p. 450-457.

P50 - ROSUVASTATIN IN HIGH CHOLESTEROL DIET SUPRESSES CYP2C11 AND CYP2C6 IN RATS

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Rosuvastatin is one of the latest statins (HMG-CoA inhibitors) used in cholesterol-lowering therapy. Several statins are metabolized by cytochrome P450 (CYP) 3A4 and can therefore interact with commonly used medications. Rosuvastatin undergoes only minor metabolism by CYP enzymes (CYP2C9 and also CYP2C19) in man. 1 The aim of the study was to investigate whether rosuvastatin at a dose 0.01% affects rat CYP2C11 and CYP2C6 (counterpart of human CYP2C9). 2, 3 Male Wistar rats were kept for 3 weeks (ad libitum) on a standard laboratory diet (STD) or on an experimental high-cholesterol diet (HCD) prepared by adding 1% (w/w) of cholesterol and 10% (w/w) of lard fat to the STD. Rosuvastatin (0.01%, w/w) was administered as a dietary supplement to the HCD. Rats were then fasted overnight, i. m. anesthetized and their liver was removed and frozen. Relative amount of CYP2C11 and CYP2C6 protein in the liver microsomes was determined by Western blotting, hydroxylating activities of these enzymes were measured by method based on formation of 4'-OH warfarin and 7-OH warfarin according to Locatelli 4 and expression of mRNA was examined by Real-Time PCR. Results obtained showed that 0.01% of rosuvastatin in high cholesterol diet significantly decreased protein levels of both observed CYP2C as well as hydroxylating activities of these anzymes and mRNA expression. CYP2C11 and CYP2C6 are taken as counterpart of human CYP2C9 form. 2, 3 The results hence indicate that the metabolism of other drugs mediated by these enzymes may be influenced by rosuvastatin (namely metabolism of warfarin by human CYP2C9).

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P51 - MOLECULAR MECHANISMS OF CYTOCHROME P450 CYP2S1 REGULATION BY CHEMICALS, ULTRAVIOLET RADIATION (UVR) AND OXIDATIVE STRESS

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Cytochrome P450 CYP2S1 was first described as an "orphan" P450, an enzyme identified in silico, predominantly expressed in extra-hepatic tissues, but with unknown function [1]. We have previously described significant individuality in cutaneous CYP2S1 expression, demonstrated regulation of CYP2S1 expression by topical retinoids, coal tar and ultraviolet radiation (UVR), and showed that CYP2S1 couples with NADPH cytochrome P450 reductase (CPR) to metabolise all-trans retinoic acid (atRA) [2]. We and others have also shown that CYP2S1 expression is significantly increased in the hyper-proliferative skin disease psoriasis [2], and in breast, ovarian and colorectal tumours [3-5]. We have now used WAVE® dHPLC analysis to show that the majority of individuality in CYP2S1 expression is not genetically determined, and suggest that inter-individual differences in CYP2S1 expression may arise from regulation of a highly inducible gene promoter by multiple stimulants, including drugs and UVR. We have identified several molecular mechanisms and key transcription factors which regulate CYP2S1 expression as an adaptive response to exposure to polycyclic aromatic hydrocarbons (xenobiotic response-element (XRE)-mediated) and retinoids (retinoic acid response element (RARE) and specificity protein 1 (SP-1) transcription factor-mediated), and have used site directed mutagenesis and promoter luciferase reporter assays to identify specific CYP2S1 promoter sequences necessary for enzyme induction. We have also investigated the wavelength dependency of CYP2S1 induction by UVR, shown that UVB wavelengths (used clinically in the treatment of psoriasis by phototherapy) most significantly induce CYP2S1 expression, and demonstrated that UVR induction is mediated by an activator protein 1 (AP-1) regulatory element in the proximal CYP2S1 promoter. Further, we have shown that the antioxidant response element (ARE) does not play a significant role in CYP2S1 regulation, but that CYP2S1 is significantly over-expressed in NRF2-null mouse embryo fibroblasts, suggestive of a novel mechanism of CYP2S1 regulation by oxidative stress. Consistent with this hypothesis, we have additionally demonstrated that CYP2S1 expression is induced by hyperbaric oxygen.

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P52 - BIOACTIVATION OF CLOZAPINE - INVOLVEMENT OF HUMAN CYTOCHROME P450S

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Clozapine is an atypical antipsychotic drug, which causes fewer extrapyramidal side effects than those experienced with typical neuroleptics. However, it is recommended only as a second line drug due to life threatening agranulocytosis occurring in 1-2% of the patients. Although the exact mechanism is not known yet, formation of reactive metabolites has been proposed as a possible explanation for this adverse drug reaction. Metabolism of clozapine to its major stable metabolites, N-desmethyl and N-oxide, is well described. However, involvement of the

various cytochrome P450s in the bioactivation of clozapine and formation of reactive intermediates was not yet investigated. Here, in vitro studies were performed to elucidate the human cytochrome P450 enzymes involved in the bioactivation of clozapine by two approaches. Commercial recombinant human P450s were used to bioactivate clozapine and reactive intermediates were measured as GSH conjugates formed in enzymatically catalysed conjugation in the presence of glutathione-S-transferase P1-1[1]. Also, inhibition of reactive intermediates and consequently GSH conjugates formation in human liver microsomes incubations by isoform selective chemical inhibitors of individual enzymes was investigated. It is concluded from both experiments that CYP3A4 has the major role in bioactivation of clozapine. Additional incubations were performed with the human liver microsomes extracted from the livers genotyped/fenotyped for P450s and the formation of GSH conjugates in different individuals was quantified. It was shown that GSH conjugation is a major pathway in clozapine metabolism. Interindividual differences and drug-drug interactions at the level of CYP3A4 might explain differences in susceptibility of patients to adverse drug reaction of clozapine.

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P53 - PHOTO-CYTOCHROME B5 – A NEW MEMBRANE CROSS-LINKING TOOL

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The catalytic activity of microsomal cytochrome P450 (CYP) monooxygenase multienzyme system might be altered by the presence of cytochrome b_5 (b_5). Depending on the CYP form, b_5 may stimulate or inhibit metabolism of a particular substrate or even change the pattern of metabolites. The mechanism underlying these effects has not been fully explained yet. The electron donation from b_5 to CYP and/or the b_5-induced conformational change in the active site have been suggested to explain the stimulatory effects, whereas the inhibition of CYP-mediated catalytic activity is attributed to the competition of b_5 and NADPH-cytochrome P450 reductase for the CYP binding site, namely at molar ratios (b_5 : CYP) exceeding the equimolar stoichiometry. To study the enigmatic role of b_5 in cytochrome P450 monooxygenase multienzyme system, analogues of b_5 such as apo-b_5, b_5 containing non-iron protoporphyrin IX or b_5 mutants are used. Our experimental approach to examine the CYP-b_5 interaction is based on photo-b_5, a b_5 analogue having methionine (Met) residues substituted for photolabile Met derivative. Met was selected since this rather rare amino acid occurs only 3 times in the b_5 sequence, all three localized in the short hydrophobic C-terminal tail, which anchors the heme-containing core of b_5 in the microsomal membrane. Recombinant photo-b_5 was expressed in the E. coli strain BL-21 (DE3) Gold. Prior to the induction of the protein expression, cells were transferred into the limiting medium (DMEM missing Met) supplemented with photo-Met (diazirine derivative). Purified b_5 sample containing > 30% of photo-b_5 was employed as a probe for mapping of CYP2B4 membrane topology. After reconstitution of CYP2B4 and photo-b_5 into artificial phospholipid membrane, the reaction mixture was photolysed (UV) to generate highly reactive intermediates (carbenes), which covalently bind to molecules (lipids, amino acid residues) in their close vicinity and thus covalently couple interacting proteins in the membrane environment. Photolyzed reaction mixture was purified on SDS-PAGE, digested with trypsin, and analyzed using high resolution LC-FT-ICR mass spectrometry. The analysis revealed three covalent CYP-b_5 oligomers composed of CYP2B4 and b_5 in 1:1, 1:2, and 2:1 molar ratios. The amount of oligomers formed was markedly increased by the presence of diamantane (CYP2B4 substrate) in the reaction mixture. This is in agreement with the evidence that the CYP substrate enhances the binding affinity for b_5. It appears that CYP and b_5 interact also within the microsomal membrane. Thus, the obtained data in conjuction with our previous results of chemical cross-linking will allow refining in silico models of CYP-b_5 interactions.

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P54 - EFFICIENT SCREENING OF P450 BM3 MUTANTS TO METABOLIZE DRUG-LIKE MOLECULES

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In the present study the diversity of a library of drug metabolizing bacterial cytochrome P450 BM3 mutants was

evaluated by an LC-MS based screening method. A strategy was designed to identify a minimal set of BM3 mutants which displays differences in regio- and stereoselectivities and is suitable to metabolize a large fraction of drug chemistry space. We first screened the activities of six structurally diverse BM3 mutants towards a library of 43 marketed drugs (encompassing a wide range of human P450 phenotypes, cLogP values, charges, and molecular weights) using a rapid LC-MS method with an automated method development and data processing system. Significant differences in metabolic activity were found for the mutants tested and based on this drug library screen, nine structurally diverse probe drugs were selected which were subsequently used to study the metabolism of a library of fourteen BM3 mutants in more detail. Using this alternative screening strategy we were able to select a minimal set of BM3 mutants with high metabolic activities and diversity with respect to substrate specificity and regiospecificity that could produce both human relevant and BM3 unique drug metabolites. This panel of four mutants (M02, MT35, MT38, and MT43) was capable of producing P450-mediated metabolites for 41 of the 43 drugs tested while metabolizing 77% of the drugs by more than 20%. This we see as the first step in our approach to use bacterial P450 enzymes as general reagents for lead diversification in the drug development process and the biosynthesis of drug(-like) metabolites.

P55 - 4-HYDROXY TRANS-RETINOIC ACID: ONE OF MAJOR AND UNIQUE METABOLITES FROM T-RA IN THE LIVER OF FETAL CYP3A TRANSCHROMOSOMIC MICE

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A human artificial chromosome (HAC) containing the CYP3A cluster (CYP3A-HAC) was constructed using the Cre/loxP-mediated chromosome-cloning system in order to generate 'humanized' trans-chromosomic (TC) mice with the human CYP3A cluster for drug screening. We have developed hCYP3A-HAC/mCyp3a knock-out mice and are now investigating to find many advantages of them for human drug screening. Although all- trans -retinoic acid (tRA), one of active metabolites of vitamin A1 (all- trans -retinol), is essential for normal human development, it is also known to cause severe birth defects. Therefore, enzymatic regulation of levels of tRA in embryonic tissues plays an extremely important role in both normal and abnormal human embryonic development. tRA is converted to 4-OH-tRA, and 4-hydroxylation of tRA might be an important protective mechanism for developing embryos. We analyzed tRA metabolites using a LC-MS/MS system (AB SCIEX QTRAP® 5500 LC/MS/MS System and AB SCIEX Triple TOF™ 5600 System). The expression of mRNA of hCYP3A7 in fetal hCYP3A-HAC/mCyp3a KO liver microsome has been confirmed in our previous investigation. In this study, a typical metabolite, 4-OH tRA was only clearly observed when the substrate was incubated with the fetal hCYP3A-HAC/mCyp3a KO mouse liver microsome, not with adult hCYP3A-HAC/mCyp3a KO, fetal/adult wild-type, and HAC-mCyp3a KO mouse liver microsomes. 4-OH tRA was also observed when the substrate was incubated with c-DNA expressed human CYPs3A4 and 3A7. We further investigate the reason of very low level of 4-OH tRA formed from the substrate incubated with the adult hCYP3A-HAC/mCyp3a KO mouse liver microsome. It was suggested that hCYP3A7 in our fetal hCYP3A-HAC/mCyp3a KO mouse liver plays a similar manner to that in human fetal liver.

P56 - FUNCTIONAL CHARACTERIZATION OF EIGHT HUMAN CYP1A2 VARIANTS: THE ROLE OF CYTOCHROME B_5

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Although substantial work has been reported on the function and effect of cytochrome b5 (b5) in cytochrome P450 (P450) mediated catalysis during the last decades, its mode of action is still not fully understood. Collectively, studies indicated that i) the first electron can only be donated by P450 reductase (CPR), ii) b5 can donate the second electron in competition with CPR, iii) CPR and b5 donate the second electron at similar rate in the formation of the reduced oxyferrous P450 and iv) the decay to the ferric ground state of P450 plus product release, is accelerated when the second electron is delivered by b5. This acceleration of the actual oxygenation step of the substrate was hypothesized to be caused by b5-mediated induction of conformational changes at P450's active site (1). To scrutinize this allosteric hypothesis, we studied the effect of b5 on eight natural occurring variants of human CYP1A2, namely T83M [CYP1A2*9], S212C [CYP1A2*12], S298R [no allele designation], G299S [CYP1A2*13], I314V [no allele designation], I386F [CYP1A2*4], C406Y [CYP1A2*5] and R456H [CYP1A2*8]. For this purpose, we used the same

approach as applied in our former study (2), co-expressing separately these polymorphic CYP1A2-variants with CPR, this time adding coexpression with human b5, in our bacterial BTC cell-model (3). All CYP1A2 strains demonstrated CYP1A2:CPR- and CYP1A2:b5-stoichimetries similar as those found in human liver. As in our previous study, a functional analysis was performed using 8 different substrates, measuring 16 different activity parameters. This heterogeneous data set was jointed with the one of our former study (2) on these 8 variants (i.e. without b5) and a multivariate analysis was applied. This analysis demonstrated that variants segregated in two distinct groups, i.e. all CYP1A2 variants coexpressed with CPR and b5 behaved functionally quite distinguishably, from the variants coexpressed with CPR alone. Moreover, variants clustered more close to the WT variant, i.e. were functionally more WT-like, upon coexpression with b5, when compared to the corresponding b5 lacking cluster. This was most striking for variant G299S, which in our former study was the most deviating variant relative to WT, which now in the presence of b5 demonstrated a very close resemblance with WT. On the other hand variants T83M and C406Y, which in our former study were found to be quite WT-like, segregated quite distinguishably from the WT cluster when in presence of b5. These three variants are located in distinct functional regions of the CYP1A2 protein based on the available CYP1A2 crystal structure (4). Collectively, our data seem to indicate that b5 has a compensatory effect on the perturbed functional capacities of these variants which can be interpreted by b5-induced allosteric attenuation of the conformational changes induced by their structural mutations. As such, b5 seems to be capable to alter the conformation of CYP1A2 in a more extensive way than only the active site architecture, as was hypothesized.

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P57 - CYTOCHROME P450 ACTIVE SITE ACCESS AND EGRESS PATHS OPENINGS DIFFER IN THEIR MEMBRANE POSITIONS

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Human Cytochromes P450 (CYPs) are membrane-anchored proteins located mostly in endoplasmic reticulum or mitochondria. The human CYP consists of an N-terminal transmembrane anchor and a catalytic cytoplasmic domain. The structure of the CYP catalytic domain has rather conserved fold according to X-ray experiments.[1] However, position of CYP on membrane remains still debatable. Recently, our group and others have published models of membrane anchored CYP2C9 and CYP3A4, based on atomistic molecular dynamics simulations.[2-4] Here, we extend the set of models of membrane-anchored human CYPs, which are relaxed on 100+ ns time scale. The set of six membrane-anchored CYPs enables us to identify common features and variations in positioning of individual CYPs on membranes. We have further computed membrane positions of typical substrates and corresponding oxidative products by a recently developed method.[5] We also compared positions of these compounds with the positions of the entries of the enzyme's active site access/egress paths, which were calculated by a recently released MOLEonline 2.0 software.[6] We show that the entry to the substrate access channel is facing the membrane interior while the exit of the product egress channel is situated above the interface pointing toward the water phase. The positions of openings of the substrate access and product egress channels math the free energy minima of substrates and products, respectively.

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P58 - WHAT IS THE CRUCIAL BINDING FEATURE FOR METABOLIC SPECIFICITY OF BIOSYNTHETIC MONOOXYGENASES CYTOCHROME P450 17?

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Cytochrome P450 (CYP) 17 has been emerging as a major target in prostate cancer for its central role in the biosynthesis of testosterone. Being biosynthetic monooxygenases of endogenous substances, CYP17 is distinguished from Xenobiotic-metabolizing CYP enzymes because it is highly specific in substrate recognition and metabolism regioselectivity. To explore the possible structural feature that contributes to the metabolic specificity, a computational analysis has been carried out both through ligand-based and structure-based modeling. A predicable 3D-QSAR model was first developed with CoMFA method based on a dataset of 76 synthesized inhibitors, demonstrating a Q^2 of 0.54 and a conventional r^2 of 0.90. The generated electrostatic and steric contours around the inhibitor suggested the active site is plain and compacted. The red contour located upon the substituent of imidazole indicated that this area shall interact with some positive electrostatic residue. To validate the ligand-based speculation, a molecular model of CYP17 was then developed by homology modeling to analyze the detailed feature of the active site bound with the substrate. With 27.5% of sequence identity, the crystallized structure of CYP2C9 (PDB Code: 1R9O) was used as the template to build the model. As a result, a reliable model was obtained whose reasonableness is of 98.5% in Protable analysis. And molecular docking was further used to validate the reasonableness of the homology model by generating rational bioactive poses of the drug abiraterone and the natural substrate pregnenolone. The structure-based analysis suggested that he compacted active site was constituted with residues Thr306, Ala302, Gly301, Ile 371, Arg239 , Phe114, Ile206, Leu209, Thr101 and Leu102, which droved the natural substrate to adopt an orientation above the Heme with its 17αposition adjacent to Fe=O in 3.11Å. Such an orientation is easy for C17 to undergo hydroxylation and the followed cleaving of C17-20. While for the inhibitor abiraterone, with the common substructure of steroid, occupied the substrate binding area but presented none of positions to Fe=O. The docking results also indicated that both the substrate and the inhibitor formed hydrogen bond with the positive electrostatic residue Arg239, which confirmed the 3D-QSAR speculation. In conclusion, both the ligand-based and structure-based models suggested that the compacted geometry of the active site as well as special distribution of key residues like Arg239 play significant role in metabolic specificity of CYP17. Such a feature in active site drives the natural substrate pregnenolone can adopt a bioactive pose with C17 positioned to Fe=O when binding with the enzyme.

P59 - INTERACTIONS OF INHIBITOR MOLECULES WITH THE HUMAN CYP2E1 ENZYME ACTIVE SITE

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CYP2E1 is an important enzyme oxidizing ethanol as well as several drugs and other xenobiotics in the human liver. The aims of this study were: (1) to determine the inhibition potency of a large set of compounds against human CYP2E1 and to create a new inhibition structure-activity database and (2) to analyse the interactions between these inhibiting compounds and the CYP2E1 active site by molecular docking and 3D-QSAR methods. The IC_{50} values of 47 compounds were determined based on their ability to inhibit oxidation by CYP2E1 of MFC to HFC. Recombinant human CYP2E1 was used as the enzyme source to exclude any interference from other CYP forms. The IC_{50} values for the tested compounds varied from 1.4 mM for γ-undecanolactone to over 46 mM for glycerol. The most important interactions for binding of inhibitors were identified by docking and key features for inhibitors were characterized via the COMFA model. Since the active site of CYP2E1 is flexible, long chain lactones and alkyl alcohols fitted best into the larger open form while the other compounds fitted better in the smaller closed form of the active site. The present docking analysis showed that the active site of CYP2E1 consists of two hydrogen-bonding sites (Thr^{303} and Asn^{206}). The interaction of larger inhibitors with Asn^{206} was important only in the open form of the active site. Most of the lactones, aromatic alcohols and solvents such as DMSO and acetone formed a hydrogen bond with Thr^{303}. The created CoMFA model supported the docking results. The CYP2E1 CoMFA fields showed that hydrogen-bond acceptor/donor interactions are important near the Thr^{303} residue. The sterically favoured and disfavoured regions

provided indications about the optimal volume and shape of a potent inhibitor. In conclusion, a sensitive and rapid assay to measure IC_{50} values of compounds for CYP2E1 was established and inhibition potencies of 47 compounds were determined. The molecular docking and CoMFA analyses revealed the most important interactions of inhibitors with the CYP2E1 active site. The CoMFA model accurately predicted the inhibition potency of a test set of molecules. Thus, the combination of CoMFA and docking analyses is a good approach to rationally design potent and selective inhibitors of CYP2E1.

P60 - IN VIVO EFFECT OF EPILOBIUM HIRSUTUM L. ON PROTEIN AND MRNA EXPRESSIONS OF RAT LIVER VITAMIN D3 METABOLIZING CYP27B1 AND CYP24A1 ENZYMES

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Epilobium hirsutum L. is found in moist waste ground of the Mediterranean region, Europe, Asia, and Africa. It shows analgesic, anti-microbial and anti-proliferative activity, and it is used in our country as an alternative medicine. The pharmacological effect of Epilobium hirsutum L. could be explained by the presence of polyphenolics including steroids, tannins and flavonoids in the aerial parts [1]. CYP27B1 and CYP24A1 are the members of cytochrome P450 super-family and most important enzymes involved in the metabolism of Vitamin D3. CYP27B1 and CYP24A1 are mitochondrial enzymes and also known as 25-hydroxyvitamin D3 1alpha-hydroxylase and 24-hydroxylase, respectively. CYP27B1 involves in 1α-hydroxylation of 25-OH-D3 into 1,25-(OH)2D3 while CYP24A1 involves in 24-hydroxylation of 25-OH-D3 and 1,25-(OH)2D3 which is required for the catabolism of vitamin D3 compounds [2,3]. In this study, in vivo effects of Epilobium hirsutum L. on rat liver CYP27B1 and CYP24A1 mRNA and protein expressions were investigated. To achieve this goal, 18.75mg water extract of Epilobium hirsitum L./kg body weight/day was intraperitoneally injected to male rats for 9 days. Effects of Epilobium hirsitum L. on rat liver mRNA and protein expressions were analyzed by qRT-PCR and western blotting, respectively. Injection of Epilobium hirsitum L. to rats caused 2.5 fold increase in mRNA expression of CYP27B1 with respect to controls and normalized with GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) expression as an internal reference ($p<0.005$). When rats injected with plant extract, 10% decrease in CYP27B1 protein expression was observed, but this change was found statistically insignificant. Epilobium hirsutum L. also caused 31% decrease in CYP24A1 protein expression ($p<0.0001$). The studies on mRNA expression of CYP24A1 are still under investigation. In conclusion, the Vitamin D3 metabolism by CYP27B1 and CYP24A1 may be altered due to the changes in mRNA and protein expressions of these two enzymes by Epilobium hirsitum L. extract.

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P61 - A COMPARISON OF INDUCTION OF CYP2E1 IN THE RABBIT LIVER AND KIDNEY BY ACRYLAMIDE

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Acrylamide (AA) exposure is one of the most important health problems in the world. In addition to industrial and laboratory uses, humans are exposed to varying amounts of AA inadvertently through diet. AA is a neurotoxin, mutagen and carcinogen. Recent studies showed a statistically significant positive association between the dietary intake of AA and ovarian and endometrial cancers in women (1). The epoxidation of acrylamide to its epoxide, glycidamide, by CYP2E1 is mostly attributed to be responsible for mutagenicity and carcinogenicity of acrylamide. A significant induction of CYP2E1, in parallel to N-nitrosodemethylamine (NDMA) metabolism, was shown in liver and kidney of rabbits treated with pyridine, benzene and in also alloxan-induced diabetic rabbits (2-3). CYP2E1 is also a

polymorphic enzyme (4). Administration of acrylamide (100mg/kg s.c, three times a week) to New Zealand male rabbits increased CYP2E1 protein levels significantly by 2-fold not only in the liver but in the kidney of the AA treated rabbits, when analyzed by Western blotting. While AA treated rabbit liver CYP2E1 associated p-nitrophenol hydroxylase and aniline 4-hydroxylase activities showed a significant 3.2-, and 1.8-fold increases, kidney microsomal aniline 4-hydroxylase and p-nitrophenol hydroxylase activities were increased significantly by 1.6-fold over controls. The results of this study demonstrated for the first time that AA treatment induced CYP2E1 protein level and associated enzyme activities not only in liver but also in kidney. In conclusion, increased amounts of CYP2E1 protein and associated enzyme activities in AA-treated rabbits further suggest that acrylamide affects its own metabolism in liver and kidney by influencing the expression levels of CYP2E1, and acrylamide exposure may stimulate not only formation of epoxide glycidamid, but the activation of other carcinogens such as benzene, styrene, pyridine, leading to further increase in toxicity, mutagenicity and carcinogenicity of these chemicals.

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P62 - CYTOCHROME P450 1A1 STRUCTURE AND UTILITY IN PREDICTING DRUG AND XENOBIOTIC METABOLISM

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Cytochrome P450 1A1 (CYP1A1) is an extrahepatic monooxygenase involved in the metabolism of endogenous ligands and drugs, as well as the bioactivation certain toxins and environmental pollutants. This aryl hydrocarbon hydroxylase is particularly well known for its ability to transform polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, a component of tobacco smoke, into carcinogens. CYP1A1 has functional similarities and differences with the closely related human CYP1A2 and CYP1B1 enzymes, but the structural basis remains unclear. Our hypothesis was that structural investigations into CYP1A1 ligand binding, employing both X-ray crystallography and docking studies, could be used to facilitate prediction of both overlapping and differential ligand binding and metabolism among all three human CYP family 1 enzymes. First, since no previous structures of CYP1A1 were known, a structure of a truncated, His-tagged form of the human cytochrome P450 1A1 enzyme was determined with the inhibitor a-naphthoflavone (ANF). This 2.6 Å structure reveals ANF binding within an enclosed active site, with the planar benzochromeneone core packed flat against the I helix, which composes one wall of the active site, and the 2-phenyl substituent oriented toward the catalytic heme iron. Second, comparisons with the previously determined structures of the closely related cytochrome P450 1A2 and 1B1 enzymes suggest differences among the active sites that may underlie the distinct functional capabilities of each of these enzymes. Finally, docking studies were utilized to determine the ability of CYP 1 family enzyme structures for predicting in vitro activity with various substrates.

P63 - THE MAMMALIAN CYP51: FROM MOUSE KNOCKOUT MODELS TO GENOTYPING IN HUMANS

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Lanosterol 14α-demethylase (CYP51) is a key enzyme in the late stage of cholesterol synthesis that is in adult life especially important in the liver. Complete disruption of Cyp51 leads to embryonic lethality in mice (Keber et al., JBC 2011). However, the liver conditional knockouts $Cyp51^{lox/lox}$;Alb-Cre^{+} and $Cyp51^{-/lox}$;Alb-Cre^{+} are viable and normal in outer appearance. 4 % of the male progeny experience life threatening jaundice and severe hepatomegaly between 6 and 9 weeks of age. Proliferation of small bile ducts, singular cases of hepatocyte mitosis and apoptosis are

observed, together with higher LDL-cholesterol and lower HDL-cholesterol and triglycerides compared to liver knockouts without jaundice. Also in the absence of jaundice there is sex-dependent and by age aggravating hepatomegaly and proliferation of cholangiocytes. Immunohistochemisty with anti-cytokeratin indicates proliferation of liver stem (oval) cells which is characteristic for liver regeneration and liver cancer. Omitting cholesterol from the high fat diet leads to further increased liver-to-body weight which is improved with added cholesterol. Sex-specific defects in expression of drug metabolism genes in Cyp51 liver conditional knockouts are under investigation, together with selection of liver patient groups that would be candidates for CYP51 genotyping. Since cholesterol synthesis is essential in embryonal development, we performed Initial CYP51 genotyping on population of 188 Caucasian women who had a spontaneous preterm delivery and 188 unrelated preterm infants born at <37 weeks. Within ten amplicons covering exons, untranslated regions (UTR) and intron-exon borders we identified 22 CYP51 polymorphisms, where 11 are rare novel variants. An T/G transversion in exon 3 causes potentially damaging Y145D substitution in the CYP51 substrate recognition site. Sequencing this amplicon in further 1000 premature infants shows low frequency, suggesting little contribution to preterm delivery. TaqMan genotyping of common variants in larger population is in progress, together with further sequencing of the 5' and 3'-UTRs. Our data indicate that Cyp51 and normal cholesterol biosynthesis are crucial for liver development of the mouse, suggesting that defects in cholesterol synthesis genes would affect also the hepatic drug metabolism. The relation between human CYP51 and liver disease has yet to be established. Despite the high conservation of the CYP51 gene, novel rare variants have been discovered in premature babies and mothers that gave preterm birth. It is possible that these or other CYP51 variants might in future be related to liver pathologies.

P64 - EFFORTS TO IDENTIFY ELECTRON DONORS TO ER LUMEN ORIENTED CYP2W1: A PILOT STUDY

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Cytochrome P450 (CYPs) are responsible for the metabolism of xenobiotics and endogenous compounds. NADPH Cytochrome P450 reductase (POR) is understood to be an important electron donor in CYP catalyzed reactions in the endoplasmic reticulum (ER): it transfers electron(s) from NADPH to the catalytic heme domain of CYPs, mediating dioxygen activation and consequential oxidation of substrates. The CYP2W1 enzyme is expressed in rat fetal colon and in various human tumors especially in colorectal tumors, whereas the expression in adult normal tissue is not detectable [1]. This differential in expression makes CYP2W1 an interesting target for cancer therapy. CYP2W1 has been reported to orient towards the lumen of endoplasmic reticulum, and to be glycosylated in HepG2, HEK293 CYP2W1 transfected cells and in colon tumor tissues, however, its catalytic activity remains in intact cells [2]. We ask the question whether this unique topology still enables CYP2W1 to receive reducing equivalents from POR. This study investigates the electron transfer components to CYP2W1 and the influence on its catalytic activity. The catalytic activity of CYP2W1 can not be seen in isolated microsomes which may be a consequence of its unusual orientation within the ER resulting in insufficient interaction with electron donors. Here we investigate CYP2W1 dependent oxidation using HEK293 cells stably transfected with CYP2W1. Small interfering RNA (siRNA) was used to down regulate cellular POR expression using western blot as verification and ERp29 protein as internal control. Cells were incubated with a CYP2W1-specific substrate and the metabolites were detected by UV-HPLC as described previously [2]. Stably CYP2C8 transfected HEK293 cells were used as a positive control using paclitaxel as a known substrate. Western blot showed that > 80% POR protein expression was successfully knocked down, 5 days after siRNA transfection, in both HEK293 CYP2W1 and CYP2C8 transfected cells. HPLC analysis revealed that in HEK293 transfected CYP2C8 cells there was almost 80% decrease of CYP2C8 activity in siRNA treated cells compared to control cells. However, no reduction of CYP2W1 activity was observed in HEK293 transfected CYP2W1 cells after siRNA treatment. This study suggests that POR is not involved in the electron transfer chain of CYP2W1. Further studies on other electron donors that may contribute to CYP2W1 –mediated oxidation activity are currently under investigation. References: 1. Karlgren M, Gomez A, Stark K, Svard J, Rodriguez-Antona C, Oliw E, Bernal ML, Cajal SRY, Johansson I, Ingelman-Sundberg M: Tumor-specific expression of the novel cytochrome P450 enzyme, CYP2W1. Biochem Biophys Res Commun 2006, 341:451-458. 2. Gomez A, Nekvindova J, Travica S, Lee MY, Johansson I, Edler D, Mkrtchian S, Ingelman-Sundberg M: Colorectal cancer-specific cytochrome P450 2W1: intracellular localization, glycosylation, and catalytic activity. Molecular pharmacology 2010, 78:1004-1011.

P65 - IDENTIFICATION AND FUNCTIONAL STUDIES OF CYTOCHROME P450S IN HUMAN PLATELETS

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Although cytochrome P450s (P450s) have been identified in most human tissues and cells, there is a relative lack of reports in human platelets. The present study screened for P450 expression in human platelets using reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis followed by activity assays using arachidonic acid (ARA) as a substrate. CYP1A1, 2U1, 2J2, 4A11 and 4F2 were expressed as both protein and mRNA in platelets; the previously identified CYP5A1 (thromboxane A2 synthase) was also expressed in the platelet. Ethoxyresorufin-O-deethylase (EROD) activity was significantly decreased in platelets (45%, $P < 0.001$) after treatment with the P450 inhibitor SKF-525A, suggesting that P450s were catalytically active in these cells. In addition, multiple ARA metabolites were detected in Platelets. In summary, multiple P450s were identified in human platelets. These findings suggest the possibility that the P450s may play a role in the metabolism of their substrates in platelets. Since P450s activity and expression levels are largely influenced by environmental factors, variations in the expression levels of P450s in platelets could be one important factor in the variable platelet activations derived from P450-mediated ARA metabolites.

P66 - COMPARISON OF CYP3A4 AND CYP3A5: THE EFFECTS OF CYTOCHROME B5 AND NADPH-CYTOCHROME P450 REDUCTASE ON TESTOSTERONE HYDROXYLATION ACTIVITIES

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CYP3A4 and CYP3A5 require cytochrome b5 (b5) and NADPH-cytochrome P450 oxidoreductase (CPR) in the metabolism, but little is known about differences in the effects of b5 and CPR for the optimal activities for these enzymes. The metabolism of testosterone (TT) by CYP3A4 and CYP3A5 was analyzed by various combinations of b5 and CPR in the fixed P450 amount which was purified from an E. coli expression system. CYP3A4 and CYP3A5 required 4 and 8 fold more of CPR than the P450s, respectively, for the optimal activity. The requirement of b5 for the optimal activity showed the same pattern in both CYP3A4 and CYP3A5, exhibiting a gradual stimulation of the activity reaching a maximum at 8 fold more b5 than P450. Both enzymes exhibited the same pattern of the activity profile in the various combinations. Therefore, stronger activity for CYP3A4 than CYP3A5 appears to be from the CYP3A4 protein itself and not from the different interactions with b5 and CPR. Since the relative amounts of b5 and CPR are important in the maintenance of CYP3A4 and CYP3A5 activities, different levels of these proteins in vitro and in vivo may cause altered metabolism of their substrates or misinterpretation of enzyme properties.

P67 - EFFECT OF AZOLE DRUGS ON ACTIVITY OF OXYSTEROL-7ALPHA-HYDROXYLASE, CYP7B1

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Oxysterol-7α-hydroxylase (CYP7B1) is a cytochrome P450 catalyzing the 6α- or 7α-hydroxylation of steroids and oxysterols. CYP7B1 gene is found in genomes of many organisms [1]. In mammals the highest level of mRNA was detected in brain (hippocampus) and kidney, suggesting important functions of CYP7B1 in these tissues [2]. CYP7B1 is involved in bile acid synthesis, but more importantly, in metabolism of neurosteroids and steroids, which can act as estrogen receptor ligands. Mutations of CYP7B1 gene affect bile acids synthesis and cause neuropathies [3, 4]. In the present work we studied medically important antifungal azoles as potential inhibitors of CYP7B1. These compounds are initially designed as inhibitors of fungal sterol 14α-demethylase (CYP51), but it is well known, that they can inhibit several human steroidogenic P450s. However, their effect on all human steroids metabolizing P450s is not studied. The screening results show that most azoles act as ligands for CYP7B1 in vitro and bind with very high affinity. Taking into account that some of them are able to cross blood-brain barrier (fluconazole and voriconazole) [5], we tested their effect on CYP7B1-dependent hydroxylation of neurosteroid DHEA. We found that voriconazole, miconazole, ketoconazole and clotrimazole significantly decrease the rate of production of 7α-DHEA in reconstituted

system, but fluconazole does not have significant effect on CYP7B1 activity. It was shown that voriconazole may cause some negative effects on the nervous system [6], which can be attributed to the CYP7B1 inhibition in hippocampus. Therefore, obtained results shed new light on the effects of azole therapy and should be considered in combination drug therapy. Effects of other cytochrome P450 inhibitors on CYP7B1-mediated steroid metabolism are under investigation.

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P68 - METABOLIC INVESTIGATION OF URINE AND PLASMA PROFILES OBTAINED FROM ALCOHOL-DOSED MICE USING ACCURATE MASS LC/MS/MS

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Introduction: Extreme alcohol consumption in man is socially disruptive and can lead to significant levels of liver injury. In this study a rodent "intragastric feeding model" was used together with accurate mass LC/MS/MS analysis to determine changes in the global metabolic profiles in both plasma and urine. The resulting raw data obtained was analysed by multivariate statistical analysis (using principal components analysis). Methodology: Blood plasma and urine were collected from two different groups of mice following dosing with either control or ethanol over a period of four weeks. The urine was processed with aqueous dilution and the plasma via protein precipitation. The chromatography was performed on a 2.1 x 100 mm 1.7m C_{18} column was maintained at 50C and eluted with either an acidic or basic aqueous methanol gradient over 15 minutes at 500L/min. The column eluent was monitored by positive and negative ion electrospray TOF MS. Data was collected over the mass range 50 – 1000 m/z using a mass resolution of 20,000 FWHM. The LC/MS data from the biofluid analysis was processed by peak picking and aligning followed by principal components analysis. Preliminary Results: The majority of the urine peaks eluted between 0.25 and 8 minutes the average peak width was in the order of 2.5 seconds at base giving a chromatographic peak capacity in the range of 400 for the system. The high pH separation showed a difference in the order of elution of the peaks compared to the low pH analysis and allowed for the visualization of basic compounds previously eluting the void of the chromatogram. In order to ensure the validity of the data a QC sample was bridge was analysed every 6 samples. The QC was constructed by mixing a small fraction of sample from each of the urine/plasma samples. The LC/MS data was acquired with alternating high and low collision energy TOF MS, allowing for the simultaneous collection of precursor and product ion information. The collected data was processed by peak alignment and section, the resulting peak table was processed by principal components analysis and partial least square discriminate analysis. The resulting statistical data showed a clear separation of the control groups from the dosed groups. The statistical data also showed that there was clear separation from the dosed groups on the different sampling occasions. The statistical separation was observed with both the urine and plasma data. From the data derived statistical data it was possible to identify the LC/MS peaks that contributed most significantly to the clustering of the dosed groups in the PCA analysis. The identity of these diagnostic peaks was elucidated by the use of MS/MS fragmentation pattern, accurate mass of the precursor and comparison of retention time to authentic standard. The analytes identified were mainly from the typtophan (205.0712) metabolic pathway and included kynurenic acid, xanthurenic acid. Other ions identified as markers of ethanol exposure were pimelic acid (159.032) and adipic acid (145.050).

P69 - SELECTING THE MOST RELEVANT NON-RODENT SPECIES FOR NON-CLINICAL ASSESSMENT: A DECISION TOOL COMPARING MINIPIG, DOG AND MONKEY TO MAN

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The poster provides a tool facilitating appropriate species selection in toxicity testing of drug candidates (small molecules). In the published literature comparisons focus on the undisputed importance of CYP enzymes, although phase II metabolism also has been covered. The current poster suggests including a wider range of ADME and PK/MTD information in the decision making process and summarizes this in a decision chart. The choice of the non-rodent species required for the non-clinical safety evaluation is sometimes difficult, with large interspecies differences in susceptibility to toxicity of drugs and other xenobiotics. Monkey, pig and dog are the candidates available with sufficient background data to be used in toxicology. Traditionally, the dog has been used the most, because of easy handling and availability of small breeds like the Beagle dog, but non-human primates and minipigs are increasingly used. However, here it is advocated that the choice should be based on a scientific rationale. This includes determining key ADME/PK properties and minimizing stress and pain for the animals during a study. If the drug candidate is metabolised by aldehyde oxidase (AOX1), N-acetyltransferases (NAT1 and NAT2) or CYP2C9, which are non-existent or very low in dogs, but present in pigs, the latter should be chosen based on in vitro studies with cell based systems expressing human enzymes and transporters. Results from in vivo studies like MTD and PK studies must also be included in the decision making process.

P70 - HYPERVARIABLE CYTOCHROME P450 ACTIVITY IN CHILDREN WITH SEVERE HEPATIC DYSFUNCTION

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Liver disease has been shown to influence the hepatic biotransformation in adults. In children, in which liver disease (often) has a different aetiology, few to no data are available. The impact of hepatic failure on the activity and abundance of the main CYP450 isoforms is important for predicting the need for dosage adjustments when a (new) drug is used in a child with liver disease. This study describes the characterization of liver tissue samples obtained from the explanted liver of children undergoing liver transplantation for various indications. Thirty-one patients were included in the study, twenty-four of those patients were diagnosed with biliary atresia, the most common indication of liver transplantation in (young) children. Besides the preparation of microsomes for activity determinations, DNA was extracted from the tissue. Furthermore, the patient files were consulted for the relevant pre-operative history (age, weight, medication,..). The in vitro activity of the 6 most important CYP isoforms was determined in the microsomes by the incubation with phenacetin (1A2), tolbutamide (2C6), S-mephenytoin (2C19), dextromethorphan (2D6), chlorzoxazone (2E1), and midazolam (3A4), and subsequent quantification of the metabolites with UPLC-MS/MS. Furthermore, the abundance of CYP2E1 and CYP3A4 in the microsomes was determined with an indirect ELISA. The calculated activities were compared with an average adult activity (determined in commercially available pooled microsomes). For CYP3A4, 2E1 and 1A2, an activity below 25% of the adult activity was observed in 68%, 52% and 71% of the paediatric microsomes, respectively. In about 80% of the microsomes, the activity of these 3 isoforms was below 55% of the adult activity. For CYP2C19 and 2D6, 55% of the microsomes showed an activity below 55% of the adult activity. In contrast, in 45% of the samples, the CYP2C9 activity was 115% of the adult activity or higher. The abundance and activity of CYP3A4 and 2E1 were positively correlated. The presented results suggest a differential influence on the six most important CYP isoforms of parameters such as pathology and age. Work is ongoing to partition the (hyper)variability in terms of pathology, age, genotype, and pre-operative co-medication.

P71 - NOVEL CYP 3A KNOCKOUT CHIMERIC MOUSE WITH HUMANIZED LIVER AND METABOLIC PROFILING OF NEFAZODONE FOR ACCURATE PRE-CLINICAL HUMAN PREDICTION

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[Purpose] Chimeric mice with humanized livers (PXB® mice) are a useful animal model for predicting human pharmacokinetics. However, drug-metabolizing enzymes such as mouse cytochrome P450 3a (m-Cyp3a) in m-hepatocytes and intestine of the PXB mice might affect the accuracy of these predictions. To address this issue, we developed a Cyp3a knockout (KO) chimeric mouse model with humanized liver, and evaluated this new model for human pharmacokinetic prediction using nefazodone (NEF). [Methods] Albumin enhancer/promotor-driven urokinase plasminogen activator transgenic (uPA) /SCID mice were crossed with Cyp3a KO mice to obtain Cyp3a KO/uPA/SCID mice. h-Hepatocytes were transplanted into Cyp3a KO/uPA/SCID mice, and h-albumin (h-alb) levels in mouse blood were monitored. The mRNA levels of h-cytochrome P450s (h-CYPs) and m-Cyps were determined in the liver and the intestine. NEF was orally administered to SCID mice, PXB mice, Cyp3a KO/uPA/SCID host mice (without h-hepatocyte transplants) and Cyp3a KO chimeric mice, and plasma and urine samples were collected. The samples were analyzed using LC-MS and the metabolic profile in the plasma and urine was investigated. [Result and Discussion] Similar to PXB mice, the h-alb levels in Cyp3aKO/uPA/SCID m-blood logarithmically increased until approximately 60 days after h-hepatocyte transplant. Quantitative real time RT-PCR analysis showed that hepatic h-CYP expression levels in Cyp3a KO chimeric mice were similar to those in PXB mice and confirmed the absence of Cyp3a11 expression in m-liver and intestine tissues. Probably due to the knockout of m-Cyp3a, the hepatic Cyp2b10 and 2c55 levels in Cyp3a KO/uPA/SCID host mice were respectively 8- and 61-times more up-regulated than in PXB mice, but were attenuated by the h-hepatocyte transplantation to comparable levels of Cyp2b10 and 13-times higher levels of Cyp2c55. Intestinal Cyp2b10 and 2c55 were also repressed by the h-hepatocyte transplant in Cyp3a KO/uPA/SCID mice. Among the mice tested in the current study, the human metabolite profile of NEF was most accurately expressed in Cyp3a KO chimeric mice. The in vivo metabolite profiles and pharmacokinetics of NEF in PXB and SCID mice were similar. The results indicate that the mouse Cyp3a in the liver and/or intestine significantly contributed to the metabolism of NEF in the PXB mice. The results show that the suppression of the Cyp3a activities changed the metabolic profile of NEF from mice to humans. [Conclusions] We have developed a Cyp3a KO chimeric mouse. The present data suggests that Cyp3a KO chimeric mouse with humanized liver can be expected to be a more accurate human pharmacokinetic model and a useful tool in the drug discovery and development.

P72 - SEX DIFFERENCES IN PROPOFOL BIOTRANSFORMATION, CLINICAL IMPLICATIONS

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With its rapid onset, relatively short emergence time and favorable safety profile, propofol (2,6-diisopropylphenol) has become a common and widely used intravenous sedative agent for induction and maintenance of anesthesia. However, extensive inter-individual variability in pharmacokinetic and pharmacodynamic parameters has been reported. In particular, women emerge faster from propofol anesthesia and have a higher level of consciousness at equal targeted plasma concentrations during surgery, compared to men; an effect that was hypothesized to be related to the gender differences in propofol metabolism. In the current study we determined the associations between sex, age, CYP2B6 and UGT1A9 polymorphisms with dose-, BMI-adjusted plasma area under the time curves (AUC) for propofol, its main metabolites propofol glucuronide (PG), 4-hydroxypropofol (OHP), and its subsequent glucuronide metabolites (Q1G, Q4G) in 98 patients ASA I-II scheduled for minor orthopedic (hand or foot) surgery treated with continuous propofol infusion. Plasma levels of propofol, PG, OHP, Q1G, and Q4G were determined by HPLC with fluorescence detection. CYP2B6 *4, *5, *8, *9, *14, *18 alleles and UGT1A9*3 (M33T), -275T>A and -2152C>T SNPs were analyzed by real-time polymerase chain reaction with the 5'-nuclease allelic discrimination assays. Significantly higher AUC of PG (1.5-fold, P=0.007), Q1G (2.7-fold, P<0.0001), Q4G (2.6-fold, P<0.0001) and OHP (3.7-fold, P=0.02) were found in women than men at pseudo-steady state ($AUC_{0\text{-}ss}$). To discriminate between

the observed sex-difference in metabolism of propofol and OHP due to glucuronidation or hydroxylation pathways, the dose-, BMI-adjusted $AUC_{0\text{-}ss}$ of OHP and its subsequent metabolites Q1G and Q4G were combined to better represent CYP2B6 key enzyme activity. A 1.9-higher median of combined OHP, Q1G, Q4G $AUC_{0\text{-}ss}$ was detected in women than men (P=0.0002). No significant impact of CYP2B6 or UGT1A9 polymorphisms on propofol biotransformation was observed. The data illustrate that propofol metabolism occurs at a higher rate in women, compared to men and may constitute a plausible factor that contributes to the variability in propofol disposition as well as in its pharmacological effect. The difference may be due to the regulation of propofol metabolism by sex hormones. Our finding contributes to a better understanding of the gender differences in propofol action and may thus explain to a great extent the previously described sex differences in the effect of propofol anesthesia.

P73 - THE INABILITY OF BARBER'S POLE WORM (HAEMONCHUS CONTORTUS) TO METABOLIZE IVERMECTIN

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Barber's pole worm (Haemonchus contortus) is very common gastrointestinal nematode and one of the most pathogenic parasites of ruminants (e.g. sheep and goat). The control of haemonchosis has been and still is based on the use of anthelmintics; it is usually treated with broad spectrum anthelmintics such as benzimidazoles, macrocyclic lactones, and salicylanilides. However, their massive and indiscriminate use has led to the frequent resistance of H. contortus to the main anthelmintics. Helminths' biotransformation enzymes metabolizing anthelmintic drugs may decrease drug efficacy and contribute to drug-resistance development. Therefore, the aim of our study was 1) to find and identify phase I and II metabolites of the anthelmintic drug ivermectin (IVM) formed by the Barber's pole worm and 2) to compare IVM metabolites in helminths with IVM biotransformation in sheep (Ovis aries) as host species. During in vitro incubations, microsomes-like fractions (prepared from homogenates of adult worms or from ovine liver homogenate) were incubated with 100 µM IVM and primary culture of ovine hepatocytes was incubated with 10 µM IVM. Liquid/liquid extraction was used for samples during in vitro experiments. In the ex vivo study, living H. contortus adults were incubated in RPMI-1640 medium in the presence of 1 µM IVM for 24 h. After incubation, the worms were removed from the medium and homogenized. Homogenates of worms and medium from the incubation of worms were separately extracted using solid-phase extraction. Ultra high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) was used for determination of parent drug and its metabolites. The results showed that H. contortus enzymatic system is not able to metabolize IVM. On the other hand, 16 different IVM metabolites both Phase I and II were detected in ovine samples using UHPLC/MS/MS analyses. In ovine hepatocytes, 9 different phase II metabolites of IVM were found; eight conjugates with sulphuric acid and one with glucuronic acid. We can conclude that H. contortus, as the member of roundworm group of parasites, is not able to deactivate IVM, and that its biotransformation enzymes do not contribute to IVM-resistance.

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P74 - DETERMINANTS OF CYP3A4 EXPRESSION AND METABOLIC ACTIVITY IN THE HUH7 HUMAN HEPATOMA CELL MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease affecting approximately one third of adults. For a majority of NAFLD patients, the associated metabolic syndrome necessitates drug therapy for management of diabetes, dyslipidemia and hypertension. Interestingly, while many drugs used by patients with NAFLD are eliminated from the body by hepatic drug metabolism, little is known regarding the effect of steatosis on hepatic drug metabolism and drug response. We examined Cytochrome P450 (CYP) 3A4 metabolic activity in patients with NAFLD and healthy subjects. After oral administration of midazolam, plasma drug levels were higher in NAFLD than healthy controls. On average, CYP3A4 mRNA expression was lower in liver biopsies from NAFLD patients than livers of healthy donors. In addition, hepatic CYP3A4 mRNA levels were lower in patients with more severe non-alcoholic steatohepatitis than those with simple steatosis. To examine the molecular mechanisms involved, cultured Huh7 human hepatoma cells were exposed with fatty acids to induce steatosis. CYP3A4 expression, midazolam

metabolism, as well as, lower CYP3A4 promoter luciferase reporter activity was observed in fatty acid-loaded cells. Moreover, in the high fat diet model of NAFLD, C57BL/6 mice had reduced hepatic CYP3A4 luciferase reporter activity after delivery by hydrodynamic tail-vein DNA injection technique. Levels of circulating Fibroblast Growth Factor 21 (FGF21), a hormone known to regulate lipid and glucose homeostasis, are known to be elevated in NAFLD. We found induction of FGF21 expression in steatotic Huh7 cells and decreased CYP3A4 expression in FGF21 treated Huh7 cells. We conclude that the activity of CYP3A4 is reduced in NAFLD due to steatosis-induced changes in gene transcription. These findings are expected to provide the basis for further studies aimed at optimizing pharmacotherapy in NAFLD. A role for FGF21 in regulating CYP3A4 in NAFLD requires further study.

P75 - COMPARISON OF GLIBENCLAMIDE METABOLISM IN HUMAN AND MOUSE LIVER MICROSOMES

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Objective: Glibenclamide (5-chloro-N-(4-[N-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide) is a second generation sulfonylurea drug used for the treatment of diabetes. Diabetes mellitus inflicts millions of people worldwide. The advantage of Glibenclamide over insulin includes long shelf life, administration and low cost. The objective of the present study is to (a) determine the resemblances, differences in the metabolism of glibenclamide in human and mouse and (b) to identify and quantify the recently reported ring loss metabolite in both the species. **Results:** Glibenclamide was incubated with human and mouse live microsomes for thirty minutes. Hydroxylated metabolites of glibenclamide were identified in both human and mouse. Metabolite due to the loss of cyclohexyl ring is also identified in both the species. In a recent report we have characterized the structure of newly identified metabolite due to the loss of cyclohexyl ring. Results obtained from LC-UV and LC-MS/MS suggests the differences in the biotransformation of glibenclamide in human and mouse. LC-UV-MS/MS data clearly suggests that the quantity of metabolites formed in both the species are dissimilar. Drug metabolite ratio is also different in both the species. In human liver, cyclohexyl hydroxylated metabolites are the predominantly formed metabolites followed by parent, ethyl hydroxylated metabolite and metabolite due to the loss of cyclohexyl ring. In mouse liver, parent is predominantly found followed by ethyl hydroxylated metabolite, ring loss metabolite and cyclohexyl hydroxylated metabolites. **Conclusions:** Glibenclamide is extensively metabolized in human where cyclohexyl hydroxylated metabolites are predominantly found with respect to parent and other metabolites. Total quantity of metabolites formed by human liver is twice that of the mouse. This suggests that different types of enzymes or the same enzymes with distinct activity in mouse and human could be the reason for the dissimilarities.

P76 - COMPARATIVE METABOLISM OF AILDENAFIL IN MOUSE, RAT, DOG AND HUMAN LIVER MICROSOMES

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Aildenafil, 1-{[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4,3-d] primidin-5-yl)-4-ethoxyphenyl] sulfonyl}-cis-3, 5-dimethylpiperazine, as a pyrrolopyrimidinone analogue of sildenafil and verdenafil, is a potent inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V by degrading of cGMP in the corpus cavernosum, and its longer duration of action than sildenafil in the treatment of male erectile dysfunction has been demonstrated in clinical trials[1]. To characterize and compare the metabolic pathways of aildenafil across species, the metabolism of aildenafil in mouse, rat, dog and human liver microsomes was studies by liquid chromatography tandem mass spectrometry (LC/MS/MS). The fragmentation pathways of aildenafil, the full-scan MS and MS/MS data were used to identify the metabolite structures. The metabolic profile of aildenafil in vitro were loss of a two-carbon fragment from the piperazine ring (N,N'-deethylation), the introduction of one oxygen atoms to piperazine ring with dehydrogenation, aliphatic side chain hydroxylation, mono-oxidation of the piperazine ring, pyrazole N-demethylation, O-deethylation, and no glucuronidated metabolites were observed. The similar metabolic pathways were found in mouse, rat and human liver microsomes, but the rate of metabolitse formation in mouse and rat liver microsomes were significantly different with the result in human liver microsomes. The aliphatic hydroxylation has not been found in dog liver microsomes, and the rate of formation to mono-oxidation of the piperazine ring in dog liver microsomes was also slower than other liver microsomes. The major metabolic pathway was N,N'-deethylation of piperazine ring in mouse, rat and dog liver microsomes, but was mono-oxidation of piperzing ring in human liver microsomes. Consequently, we have to limit the application of extrapolating the metabolic data of these non-clinical species model to humans before exhaustive metabolic phenotype researches.

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P77 - DISPOSITION OF FLUOXETINE IN NEWBORN LAMBS UP TO 1 YEAR OF AGE

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Disposition of Fluoxetine in Newborn Lambs up to 1 Year of Age *Timothy Chow1, Tuan-Ahn Nguyen2, Dr. Dan Rurak2, Dr. Wayne Riggs1 1 Ð Faculty of Pharmaceutical Sciences, UBC, Vancouver, BC. 2 Ð Faculty of Medicine, UBC, Vancouver, BC. The disposition of many drugs in infant life is not well studied. Drug therapy is commonly used in newborns and neonates, but drug administered to the mother during the perinatal period and passively transferred to the fetus in utero constitutes a major route of drug exposure in newborns. In utero the fetus has maternal routes for drug disposition, however newborns must rely on their own immature mechanisms. A thorough understanding of the ontogeny of drug disposition is essential to determine the development of these clearance mechanisms from birth to adult life with the goal of providing clinicians with guidelines for optimal drug dosing. In this study, sheep are used as the animal model to determine the disposition of the commonly used antidepressant fluoxetine at ~ 3 and 10 days, and 1, 3, 6, and 12 months of age. Under isoflurane anesthesia and aseptic surgical techniques, heparin bonded polyurethane catheters were implanted in a carotid artery and jugular vein in lambs at 2 days of age. Racemic fluoxetine (1mg/kg) was administered as an IV bolus injection and arterial blood samples (2ml) were collected at 5, 15, 30, 45, 60 min., and 2, 4, 6, 9, 12, 24, 36, 48, 60, and 72 hours; urine was quantitatively collected up to 72 hours. Plasma and urine samples were stored at -80oC until analysis with a validated sensitive and stereoselective LC/MS/MS assay.1 Pharmacokinetic (PK) parameters estimated include area under the curve (AUC), elimination half-life (t1/2), clearance (Cl), volume of distribution (Vd), and mean residence time (MRT). The plasma protein binding of fluoxetine and its metabolite norfluoxetine was studied by equilibrium dialysis using cellulose dialysis membrane (10kDa molecular weight cutoff) in equilibrium cells. Further, the level of alpha-acid glycoprotein, the predominant plasma protein bound by this drug, was quantified using an immunoassay. Based on plasma PK data, a possible gender difference in the disposition of fluoxetine may exist at the 3 days age group. The lambsÕ metabolic capacity for fluoxetine did not mature significantly from 3 days to 1 month. At 3 months of age, there was significant maturation in the lambsÕ metabolic capacity for fluoxetine, however there were no significant changes from 3 months to 1 year. The plasma protein binding of fluoxetine and norfluoxetine was stereoselective at all age groups, and slightly lower at younger ages. 1. Chow TW et al. A Validated Enantioselective Assay for the Simultaneous Quantitation of (R)-, (S)-fluoxetine and (R)-, (S)-norfluoxetine in Ovine Plasma Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). J. Chromatogr. B 879 (2011) 349.

P78 - SPECIES-SPECIFIC IN VITRO GLUCURONIDATION OF THE MYCOTOXIN DEOXYNIVALENOL

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Deoxynivalenol (3a,7a,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, DON; MW: 296.32 g/mol) is a trichothecene mycotoxin commonly produced by field fungi including some Fusarium strains. The toxin, also called vomitoxin, occurs frequently at considerably high concentrations in cereals and grains, especially in wheat, barley and maize (1). It is regarded as an undesirable substance in food and feed, and regulatory limits for food and feed contents as well as a temporary tolerable daily intake (tTDI) have been established by food authorities (2). DON is a very stable compound resisting storage and food processing. Chronic exposure to DON is connected to growth retardation and impairment of the immune system whereas acute toxicity is commonly associated with vomiting, diarrhoea and potentially death by circulatory shock (3). Naturally contaminated grains contain in addition to DON the plant detoxification products 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside, which can become at least partly bioavailable after ingestion by hydrolytical cleavage in the gastrointestinal tract. Intestinal and ruminal microbial activity has a major role in DON-detoxification transforming the toxin into the non-toxic de-epoxy-DON (DOM-1). Therefore, the observed variability in sensitivity to DON intake among several species is partly a consequence of their microbiological profiles. Furthermore, there are considerable species differences in DON toxicokinetics and biotransformation. Conjugation to glucuronides and elimination via the urine appears to be the major metabolism pathway although with differing efficiency. DON, DON-glucuronide, DON-sulphate, DOM-1, and DOM-1-glucuronide

have been found in ruminant urine but the metabolites have not been further characterised (4). Furthermore, the occurrence of DON-glucuronide in human urine is used as a biomarker for DON-exposure although the mechanistics of DON-glucuronidation are not yet known (5). The objective of the present study was therefore to investigate DON-glucuronidation in more detail in a number of species. Assays for in vitro metabolism using liver microsomes supplemented with UDP-glucuronic acid and detergent were established and optimised. DON-glucuronides were separated by hydrophilic interaction chromatography (HILIC) and analysed on a linear ion trap mass spectrometer. DON and three different DON-glucuronides were baseline-separated showing that DON can be conjugated at all theoretically possible positions, i.e. C-3, C-7 and C-15, in vitro. The data also revealed different DON-glucuronidation patterns in the examined species. The individual glucuronides are currently produced in larger quantities to allow their purification and chemical characterization.

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P79 - VARIATIONS IN HUMAN DRUG AND STEROID METABOLISM CAUSED BY MUTATIONS IN NADPH P450 OXIDOREDUCTASE

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Mutations in P450 oxidoreductase (POR, CPR, CYPOR) cause a range of human diseases. Since the initial observation of mutations in POR affecting activities of CYP17A1 and causing disruption of steroid metabolism, more than 60 variations in POR gene in humans have been identified. Most of the POR variants have been tested to support the 17-hydroxylase and 17,20 lyase activities of CYP17A1 (1-3) but recently activities of some other steroidogenic P450s (CYP19A1 and CYP21A2) as well drug metabolizing P450s (CYP3A4 and CYP2D6) and other partner proteins of POR like cyt b5 and heme oxygenase have been reported by us and other laboratories (4-9). Some general conclusions can be drawn about POR mutations directly affecting cofactor (FMN, FAD and NADPH) binding. However, here are differences in effects of POR variants on different redox partners. We have sought to examine the biochemical and clinical changes caused by specific POR variants by correlation of enzymatic, protein-protein interaction and clinical data. A detailed analysis of new POR variants causing changes in interaction with individual P450s and other redox partners and specific effects on activities as well as role of other reductases on metabolism in humans will be presented.

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P80 - ORAL ABSORPTION AND EXCRETION OF ICARITIN, AN AGLYCONE AND ALSO ACTIVE METABOLITE OF PRENYLFLAVONOIDS FROM THE CHINESE MEDICINE HERBA EPIMEDII, IN RATS

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Icaritin (ICT) is a main aglycone and also active intestinal metabolite of prenylflavonoids from the Chinese medicine Herba Epimedii. In the present study, the oral absorption and excretion of this compound was investigated using rats for exploring its fate in the body, so as to better understanding its in vivo pharmacological activities. The free (parent) and total (parent plus conjugated metabolites) ICT concentrations in rat plasma, urine and bile, after intravenous (i.v.) and oral administration both at 5 mg/kg, were determined before and after enzymatic hydrolysis with β-glucuronidase/sulphatase, respectively, by a HPLC-UV method. The results showed that free ICT plasma concentration after i.v. dose was rapidly decreased with average $t_{1/2}$ of 0.43 h, while the total ICT concentration was decreased slowly with $t_{1/2}$ of 6.86 h. The area under the curve of ICT conjugated metabolites was about 11-fold higher than that of free ICT. The majority of ICT in the body was excreted from the bile with 68.05% of dose over 8 h after i.v. dosing, in which only 0.15% was in parent form. While very little amount of ICT was excreted from the urine with 3.01% of dose over 24 h, in which the parent form was 0.62%. After oral administration, very little amount of parent ICT was detected only in 0.5, 1 or 2 h plasma samples with the concentration less than LOQ, however, its total plasma concentration after enzymatic hydrolysis treatment was at relative high level with average maximum concentration of 0.49 mg/ml achieved at 1 h post dose. The oral bioavailability of ICT was 35% of dose, estimated by its total plasma drug concentrations. It is concluded that ICT can be easily absorbed into the body, and then rapidly converse to its conjugated metabolites, and finally removed from the body mainly by biliary excretion.

P81 - RAPID QUANTITATIVE METHOD DEVELOPMENT FOR DRUG METABOLITES (DMS) IN HUMAN PLASMA USING ISOTOPE DILUTION

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Quantitative analysis of DMs can be accomplished by: 1) Synthesis of authentic standards of the DMs or by 2) Synthesis of [^{14}C]drug and generating DMs with the same specific activity in vitro. In the second approach, stock solutions calibrated in this manner can then be used as standards to construct standard curves for analysis of unknowns by MS. The second approach has many drawbacks that are addressed if an additional step is included, the topic herein, by obtaining [$^{13}C_x$]drug and generating DMs with the same label in vitro. The materials generated allow for stable-isotope dilution mass spectrometry to be employed in the analysis of DMs. The key concept of this paper is to demonstrate the enabling advantages of SID-MS for this particular application: Isotope dilution is a I) definitive method with precision, accuracy and precision of definable uncertainty values, the only assumption is that equilibrium is achieved at initial addition of the stable isotope labeled analyte, II) the only measurement is the isotope ratio, which also defines the accuracy of the method, and III) loss of analyte by breakdown, or loss from sample (recovery, surface adsorption), or typical error sources from mass spectrometry variables (matrix effects), does not alter this ratio measurement. All of these characteristics make the overall method very simple and rugged relative to non-isotope dilution MS methods (Guidelines for achieving high accuracy in isotope dilution mass spectrometry, IDMS. Eds: M. Sargent, C. Harrington, R. Harte. The Royal Society of Chemistry, Cambridge, 2002). Testosterone (T) and diclofenac (D) were chosen for mass defect characteristics, with T representing an endogenous molecule with a natural product-like empirical formula (mass defect) and D representing a drug with a clearly atypical natural product empirical formula (mass defect). [^{14}C]T, [$^{13}C_3$]T, [^{14}C]D and [$^{13}C_6$]D, obtained from

commercial sources, were metabolized separately in vitro to produce test metabolites. LC-RAM analysis was used to determine the concentration of the test metabolites in the terminal incubate. Dilutions of the incubates containing 6β-hydroxy-T, [$^{13}C_3$]6β-hydroxy-T, 4′-hydroxy-D and [$^{13}C_6$]4′-hydroxy-D were used to make standard curves. Plasma samples were prepared by "dilute-and-shoot" and analyzed by LC-MS using SCIEX 4000 and Thermo Orbitrap instrumentation. It was assumed that the amounts of [$^{13}C_3$]6β-hydroxy-T and 2 µM [$^{13}C_6$]4′-hydroxy-D produced were similar (the absolute amount added to each sample in unimportant). Dilutions and standard curves were prepared in human plasma. Analysis of the DMs by SID-MS using a Thermo LTQ Orbitrap exhibited the characteristic specificity and reproducibility expected for SID methods. Not unexpectedly, quantitative analysis by HRMS was simpler than analysis by tandem quadrupole MS.

P82 - SELECTION OF DRUG CANDIDATES USING RAPID PK SCREENING

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Nowadays, the inclusion of high throughput screening methods for absorption, distribution, metabolism and elimination is a golden standard in most lead optimization programs. The addition of these assays has greatly facilitated early elimination of compounds with poor pharmacokinetic properties and low bioavailability. However, despite advantages in in vitro technologies and in silico approaches, in vitro to in vivo extrapolation and predictive power of these approaches for in vivo pharmacokinetic properties is not always reliable and accurate. In vivo PK studies are critical to ensure compounds have appropriate pharmacokinetic properties to be evaluated in preclinical pharmacology and safety studies. Traditionally they are still conducted in a low throughput manner and are therefore often bottlenecks in drug discovery projects in many pharmaceutical companies. To overcome this, we implemented rapid PK screening approaches to be used in the drug discovery programs of pharmaceutical companies. Several study designs in rat and dog were developed, which typically include both IV and PO arms or just one PO arm and 2 animals per arm. Compounds are dosed single, or cassette dosing can be used, in which 3-6 compounds are dosed simultaneously. Blood is sampled at 8 timepoints for dogs and at 6 timepoints for rodents. In case of intravenous dosing in rodents, the site of dosing is different compared to the site of blood sampling (e.g. dosing via femoral vein and sampling via tail vein). The rapid screening PK approach has several integrated components, including standardized formulations and protocols for dosing and sampling; sample pooling across rodents to reduce number of samples; generic LC/MS/MS method with automated tuning; automated plasma sample preparation using a Hamilton Microlab star and Tomtec Quadra 4 SPE; non-compartmental PK evaluation using WinNonlin; and reports generated using Excel. A typical work flow has a turn-around time of less than 2 weeks, from compound receipt to report availability. With this approach over more than 1000 compounds were screened last year for the pharmaceutical industry. Approximately 300 compounds were screened in a rapid screening PK assay in rats, with one PO arm only. Based on set cut-off values for the AUC, about ~30% of the screened compounds were eliminated due to too low AUC values, implying low bioavailability. The rapid PK screening assay therefore proves to be a very efficient deselection tool in pre-selection and prioritization of drug candidates for the pharmaceutical industry.

P83 - A NOVEL APPROACH TO THE STORAGE AND RETRIEVAL OF INFORMATION IN XENOBIOTIC METABOLISM STUDIES TO IMPROVE DECISION MAKING AND KNOWLEDGE SHARING BETWEEN SCIENTISTS, FACILITIES AND LOCATIONS

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Current informatics approaches for xenobiotic studies limit the progress of scientists due to the restrictions of predefined software workflows and the difficulty on passing on one's knowledge to peers. The ability to share discoveries and knowledge on the biotransformations of NCE's/classes for universal use in an organization and to feed previously discovered information into today's experiments holds the potential to unlock the potential of historical data – the pharmaceutical industries most important asset. The approach defined in this paper is a system that uses the latest generation hardware and informatics to accurately screen samples, confidently interrogate datasets and efficiently disseminate data in a highly flexible format. This system allows users to elucidate metabolic pathways, store, access and interrogate each other's data and benefit from previous experience, bringing an encyclopedia of knowledge from the organization's best scientists to bear on the pertinent challenges. The samples were analyzed on accurate mass MS platforms equipped with sub 2uM LC systems. Metabolite analysis of several

compounds was performed using a prototype version of a scientific information system software. The software contains a scientific library function which is used to enter and retrieve information about the compounds being analyzed and is used as an interface to disseminate information. The samples were automatically processed to provide a comprehensive list of metabolites which can then be filtered or interrogated to suit both the analytical and reporting needs of the user. Metabolite data was analyzed using novel peak picking algorithms with advanced multicore processing capabilities. Processing takes advantage of built in scientific library functionality which manages both compound and metabolite identification specific criteria and allows users to define and generate metabolite relationships within a single LCMS software package. Users are able to both build and retrieve metabolite relationship information across datasets and samples that were historically difficult to manage as a single report. Integration of in silico tools, such as; metabolite prediction, isotopic pattern analysis and intelligent mass defect filtering will be discussed. We will also demonstrate how drug metabolism departments will have the ability to manage instrument and server assets remotely across the network. This network based infrastructure also facilities scientifically sharing and disseminating data and knowledge across labs and across networks. DMPK datasets are processed for both generating qualitative structural and relationship pathways as well as for quantitative analysis and putting the datasets into meaningful context within a single, integrated processing and reporting environment.

P84 - THE INFLUENCE OF DACARBAZINE ON HEMATOLOGICAL AND HISTOLOGICAL CHANGES IN HAMSTERS WITH FIBROSARCOMA

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Introduction: Dacarbazine is widely used cytostatic drug in monotherapy or adjuvant therapy of soft tissue sarcomas. It works by methylation of DNA and inhibition of synthesis of RNA, purines and proteins. Side effects of dacarbazine therapy include gastrointestinal, liver and kidney problems, myelosuppression and pneumonia. **Objective:** The aim of our experiment was to study acute hematologic and histological changes caused by dacarbazine on hamsters with previously induced fibrosarcoma. **Materials and methods**: In experiment we used Golden Syrian hamsters. Animals are divided in one control and four experimental groups. On the 1st day of experiment all of animals were given trypsinized cells from Baby Hamster Kidney (BHK) culture subcutaneously, which are tumorogenes for Syrian hamsters. On the 14th day animals in experimental groups had received different doses of dacarbazine by intraperitoneal injection: 1.200, 1.400, 1.600 and 2.000 mg/m^2. Seven days later all hamsters were sacrificed by urethane anesthesia. Blood samples from hamsters were collected for further analysis. We used Neubauer's chamber and appropriate reagents (Türk, Hayem and Dacie) in order to count leukocytes, erythrocytes and thrombocytes. Blood samples were also stained with brilliant cresyl blue and Giemsa in order to count reticulocytes and to differ leukocytes. Their heart, lung, liver, kidney, small intestine, spleen and tumor samples were proceeded. Tissue samples were fixed in buffered formalin, dehydrated with ethanol and xylene, embedded in paraffin, cut on microtome and stained with H&E. **Results**: Induced fibrosarcoma per se caused anaemia, thrombocytopenia and contrary to previously, leukocytosis, which was mainly based on neutrophilia and monocytosis. Experimental groups had shown anaemia, thrombocytopenia and leukopenia, which was basically only lymphocytopenia, but all other leukocytes were close to lower referent level. The numbers of all blood cell types were lowered in proportion to dacarbazine dose. Reticulocytes had vanished from blood when maximum dose of dacarbazine had been used. Dacarbazine caused mild hepatitis: parenchymal degeneration of hepatocytes with portal mixed inflammatory infiltrates and foci of inflammatory cells inside liver lobules. There were no significant morphological changes on other proceeded organs. Tumor samples showed enlargement of nucleus, pale nucleoplasma and lowered number of enlarged nucleoli. Number of mitosis in tumor was lowered in proportion to dacarbazine dose. **Conclusion**: Dacarbazine has powerful general myelosuppressive effect, but lymphocytes and erythrocytes are mostly affected. The single dose of 2.000 mg/m^2 induces complete suppression of red bloodline in hamsters. Mild hepatitis and changes in tumor cells were main morphological features of acute organotoxic and tumorotoxic effect of dacarbazine single dose.

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P85 - ANALYSIS OF THE REPAGLINIDE CONCENTRATION INCREASE BY GEMFIBROZIL AND ITRACONAZOLE BASED ON THE INHIBITION OF HEPATIC UPTAKE TRANSPORTER AND METABOLIC ENZYMES

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Drug-drug interactions at metabolic enzymes have long been investigated, including rigorous attempts at quantitative predictions of resulting changes in drug concentrations. Recent advances in the role of transporters in pharmacokinetics have suggested that drug-drug interactions at transporters could also affect the drug disposition. As an example, the area under the plasma concentration-time curve of repaglinide is reported to increase 1.4-fold by co-administration of itraconazole, 8.1-fold by gemfibrozil, and 19.4-fold by both. The present study analyzed this interaction based on a physiologically based pharmacokinetic (PBPK) model incorporating the inhibition of hepatic uptake transporter and metabolic enzymes involved in repaglinide disposition. First, the plasma concentration profiles of inhibitors (itraconazole, gemfibrozil and gemfibrozil glucuronide) were reproduced by a PBPK model to obtain their pharmacokinetic parameters. The plasma concentration profiles of repaglinide were then analyzed by a PBPK model, together with those of the inhibitors, assuming a competitive inhibition of CYP3A4 by itraconazole, mechanism-based inhibition of CYP2C8 by gemfibrozil glucuronide, and inhibition of organic anion transporting polypeptide (OATP) 1B1 by all 3 compounds. The results suggested that the reported concentration increase of repaglinide, suggestive of the synergistic effects of co-administered inhibitors, can be quantitatively explained by the simultaneous inhibition of OATP1B1-mediated hepatic uptake and CYP2C8- and CYP3A4-mediated metabolism of repaglinide. To our knowledge, this is the first report of analyzing the transporter-based drug-drug interactions using a PBPK model. The present model is expected to be applicable to quantitative predictions of other drug-drug interactions involving both transporters and metabolic enzymes, providing valuable information for increased efficiency in drug development and avoidance of toxic interactions in clinical practice.

P86 - VALIDATION OF AN IN VIVO PHENOTYPING COCKTAIL FOR THE EARLY DETECTION OF THE INDUCTIVE OR INHIBITORY POTENTIAL OF NEW DRUGS

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The aim of this study was to develop a practical approach for an early detection of inductor or inhibitory potential of new drugs using an in vivo phenotyping cocktail. In the first part of the study, 24 volunteers received caffeine (CYP1A2), S-mephenytoin (CYP2C19), tolbutamide (CYP2C9), dextromethorphan (CYP2D6) and chlorzoxazone (CYP2E1), which are regarded as model substrates for several cytochrome P450 enzymes. In addition, endogenous cortisol metabolism was monitored for CYP3A4 activity. The substrates were monitored once individually and once simultaneously to study possible interactions. After this baseline activity determination, the volunteers were challenged with a known (selective) inducer (rifampicine, phenytoin and omeprazole) for 10 consecutive days. On Day 10, the administration of the inducer was immediately followed by administration of the cocktail. The inhibitors, ketoconazole, fluvoxamin and paroxetine were dosed orally only on Day 10, followed by administration of the cocktail. The changes in baseline activity were monitored. For practical considerations, a single time point for the determinations of the activities was selected. Baseline activity differed between individual and cocktail administration. However, the cocktail was able to reflect the inductive or inhibitory potential of the known inducers and inhibitors. A correlation was seen between analysis at only one time point and the analysis of all samples across time. In conclusion, the described cocktail enables the early detection of the inductive or inhibitory potential of a co-administered drug on the main CYP isoforms, with the exception of cortisol metabolism as a measure for CYP3A4 activity. By selecting a single sampling time point, this can be done in a practical manner during phase I multiple dose studies. The obtained results help in the set up of further interaction studies.

P87 - A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING APPROACH FOR REPAGLINIDE CLEARANCE AND DRUG-DRUG INTERACTIONS: IMPACT OF IN VITRO SYSTEM AND ROLE OF TRANSPORTER-METABOLISM INTERPLAY

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Repaglinide, an orally administrated insulin secretagogue, is commonly prescribed to patients with diabetes type 2. Clinical data indicate the importance of both active uptake via OATP1B1 and multiple metabolic pathways in its pharmacokinetic behavior. The current study characterized the formation of four major repaglinide metabolites, namely M1 (2-Despiperidyl-2-amino repaglinide), M2 (2-Despiperidyl-2-(5-carboxypentylamine)), M4 (3'-hydroxy repaglinide) and repaglinide acyl-β-D-glucuronide. The metabolism was characterized by monitoring the formation of these metabolites in a range of in vitro systems (pooled cryopreserved human hepatocytes, human liver microsomes and liver S9 fractions) over a 0.5 to 150 µM repaglinide concentration range using LC-MS/MS [1]. M2 was identified as the major metabolic pathway in hepatocytes and S9 and estimations of fm_{CYP} values resulted in comparable contribution of CYP2C8 and CYP3A4 (each <50%) to repaglinide metabolism. This metabolism data were implemented in a physiologically-based pharmacokinetic (PBPK) model [2] constructed in Matlab v.7.10. Initial model validation was performed using an i.v. infusion dose (2 mg). Use of metabolism data alone resulted in a considerable under-prediction (<5%) of observed in vivo clearance regardless of the source of in vitro metabolic data, consistent with general trends reported in the literature [3]. Inclusion of in-house active uptake data and intracellular binding into the PBPK model improved prediction of the i.v. clearance, but the estimate still represented <20% of the observed in vivo clearance. Intrinsic clearance for active uptake (79 µl/min/10^6 cells) was approximately 13-fold higher than total metabolic clearance (6.2 µl/min/10^6 cells). Optimization of the uptake V_{max} by 12 to 15-fold (depending on the use of HLM, S9 or hepatocyte metabolic data in the model) was required in order to recover the observed in vivo profiles. Use of metabolic data and optimized hepatic uptake clearance was subsequently applied for prediction of repaglinide profiles following oral dosing (0.25-4 mg). Pharmacokinetic parameters (CL, AUC and C_{max}) predicted by the PBPK model were within 2-fold of the observed data; trends were consistent across doses investigated. The PBPK model presented in the current study can be used in the assessment of complex drug-drug interactions involving inhibition of metabolizing enzymes together with hepatic uptake transporters previously reported for repaglinide.

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P88 - VALIDATION OF ENDOGENOUSLY EXPRESSED OCT2 IN HUMAN RENAL PROXIMAL TUBULAR CELL MODEL AS A TOOL FOR COMPOUND SCREENING

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Purpose: Substrate specificity of drug transporters is an important aspect in drug development to predict drug-drug interactions and drug toxicity. High expression of drug transporters located at the renal proximal tubular epithelium play a seminal role in renal drug excretion. Most in vitro assays in drug development are based on animal models or cell lines over-expressing one particular drug transporter. Because of species differences and cooperation between apically and basolaterally expressed transporters, representative human cell lines expressing endogenous drug transporters are required. Recently, our group developed a human conditionally immortalized proximal tubular epithelial cell line (ciPTEC) expressing functional drug transporters, including the basolateral organic cation transporter 2 (OCT2; SLC22A2) [Wilmer et al Cell Tiss Research 2010]. Here, we demonstrate the use of ciPTEC in investigating the transporter interactions of known human OCT2 substrates. **Methods:** Using a concentration range

of fluorescent model substrate ASP, the Km and Vmax for OCT2-mediated ASP uptake could be determined in both proliferating and matured ciPTEC. To validate OCT2-mediated transport and inhibition for pharmacological compounds in screening mode, cells were pre-incubated with several known substrates at a concentration range of 5-5000 µM; tetrapentylammonium (TPA), cimetidine, metformin or quinidine for 30 min prior to incubation with ASP (25µM) for an additional 20 min in presence of the potential inhibitors. The fluorescence intensity was measured on a Cytofluor 4000 fluorescence reader and IC_{50} values were calculated using Graphpad Prism (version 5.03). The inhibition constant (K_i) was determined by performing the uptake with ASP at 10, 20 and 30 µM in addition to the respective inhibitor at the concentration as indicated. Expression of OCT2 was analyzed using Western blotting. **Results:** Using Western blotting, OCT2 protein was detected in total cell lysate of ciPTEC at ~66 kDa. The Vmax of ASP in matured ciPTEC cultured for 7 days at 37°C was significantly higher compared to proliferating ciPTEC (5623 ± 322RFU in matured and 4389 ± 457 RFU in proliferating cells, $P<0.05$). All compounds tested significantly inhibited the cellular uptake of ASP by OCT2 (calculated IC_{50} values: TPA 16±2; metformin 3954±1245; cimetidine 8±2; quinidine 28±5 µM). Dixon plots were analyzed by linear regression and were consistent with competitive or mixed-type inhibition for all tested compounds (Ki values: TPA 13±2; metformin 4271±522; cimetidine 36±4; quinidine 40±5 µM). **Conclusions:** Pharmacological OCT2 substrates can be analysed accurately using the human ciPTEC model and the data are suitable to be used in DDI-predictions.. The human origin and endogenous expression of other drug transporters, such as MRP4, P-glycoprotein and BCRP in this cell line can be used to study vectorial transport of compounds to better understand renal drug handling.

P89 - ANTHOCYANIDIN PELARGONIDIN DOES NOT INHIBIT HUMAN LIVER CYTOCHROMES P450 AT PHYSIOLOGICALLY RELEVANT CONCENTRATIONS

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Anthocyanins are subclass of flavonoids occurring in fruits, vegetables and certain beverages. The structure of anthocyanins consists of sugar molecules bound to a flavylium cation (anthocyanidin). Recent research has shown that anthocyanins have remarkable health beneficial effects (antiinflammatory, anticancerogenic, antiviral, antiproliferant, normolipidemic and normoglycemic [1]). As compounds exhibiting often a beneficial effect to human health they are often coadministered with drugs. The question however is whether (although having relatively low bioavailability ranging in plasma from 10^{-7} to 10^{-8} M) they can affect the activity of the main enzymes of drug biotransformation, human liver microsomal cytochromes P450. This study is aimed at the assessment of the inhibition of specific activities of six cytochrome P450 (CYP) enzymes (CYP1A2 with substrate ethoxyresorufin, CYP2A6 with coumarin, CYP2B6 with 7-ethoxy-4-(trifluoromethyl)coumarin, CYP2D6 with bufuralol, CYP3A4 with testosterone and CYP2E1 with chlorzoxazone), by antocyanidin pelargonidin. All activities were detemined according to established protocols [2]. Human hepatic microsomes were used as system for testing the respective enzyme activities. The activities of CYP enzymes were inhibited in a dose-dependent manner, however, at the highest concentration of pelargonidin used (100 microM) the maximal extent of inhibition was down to 55 % only in the case of CYP3A4 activity. CYP1A2 and CYP2D6 were inhibited at the same concentration down to 75% of the remaining activity. Other activities were inhibited in an even less significant extent. These (although weak) effects on individual CYP activities were confirmed in an in vitro experiment analyzing the influence of pelargonidin to formation of warfarin metabolites. Taking into account the low bioavailability of anthocyanidins (see above), it can be reasonably expected that an usual intake of fruits and vegetables or beverages does not pose any risk based on an unwanted interaction with a concomitantly taken drug.

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P90 - APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING AND SIMULATION FOR WAIVER OF ORTERONEL DRUG-DRUG INTERACTION TRIALS

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Orteronel (TAK-700) is an oral, selective, nonsteroidal 17,20-lyase inhibitor currently in clinical developmentas a treatment for men with castrate-resistant prostate cancer (CRPC). In an in vitro human liver microsomal Cytochrome P450 (CYP) inhibition study, TAK-700 showed weak inhibition toward CYP1A2, 2C8, 2C9, and 2C19 with IC_{50}s of 17.8, 27.7, 30.8, and 38.8 uM, respectively. In contrast, TAK-700 did not inhibit CYP2B6, 2D6, and 3A4 (IC_{50}s > 100 uM). Following the FDA DDI guidance, Physicologically-Based Pharmacokinetic (PBPK) modeling and simulation was performed in this study to assess the DDI potential of TAK-700 with sensitive CYP substrates/drugs: theophylline for CYP1A2, repaglinide for CYP2C8, S-warfarin for CYP2C9, and omeprazole for CYP2C19. The PBPK model was established for TAK-700 using Simcyp® (Version 11) with in vitro ADME data and a population of 100 healthy subjects (age 50-65 years, 50% male). The model was then refined with clinical data. Simulation was performed with 4 probe substrates suggested by the FDA for the corresponding CYPs. PK at steady-state for both TAK-700 and the probe substrates were used to reflect the usage of TAK-700 and probe substrate in clinics. These PBPK simulation results, show in the Table below, suggest that TAK-700 is unlikely to cause clinical meaningful DDI when it is co-administrated with drugs that metabolized CYP1A2, 2C8, 2C9, and 2C19 as well as CYP2B6, 2D6, and 3A4. The increase of AUC of these drugsin the presence of TAK-700 is less than 25%. Therefore, clinical DDI trials can be waived according to the new FDA guidance.

TAK-700 DDI Potential as a Perpetrator Using Simcyp Simulation

CYP	Substrate	Dose	TAK-700 IC_{50} (uM)	Geometric Mean AUC ratio (5th - 95th)
1A2	theophylline (SV)	125 mg TID	17.8	1.23 (1.13 - 1.34)
2C8	repaglinide (SV)	0.25 mg BID	27.7	1.13 (1.05 - 1.22)
2C9	S-warfarin (SIM)	10 mg QD	30.8	1.19 (1.13 - 1.28)
2C19	omeprazole (SV) enteric-coated	20 mg BID	38.8	1.24 (1.13 - 1.38)

P91 - MANAGING DIGOXIN DRUG INTERACTION POTENTIAL BY RECEIVER OPERATING CHARACTERISTICS ANALYSIS

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Background: Digoxin, an orally administered cardiac glycoside, has a narrow therapeutic window and is susceptible to transporter-mediated drug interactions (DDI). Recent in vitro methods to predict potential clinical digoxin DDIs of new candidate drugs have centered on the ability to inhibit P-glycoprotein (P-gp, MDR1). The objective of this study was to apply statistical Receiver Operating Characteristic (ROC) analysis to define new in vitro cut-off values for theprediction of digoxin DDIs using P-gp expressing cell lines and vesicles. **Methods:** Twenty-two commercial laboratories collaborated to generate IC_{50} data in three cell systems, plus vesicles, using four equations and multiple nonlinear regression programs. P-gp inhibition data were fitted to several logistic and nonlinear regression equations, and statistics were applied to identify robust data for variability analysis. ROC analysis was conducted utilizing all in vitro IC_{50} values generated by all companies and for individual laboratories. **Results:** Principal component analysis indicates that lab to lab differences were the main source of variability. Utilizing all company IC_{50} values, the ROC analyses generated new in vitro cut-offs of the inhibitor maximum concentration at steady state

(I) divided by P-gp inhibitory potency (IC_{50}) is > 0.03, or the nominal gut concentration (I_2) divided by IC_{50} is > 45 to predict that a clinical digoxin interaction study is needed (ROC area under the curve [AUC'] ~ 0.8). Individual laboratory ROC analyses performed similarly to the "all company analysis" indicated that a company can utilize their own in vitro cut-off values to define whether a clinical digoxin study is needed, provided enough data points are available for a robust ROC analysis. **Conclusion:** We propose that digoxin DDI potential may be better predicted by applying the ROC analysis to determine in vitro I/IC_{50} and I_2/IC_{50} cut off values to anticipate potential DDIs with digoxin.

P92 - WHAT IS THE CONTRIBUTION OF CYP2B6 TO BUPROPION METABOLIC CLEARANCE? IMPLICATIONS FOR THE PREDICTION OF CYP2B6 MEDIATED DRUG-DRUG INTERACTIONS

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Bupropion, an anti smoking and antidepressant drug, undergoes extensive hepatic metabolism via oxidative and reductive pathways to three principal metabolites; hydroxybupropion threohydrobupropion and erythrohydrobupropion. The t-butyl hydroxylation pathway appears to be the major metabolic route since peak plasma concentrations of hydroxybupropion are about 5- and 3-fold greater than those of parent drug and threohydrobupropion, respectively[1]. Studies with human liver microsomes have shown that the primary enzyme involved in bupropion hydroxylation is CYP2B6, with minor contributions from CYP2E1 and CYP2C19[2,3]. Thus, bupropion hydroxylation is recommended for use in CYP2B6 phenotyping and drug-drug interaction (DDI) studies[4]. However, the results of a recent in vitro study appear to contradict the in vivo finding that the fractional contribution (fm) of hydroxylation (via CYP2B6) to the overall metabolic clearance of bupropion is significant; indeed, the data indicate that reductive (via carbonyl reductase enzymes) and oxidative pathways contribute 99% and 1% to the metabolism of bupropion, respectively[5]. The aim of this study was to use a modelling and simulation approach to obtain an estimate of fm_{CYP2B6} that could then be applied for 'a priori' assessment of the CYP2B6 DDI potential of bupropion. Relevant in vitro and in vivo data were incorporated into a mechanistic physiologically based pharmacokinetic (PBPK) model within Simcyp (Version 11.1) to simulate the plasma concentration time profiles of bupropion and hydroxybupropion. As there was uncertainty regarding the fm of the hydroxylation pathway (and hence fm_{CYP2B6}), a sensitivity analysis was performed using a range of fm_{CYP2B6} values (0.01 to 1) to assess the impact of this variable on the plasma exposure of both parent and metabolite. The fm_{CYP2B6} value that allowed recovery of observed bupropion and hydroxybupropion plasma concentration time profiles was then used for prediction of the DDI between bupropion and the CYP2B6 mechanism based inhibitor ticlopidine[6]. Assuming a value of 0.6 for the fm_{CYP2B6} of bupropion, predicted geometric mean AUC ratios of bupropion and hydroxybupropion were 1.90 and 0.11 respectively. These were consistent with observed values of 1.78 and 0.13 for bupropion and hydroxybupropion respectively[6]. Use of an fm_{CYP2B6} of 0.01 (as reported[5]), resulted in a predicted lack of interaction between ticlopidine and bupropion and a 54-fold underprediction in plasma levels of hydroxybupropion. The results of this study demonstrate the utility of sensitivity analysis in conjunction with PBPK modelling to provide reasonable estimates of parameters associated with substantial uncertainty, thus enabling prospective assessment of metabolic DDI. Further in vitro studies are warranted to confirm the fm_{CYP2B6} of bupropion.

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P93 - CLASSIFICATION OF 331 DRUGS INVOLVED IN SIGNIFICANT PHARMACOKINETIC DRUG-DRUG INTERACTIONS

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[Background] Important drug-drug interactions (DDIs) have been alerted by particular drug pairs in many cases as documented typically in the product labeling. However, it is impractical to raise alerts for all possible drug pairs with significant DDIs by this means simply because numerous drugs are being involved in pharmacokinetic DDI. [Methods] In this study, based on literature information, we selected and systematically classified 331 drugs (including some redundancy) which are prescribed often and involved in significant pharmacokinetic DDIs in human to overcome the situation by raising alerts for group of drugs. Classifications were made by the mechanism of DDI (molecular species of metabolizing enzyme, transporter, etc), and by the degree of AUC changes conforming to PISCS (Hisaka A, et al. Clin Pharmacokinet, 2009: 48: 653-66). In addition, drugs those with higher safety concerns possibly caused by small pharmacokinetic changes were marked. [Results and Discussion] In this survey, number of drugs involved in DDIs with changes in AUC similar or more than double was 289, which includes substrates, inhibitors and inducers of metabolizing enzymes and transporters. By conforming PISCS, it became possible to estimate significance of DDIs for any combinations of these drugs. Among them, 191 drugs were involved in CYP-mediated DDIs, 73 drugs were transporter-mediated, 25 drugs were conjugation-mediated, and 40 drugs were associated with pH changes or chelate formations in the GI tracts. Molecular species responsible for CYP-mediated DDIs were CYP3A, 2D6, 2C9, 1A2, 2C19, and those for transporter-mediated DDIs were MDR1, OATP (1B1 and 1B3), OAT (2 and 3), intestinal OATP, in the decreasing order of number of drugs, respectively. Number of victim drugs involved in highly significant DDIs (associated with similar or more than 5-fold change in AUC) was 56 which included 45 CYP-mediated and 5 transporter-mediated DDIs. These drugs are corresponded to the sensible substrates of CYP which was classified in the draft guidance of DDI by US-FDA updated in 2012. In addition to this class, 66 CYP substrates were listed which associated with 2 to 5-fold change in AUC in this study, but these drugs were not classified clearly in the draft guidance. Overall, the list would be informative for appropriate management of pharmacokinetic DDIs in the clinical settings. Particular care is necessary for DDIs involving CYP substrates especially for CYP3A, since numbers of drugs associated with significant DDIs were apparently more than other categories. On the other hand, molecular species may need to be clarified more convincingly to achieve appropriate clinical managements for drugs involved in transporter-mediated DDIs.

P94 - REACTIVE OXYGEN SPECIES SCAVENGING AND DETOXIFICATION POTENTIALS OF BLIGHIA SAPIDA POLYPHENOLIC EXTRACT IN RAT MICROSOMES

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Polyphenolic extract from Blighia sapida was evaluated for reactive oxygen species scavenging and detoxification potentials in male rat microsomes. The polyphenolic extract was obtained by successive extraction of freeze-dried B. sapida arillil in hexane, ethylacetate and methanol. Reactive oxygen species scavenging potentials of the polyphenolic fractions of B. sapida arillils (0.2 Ð 1.0 mg/mL) was investigated using DPPH radical, superoxide ion, hydrogen peroxide, and hydroxyl radical and ferric ion reducing system. The detoxification of reactive oxygen was evaluated in diethyl nitrosamine induced redox imbalanced in rat liver microsomes. Rat microsomes were divided into five groups (A-E). Group A was treated with the vehicle. Group B, D and E microsomes were treated with 25 mM DEN. In addition to the DEN treatment, group D and E microsomes were treated with 10 mg/mL of B. sapida polyphenolic extract and vitamin C respectively. Group C microsomes received only B. sapida polyphenolic extract treatment at 10.0 mg/mL. DPPH sprayed TLC based polyphenolic compounds detection showed the presence of two spots with DPPH scavenging activity with Rf value 0.425 and 0.850. UV-visible spectrum and gas chromatography analysis of B. sapida polyphenolic extract shows seven different peaks with 2 prominent peaks. B. sapida polyphenolic extract at 1.0mg/mL scavenged the DPPH, superoxide ion, hydrogen peroxide, and hydroxyl radical at 60, 67, 63, and 57%, respectively, while ferric ion was significantly reduced. Reactive oxygen species detoxifying enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose 6- phosphate dehydrogenase) activities were significantly induced by B. sapida polyphenolic extract. This induction significantly

attenuated the increase mediated by diethyl nitrosamine treatment and compared favourably with vitamin c. Diethyl nitrosamine mediated elevation in the concentrations of oxidative stress biomarkers; malondialdehyde, conjugated dienes, lipid hydroperoxide, protein carbonyl protein hydroperoxide and percentage DNA fragmentation were significantly lowered by B. sapida polyphenolic extract. Overall, the results of the present study indicated that the polyphenolic fractions from B. sapida arillils elicited reactive oxygen species scavenging and detoxification potentials. In addition, the polyphenols also prevented the peroxidation of microsomal lipids, oxidation of microsomal proteins as well as fragmentation of microsomal DNA component. Thus, showing the possible prophylactic potentials of B. sapida arillils.

P95 - COMPLEX EFFECTS OF DIETARY PHYTOCHEMICAL SULFORAPHANE ON CYP3A4 GENE EXPRESSION

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Sulforaphane (SFN) is a phytochemical present at high concentrations in some cruciferous vegetables, especially broccoli. SFN has been found as a naturally occurring Pregnane X receptor (PXR) antagonist that down-regulates both basal and inducible cytochrome P450 3A4 (CYP3A4) expression in primary human hepatocytes (Zhou et al. 2007). PXR is a xenobiotic sensor (xenosensor) that regulates numerous genes involved in xenobiotic metabolism via transcriptional regulation. CYP3A4 is the most important target gene of PXR metabolizing about 50% of all drugs, xenobiotics and some endogenous sterols. Nevertheless, the effect of SFN on CYP3A4 expression has been proposed to be more complex that simple SXR antagonism. Here, we report our preliminary data showing that SFN down-regulates nuclear receptors PXR, RXRa and CAR mRNAs. We show that SFN inhibits PXR-mediated activation of CYP3A4 gene reporter construct in HepG2 cells. At the same time, however, SFN was able to activate the reporter construct in the absence of rifampicin, a model PXR ligand, both in the presence or absence of cotransfected PXR cDNA. SFN is not an inhibitor of CYP3A4 catalytic activity. These results indicate more complex effects of SFN on CYP3A4 gene expression and suggest that dietary exposure to SFN from cruciferous vegetables such as broccoli could contribute to the large interindividual variability in basal and inducible expression of CYP3A4 gene.

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P96 - PLATEABLE CRYOPRESERVED HUMAN HEPATOCYTES FOR THE ASSESSMENT OF CYTOCHROME P450 INDUCIBILITY : EXPERIMENTAL CONDITION-RELATED VARIABLES AFFECTING THEIR RESPONSE TO INDUCERS

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The aim of the present study was to assess the stability of cryopreserved human hepatocytes over 5 years and to explore experimental condition-related variables such as seeding density, culture matrix and medium, start and duration of treatment that could potentially affect the quality of cultures and their response to cytochrome P450 (CYP) inducers. About 50% of batches (63 / 125) of cryopreserved human hepatocytes were plateable after thawing. Of those, 17 batches showed reproducible recovery, viability and plateability (less than 5% intra-batch variability) up to 5 years. When cultured in collagen home-coated 48-well plates at a seeding density allowing 70% confluence, cryopreserved human hepatocytes displayed activities equivalent to fresh counterparts. Their response to CYP inducers was maximal and equivalent to fresh for an incubation of 72h starting at Day2 or Day3 after plating when cultured in modified HMM. The number of cryopreserved human hepatocytes could be further reduced by using a cocktail of CYP substrates for the assessment of their inducibility. In conclusion, experimental condition-related variables, such as seeding density, culture matrix and medium, start and duration of treatment, which affect the response of plateable thawed cryopreserved human hepatocytes to cytochrome P450 inducers can be reduced by optimizing critical steps of the protocols.

P97 - A TRANSGENIC MOUSE MODEL TO STUDY EXPRESSION OF THE HUMAN GLUTATHIONE TRANSFERASE P GENE

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Despite being identified as a significant factor in the development of drug resistance in tumour cell lines, and over-expressed in a range of both animal and human cancers, relatively little progress has been made to determine the in vivo function(s) of glutathione transferase P (GSTP), particularly in humans. We have previously demonstrated the role of AP-1 in the regulation of human GSTP (hGSTP) in vitro, and have developed a mouse model devoid of Gstp1/p2 in which we have shown an increased susceptibility to chemically-induced (skin, lung) or genetically-driven (colon) tumours. We now report on the generation of a transgenic reporter mouse line - hGSTP1::LacZ^{ROSA26} - which accurately recapitulates the expression profile of the human enzyme, and have used this model to investigate how this enzyme is regulated in vivo. Basally, hGSTP expression was found, at varying levels, in the crypts and villi of the small and large intestine, the bronchiolar epithelial cells of the lung, the epidermis and hair follicles of skin, the gall bladder epithelium, the choroid plexus in the brain, the biliary epithelium in the liver, and at a low level throughout the heart and kidney. Expression could be induced to varying degrees in different tissues by the antioxidants ethoxyquin (EQ) or butylated hydroxyanisole (BHA), although genetic deletion of the Nrf2 transcription factor, which regulates expression of a battery of genes protective against oxidative stress through the antioxidant response element (ARE), led not to an attenuation of hGSTP1 expression but increased expression on an Nrf2 null background. However, further investigation using mouse embryonic fibroblasts derived from hGSTP1::LacZ^{ROSA26} mice +/- Nrf2, treated with tert-butylhydroquinone, found that while the ARE undoubtedly plays a role in the regulation of hGSTP, other factor(s) such as AP-1 are capable of controlling the expression of this enzyme.

P98 - EVALUATION OF CYP3A4 INDUCTION BIOMARKERS: A CASE STUDY OF A CANDIDATE DRUG

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Multiple in vitro tools have become available to gain insight in the potential DDI liabilities of NME candidates. Although the majority is focused on inhibition of metabolic and transport related enzymes, there are also well accepted assays for induction of the main CYPs. In most cases, when major induction is seen, this is built in to the criteria for lead optimization and the liable compound will not progress. In the case of this candidate drug, the compound progressed to NME declaration and Phase 1 since at the time early induction screens were not routinely used, and preclinical in vivo PK data did not show signs of induction. A post-NME study with cryopreserved human hepatocytes showed a major potential for 3A4 induction, exceeding the positive control rifampicin. The induction correlated with a post-hoc study using the cell-based human PXR reporter assay, whereas the rat PXR assay was negative. Since the compound also showed a very high plasma protein binding, it was not immediately apparent what the clinical impact would be. Physiologically Based Modeling and Simulation based on the in vitro induction data led to a prediction of ~3-fold decreased exposure of midazolam. To obtain an early clinical read-out, and to explore the usefulness of CYP3A4 biomarkers, it was decided to include two markers in Phase 1: one well accepted, urinary 6-β-OH-cortisol, (6-HC) one less established, plasma 4-β-OH-cholesterol (4-HCh).

P99 - EXAMINATION OF EXTRACTS FROM FLAVORED MINERAL WATERS ON AHR-CYP1A1 SIGNALING PATHWAY IN HUMAN HEPATOCYTES AND IN HUMAN HEPATIC AND INTESTINAL CANCER CELLS

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A variety of xenobiotics such as terpenes, flavonoids, polyphenols etc. are taken in the diet and they can interfere with regulatory pathway of drug metabolizing enzymes in humans. This can result in food-drug interaction, which is an emerging phenomenon, comprising pharmacokinetic or toxicokinetic interaction between food constituents and drugs. Xenobiotics-mediated food-drug interactions include inhibition of enzymes and transporters, and induction of drug metabolizing enzymes, particularly cytochromes P450, where the most prominent and important inducible cytochromes are CYP1A and CYP3A4. The induction occurs via xenoreceptors PXR and AhR with consequence in expression of AhR- and PXR- driven genes. In the current study, we have examined extracts from 28 different

flavored mineral waters for their capabilities to activate AhR receptor and to induce CYP1A genes. Primary cultures of human hepatocytes and cancer cell lines HepG2 (hepatic) and LS174T (intestinal) were used as in vitro models. Techniques of RT-PCR, western blot and gene reporter assays were employed to determine the expression of CYP1A mRNA, protein and activation of AhR, respectively. We have identified four mineral waters which activated AhR and/or induced CYP1A1. We observed some discrepancies between the data from cancer cell lines and human hepatocytes, probably due to extensive metabolism of mineral waters constituents in hepatocytes. This study is the first report in effects of flavored mineral waters on the expression of drug-metabolizing enzymes. These data imply a potential of some mineral waters to cause food-drug interaction. In addition, activation of AhR-CYP1A1 signaling may result in chemically-induced carcinogenesis and alteration of intermediary metabolism.

P100 - DIFFERENTIAL INDUCTION OF CYPS IN VARIOUS ORGANS OF RAT BY AROCLOR AND PHENOBARBITAL

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CYPs are the key enzymes in Phase I drug biotransformation, where the reactivity of parent compound is generally increased in order to undergo Phase II conjugation reactions. Most reactive metabolites (RM) are formed following Phase I reactions, although some relatively rare bioactivations occur following Phase II. Drug development process requires early detection of potentially toxic reactive metabolites. This is usually done by incubation of the parent compound with microsomes from rodents. However, the quantity of metabolites is usually too low in order to allow identification of the chemical structure, and isolating sufficient amounts for further toxicodynamic/mechanistic studies. Contemporary strategy is inducing the gene expression of CYPs beforehand, and obtaining microsomes afterwards. In the present study, we compared the result of two CYP inducing agents, aroclor and phenobarbital (PB), on different organs in rat. Two groups of animals were dosed with one compound, as well as a control group included. Liver, kidney, heart, brain and lung tissues were dissected at the end of the experiment, and microsomes were prepared. CYP1A1 and CYP2B1/2 activities were determined. PB caused about 4 fold increase in CYP 1A1 activity compared to controls in liver, while aroclor caused 80 fold increase in the same organ. In all other organs, aroclor caused more pronounced effect on CYP1A1 activity compared to PB. On the other hand, CYP2B1/2 activity was induced more with PB compared to aroclor in liver. Induction was also higher in lung with PB, while activities of the other organs were comparable. Liver and kidney CYP activities of control and induced rats compared to human organ activities. The effect of CYP inductions on drug metabolism were checked with the formation of N-acetyl-p-benzo-quinonimine (NAPQI) from paracetamol.

P101 - SIMULTANEOUS EVALUATION OF INFLUENCE OF BREVISCAPINUM ON CYP450 ISOFORMS BY COCKTAIL PROBE DRUGS

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BACKGROUND: Breviscapinum (Lifeflower) is extracted from the traditional Chinese herb Erigeron Breviscapus (vant.) Hand. Mazz. It is widely used in clinic to treat cardiovascular diseases and cerebrovascular injury. It is often co-administered with other drugs, so it is very important to evaluate its influence on CYP isoforms to predict the drug-drug interactions. OBJECTIVES: To evaluate the effect of breviscapinum on the activities of CYP450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 with a six-probe cocktail in rats. METHODS: The rats were randomly divided into four groups, the control group, the low, middle and high dose breviscapinum groups. The rats in each group were given intraperitoneally normal saline 5ml/kg, breviscapinum 1.8mg/kg, 5.4mg/kg, and 16.2mg/kg, respectively, once a day for seven days. On the eighth day, the six-probe cocktail including caffeine (2.5mg/kg), tolbutamide (2.5mg/kg), omeprazole (10mg/kg), metoprolol (10mg/kg), chlorzoxazone (5mg/kg) and midazolam (5mg/kg) was intravenously injected to all the rats. Before and after injection, the blood samples were collected at a series of time-points and the concentrations of six probe drugs in plasma were determined by a HPLC method with UV detection in a single run. The main pharmacokinetic parameters were calculated by the DAS 2.0 software (Drug And Statistics 2.0 edition, provided by Chinese Pharmacological Society). RESULTS: The AUCs of metoprolol were 92.60 +/- 11.96 mg/L/h, 102.27 +/- 13.87 mg/L/h, 137.21 +/- 14.68 mg/L/h in high, middle and lower dose breviscapinum group, respectively, which were significantly higher than that in control group (66.72+/-22.78 mg/L/h, $P<0.05$). The t1/2s of metoprolol were 9.24 +/- 2.95 h and 10.34 +/- 3.41 h in middle dose and high dose breviscapinum group, respectively, which were significantly higher than that in control group (5.31+/-2.42 h, $P<0.05$). The CLs of metoprolol were 0.113+/-0.012 L/h/kg, 0.104+/-0.011 L/h/kg and 0.101+/-0.009 L/h/kg,

respectively, which were significantly lower than that in control group (0.163 +/- 0.031 L/h/kg, P<0.05). Meanwhile, the AUC and t1/2 of midazolam were significantly higher in middle and high dose breviscapinum group than those in control group (12.70+/-2.48 mg/L/h, 16.75+/-5.12 mg/L/h vs 8.95+/-0.72 mg/L/h; 7.11+/-3.00 h, 8.26+/-2.59 h vs 4.11+/-1.53 h, P<0.05) and CLs of midazolam were significantly lower (0.292 +/- 0.104 L/h/kg, 0.261 +/- 0.110 L/h/kg vs 0.43 +/- 0.04 L/h/kg; P<0.05). There were no significant differences for the main pharmacokinetic parameters of midazolam between low dose breviscapinum group and control group (P>0.05). Compared with control group, the main pharmacokinetic parameters of caffeine, tolbutamide, omeprazole, and chlorzoxazone have no significant differences in three dose breviscapinum groups (P>0.05). CONCLUSION: Breviscapinum can inhibit the activity of CYP2D6 and higher than 5.4mg/kg dose of breviscapinum can inhibit the activity of CYP3A4. Breviscapinum has no influence on the activities of CYP1A2, CYP2C9, CYP2C19 and CYP2E1. The results suggest that when breviscapinum is co-administered with the drugs metabolized by CYP2D6 and CYP3A4, the dosage of both drugs should be adjusted.

P102 - STRUCTURE-ACTIVITY RELATIONSHIPS OF FLAVONOIDS AS INHIBITORS OF PURIFIED HEPATIC NADH-CYTOCHROME B5 REDUCTASE

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NADH-cytochrome b5 reductase is a clinically and toxicologically important enzyme as it is involved in cytochrome b5 mediated metabolism of endogenous substrates and in cytochrome P450 catalyzed drug metabolism. NADH-cytochrome b5 reductase, either alone or together with cytochrome b5, also catalyzes the reductive metabolism of a variety of drugs and xenobiotics including some anticancer drugs, amidoximes, hydroxylamines and heterocyclic amines, and therefore plays an important role in the bioactivity or toxicity of these chemicals. Since the modulation of cytochrome b5 reductase by natural products may lead to alterations in drug toxicity, prodrug bioactivation and carcinogenic activity of various compounds, we attempted screening of naturally occurring flavonoids of variable structure belonging to different classes as putative inhibitors of cytochrome b5 reductase. The inhibitory properties of flavonoids on b5 reductase were analyzed using purified enzyme from bovine liver microsomes, which shares a high degree of homology with human counterpart, and potassium ferricyanide as final electron acceptor. Although, our preliminary studies with apigenin, quercetin, naringenin, (+)-catechin, quercitrin, rutin and naringin identified quercetin as a potent inhibitor of b5 reductase with an IC_{50} value of 1.07 µmol/L, analysis of inhibitory properties of a number of additional flavonoids including luteolin, chrysin, myricetin, morin, (-)-epicatechin, (+)-taxifolin and luteolin-7-O-glucoside demonstrated that the flavone luteolin was almost tenfold more potent than quercetin in inhibiting b5 reductase with an IC_{50} value of 0.11 µmol/L. The inhibitory potency of all the remaining flavonoids was found to be in decreasing order from (+)-taxifolin (IC_{50}:234 µmol/L), rutin (IC_{50}:56.7 µmol/L), apigenin (IC_{50}:36.1 µmol/L), (+)-catechin (IC_{50}:4.47 µmol/L), (-)-epicatechin (IC_{50}:3.22 µmol/L), luteolin-7-O-glucoside (IC_{50}:2.35 µmol/L), myricetin (IC_{50}:1.5 µmol/L), quercitrin (IC_{50}:1.23 µmol/L) and morin (IC_{50}:0.81 µmol/L). Based on these results, while (+)-taxifolin, rutin and apigenin appeared to be poor inhibitors of cytochrome b5 reductase, the remaining flavonoids produced a considerable inhibition of enzyme activity. Naringenin, naringin and chrysin, on the other hand, were inactive within the concentration range tested. Detailed analysis of structure-activity relationships drawn from screening experiments revealed that flavonoid structures seem to be closely related to the degree of inhibition. Flavonoids containing two hydroxyl groups in ring B and a carbonyl group at C-4 in combination with a double bond between C-2 and C-3 produced a much stronger inhibition, whereas substitution of a hydroxyl group at C-3 was associated with a less inhibitory effect. The findings of this study indicate that inhibition of the activity of cytochrome b5 reductase by flavonoids with physiologically relevant IC_{50} values may have possible implications in toxicity or bioactivity of certain drugs and carcinogens in terms of potentially significant flavonoid-drug or flavonoid-xenobiotic interactions.

P103 - A SIMPLIFIED APPROACH TO PREDICT CYP3A MEDIATED DRUG-DRUG INTERACTIONS AT EARLY DRUG DISCOVERY: VALIDATION WITH CLINICAL DATA

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Purpose. Many factors contribute to P450 mediated drug-drug interactions (DDI), such as protein and microsomal binding, transporter-enzyme interplay and tissue distributions. It is unclear how many of them are critical for the

early DDI predictions. The present study evaluates which factors have to be incorporated into a simplified approach to provide reasonable CYP3A mediated DDI predictions at early drug discovery stage. **Methods.** In vitro CYP3A IC50 and Ki values were obtained from a high-throughput assay using human liver microsomes (HLM) and hepatocyte preparations for 10 well-documented drugs covering from low to high CYP3A mediated DDIs (indexed by the change of area under the concentration curves, AUC). Plasma and microsomal protein binding were determined using standard equilibrium dialysis methods. In vitro hepatocyte partition coefficient (Kp) was estimated by the ratio of drug concentration in the suspended cells over that in the incubation medium. Human plasma concentration and AUC were retrieved from literature. DDI predictions were performed using an equation incorporating the fraction of the substrate metabolized by CYP3A (Ito et al., 2005; Obach et al., 2006) with the maximum drug concentration in the plasma (Cmax) and Ki values as corrected as follows: 1) total Cmax; 2) free drug (fu) corrections for both Cmax and Ki in HLM; 3) Cmax corrected by hepatocyte Kp; 4) Cmax corrected by both hepatocyte Kp and fu. **Results.** Based on the Ki data from HLM, approach 1 and 3 provided a prediction of DDI within 2-fold of the observed clinical values for 9 out of 10 drugs. In addition, the likelihood of DDI predicted using these simplified methods also agreed well with the updated FDA guidelines (2012), with a significant correlation between predicted AUC changes and the [I]/ Ki ratios (r^2 =0.982 and 0.894). In comparison, approach 2 and 4 (fu corrections) led to an underprediction of DDI (>3-fold error for 5/10 and 2/10 drugs). Data from the hepatocytes showed generally lower prediction accuracy, with approach number 3 as the best (7 out of the 10 drugs predicted within 2-fold of clinical observed DDIs). **Conclusions.** CYP3A mediated DDIs can be predicted with a high level of accuracy based on Ki estimates from HLM data and the total plasma Cmax of the inhibitors. This approach should be widely applicable to the assessment of clinically significant DDI risks in early drug discovery programs.

P104 - THREE NEW SHRNA EXPRESSION VECTORS MEDIATED INHIBITION OF CYP3A4

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RNA interference (RNAi) is useful for selective silencing the expression of a target gene of interest. The cytochrome P450 3A4 (CYP3A4) plays an important role in drug metabolism, which metabolizes approximately 50% of drugs in clinical use. In the present study, we aimed to develop a short hairpin RNA (shRNA) based RNAi approach to modulate CYP3A4 expression.. Three new shRNAs (S1, S2 and S3) were designed to target the coding sequence (CDS) of CYP3A4, cloned into a shRNA expression vector, and tested in different cells. Interestingly, the mixture of three shRNAs caused a significantly higher level (55%) of reduction of CYP3A4 CDS-luciferase activity in both CHL and HEK293 cells, whereas individual shRNAs only led to a lower extent (<30%) of suppression. Endogenous CYP3A4 expression in HepG2 cells was decreased about 50% at both the mRNA and protein level after transfection of the mixture of three shRNAs. In contrast, CYP3A5 gene expression was not altered by the shRNAs, supporting the selectivity of CYP3A4 shRNAs. In addition, HepG2 cells transfected with CYP3A4 shRNAs were less sensitive to Ginkgolic acids, whose toxic metabolites are produced by CYP3A4. These results demonstrate that vector-based shRNAs are able to modulate CYP3A4 expression in cells through their actions on CYP3A4 CDS, and CYP3A4 shRNAs may be utilized to define the role of CYP3A4 in drug metabolism and toxicity.This work was supported by National Major Projects of China (2012ZX09506001-004) and Nature Scientific Found of China (81173120).

P105 - THE INHIBITORY EFFECT OF SANGUINARINE ON CYTOCHROME P450 ISOFORMS IN HUMAN LIVER MICROSOMES

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Sanguinarine (SAG, CAS: 2447-54-3), a benzo[c]phenanthridine alkaloid mainly distributed in Sanguinaria canadensis, Chelidonium majus and Macleaya cordata, has drawn much attention due to its intended effects (e.g., antimicrobial, anti-inflammatory and antitumor activities) and adverse effects (e.g., genotoxicity, hepatotoxicity) in recent years [1]. Interestingly, several studies show that both positive and negative effects of SAG seem to be associated with the inhibitory and inductive effects of SAG on cytochromes P450 system [2, 3]. In the light of this crucial clue and a previous study which has reported the inhibitory effect of SAG on human CYP1A [4], the present study intends to investigate the inhibition effects of SAG on six major CYP isoforms (CYP3A4, 2A6, 2D6, 2C9, 2C8 and 2E1) using human liver microsomes. The $[I]/([I]+K_i)$ values and $\{K_{deg}+([I]\cdot K_{inact})/([I]+K_I)\}/K_{deg}$ values are employed respectively to

predicate the potential magnitudes of reversible and irreversible (mechanism-based) inhibition induced by SAG. The results showed that SAG competitively inhibited CYP3A4 and 2C9 with IC_{50} (K_i) of 3.2 μM (2.2 μM) and 6.5 μM (2.6 μM) respectively, and noncompetitively inhibited CYP2C8 with IC_{50} (K_i) of 12.1 μM (8.8 μM). Moreover, SAG exhibited time-dependent inhibition (TDI) against CYP3A with K_I of 5.5 μM and K_{inact} of 0.03 min^{-1}. Nevertheless, SAG displayed little inhibitory activity against CYP2A6 and 2E1. According to the previous studies [5, 6], SAG peak plasma concentrations are rang form 8.4 μM to 23.5 μM in human. On the basis of $[I]/([I]+K_i)$ values calculated using mean peak total plasma concentration (16 μM) of SAG, 88%, 86% and 65% inhibition of CYP3A4, 2C9, and 2C8 substrates can be forecasted. Additionally, based on the $\{K_{deg}+([I]\cdot K_{inact})/([I]+K_I)\}/K_{deg}$ values calculated using the $K_{deg\ (CYP3A4)}$ value of 0.00016 min^{-1}, approximately 100% inhibition of CYP3A4 substrates can be predicated. It should be noted that compounds with methylenedioxyphenyl groups could exhibit TDI to cytochrome P450 via generation of cabene metabolites, which is the possible mechanism of the SAG-induced TDI towards CYP3A4. Upon consideration of the potent inhibitory effects of SAG on CYP3A4, 2C9 and 2C8, and above all the wide utilization of SAG-containing herbs as folk medicines in China, Europe and South America [1], sufficient attention should be given to avoid the probable drug-drug interactions between SAG/SAG-containing products and substrates of these CYP enzymes.

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P106 - BAICALEIN, SCUTELLAREIN AND THEIR GLUCURONIDES ARE POTENT INHIBITORS OF HUMAN LIVER CATECHOL-O-METHYLTRANSFERASE

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Abstract: Catechol O-methyltransferase (COMT), the enzyme mediated methylation of catechols to corresponding O-methylated catechols, played an important role in the biotransformation of the dopaminergic drug levodopa. Inhibition of human peripheral COMT is an important therapy approach in Parkinson's disease, due to potent COMT inhibitors can slow down the metabolic fates of levodopa and dopamine, ultimately enhancing the dopamine level in brain[1-2]. It has been found that many flavonoids in tea, berries and herbals, have a positive effect and protect against Parkinson's disease, but its action mechanism has not been fully elucidated yet. In this study, the inhibitory affects towards human COMT by two natural flavonoids (Baicalein, Scutellarein) and their Glucuronides (Baicalin and Scutellarin) were first investigated by using human liver cytosol (HLC), while the O-methylation of 3,4-Dihydroxyphenylacetic acid was used as probe reaction for determination the COMT activity. All investigated compounds showed potent inhibitory effects towards the formation of O-methylated metabolites of 3,4-Dihydroxyphenylacetic acid in a concentration-dependent manner, and the IC_{50}values of Baicalein, Scutellarein, Baicalin and Scutellarin for COMT were 36 nM, 29 nM, 230 nM, and 44 nM respectively. Furthermore, both Lineweaver–Burk and Dixon plots demonstrated that inhibition of human liver COMT by these four flavonoids gave the best fit for types of non-competitive inhibition, and the K_i values of Baicalein, Scutellarein, Baicalin and Scutellarin were 18.44 nM, 21.86 nM, 96.95 nM, 58.25 nM respectively. These results demonstrated that Baicalein, Scutellarein and their Glucuronides can strongly inhibit the activity of human COMT, and the inhibition effects toward COMT by these natural flavonoids provide one of potential explanations for why natural flavonoids can give a positive effect for patients with Parkinson's disease.

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P107 - SELECTION OF DRUG CANDIDATES USING HIGH-THROUGHPUT SCREENING OF REVERSIBLE AND TIME-DEPENDENT CYP INHIBITION

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In the pharmaceutical industry, cytochrome P450 (CYP450) inhibition studies are generally included in the standard screening strategy of drug candidates. The results of these screening assays are used for selection purposes, prioritization of drug candidates or to predict possible drug-drug interactions of a specific compound. The last years, time-dependent inhibition is receiving increased attention since this could lead to clinically relevant drug-drug interactions. To screen drug candidates for reversible and time-dependent inhibition in an early stage of development, we implemented an IC_{50} shift method adapted from the methods published by Grimm et al. (1) (among others). **Methods**: Drug candidates were tested for reversible and time-dependent inhibition properties using two different incubations performed simultaneously. Drug candidates were incubated at 6 concentrations (in duplicate) with human liver microsomes with or without a pre-incubation of 30 minutes at 37°C. Enzymatic reactions were started immediately (reversible inhibition) or after 30 minutes pre-incubation (time-dependent inhibition) by the addition of a probe substrate. CYP inhibition was investigated for the human CYP isoforms 1A2, 2C8, 2C9, 2C19, 2D6 and 3A4/5 with appropriate substrates and inhibitors using LC-MS analysis with validated methods. IC_{50} values obtained from pre-incubated samples were compared with IC_{50} values obtained from the incubations without pre-incubation (co-incubations) and the fold-shift was determined. From selected drug candidates with time-dependent inhibition observed, additional k_{inact}/K_I experiments were performed with incubations at five different time points in screening mode. **Results**: All known time-dependent inhibitors showed an IC_{50}-fold shift of at least 2.5 with a 30-minute pre-incubation. The assay was used for screening and selection of hundreds of compounds for the pharmaceutical industry. Based on all results obtained, compounds could be prioritized using the IC_{50} fold shift in “green zone” (no time dependent inhibitor, no TDI), the “grey zone” (possible TDI) and the “red zone” (TDI). By transferring the screening assay to a TECAN Freedom Evo automated workstation, selection of series of compounds can be performed within a turnaround time of only 2 weeks. **Conclusions**: This combination of time-dependent and reversible screening assay has proven to be a helpful tool in pre-selection and prioritization of drug candidates for the pharmaceutical industry. **References**: (1). Grimm SC, Einolf HJ, Hall SD, He K, Lim H-K, Ling K-HJ, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, van Horn R, Wang RW, Wong YN, Yang TJ, Obach RS. The conduct of in vitro studies to address time dependent inhibition of drug-metabolizing enzymes: a perspective of the pharmaceutical research and manufacturers of America. Drug Metabolism and Disposition 37, p. 1355-1370 (2009).

P108 - VALIDATION OF A NEW IN VITRO ASSAY TO ASSESS THE INHIBITORY POTENTIAL OF NEW CHEMICAL ENTITIES (NCE) AGAINST EPOXIDE HYDROLASE, A KEY ENZYME IN THE METABOLISM OF CARBAMAZEPINE

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Carbamazepine is one of the most commonly prescribed antiepileptic drugs. As it is frequently given in combination with other drugs, this could lead to drug-drug interactions (DDI) with carbamazepine as drug victim or as drug perpetrator. One of the well-known drug-drug interactions with carbamazepine is related to the inhibition of epoxide hydrolase. Indeed, the neuro-toxic metabolite carbamazepine-10,11-epoxide produced by CYP3A4, is detoxified by epoxide hydrolase. Consequently, inhibition of this enzyme could significantly increase the circulating level of epoxide carbamazepine and therefore cause severe CNS adverse events. It is therefore important to detect this potential liability of NCE intended to be co-administered with carbamazepine. Classically, styrene oxide was used as probe marker for epoxide hydrolase activity. However, it is a R45 compound (carcinogen) requiring safety precaution to handle it. With the commercial availability of carbamazepine epoxide, a new assay was developed for measurement of epoxide hydrolase activity, in human liver microsomes (HLM) and human hepatocytes suspensions, in order to predict the potential risk of In Vivo DDI with carbamazepine. The metabolite 10,11-dihydroxycarbamazepine, was measured by a LC-MS/MS method. Formation of 10,11-dihydroxycarbamazepine was linear up to 60 minutes and 1 mg protein/mL, in human liver microsomes and up to 240 minutes, in human hepatocytes suspensions. Accurate determination of the Km value could not be performed, due to solubility limitations of carbamazepine 10, 11-epoxide. The formation of 10,11-dihydroxycarbamazepine was approximately 13-fold higher in human hepatocytes, in comparison to HLM,

when activities were normalized per gram of liver. Investigations with human liver cytosol and recombinant microsomal and cytosolic human epoxide hydrolase showed that the two isoforms were able to catalyze the hydrolysis of carbamazepine-10,11-epoxide. Nevertheless, the activity observed in hepatocytes was still 6-fold more important, in comparison with the sum of activities observed in HLM and human liver cytosol. In order to further explore these differences between HLM and hepatocytes, the uptake of carbamazepine epoxide into hepatocytes was determined. The results showed that the total intracellular concentrations of carbamazepine epoxide were about 8-fold higher than those observed in the extracellular media, which is consistent with the apparent higher activity observed in hepatocytes. Finally, progabide was investigated as a reference inhibitor of epoxide hydrolase, as significant clinical interactions between progabide and carbamazepine have already been reported. Progabide caused a significant inhibition of epoxide hydrolase with IC_{50} values of 19 µM and 1.1 µM, in HLM and hepatocytes, respectively. Using free C_{max} of progabide (ca 0.3µM), the IC_{50} obtained in hepatocytes better predicted clearance inhibition of carbamazepine epoxide observed in vivo. In conclusion, the present study showed that this assay could be used to investigate the inhibitory potential of NCE against epoxide hydrolase. In addition, human hepatocytes seemed to be more predictive than HLM for the prediction of the observed In Vivo DDI between progabide and carbamazepine. However, this better predictability of human hepatocytes should be further explored using other known inhibitors of epoxide hydrolase.

P109 - EFFECT OF AMARANTHUS LIVIDUS AGAINST CARBON TETRACHLORIDE-INDUCED KIDNEY DAMAGE IN RATS

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The present study was conducted to evaluate the protective effect of the water extract of the Amaranthus lividus L., widely used as a vegetable in West Black Sea Region of Turkey, on carbon tetrachloride (CCl4)-induced oxidative stress in the kidney tissues of rats. A. lividus was collected from Bartin, Turkey. The stems with leaves and flowers of A. lividus were extracted in boiling water. The extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator. Male Wistar albino rats were pretreated with the water extract of A.lividus (250 and 500 mg/kg body weight; p.o.) once daily for 9 days and then on the 10th day CCl4 (1.5 mL/kg body weight; i.p.) in olive oil (1:1, v/v) was applied. All rats were sacrificed 24 h after CCl4 administration, kidneys were dissected out and used for biochemical studies. Antioxidant status in kidney tissues was estimated by determining the activities of the antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and myeloperoxidase (MPO); as well as by determining the levels of lipid peroxidation (LPO). The results showed that CCl4 administration to the rats caused a remarkable increase in LPO levels, MPO and SOD activities also significant decrease in CAT activities compared with the control group. Pretreatment with A. lividus water extract (250 and 500 mg/kg body weight) successfully prevented the elevation of MPO activity and LPO levels as well as significantly prevented the decrease of CAT activity in CCl4 treated rats as compared to CCl4 group. However, no significant difference in SOD activity was observed between the A. lividus pretreated and CCl4-intoxicated rats. This study indicates that A. lividus water extract pretreatment may be useful for the prevention of CCl4-induced oxidative stress in kidney tissue, which probably by both the increase of antioxidant-defence system activity and the inhibition of lipid peroxidation.

P110 - INHIBITORY EFFECTS OF THAI MEDICINAL PLANTS ON HUMAN UGT1A1 AND UGT2B7 ACTIVITIES

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UDP-glucuronosyltransferase (UGT) enzyme superfamily is one of the major conjugative enzymes that involved in detoxification and metabolic pathway for endogenous and xenobiotic compounds. In human, UGT enzyme superfamily is classified into UGT1 and UGT2 families[1]. Among UGT1 and UGT2 enzymes, UGT1A1 and UGT2B7 are the major isoforms responsible for drug conjugation[2]. The herbal extract components as well as herbal supplements have been reported to inhibit UGT enzymes activities which may consequently lead to drug interactions[3, 4]. However, the data concerning the interaction between Thai medicinal plants and UGT enzymes is still limited. Therefore, in the present study, the inhibitory effects of Thai medicinal plants on the UGT activities of human were investigated in order to predict potential herb-drug interactions. AZT and SN38 glucuronosyltransferase activities in human liver microsomes were measured by HPLC as a selective marker of UGT2B7 and UGT1A1, respectively. The ethanolic and aqueous extracts of 13 commonly used Thai medicinal plants

were incubation with human liver microsomes for their potential to inhibit UGT1A1 and UGT2B7 activities. Among these medicinal plants, the ethanolic extract of M. hortensis, K. parviflora, R. nasutus and C. longa showed strong inhibition toward UGT1A1 with an IC_{50} values less than 10.0 µg/mL. The ethanolic extract of A. paniculata and C. longa were potent inhibitor of UGT2B7 with IC_{50} values 12.5 ± 7.1 and 11.2 ± 4.3 µg/mL, respectively. The aqueous extracts of these plants showed inhibition of UGT activities less than ethanolic extracts. Results from this present study reveal that some Thai medicinal plants were strong inhibitors of human UGTs.

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P111 - PROBE-DEPENDENT DIFFERENCES IN CYP2C9 INHIBITION WHEN USING WARFARIN AND DICLOFENAC: HETEROACTIVATION OF CYP2C9

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In our high-throughput, CYP inhibition assay, diclofenac was considered as an alternative CYP2C9 probe substrate to warfarin. However, for a proprietary class of compounds the CYP2C9 inhibition was observed to be probe-dependent since the IC50 values were greater (less potent) with diclofenac compared to lower IC50 values (potent inhibition) with warfarin. In CYP inhibition assays, warfarin is commonly used as a CYP2C9 probe substrate and the CYP2C9-mediated metabolite, 7-hydroxywarfarin, is monitored by LC-MS/MS. Warfarin is also hydroxylated by CYP3A4 (4Õ-hydroxywarfarin) and CYP1A2 (6- and 7-hydroxywarfarin), which results in multiple +16 amu oxidative metabolites with the same MRM transition as the CYP2C9-mediated 7-hydroxywarfarin. Typically LC methods can separate these metabolites, but in our high-throughput assay, we use the RapidFire (direct flow injections) and the lack of separation of oxidative metabolites poses some challenges. To avoid compromised data quality, we evaluated diclofenac as a CYP2C9 probe. Interestingly, for a set of structurally related proprietary compounds, IC50 values were probe substrate-dependent. However, for sulfaphenazole, a potent and well-studied CYP2C9 inhibitor, no difference in the IC50 values was observed between the two probes. This suggests that the proprietary compounds have physical/chemical properties and/or bind within CYP2C9 in such a way that lead to the probe sensitivity of CYP2C9 inhibition. CYP2C9 has been reported to have two binding sites: the active site near the haem and the warfarin binding pocket (Williams et al. Crystal structure of human cytochrome P450 2C9 with bound warfarin. Nature 424:464-468 (2003)). We hypothesize that the proprietary compounds are binding at the Ôwarfarin binding pocketÕ, which results in the activation of CYP2C9 and/or facilitates compounds such as diclofenac to bind at the ÔhaemÕ binding site. Therefore, the activation of CYP2C9 activity and increased rate of metabolism of diclofenac results in the observed higher IC50 values and less potent inhibition of CYP2C9. Sulfaphenazole did not show a difference in CYP2C9 inhibition between probes as it binds at the active site near the haem and does not activate CYP2C9. From the studies, a structural activity relationship was identified to mitigate the CYP2C9 inhibition liability.

P112 - INVOLVEMENT OF METABOLIC INTERMEDIATE COMPLEX IN INHIBITION OF CYP3A4 BY SERTRALINE

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Clinically significant drug interactions associated with serotonin reuptake inhibitors (SSRIs) have been reported. Although SSRIs, fluvoxamine, fluoxetine and paroxetine, are structurally unrelated, they have a common property that inhibits cytochrome P450 (P450). Since paroxetine has a methylenedioxy substituent, it can inactivate P450 via the formation of a carbene metabolic intermediate complex (MIC). On the other hand, fluoxetine has a secondary amine group and can form a nitrosoalkane MIC with P450. Sertraline, another SSRI, is also reported to cause drug interactions and inhibit P450s competitively, but to a lesser extent than other SSRIs. In the present study, we investigated the roles of metabolites in inhibition of P450 by sertraline. Because sertraline also has a secondary amine group, we further examined whether it forms MIC with P450. Human liver microsomes were preincubated with sertraline in the presence of NADPH, followed by assay of the microsomal oxidation activities with the probe substrates: testosterone for CYP3A4; propranolol for CYP1A2 and CYP2D6; diclofenac for CYP2C9. In some studies, liver microsomes of untreated and dexamethasone-treated male Wistar rats were used. The reaction mixtures including the microsomes were also subjected to difference spectra with and without sertraline, which were monitored from 500 to 400 nm, to determine MIC formation by sertraline. Preincubation of human liver microsomes with sertraline in the presence of NADPH resulted in time-dependent inhibition of CYP3A4. On the other hand, no significant time-dependent inhibition was observed for other P450 enzymes. N-Desmethylsertraline (NDS), a major metabolite generated by P450, inhibited CYP3A4 more potently than sertraline, whereas the inhibition required a higher NDS concentration than that generated from sertraline. Preincubation of microsomes with NDS did not potentiate the CYP3A4 inhibition by NDS. These suggest that time-dependent inhibition of CYP3A4 is caused by the inactivation through the sertraline metabolism that is independent of NDS generation. The pseudo-first order kinetics, the requirement of NADPH, and the lack of protection by the addition of glutathione in the inactivation demonstrate that sertraline is a mechanism-based inhibitor of CYP3A4. Similar to fluoxetine, difference spectra with and without sertraline in the presence of NADPH gave a Soret peak at 456 nm, which was more prominent when using the microsomes from dexamethasone-treated rats, suggesting CYP3A-dependent formation a nitrosoalkane metabolite to form MIC with P450. On the other hand, difference spectra with NDS revealed that NDS did not form MIC, but rather attenuated the MIC formation by sertraline. In conclusion, it is suggested that sertraline is a mechanism-based inhibitor of CYP3A4. Sertraline can be oxidized into a metabolite to form MIC with CYP3A4, which is involved in the time-dependent CYP3A4 inhibition.

P113 - INTERACTION OF AZOLE-CONTAINING DRUGS WITH HUMAN RECOMBINANT POLYMORPHIC VARIANTS OF CYP2C9 AND CYP2C19

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Cytochrome P450s are a superfamily of oxygen-activating enzymes that carry out an enormous range of metabolic reactions on endogenous and exogenous substrates in processes both beneficial and deleterious to the organism. Annotation of the human genome has revealed the presence of some 57 human P450 genes, but less than a dozen of these play important roles in the hepatic clearance of drugs. CYP2C9 and CYP2C19 are the most important CYPs in the CYP2C subfamily, which comprises also CYP2C8, CYP2C18. It has been estimated that CYP2C9 is responsible for the metabolic clearance of up to 15% of all drugs that undergo Phase I metabolism including drugs with narrow therapeutic indices, such as (S)-warfarin, tolbutamide and phenytoin. For CYP2C9, a number of genetic variants have been characterized including the two most common variants which produce intact enzyme with reduced enzymatic activity arising from amino acid substitutions that are at positions critical for activity. The wild-type CYP2C9 has Arg144 and Ile359, with variants being CYP2C9*2 (with Cys144) and CYP2C9*3 (with Leu359). CYP2C19 is a clinically important enzyme responsible for the metabolism of a number of therapeutic drugs, such as S-mephenytoin, anti-ulcer drugs such as omeprazole, certain antidepressants, and the antimalarial drug proguanil. Genetic polymorphisms in this enzyme (D92E, I331V, H144R and R410Ñ) result in alteration of metabolism of these drugs. We have developed a technology for producing preparations of recombinant human CYP2C9, CYP2C19 as well as their polymorphic variants. We investigate the binding of number therapeutical azole-containing drugs with CYPs, listed above. Azoles, prescribing together with other therapeutically administrated drugs (substrates for CYPs), can influence the pharmacokinetics and pharmacodynamics of these drugs, leading to the development of a significant

number of adverse drug reactions. The polymorphic forms of P450s are also responsible for the development of adverse drug reactions. In vitro systems (heterologous expressed individual CYPs and combinations of enzymes) could be very useful in predicting effects of the CYP2C9 and CYP2C19 and their polymorphic variants on metabolism in vivo of various types of drugs in individuals, carriers of a particular combination of variants of enzymes. This approach allows us to determine the contribution of individual polymorphic forms of human enzymes in the metabolism of the most common and newly developed drugs and to make recommendations (on the basis of molecular diagnostic) for the most efficient doses in drug therapy of diseases, as well as find out mechanisms of the individual toxicity of drugs or food components.

P114 - COMPARISON OF METABOLIC STABILITIES FOR XENOBIOTICS BETWEEN STUDIES WITH HEPATIC AND CUTANEOUS SUBCELLULAR FRACTIONS

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Skin is mainly considered as a biological barrier against xenobiotics. Skin is also recognized to be a site of metabolism. Consequently, skin metabolism study of cosmetic ingredients becomes an important issue in the assessment of their biotransformation and local exposure. Because hepatocyte subcellular fractions are widely used in the hepatic metabolism prediction, subcellular fractions of a reconstructed human skin model (full thickness model of Episkin^{TM}) were used to test the skin metabolism. This strategy comes within the scope of L'Oreal effort in the development of new in vitro alternative methods to animal testing. In this work, we compared metabolic stabilities (half-life time) of xenobiotics in the presence of cutaneous and hepatic subcellular fractions. Results confirmed that cutaneous subcellular fractions had a lower catalytic activity level than hepatic fractions for numerous compounds but that biotransformations could occur at the skin level for peculiar chemicals. For example, the cutaneous stability of Nicardipine which is a positive control used for probing Cytochrome P450-dependent monooxygenases activity, confirmed that skin is much less equipped of these enzymes than liver. However, a skin specificity could be observed with N-acetylation of p-aminobenzoic acid and N-acetyl-p-phenylenediamine compared with the liver. To conclude, although xenobiotics skin metabolism is weaker than that of the liver, results presented here confirmed the interest in studying their cutaneous metabolism because skin can be metabolically active for specific compounds and the site of a first pass effect for such compounds.

P115 - USE OF IN VITRO CLEARANCE DATA AND PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING FOR PREDICTION OF PROPOFOL BLOOD CONCENTRATION PROFILES AND CLEARANCE

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Renal metabolism has been suggested as an important contributor to the systemic clearance of propofol [1]. Previously, predictions of in vivo clearance have not assessed the accuracy of hepatic or extrahepatic clearance in isolation. The aim of this study was to characterise in vitro hepatic and extrahepatic glucuronidation and CYP intrinsic clearance (CL_{int}) of propofol. These data were used to predict propofol in vivo clearance and blood concentrations using physiologically-based pharmacokinetic (PBPK) modelling. In vitro CL_{int} values were determined using the substrate depletion approach at $[S] << K_m$ in human liver (HLM) and kidney (HKM) microsomes in the presence and absence of 2% bovine serum albumin (BSA) and scaled using 40 and 12.8 mg protein/g tissue for HLM and HKM, respectively [1]. Generated in vitro data were implemented in a whole body PBPK model [2] using Matlab v 7.12.0. The model was validated with clinical data over a range of doses (3 to 9 mg/kg/h infusions) where blood concentration data were available up to 10 h post-dose. In addition, blood concentration data during the anhepatic phase of liver transplantation were used to assess the accuracy of renal clearance prediction in isolation. BSA increased propofol UGT CL_{int} by 3- and 11-fold in HLM and HKM [1], whereas it had minimal impact on CYP CL_{int}. In HLM, propofol CYP CL_{int} (+BSA) was approximately 2.6-fold higher than UGT CL_{int}; in contrast, CYP CL_{int} in HKM was minimal. Use of In vitro CL_{int} in the presence of BSA improved prediction accuracy compared to data obtained without BSA, but still resulted in under-estimation of clearance (predicted clearance was 34% of the observed value). The PBPK model under-predicted renal to a greater extent than liver extraction; predicted extraction ratios were 0.07 and 0.42 for kidney and liver, compared to 0.43 - 0.87 and 0.76 - 0.98 estimated from reported renal and hepatic vein concentrations in vivo. Similarly, use of in vitro renal CL_{int} data (+BSA) in isolation over-predicted

propofol blood concentrations in anhepatic patients due to a 12-fold under-estimation of clearance. Optimisation of the renal glucuronidation and subsequent use to predict clearance in intact patients significantly improved prediction accuracy, resulting in predicted clearance within 2-fold of observed values. The under-estimation of renal glucuronidation clearance observed in the current study highlights the need for further refinement of the assessment of the contribution of extrahepatic clearance mechanisms and the adequacy of current scaling factors. In addition, poor prediction of renal metabolism may suggest that well-stirred assumptions may not be appropriate for the kidney and more complex models need to be considered.

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P116 - REGIOSELECTIVITY OF METHYLATION OF LUTEOLIN AND QUERCETIN BY HUMAN CATECHOL-O-METHYLTRANSFERASE

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Background: Catechol-O-methytransferase(COMT, EC2.1.1.6) can transfer a methyl group to one of hydroxyls in catechol ring, in which there was a preference of meta-methylation in the most known COMT substrates, such as dopamine(meta-/para-:4.3) and levodopa (meta-/para-:15.4). Though catechol containing flavonoids are good substrates of COMT, there was lack of information on their regioselectivity of methylation. The present study aims to elucidate the regioselectivity of methylation of two typical flavonoids, luteolin(3',4',5,7-tetrahydroxy flavone) and quercetin(3,3',4',5,7-pentahydroxy flavone) by human COMT, and determine their meta/para methylated metabolite ratios in vitro and in vivo. **Methods:** Human recombinant COMT was used to study the formation kinetics of meta-methylation and para-methylation of luteolin/quercetin and human liver microsomes were applied to evaluate the stability of the meta-MeO- and para-MeO-luteolin/quercetin, while a series of typical inhibitors of CYPs were utilized to identify the subtype of CYPs contributing to further metabolism of meta-MeO- and para-MeO-luteolin/quercetin. The methylated metabolites of luteolin/quercetin in human urine(0-4h) collected after oral administration of 1 mg/kg luteolin or 3.8 mg/kg quercetin were analyzed with HPLC after acidic hydrolysis. **Results:** V_{max} values(nmol mg protein^{-1}min^{-1}) for formation of meta-MeO-luteolin(839.5), para-MeO-luteolin(1021), meta-MeO-quercetin(203), para-MeO-quercetin(94.6) and K_m values(μM) of meta-MeO-luteolin(0.941), para-MeO-luteolin(0.471), meta-MeO-quercetin(2.84), para-MeO-quercetin(3.47) were obtained, respectively. The ratio of meta-MeO/para-MeO of $CL_{int}(V_{max}/K_m)$ were 0.41(for luteolin) and 2.6(for quercetin). Meta- and para-MeO-luteolin/quercetin were also detected in human urine after dosing of luteolin/quercetin, but the ratio of the meta- to para-mathylated metabolite was approximate to 1. Human liver microsomes showed preferential to metabolize para-methylated luteolin/quercetin over meta-methylated luteolin/quercetin and the inhibitor of CYP1A2 (α-naphthoflavone) inhibited the metabolism of para-methylated luteolin/quercetin markedly. **Conclusion:** Quercetin showed a preference of meta-methylation by human COMT which was consistent with the most of known COMT substrates, while luteolin was a scarce substrate with a preference of para-methylation by human COMT. CYPs, especially CYP1A2, preferentially metabolized the para-MeO-luteolin/quercetin, which partially resulted in the different ratios of meta-/para- methylated metabolites between in vivo and in vitro. Our study gave new information on regioselectivity of methylaiton of flavonoids by human COMT, which deeply explained the metabolism pathway of luteolin and quercetin or other catechol containing compounds.

P117 - HUMAN CYP2C8 IS POST-TRANSCRIPTIONALLY REGULATED BY MIRNAS 103 AND 107 IN HUMAN LIVER

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CYP2C genes are transcriptionally regulated by xenobiotic nuclear receptors. We show for the first time that CYP2Cs are also regulated post-transcriptionally. Using an on-line search engine, we found potential recognition sites for micro-RNAs (miRNAs) 103 and 107 in the 3' UTR region of human CYP2C8 (CYP2C8MRE). Moreover, CYP2C8 protein

as measured by immunoblot analysis did not correlate with CYP2C8 mRNA measured by qPCR in human liver samples. Instead, the translational efficiency of CYP2C8 (protein/mRNA) was inversely correlated with the expression of miRNAs 103 and 107. When three copies of the putative recognition elements from CYP2C8 were inserted downstream of a luciferase expression reporter, transfection with precursors for miR103 or 107 into primary hepatocytes decreased luciferase activity while transfection of antisense oligonucleotides (AsOs) to silence miRNA 103 and 107 expression increased luciferase activity. As expected, there was no effect of the precursors or AsOs when three copies of the putative reverse constructs were inserted in the reporter plasmids. Moreover, when precursors for miRNA 103 or 107 were transfected into primary human hepatocytes, CYP2C8 protein was decreased, while AsOs for miRNA 103 or 107 increased the CYP2C8 protein levels as judged by immunoblots. Neither precursors nor AsOs affected CYP2C8 mRNA, indicating the effect was post-translational. Changing the miRNA levels perturbed metabolism of a CYP2C8 substrate. Putative miRNA motifs were also found in the 3Õuntranslated region of CYP2C9 and CYP2C19, suggesting the same miRNAs could regulate other members of CYP2C family albeit to a lesser degree than CYP2C8. These results clearly show that CYP2Cs are regulated post-transcriptionally by miR103 and miR107.

P118 - WNT/BETA-CATENIN PATHWAY CONTROLS CYP2E1 AND OTHER DRUG METABOLIZING ENZYME INDUCTION IN PRIMARY HUMAN HEPATOCYTES

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The liver displays a remarkable property known as functional zonation. Periportal hepatocytes (zone 1) located around the portal triad, mostly ensure gluconeogenesis and urea formation. Pericentral hepatocytes (zone 3), surrounding the efferent centrolobular vein, are implicated in glycolysis, glutamine synthesis and detoxication. Midlobular hepatocytes (zone 2), represent a less well-defined cell population. Consequently, most of the major hepatic functions exhibit different activity within the periportal and perivenous zone gradient conditioned by blood flow direction, oxygen and circulating molecules gradients and extracellular matrix composition. Recently, Wnt/b-catenin/APC (Adenomatous polyposis coli) pathway has been identified as a major regulator of this functional zonation with maximum activation in zone 3 [1]. b-catenin expression and localization is tightly regulated. In the absence of Wnt ligands stimulation through frizzled receptor, b-catenin is phosphorylated by a protein complex including glycogen synthase kinase 3b (GSK3b) leading to its degradation by the proteasome. In the presence of Wnt ligands, b-catenin remains unphosphorylated, accumulates in the cytosol, translocates to the nucleus and transactivates its target genes. Interestingly, data from the literature, based on histochemistry detection of liver tissue sections in rodent [2, 3] and human liver or hepatoblastoma [4], demonstrate that CYP2E1, CYP1A2, P450 oxydo-reductase (POR), constitutively activated receptor (CAR) and aryl hydrocarbone receptor (AhR) are mainly expressed in zone 3 of liver lobule, where Wnt/b-catenin pathway is mostly activated. In primary culture, human hepatocytes (HH) loose their zonal organization and some of their detoxication functions. We therefore decided to evaluate the impact of Wnt/b-catenin pathway activation on HH phenotype in vitro. HH were cultured in the absence or presence of b-catenin activators: recombinant mWnt3a (Frizzled receptor activator) or CHIR99021 (6-((2-((4-(2,4-dichlorophenyl)-5-(4methyl-1H-imidazol-2-yl)pyrimidin-2-yl)amino)ethyl)amino)nicotinonitrile, GSK3b inhibitor). A global transcriptional analysis using microarray approaches after CHIR99021 stimulation demonstrated that the expression of CYP2E1, CYP1A2, POR and CAR was restored. CYP2E1 protein expression and chlorzoxazone-mediated metabolism were also induced. In parallel, OATP1B3 expression and activity, and the number of bile canalicular structures were increased. Similar results were obtained using Wnt3a treatment. Wnt/b-catenin pathway appears therefore to control primary HH phenotype in vitro.

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P119 - MUTATIONAL ANALYSIS OF A PUTATIVE GLUCOCORTICOID RESPONSE UNIT OF THE RAT CYP2B2 GENE

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CYP2B genes are dramatically induced in rat and mouse liver by phenobarbital as well as by dexamethasone (DEX), a synthetic glucocorticoid and a ligand of the glucocorticoid receptor (NR3C1, GR). While phenobarbital responsiveness of CYP2B genes can only be observed in animals or in primary hepatocytes, DEX responsiveness can also be studied using established cell lines. A glucocorticoid response unit comprising several glucocorticoid response elements (GREs), GRE half-sites and putative binding-sites for accessory factors clustered in three regions within 2.5 kb of the CYP2B2 5' flank has been postulated to be responsible for DEX responsiveness in cell culture[1,2]. These regions are the phenobarbital-response unit (PBRU), a segment centered around −1.2 kb and another close to the minimal promoter. Using deletants and GRE inactivation mutants of a CYP2B2 reporter construct transfected into the human HepG2 cell line, we evaluated the roles of 8 GREs and GRE half sites in DEX responsiveness. As these cells do not contain any active GR, each construct was cotransfected with an expression vector for human GR. We show that a newly identified GRE (GRE2A) located from −1163 to −1149 is critical in conferring DEX responsiveness. Indeed, mutations inactivating this GRE completely abolished DEX responsiveness. Inactivation mutations of the two other GREs located in this cluster diminished but did not abolish DEX responsiveness of the reporter gene. Mutational inactivation of one GRE half-site located near the basal promoter also almost completely abrogated DEX responsiveness. The two other GRE half-sites located in this region appeared not to be involved. Inactivation of the 2 GRE half-sites located within the PBRU did not diminish DEX responsiveness. It is noteworthy that elements responsible for conferring DEX responsiveness in cultured cells are distributed throughout the CYP2B2 5'-flank whereas elements conferring phenobarbital responsiveness in rat hepatocytes are concentrated within the PBRU. These observations, plus the fact that DEX responsiveness is maintained in CAR-negative mice[2,3], that PB-responsiveness is maintained in GR-negative mice[4], and that phenobarbital responsiveness can only be observed in primary hepatocytes, all demonstrate that phenobarbital and DEX responsiveness of murine CYP2B genes involve distinct molecular mechanisms.

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P120 - OKADAIC ACID INHIBITS THE TRICHOSTATIN A-MEDIATED INCREASE OF HUMAN CYP46A1 EXPRESSION IN A SP3-ERK1/2-DEPENDENT PATHWAY

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The CYP46A1 gene, codes for the cholesterol 24-hydroxylase, a cytochrome P450 specifically expressed in neurons, and responsible for the majority of cholesterol turnover in the central nervous system. Previously, we have demonstrated the critical participation of Sp transcription factors in the basal expression of this gene (1), and in the CYP46A1 response to histone deacetylase inhibitors (HDACi) (2). In this study we investigated the involvement of intracellular signaling pathways in trichostatin A (TSA)-mediated induction of CYP46A1 expression. Our results show that pre-treatment of SH-SY5Y neuroblastoma cells with chemical inhibitors of the mitogen activated kinase kinase 1 (MEK1) significantly potentiates the TSA-dependent induction of CYP46A1, while inhibition of protein phosphatases by okadaic acid (OA), or overexpression of MEK1, partially impairs the HDACi effect. Immunoblotting revealed that

TSA treatment decreases ERK1/2 phosphorylation, which is reversed by pre-treatment with OA. Interestingly, chromatin immunoprecipitation assays (ChIP) revealed that OA does not affect the CYP46A1 promoter histone hyperacetylation observed upon TSA treatment, but it reverts the decrease in Sp3 binding activity to the SP-RE sites of the proximal promoter, as observed by gel-shift assay. ChIP and Re-ChIP analysis demonstrated that TSA induces the release of p-ERK1/2 from the CYP46A1 proximal promoter, while pre-treatment with OA restores the co-occupancy of Sp3-ERK1/2 in the same promoter fragments. With our work we demonstrate for the first time the participation of MEK-ERK1/2 signaling pathway in HDACi-dependent induction of cytochrome P450 gene expression, underlying the importance of this regulatory signaling mechanism in the control of brain cholesterol elimination. (1) Milagre I, Nunes MJ, Gama MJ, Silva RF, Pascussi JM, Lechner MC and Rodrigues E (2008). Transcriptional regulation of the human CYP46A1 brain-specific expression by Sp transcription factors. J Neurochem, 106: 835-849. (2) Nunes MJ, Milagre I, Schnekenburger M, Gama MJ, Diederich M and Rodrigues E (2010). Sp proteins play a critical role in histone deacetylase inhibitor-mediated derepression of CYP46A1 gene transcription. J Neurochem, 113: 418-431. This work was supported by Fundação para a Ciência e Tecnologia (Projects PTDC/SAU-GMG/64176/2006 and PEst-OE/SAU/UI4013/2011, and PhD grants SFRH/BD/41848/2007 to MJN and SFRH/BD/27660/2006 to IM).

P121 - BASAL GENE EXPRESSION OF DRUG METABOLIZING ENZYMES, TRANSPORTERS AND NUCLEAR RECEPTORS IN HEPATOCYTE-LIKE CELLS DERIVED FROM HUMAN FETAL LIVER MULTIPOTENT PROGENITOR CELLS

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Alternative sources of primary human hepatocytes (PHH) are being sought for drug metabolism testing in drug discovery and development due to limitations of PHH in terms of availability, cost and batch-to-batch variability. HuH-7, a hepatoma cell line, is commonly used as an alternative drug metabolism model since it is sustainable, stable and easy to manipulate. However, its drug metabolism phenotype is not comparable to that of PHH[1]. In recent years, scientists are exploring stem cells as an alternative source of hepatocyte-like cells (HLC). This study aims to evaluate the basal gene expression of drug metabolizing enzymes (DMEs), transporters and nuclear receptors in HLC derived from human fetal liver multipotent progenitor cells (hFLMPC) in comparison with HuH-7 and PHH. Total RNA was isolated and reverse transcribed from three batches each of HLC derived from hFLMPC and HuH-7, and two cryopreserved PHH samples. Quantitative real-time PCR was performed for 22 cytochrome P450 (CYP), Phase II, transporter and nuclear receptor genes. Dissociation curve analysis and gel electrophoresis were performed subsequently to verify the specificity of the amplification. Six reference genes were validated across all eight samples and the best pair of stably expressed genes were found to be GAPDH and TBP (M=0.4215). The geometric mean of the C_q of GAPDH and TBP was used for normalization. The fold change in mRNA expression levels of each target gene were compared against the average ΔC_q from the two PHH samples and calculated using the $\Delta\Delta C_q$ method. The mRNA expression levels of all genes except BCRP, GSTM1 and PXR in HLC were higher, ranging from 2.8- to 103-fold, compared to HuH-7. However, in comparison with PHH, the mRNA expression levels of all genes in HLC were markedly lower except for NAT1 (1.07-fold) and P-gp (1.82-fold). HuH-7 expressed 1.95-fold of BCRP compared to PHH which could possibly be attributed to its cancerous origin. A principal component analysis (PCA) revealed the distinct clustering of HLC, HuH-7 and PHH that was characteristic of each genotype ($R^2(X) = 0.958$, $Q^2(cum) = 0.691$). Although the HLC samples were derived from three different fetal sources ranging from 17-22 weeks of gestational age, their tighter clustering within the PCA scores plot suggested a greater degree of homogeneity in terms of genotype as compared to adult PHH. A partial least square discriminant analysis further revealed that NAT2, CAR, BCRP and CYP2D6 contributed significantly to the class differentiation between HLC and PHH (VIP>1.20). The superior DME, drug transporter and nuclear receptor profile of HLC in comparison to HuH-7 and its minimal batch-to-batch variability hold promise that HLC would serve as a good alternative to hepatoma cell lines for drug metabolism testing. While the metabolic genotype of HLC is distinct from PHH and further optimization of HLC differentiation may be warranted, the functional drug metabolism phenotype of the HLC model should nevertheless be established.

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P122 - REGULATION OF PXR FUNCTION BY POST-TRANSLATIONAL MODIFICATION

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[Purpose] Human UDP-glucuronosyltransferase (UGT) 1A1 is a critical enzyme responsible for detoxification and metabolism of endogenous and exogenous lipophilic compounds such as potentially neurotoxic bilirubin. We have found that CDK inhibitor roscovtine markedly stimulated the expression of UGT1A1 mRNA and protein, whereas it slightly stimulated the expression of CYP1A1, CYP2B6, and CYP3A4 mRNAs and proteins in HepG2 cells. In the present study, we investigated how the expression of UGT1A1 is enhanced by roscovitine in a ligand-independent manner, that is, through post-translational modification of PXR linked to cell signals. [Methods] To investigate the transactivation mechanism of UGT1A1 induction by roscovitine, we investigated the transcriptional activities of distal (-3570/-3180) enhancer motif linked to proximal (-165/-1) promoter motif placed in front of the reporter luciferase gene in HepG2 cells in the presence of exogenously expressed CAR, PXR, or AhR/ARNT. Further, HepG2 cells transfected plasmids expressing CAR, PXR (wild-type and phosphomimetic and phosphodeficient mutants), and AhR/ARNT were stimulated by a ligand-dependent manner (rifampicin) or a ligand-independent manner (roscovitine). Expression and localization of drug-metabolizing enzymes and nuclear receptors was analyzed by Western blot and RTQ-PCR, or using a confocal microscope. Association with nuclear receptor-associated proteins in the cytoplasm and the nucleus was analyzed using coimmunoprecipitaion and plasmids expressing YFP-nuclear receptor fusion protein. [Results and Discussion] PXR-mediated transactivation of the reporter gene in the absence of rifampicin was enhanced by roscovitine in a dose-dependent manner and peaked at 5×10^{-6} M roscovitine. PXR-mediated transacriptional activity of the reporter gene in the absence of rifampicin by roscovitine was more prominently enhanced, compared with the basal-, CAR-, and AhR/ARNT-mediated transacriptional activities. To determine whether the regulatory mechanism of UGT1A1 gene expression through PXR stimulation by roscovitine is different from that by PXR ligand rifampicin, we were prompted to investigate effects of phosphomimetic mutations of PXR on UGT1A1 and CYP3A4 gene expression in the cells stimulated by roscovitine and rifampicin. Phosphomimetic mutations at positions T57, T290, S350, and T408 attenuated the induction of UGT1A1 and CYP3A4 mRNAs by roscovitine, while phosphorylation of the residues T57, T290, S305, and T408 was involved in the suppression of PXR activation in the cells stimulated by rifampicin. Transfection with anti-CDK2 siRNA but not anti-CDK1 and CDK5 siRNAs led to stimulated expression of UGT1A1. Phosphomimetic mutant at S350 of PXR was detected in the nucleus, and co-transfection with co-activator SRC-2 but not SRC-1 recovered the PXR activity. T290D- and T408D-mutated YFP-PXR fusion proteins retained in the cytoplasm and were not translocated to the nucleus of the cells stimulated with roscovitine. Coimmunoprecipitation analysis revealed the altered extent of binding of the mutated PXR with PXR-retaining protein in the cytoplasm and with HDAC in the nucleus. [Conclusions] These results indicate that phosphorylation at positions T290 and T408 by PKA/PKC retained PXR protein in the cytoplasm, and that roscovitine stimulated expression of UGT1A1 and CYP3A4 through inhibiting CDK2, which phosphorylated PXR at S350 to suppress the transactivation in the nucleus, that is, the binding of PXR with RXR and the deacetylation of PXR.

P123 - DETERMINATION OF CELLULAR SIGNALING PATHWAYS MODULATED BY EXPOSURE OF HUMAN INTESTINAL CELLS TO CYLINDROSPERMOPSIN

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Cylindrospermopsin (CYN) is a cyanotoxin produced by some cyanobacteria strains. Due to eutrophization, these microbes are able to massively proliferate resulting in a significant release of CYN in freshwater. This phenomenon leads to a population health problem associated with this toxin because of consumption of contaminated food (seafood and vegetables) and ingestion of contaminated water, also via the drinking water during swimming and other water activities. Although some studies indicated that CYN could inhibit protein synthesis, information concerning the mechanisms of action associated with toxic effects caused by this toxin remains unclear. The objective of this study was to investigate the cellular signaling pathways modulated by exposure of human intestinal cells to CYN. We performed a transcriptomic experiment using whole-genome DNA microarrays. We used Caco-2

cells as a human intestinal cells model. The differentiated Caco-2 cells were exposed for 24 h at 1.5 µM of CYN. After extraction of total RNA, nucleic acids were labeled with Cyanine 3 and hybridized on slides. Compared with the control, only genes with expression level above 2 or below 0.5 with a P value less than 0.05 were selected. Using these selected genes, an analysis of biological functions modulated by exposure to CYN was firstly performed, and secondly an individual analysis of genes expression was also included. The results indicated that 522 genes had their expression increased and 50 genes had their expression decreased significantly. The later analyses were only performed on overexpressed genes. The results indicated that exposure to CYN resulted in an upregulation of the transcriptional activity. Individual analysis of genes indicated that they also encoded proteins involved in DNA recombination. Our results on modulated functions and individual genes expression were consistent and indicated that exposure to CYN resulted in a strong upregulation of gene expression mechanisms.

P124 - REGULATION OF P2Y12 EXPRESSION BY TESTOSTERONE IN MAGAKARYOTIC DAMI CELLS

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P2Y12 is an important G protein-coupled receptor that is involved in ADP-induced platelet aggregation, which is essential for normal haemostasis. Gender differences in the incidence of cardiovascular disease have been proposed to be linked to the effects of sex hormones on cardiovascular-related genes. We examined the influences of testosterone and 17β-oestradiol on P2Y12 gene expression in megakaryocytic DAMI cell line. Altered levels of P2Y12 mRNA, protein, and the cAMP-dependent vasodilator-stimulated phosphoprotein-Ser157 (VASP-Ser157) phosphorylation were investigated after treatment with 17β-oestradioal or testosterone as compared to the control groups. Quantitative Real-Time PCR revealed that the P2Y12 mRNA levels were increased by testosterone in a dose-dependent manner, whereas 17β-oestrodiol had no effect on P2Y12 gene expression. Induction of the P2Y12 protein by testosterone was found in Western blots of the proteins isolated from testosterone-treated cells. Testosterone-mediated P2Y12 expression was repressed at both the transcriptional and translational levels by the anti-androgen receptor bicalutamide. Treatment with testosterone also resulted in a decrease in the level of VASP-Ser157 phosphorylation, as compared to the control group. The decrease in the level of VASP-Ser157 phosphorylation was reversed by bicalutamide. These findings suggest a novel pathway for testosterone regulation of P2Y12 expression in a megakaryocytic DAMI cell line. Further studies using primary human megakaryocytes and platelets could be necessary to know the effect of hormones on the P2Y12 expression in circulating platelets.

P125 - BONE-SPECIFIC DELETION OF CYTOCHROME P450 REDUCTASE IN MICE RESULTS IN ANTLEY-BIXLER SYNDROME-LIKE CRANIOFACIAL AND BONE MASS DEFECTS: POSSIBLE ROLE OF FIBROBLAST GROWTH FACTOR SIGNALING

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NADPH-cytochrome P450 oxido-reductase (POR) is the only known electron donor for cytochromes P450, dehydrocholesterol reductase, and heme oxygenase. Genetic screening of patients revealed the presence of mutations in POR, severe forms of which lead to developmental defects including sexual ambiguities and various bone defects, such as craniosynostoses and mid-face hypoplasia, originally described by Antley and Bixler (1975). The POR deficiency patients also present with ambiguous genitalia and abnormal steroidogenic profiles. Complete deletion of POR in mice leads to embryonic lethality. To probe the possible roles played by POR during bone development, we have generated conditional knockout (CKO) mice by crossing $Cpr^{lox/lox}$ and Dermo1 Cre mice. Since the Dermo1 Cre recombinase is active in mesenchymal cells (from 9.5 dpc), POR is deleted in both chondrocytes and osteoblasts in the CKO mice. Here we report the first mouse model showing craniofacial abnormalities upon deletion of POR in bone. Differential staining of CKO mouse skulls shows premature fusion of spheno-occipital synchondroses and anterior intraoccipital synchondroses in the base of the skull. Type III tooth malocclusion was noticed in adult CKO mice. Micro CT analysis of the long bones showed that deletion of POR leads to a reduction in the trabecular

bone mineral density compared to age- and sex-matched littermate controls. No apparent defect was noticed in the cortical bone thickness. As fibroblast growth factor (FGF) signaling plays a critical role in skeletal development and mutations in FGF receptors are associated with syndromes exhibiting skeletal abnormalities, including craniosynostoses, we probed for proteins in the FGF signaling pathway using bone sections from wild type and CKO litter mates. Preliminary data suggest that deletion of POR increases the pAkt level and the downstream Sprouty2 level. Sprouty2 has an inhibitory effect on FGF signaling. Gli2, a zinc finger protein acting as a transcription factor downstream of FGF signaling, is decreased in the CKO mice compared to wild type controls. These data suggest a possible connection between POR activity and FGF signaling, recapitulate the human POR deficiency phenotype and offer an approach to study the sequelae of POR mutations.

P126 - THE ARYL HYDROCARBON RECEPTOR MEDIATES INCREASES IN EICOSANOID LEVELS ELICITED BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN SEVERAL ORGANS OF THE MOUSE

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Eicosanoids are derivatives of twenty carbon polyunsaturated fatty acids, particularly arachidonic acid (AA). Equivalent derivatives of eighteen and twenty-two carbon unsaturated fatty acids, such as linoleic (LA) acid and docosahexaenoic acid (DHA), may have similar biological activities. Arachidonic acid (and the other aforementioned polyunsaturated fatty acids) are metabolized via three pathways: the cyclooxygenase, lipoxygenase and cytochrome P450 epoxidation/hydroxylation pathways (although cytochromes P450 are or can be involved in all three pathways). The least is known about the last pathway. Many mammalian cytochromes P450 including CYP1A1, CYP1A2 and CYP1B1 exhibit arachidonic acid epoxidation and/or hydroxylation activities. The immediate products of the epoxidation/hydroxylation pathway of arachidonic acid metabolism include four cis-epoxyeicosatrienoic acids (EETs), the “terminal” hydroxides, 16-HETE, 17-HETE, 18-HETE, 19-HETE and 20-HETE, and certain “midchain” hydroxides, including 5-HETE, 8-HETE, 12-HETE and 15-HETE. The midchain hydroxides are also products of the lipoxygenase-catalyzed metabolism of arachidonic acid. Several of the cis-epoxyeicosantrienoic acids and hydroxides, including some of the terminal hydroxides (particularly 20-HETE), exhibit potent biological activities. 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) elicits a wide variety of toxic, teratogenic and carcinogenic responses in animals and in humans. Most if not all the effects of TCDD are mediated by the aryl hydrocarbon receptor (AHR), which after binding TCDD, activates transcription of a large number of genes, among which CYP1A1, CYP1A2 and CYP1B1 are generally the most inducible. Many if not most of the biological effects of the AHR are probably mediated by its effect on these transcriptional responses. We report here a “lipidomics” approach to quantitate up to twenty-three eicosanoids and three polyunsaturated fatty acids in five different organs/tissues from TCDD-treated and untreated mice. Our results demonstrate that the levels of eicosanoids derived from the cytochrome P450-dependent epoxidation/hydroxylation pathway were widely and markedly increased by TCDD treatment, and that the levels of some of mid-chain hydroxides and other metabolites generally considered to be products of the lipoxygenase pathways were also increased, although there were differences in these regards between organs/tissues. TCDD also increased the levels of the esterified forms of the eicosanoids in the liver in parallel with the corresponding free forms. Products of the cyclooxygenase pathway were generally not affected by TCDD treatment. Analysis of an Ahr-/- null mouse demonstrated that the changes in eicosanoids levels elicited by TCDD are dependent upon the AHR. TCDD increased the mRNA levels of CYP1A1, CYP1A2, CYP1B1 and the PLA2G12A form of phospholipase A2 to varying degrees in the different organs, and these increases correlated with some but not all the changes in eicosanoids levels in the organs. In the absence of TCDD, wild-type and AHR-/- knockout mice exhibited similar levels of the eicosanoids, indicating that the alterations in phenotype of the Ahr null in the absence of TCDD are not ascribable to changes in eicosanoid levels. Altogether, these studies lay the foundation for future experiments addressing the potential role of eicosanoids in mediating the toxic effects of TCDD and other ligands of the AHR.

P127 - METABOLOMIC APPROACHES TO GASTRIC CANCER

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Gastric cancer is one of the most common malignant tumors and the second leading cause of cancer-related death worldwide, but useful biomarkers for prognosis and pathological advance are still unavailable. In this study, nontargeted metabolomic analyses were performed on plasma collected at the different cancer stages using liquid chromatography in combination with mass spectrometry. Metabolite profiles were compared focusing on molecules that were associated with gastric cancer development. The plasma levels of metabolites associated with lipid, aromatic amino acids (tyrosine and tryptophan) and nucleoside were notably changed in malignant gastric cancer. Especially, alteration in endocannabinoid, neuromodulatory lipids may be pathophysiological characteristics in gastric cancer. The change of aromatic amino acid metabolism can be closely related to variation of the neurotransmitters like epinephrine in patients, which affects gastric function. Furthermore, the plasma concentration of the fragment peptide derived from gastrin was changed in malignant cancer patients, which implies that endocrine systems may play a critical role in cancer pathology. According to advancement of gastric cancer, the metabolites related to fatty acid oxidation were prominently affected, which implies that energy metabolism can be changed in gastric cancer progress. As these endogenous metabolite profiles were obtained by comparing the plasma metabolites among the patients at the different stage, they could be characteristic for gastric cancer advancement. Additionally, these metabolites could be used as biomarkers for gastric cancer and could provide valuable tools for studying the mechanism(s) on pathophysiology of gastric cancer.

P128 - MICRORNAS AS BIOMARKERS OF LIVER INJURY: PROFILES IN PLASMA AND LIVER FROM RAT MODELS

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Acute liver injury is classified into three types: hepatocellular injury, cholestasis, or mixed type. Chronic liver injury is a progressive disease showing increasing severity such as steatosis, steatohepatitis, fibrosis, cirrhosis and cancer. For the diagnosis of liver injury, ALT, AST, ALP and T-Bil in the blood are commonly used. However, these parameters cannot thoroughly identify the type of liver injury. In addition, these parameters may show increases with extrahepatic injury such as muscle damage or cardiac injury. Moreover, ALT is not always correlated well with the histomorphologic data of liver. In this study, we compared the changes of the plasma miRNA expressions by acute liver injury (hepatocellular injury or cholestasis) and chronic liver injury (steatosis, steatohepatitis and fibrosis) using rat models made by the administration of chemicals or special diets in order to evaluate whether plasma miRNAs can be markers that can distinguish the different types of liver injury. We found that the plasma miRNA expressions were dramatically changed in rats with acute liver injury, whereas they were relatively mild in rats with chronic liver injury. Interestingly, the expression profiles were different between the acute and chronic injuries, and further between the pericentral and periportal hepatocellular injuries. We identified miRNAs whose expression was typically changed in each type of liver injury. It is notable that, in acute liver injury models, the plasma level of miR-122, the most abundant miRNA in the liver, was more quickly and dramatically increased than the plasma ALT level, reflecting the extent of hepatocellular injury. A previous report suggests that the increased miRNAs in plasma would be originated from the damaged cells, that is, the miRNA levels in liver were decreased. To assess the expression profile of miRNAs in liver, we isolated the pericentral or periportal region of liver from the rats with hepatocellular injury by laser microdissection. Interestingly, we found that the levels of a set of miRNAs in control rats were different between the pericentral and periportal region. In hepatocellular injury models, the changes of hepatic miRNA expression were not limited at injured region, and half of miRNAs whose expression was dramatically changed in plasma were not changed in liver with hepatocellular injury. Taken together, plasma miRNA profiles would be good biomarkers of liver injury, although the profiles would not simply reflect the changes of profiles in liver.

P129 - IDENTIFICATION OF NOVEL TRANSLATIONAL URINARY BIOMARKERS FOR ACETAMINOPHEN-INDUCED ACUTE LIVER INJURY BY PROTEOMIC PROFILING

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The leading cause of acute hepatic damage is drug-induced liver injury (DILI). Currently, there is a lack of adequate predictive biomarkers for DILI. Here, we describe a translational approach using proteomic profiling for identification of urinary proteins related to acute liver injury induced by acetaminophen (APAP). Male FVB mice were given a single intraperitoneal dose of APAP (0-350 mg/kg bw; n=6-30 per dose), followed by 24 hour urine collection. Subsequently, animals were sacrificed for collection of plasma and kidney and liver tissue. Additionally, two urine samples were collected from a patient with APAP intoxication. Urine samples were profiled using matrix associated laser desorption/ionization (MALDI) time-of-flight mass spectrometry. Differential protein masses were identified with MALDI LTQ and LC/MS-MS techniques and confirmed using specific antibodies. APAP treatment in mice resulted in centrilobular hepatic necrosis and a dose-dependent increase in plasma alanine aminotransferase (ALT) levels ($p<0.0001$). Proteomic profiling resulted in the identification of twelve differential urinary proteins, including superoxide dismutase 1 (SOD1), carbonic anhydrase 3 (CA3), and calmodulin (CaM) as novel biomarkers for APAP-induced liver injury. Urinary protein levels of SOD1 and CaM were closely associated with increasing plasma ALT levels ($p<0.001$ and $p<0.01$, respectively). This is in accordance with recent evidence that SOD1 and CaM mediate early events causing APAP-induced liver injury. Moreover, hepatic mRNA expression of sod1 and calm2 were significantly decreased with increasing APAP-induced liver injury ($p<0.05$), probably reflecting a defense mechanism to prevent aggravation of liver injury. Importantly, we showed the presence of CA3, SOD1 and CaM in human urine after APAP intoxication. Using a translational proteomics approach we have identified CA3, SOD1 and CaM as potential non-invasive urinary biomarkers for human APAP-induced liver injury.

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P130 - AN SIRNA SCREEN IDENTIFIES NOVEL KINASES AND UBIQUITINASES THAT CONTROL SYNERGISTIC PRO-APOPTOTIC RESPONSES BY HEPATOTOXIC DRUGS AND TNFA

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Drug-induced liver injury is an important clinical problem, which involves cytokine-mediated signaling. In hepatocytes TNFα synergizes with various hepatotoxic drugs (e.g. diclofenac, carbamazepine and ketokonazole) that all cause idiosyncratic liver failure in the clinic. We demonstrated that this synergy is related to perturbations of TNFα-mediated NF-κB signaling. Hepatotoxic drugs reduced the amplitude and frequency of NF-κB cytoplasm-to-nucleus oscillation, while knockdown of p65/RelA sensitized against this drug/TNFα liver cell death. Gene expression profiling identified Nrf2 signaling, endoplasmic reticulum (ER) stress responses and death receptor signaling as key components of the cellular stress response caused by hepatotoxic drugs. Follow-up studies confirmed the functional relevance of these pathways in the control of the drug/TNFα pro-apoptotic synergy. Since cellular stress responses modulate the post-translational protein modification, mostly through phosphorylation or ubiquitination, and thereby protein activity/function, the identification of novel critical kinases and ubiquitinases that mediate the drug/TNFα synergistic pro-apoptotic response is essential. We performed a siRNA-based knockdown screen for drug/TNFα hepatocyte cell killing using diclofenac as a model drug. Out of ~1600 kinases/ubiquitinases evaluated, we identified 92 candidate genes that strongly affected cell survival: 8 genes for TNFα alone, 32 genes for diclofenac alone, and 52 genes with diclofenac/TNFα combination. We will further discuss follow-up studies on the role of these candidate genes in controlling the cellular stress response as well as the TNFα-mediated pro-apoptotic and NF-κB signaling. We propose that these candidate genes are likely critical determinants in the clinical susceptibility for idiosyncratic drug-induced liver injury and/or other liver injury responses that involve cytokine signaling, including liver carcinogenesis.

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P131 - NRF2 IN THE ADAPTIVE RESPONSE TO CHEMICAL STRESS – DEVELOPMENT OF NOVEL BIOMARKERS OF CHEMICALLY-INDUCED STRESS

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Nrf2 is a transcription factor that regulates the expression of a number of cytoprotective proteins including antioxidants and enzymes involved in conjugative drug metabolism and glutathione synthesis. Nrf2 knockout (KO) mice have been shown to be more susceptible to the toxicity associated with a number of different drugs in a variety of organs. However there are currently no biomarkers that can be used to assess the importance of Nrf2 in drug toxicity in man. By use of the Nrf2 KO mouse model together with a pharmacological Nrf2 induction model using the triterpenoid CDDO-Me (methyl-2-cyano-3, 12-dioxooleana-1, 9-diene-28-oate), we aimed to investigate the proteomic and metabolomic profiles of Nrf2 modulation in order to identify candidate biomarkers of chemical and oxidative stress. In a previous study in our lab, proteomic analysis using isobaric tags for absolute and relative quantitation (iTRAQ) identified a number of proteins that were differentially expressed in the livers of WT and Nrf2 KO mice[1]. While expression of enzymes associated with glutathione metabolism was significantly down-regulated in Nrf2 KO mice, enzymes in the fatty acid metabolism pathway were identified as the group of proteins most significantly up-regulated in the absence of the Nrf2 gene. In our present study, fatty acid profiling using GC-FID showed that Nrf2 deficiency resulted in an increase in the majority of fatty acids in the livers of the Nrf2 KO mice, including a 3-fold increase in palmitic acid and a 2-fold increase in oleic acid. Western blot analysis of liver homogenates from WT mice treated with 3mg/kg CDDO-Me identified a significant increase (2-fold; $P<0.001$) in the expression of the Nrf2 regulated protein NAD(P)H:quinone oxidoreductase (NQO1), and a concurrent decrease (1.8-fold; $P<0.05$) in ATP citrate lyase (ACL), a protein key for fatty acid synthesis. No significant difference was seen in the expression of either protein in CDDO-Me treated Nrf2 KO mice. Work to perform iTRAQ analysis on the livers of CDDO-Me treated WT and Nrf2 KO mice is currently ongoing. Results to date support the emerging evidence for a role of Nrf2 in the regulation of lipid metabolism and suggest that proteins and metabolites of the drug metabolism and fatty acid pathways have the potential to serve as biomarkers of Nrf2 modulation. The potential translational utility of such biomarkers will subsequently be investigated using freshly isolated human hepatocytes.

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P132 - THE DEVELOPMENT OF A METHOD FOR IN-SOLUTION HYBRID CAPTURE OF BISULFITE CONVERTED DNA FOR TARGETED BISULFITE SEQUENCING OF 175 ADME GENES

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DNA methylation is one of the most important epigenetic alterations involved in the control of gene expression. A gold-standard method for the study of DNA methylation at a single nucleotide resolution utilizes Bisulfite DNA treatment followed by sequencing. Next-generation sequencing of bisulfite-converted DNA is the method of choice to investigate DNA methylation profiles at the genome-wide scale. Sequencing of hundreds of human methylomes however, remains an expensive procedure. Furthermore, researchers often prefer to investigate a subset of genes of interest. Thus, the use of DNA target enrichment techniques together with bisulfite conversion of DNA can allow epigenetic analysis of a focused region of the human genome. Currently, such methods are poorly reported in the literature due to difficulties arising from bisulfite converted DNA. We developed a novel protocol for the hybrid capture of bisulfite converted gDNA using custom Agilent SureSelect Target Enrichment system. In the pilot experiment, we initiated the protocol with 4 different gDNA samples isolated from adult human livers and prepared 4 bisulfite-converted NGS libraries, which were enriched for 4 Mb genomic region of interest (covering non-repetitive intervals of coding and regulatory regions of 175 ADME genes). Sequencing of these target enriched bisulfite libraries on Illumina HiSeq2000 allowed us to reliably quantify methylation levels of more than 50.000 CpG sites in the selected genes. Preliminary data shows that specific CpG sites are variably methylated among individuals and this variability is found in almost all ADME genes assessed. Such variable DNA methylation patterns can provide

valuable information regarding ADME gene regulation and aid in understanding interindividual differences in drug metabolism and transport.

P133 - VALIDATION OF THE ZEBRAFISH EMBRYO AS AN ALTERNATIVE MODEL FOR HEPATOTOXICITY TESTING

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Hepatotoxic properties of compounds are traditionally tested using in vivo animal models, which have a number of ethical, economic and scientific disadvantages. Development of alternative systems for hepatotoxicity is therefore imperative. One of the main purposes of this project is to validate the zebrafish embryo (ZFE), which is considered as a non-licensed animal model until 5 days post fertilization as an alternative testing model for hepatotoxicity. To this end, ZFEs were exposed to 12 model hepatotoxicants/controls, including those inducing three defined phenotypic endpoints, necrosis, steatosis, and cholestasis. After histopathological characterization and cross-validation against the phenotype induced in adult zebrafish liver, toxicogenomic profiles of the individual compounds were generated. These expression profiles were compared to the profiles obtained in other alternative models, including the adult zebrafish, mice and rats. Preliminary analysis after Cyclosporine A exposure suggested that the hepatotoxic expression profile in the ZFE match better with that in the mouse liver than profiles obtained in human hepatocyte cell lines (HepG2 and HepaRG), which are another alternative model under investigation. Finally, combined analysis of RNA - deep sequence technology and microarray results may define a limited set of expression markers that when combined may predict hepatotoxicity. These markers will be considered for generation of a transgenic reporter zebrafish.

P134 - EXPLORING THE INTERACTION OF HOST AND GUT MICROBIOME IN THE TOXICOKINETICS OF TACRINE

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Gut microbiome is known to possess a diverse range of biological activities and influences the metabolism of drugs. Mechanistically, the gut microbiome could affect drug disposition by direct metabolism of the xenobiotics[1] or indirect interaction with the host enzymatic system through metabolites of gut microbial or mammalian-microbial co-metabolic origin.[2] Such interactions between the host and gut microbiome may culminate in wide-ranging metabolic and toxicological consequences. Tacrine (THA) was the first drug marketed for the treatment of Alzheimer's disease but it lost favor due to its high incidence of hepatotoxicity and the emergence of safer and more efficacious alternatives. Large inter-individual variability in THA's disposition profile and susceptibility to THA-induced transaminitis was observed clinically and preclinically but little is known with regards to the contribution of the host-gut microbiome interaction on this phenomenon.[3] In this study, we investigated the interaction of host and gut microbiome and its effect on the toxicokinetic (TK) profiles of THA-dosed Lister hooded (LH) rats. An acute dose of THA (20 mg/kg) was administered to male LH rats (n=28) that were monitored over 7 days. Clinical chemistry measurements were performed to assess for THA-induced hepatotoxicity. LC/MS/MS was used to determine the in vivo TK profile of THA-dosed LH rats. Global GC/TOFMS metabonomics was applied to profile the urinary metabolome of THA-dosed rats and screen marker metabolites of host, gut microbial or co-metabolic origin that were predictive of the metabotypes associated with toxicity and disposition. Large inter-individual variability in toxicological susceptibility and TK profiles of THA-dosed rats was observed. Significant elevation ($p<0.05$) in aspartate aminotransferase (AST) revealed manifestation of hepatotoxicity in THA-dosed rats where twelve of the twenty-eight THA-dosed rats displayed AST more than three times the mean of VEH-dosed rats. The twelve rats were further classified as extreme, moderate or mild responders depending on the duration of transaminitis. Extreme responders were found to have poorest THA clearance (CL= 1.27±0.34 L/h) and highest maximum plasma THA concentration (C_{max}= 0.64±0.18 mg/L). Principle component analysis (PCA) of urinary metabolome within the period of 8-24h post-treatment where the highest aminotransferase (AST and alanine aminotransferase (ALT)) elevation was detected, revealed distinct clustering of responder groups (R^2X=0.567 and Q^2(cum)=0.332). Combinatorial analyses of partial least squares discriminant analysis (PLS-DA) (R^2X=0.697, R^2Y=0.975 and Q^2(cum)=0.829) and Pearson correlation ($r>0.60$) uncovered a list of marker metabolites that characterized the responder metabotypes

and correlated strongly with AST/ALT elevation. Marker metabolites of microbial and co-metabolic origin (e.g. indoleacetic acid, nicotinamide and fumaric acid) suggested the involvement of host and gut microbiome interaction on the TK profile of THA-dosed LH rats. Our study suggested that the interaction between host and gut microbiome plays an important and profound role in determining the TK profiles of drugs such as THA.

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P135 - GLOBAL GAS CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETRY (GC/TOFMS)-BASED METABOTYPING OF LYOPHILIZED HUMAN FECES

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Gas chromatography mass spectrometry (GC/MS)-based metabonomics is a powerful “hypothesis-free” system biology approach for the profiling of endogenous metabolic biomarkers of human diseases. In the characterization of lower gastrointestinal tract (GIT) diseases, such as ulcerative colitis, Crohn’s disease and colorectal cancer, feces represents a viable biological matrix (1). Its direct contact with and transient stay in the colon and rectum enriches important molecular information that provides insights on the health/disease status of the lower GIT and unique metabolic interaction between the host and symbiotic microbes. While GC/MS-based metabolic profiling of fecal water has been reported (2), the profiling of complete fecal material has not been explored. In addition, it remains unclear the influence of blood in stool on the fecal metabotype. In this study, a gas chromatography/time-of-flight mass spectrometry (GC/TOFMS) method was developed for the global metabonomic profiling of human feces. Fecal and fecal water metabotypes were profiled and compared. In addition, the effects of occult (1mgHb/g feces) and gross (100mgHb/g feces) levels of human blood on the fecal metabotype were investigated. In our optimized method, each lyophilized fecal sample (80mg) was ultra-sonicated with 1 mL of extraction solvent [methanol/water in the ratio of 80:20 (v/v)] for 30 min, followed by centrifugation, collection of supernatant, drying and derivatization using N-methyl-N-trifluoroacetamide (MSTFA) for 45 min. A correlated polynomial relationship between integral peak response and the concentration of fecal extracts was established (R^2=0.9635). The detected fecal metabolites ranged from endogenous metabolites associated with host, gut microbes and microbial-host co-metabolism to exogenous xenobiotics. Lyophilized feces demonstrated a more comprehensive metabolic coverage compared to fecal water, particularly a higher number of non-polar metabolites were detected in the former. Unsupervised principal component analysis (PCA) revealed a clustering trend between neat feces and feces spiked with occult blood (1mgHb/g feces), suggesting that occult blood exerts a negligible effect on the fecal metabotype. On the contrary, feces spiked with gross level of blood (100mgHb/g feces) were clearly distinct from neat feces in the PCA scores plot (R^2X=0.868 and Q^2(cumulative)=0.744), confirming the potential confounding effect of gross GIT bleeding on fecal metabotype. Based on partial least square discriminant analysis, discriminating metabolites related to blood in feces were further uncovered (e.g. elevated serine, proline and glycine and decreased propanoic acid, 2-phenylacetic acid and 3-hydroxybutyric acid). In conclusion, a novel GC/TOFMS method was developed for the metabonomic profiling of lyophilized human feces where its metabotype was confirmed to be more comprehensive than fecal water. For the first time, we elucidated the effect of occult and gross bleeding on the fecal metabotype. This finding is pertinent as it would guide future interpretation of fecal metabonomic data, particularly in GIT diseases where bleeding is prevalent such as colorectal cancer.

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P136 - GLOBAL PROTEOMIC ANALYSIS OF HUMAN LIVER WITH FOCUS ON ADMET PROTEIN EXPRESSION

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ADMET-proteins play an important role in the absorption, distribution, metabolism, excretion and sometimes also in the toxicity of xenobiotic and endogenous compounds. Quantitative information on the expression levels of these proteins can help to improve the understanding and prediction of the pharmacokinetics and safety profiles of new drugs. Here, we determined the global protein expression levels in membrane fractions of human liver (n=12) and isolated human hepatocytes (n=8) using a filter-aided sample preparation (FASP) based proteomics approach.[1] Out of more than 4000 proteins detected in the mass spectrometric analysis of the liver samples, 173 ADMET proteins were identified, including 37 cytochrome P450 (CYP) enzymes and 30 transport proteins. As expected, there was a good correlation between the expression levels of ADMET proteins in human liver and in human hepatocytes (r^2= 0.86). Cell specific markers (CD68 for Kuppfer cells, von Willebrand factor for endothelial cells, and CK19 for cholangiocytes) indicated a proper isolation of hepatocytes without contamination of other cell types. The expression levels of important phase I metabolizing enzymes were compared and CYP2C9 was identified as the most highly expressed CYP enzyme in the liver samples, which is in line with a recent publication. For CYP2C9 and CYP2C8, six individuals had much higher expression levels of both these enzymes than the other group of individuals. This could be a result of polymorphisms in these genes or, more likely, induction by e.g., medication or food since their gene expressions are regulated by the same nuclear receptors. CYP2D6 also displayed a high (n=4) and low expressing group of individuals (n=8). This is consistent with the fact that CYP2D6 is known to be highly polymorphic resulting in variations in the metabolic capacity of the enzyme. Among the transporters that are important for drug uptake and efflux, organic anion transporting polypeptide (OATP) 1B1 and bile salt export pump (BSEP) were identified as the most highly expressed uptake and efflux transporter, respectively. The inter-individual variability in OATP1B1 expression, displayed a four-fold difference. Interestingly, OATP1B3 was identified as one of the transporters with the largest inter-individual variation in abundance, revealing a 30-fold difference between the lowest and the highest expression level. The data obtained in this project is currently used to quantify and improve predictions of drug transport processes and drug-drug interactions in the human liver. In our first study, the contribution of three OATPs (OATP1B1, OATP1B3 and OATP2B1) to atorvastatin uptake and inhibition in the human liver was predicted.[2]

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P137 - EXPRESSION OF CYTOCHROME P450 3A4 AT MRNA LEVELS AND DNA DAMAGE INDUCED BY AFLATOXIN B1 IN HEPATOCYTE-LIKE CELLS AND THEIR PROGENITOR MESENCHYMAL STEM CELLS AND CD34+ CELLS ISOLATED FROM HUMAN UMBILICAL CORD BLOOD

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Despite the progress in differentiation process and characterization of specialized cells derived from stem cells, the changes in drug metabolizing system during hepatogenic differentiation is not fully understood. In the present study, hepatogenic differentiation of mesenc hymal stem cells (MSCS) isolated from human umbilical cord blood (UCB) was induced in presence of specific growth factors and differentiation was monitored by measuring liver specific markers such albumin and alpha-fetoprotein (AFP). Differentiation was carried out in presence of aflatoxin B1 (AFB1) and expression levels of cytochrome P450 3A4 (CYP3A4) as well as DNA damage was estimated by Comet assay. The results showed that the expression CYP3A4 gene at the mRNA levels on days 0 and 14 was assessed in differentiated hepatocyte-like cells and their non-differentiated state by reverse transcription polymerase chain reaction (RTPCR). There were no detectable levels of CYP3A4 in non-differentiated MSCs and CD34+, whereas it was predominantly expressed in hepatocyte-like cells recovered on day 15 of differentiation. Expression of the enzyme

was associated with the extent of DNA damage in cells before and after differentiation. DNA damage in cells treated with AFB1 was measured in terms of Arbitrary Units (AU) and the % DNA in the tail in a comet assay. The background level of DNA damage was always significantly lower in control (untreated) cells than in those treated with AFB1. The maximum level of DNA in the tail was found to be 11% in non-differentiated MSCs (35.5AU). Exposure of either cell type to AFB1 caused a dose-dependent increase in DNA damage, as judged by visual scoring of comets. The DNA damage due to AFB1 was greater in CD34+ cells (3–5 fold) and MSCs (2.5–3.5 fold) than in the corresponding controls. The DNA damage caused by AFB1 in CD34+ stem cells was also greater than that measured in MSCs. AFB1-induced DNA damage in hepatocyte-like cells and their progenitor stem cells showed that the differentiated MSCs were 1.5–2 times more sensitive to AFB1 toxicity than were un-differentiated MSCs. The background DNA damage in differentiated MSCs untreated with AFB1 (control)was also greater than that measured in their un-differentiated counterparts. The DNA damage response in hepatocytes derived from MSCs exhibited a dose dependent pattern. The DNA damage in un-differentiated CD34+ cells were greater (86%) than that measured in hepatocytes derived from these cells. In conclusion these data show that the extent of DNA damage induced by a mutagen in varies depending on the stage of differentiation and the type of their metabolic activation capacity.

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P138 - EFFECT OF ISOTHIOCYANATES ON EXPRESSION OF CYTOCHROME P450, PHASE II ENZYMES, AND ACTIVATION OF NRF2 IN PRIMARY RAT HEPATOCYTES

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Glucosinolates have shown a low bioactivity as such, but once converted by myrosinase enzyme (also present in intestinal flora), they originate isothiocyanates (ITCs) [1], which induce antioxidant enzymes through the activation of nuclear factor E2-related protein (Nrf2) receptor. Primary cultures of rat hepatocytes were used to investigate whether and how eight isothiocynates (ITCs) with aromatic and aliphatic structures, derived from hydrolyzed glucosinolates, were able to modulate cytochrome P450 (CYP), antioxidant/detoxifying enzymes and to activate the Nrf2 transcription factor. The aromatic ITCs, unlike the aliphatic ones, at 40 µM increased the transcription of CYP1A1 and 1A2 mRNA and the associated ethoxyresorufin O-deethylase activity after 24 hours of treatment. On the other hand, all the ITCs at 40 µM caused a striking and similar transcriptional repression of CYP3A2, and the corresponding benzyloxyquinoline debenzylase activity. In the same culture conditions, most of the antioxidant/detoxifying enzymes were significantly up-regulated by 40 µM ITCs and in particular, NAD(P)H:quinone oxidoreductase and heme oxygenase-1. As for the Nrf2, a partial translocation of its protein from the cytosol to the nucleus was revealed by immunoblotting after 1h of treatment for all the ITCs tested. The ability of ITCs to induce the antioxidant and phase II enzymes did not appear to be affected by their hydrophilicity. Taken together, these results show that these ITCs are effective inducers of ARE/Nrf2-regulated antioxidant/detoxifying genes and have the potential to inhibit the bioactivation of carcinogens dependent on CYP3A2 catalysis.

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P139 - BILE SALT TOXICITY IN SANDWICH-CULTURED RAT HEPATOCYTES (SCRH): ROLE OF GLYCINE CONJUGATES

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Introduction: Supraphysiological concentrations of bile salts are known to mediate apoptosis and (or) necrosis in hepatocytes[1]. Bile salt secretion by hepatocytes into the canalicular space is primarily mediated by the Bile Salt Export Pump (BSEP; ABCB11). Development of bile canaliculi in SCRH takes several days[2]. Therefore, we hypothesize that bile salt toxicity will decrease with culture time because of increased biliary excretion with culture time. **Methods:** The in vitro bile salt toxicity was determined in day-1 and day-3 SCRH. Toxicity was assessed by reduced urea cycle function in SCRH. The intracellular and intracanalicular concentrations of bile salts were measured after extracellular exposure to chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) by UHPLC-MS/MS. **Results:** Bile salts can be ranked in decreasing order of toxicity: glycine conjugated > unconjugated > taurine conjugated bile salt, as measured in day-1 cultures. CDCA, DCA, and glycochenodeoxycholic acid (GCDCA) exerted higher toxicity in day-1 SCRH than in day-3 SCRH. Intracellular concentrations of glycine conjugates of CDCA and DCA were much lower in day-3 than day-1 SCRH. However, the concentrations of unconjugated bile salts were found to be higher in day-3 cultures. The formation of glycine and taurine conjugates was found to depend on the time of incubation. The amount of glycine conjugates increased with incubation time (5 min-4 hr) while for taurine conjugates reverse trend was observed. Decreased intracellular glycine conjugate concentration in day-3 compared to day-1 SCRH appears to play a role in the observed toxicity difference of bile salts between day-1 and day-3 SCRH. The decrease in intracellular concentration of glycine conjugates may be explained primarily by decreased conjugation and also by increased excretion in day-3 SCRH versus day-1 SCRH.

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P140 - THE FUNCTIONALITY OF UPTAKE TRANSPORTERS IN PRIMARY HEPATOCYTES OF DIFFERENT SPECIES CAN BE VERIFIED BY ESTRONE-3-SULFATE

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Introduction: Primary mammalian hepatocytes are used for several in vitro applications like testing of drug metabolism, toxicity and transporter assays. However, little is known about species specific differences or similarities in the activity of uptake and efflux transporter. Therefore, we started a species specific characterization and compared the uptake of different substrates in hepatocytes of species used for drug testing. Materials & Methods: Human, rat, dog and monkey hepatocytes were incubated in serum free media. 2-4 days after cell isolation a time and concentration dependent uptake of [^{3}H]- estrone-3-sulfate (E_3S), -bromosulfophthaleine (BSP), -digoxine (Dig) and -taurocholic acid (TA) at 4°C and 37°C was measured using liquid scintillation counting. Competition assays were performed using rifampicin. Results: All hepatocytes showed at 37°C a time-dependent and saturable increase in E_3S and BSP uptake compared to the uptake at 4°C. By contrast, Dig and TA showed a time-dependent and saturable transport only in hepatocytes of humanoids and rats, but not in dog. E_3S revealed a high affinity to human (Km = 12.9 ± 10.1 µmol/l; Vmax = 84.2 ± 30.3 pmol/mg × min), monkey (Km = 8.2 ± 2.3 µmol/l; Vmax = 31.3 ± 3.6 pmol/mg × min) and dog (Km = 3.3 ± 2.3 µmol/l; Vmax = 10.0 ± 2.1 pmol/mg × min) but not to rat hepatocytes. For BSP and Dig no pharmacokinetic data could be calculated and TA was transported only into hepatocytes of monkey (Km = 17.5 ± 12.5 µmol/l; Vmax = 5.8 ± 2.1 pmol/mg × min). Rifampicin clearly inhibited the E_3S uptake in monkey and dog

hepatocytes while uptake of E_3S in rat hepatocytes was not influenced. Discussion: Our data suggest that from all tested substrates E_3S is the most suitable substrate to verify the functionality of uptake transporters in primary hepatocytes of humanoids and dogs, but not of rats. However, none of the tested substrates can be used to verify the functionality of uptake transporters in primary rat hepatocytes. This species specific differences in hepatocellular uptake transporter activities may contribute to species specific differences very often observed when drug metabolism and toxicity are analyzed.

P141 - PRE-VALIDATION STUDY USING THE HUMAN HEPATOMA HEPARG CELLS FOR DETECTING GENOTOXIC COMPOUNDS WITH THE CYTOKINESIS-BLOCKED MICRONUCLEUS ASSAY

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Many chemical carcinogens require metabolic activation to form genotoxic compounds. Current in vitro models used induced-rat liver S9 for detecting pro-genotoxic compounds. Also, the low specificity of mammalian cells for carcinogenicity prediction requires the development of more predictive in vitro models. Among the human cell culture models mimicking human liver metabolism, the hepatoma HepaRG cells are certainly one of the most promising in vitro model for genetic toxicology. They express most of liver-specific functions (pahse I and II enzymes, nuclear receptors ...). In order to determine the sensitivity and the specificity of the cryopreserved HepaRG model for detecting genotoxic carcinogens, the responses induced by a list of selected compounds including genotoxic and non-genotoxic (ENU, Etoposide, Taxol, Cadmium chloride, 4-NQO, 7,12- Dimethylbenzanthracene, 2-acetylaminofluorene, 2,4-diaminotoluene, Styrene, Estragol, Eugenol, Ethanol, D-limonene, Di-(2-éthylhexyl)-phthalate, Methyl carbamate, Tetracholorethylene, D-mannitol, EDTA) was investigated with the cytokinesis-blocked micronucleus assay. Cryopreserved HepaRG cells were seeded in 24-well plates in the presence of 2% of DMSO. At day 7, after complete differentiation of HepaRG cells into hepatocytes and biliary cells, the cells were exposed for 24-h to the compounds. The results showed high sensitivity and specificity of the HepaRG model for detecting genotoxic chemicals.

P142 - SYSTEMS BIOLOGY APPROACH IDENTIFIES CSNK1A1-CONTROLLED WNT SIGNALING AS A NOVEL MODULATOR OF THE DNA DAMAGE RESPONSE IN PLURIPOTENT STEM CELLS

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In pluripotent stem cells, DNA damage triggers repair mechanisms, loss of pluripotency, and apoptosis, which acts as a safeguard to exclude cells containing damaged DNA from the lineage. The DNA damage response (DDR) signaling network that ensures that the response is proportional to the severity of the damage remains unresolved. Here, an RNAi screen targeting all kinases, phosphatases, and transcription factors was combined with global transcriptomics and phosphoproteomics to map the DDR in embryonic stem cells (ESC) treated with the DNA cross linker, cisplatin (CP). Integrated signaling networks were derived from shared overrepresented canonical pathways implicated in DNA damage repair, cell cycle & survival, and differentiation. Experimental probing of these networks identified a novel mode of DNA-damage induced Wnt/β-catenin signaling that constrains apoptosis. In contrast to loss-of-pluripotency and apoptosis, which are mediated by p53; silencing or deletion of the p53 gene demonstrates that genotoxic stress elicits Wnt signaling in a p53-independent manner. Instead, the latter occurs through downregulation of suppressors of Wnt/β-catenin signaling, including Csnk1a1 (CK1α). Our findings reveal a balance between p53-signaling that triggers elimination of stem cells and Wnt/β-catenin signaling that attenuates this response to tune the outcome of the DDR.

P143 - MULTI-PARAMETRIC PHENOTYPE PROFILING OF 3D CELL CULTURES FOR THE CLASSIFICATION OF BIOLOGICALLY ACTIVE MOLECULES

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3D cell cultures are gradually replacing the animal model and 2D cell cultures, to study cellular processes, especially for the study of tissue development where the 3D culture environment allows for the spatial organization of cells. The complex biological phenotype of 3D cell cultures can be captured by high content imaging, providing rich information on the effect of a treatment on biological processes that are relevant to toxicity. We have developed a high-throughput screening platform to examine the effects of a broad range of molecules on tissue development and tumor cell invasion. By using multi-parametric profiling of the cellular phenotypes and multi-parametric comparison, the biologically active molecules that influence cellular phenotype can be automatically identified. To be able to characterize the phenotypic influence induced by different molecules, pattern recognition methods were used to classify molecules according to the phenotype they induced and establish a phenotypic pattern for each class. We discovered that molecules which inhibit the same target influence the cell phenotype in the same pattern, which revealed the potential to develop our methodology into a high throughput compound screen for target specificity.

P144 - EVALUATION OF ULTRA-FAST ONLINE SPE/MS FOR THE EFFICIENT SCREENING OF CYTOCHROME P450 INHIBITION USING CASSETTE ANALYSIS

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The cytochrome P450 (CYP) enzymes are involved in many drug metabolism transformations and account for a significant number of bioactivation and metabolic reactions. The presence of some drugs can interfere with the metabolic activity of CYP enzymes causing drug-drug interactions. Analysis of potential drug-drug interactions caused by inhibition of the enzymatic activities of CYP enzymes plays an important role in the drug discovery process. The ever increasing need to evaluate a large number of samples and the need to eliminate weaker candidates in the initial phase of the drug discovery process, has created a bottleneck at LC/MS/MS analysis. The use of an ultra-fast online SPE/MS system coupled to an Agilent QqQ and Agilent QTOF (the Agilent RapidFire High-throughput Mass Spectrometry systems) has found utility for high throughput CYP inhibition screening. We evaluated this system for the analysis of pooled samples across several CYP450 isoforms in a single cassette injection mode to provide an even faster and more cost-effective screen. FDA recommended CYP drug probe substrates (CYP2C9/diclofenac; CYP2D6/dextromethorphan; CYP3A4/midazolam) were incubated individually with human liver microsomes (0.25mg/ml) and an eight point dose response curve with respective known inhibitors starting at 100 µM with subsequent 10X dilutions. The reaction was started by the addition of NADPH and stopped with 2X volume of acetonitrile containing respective deuterated internal standards. Individual as well as pooled samples were analyzed at a rate of 7-10 seconds per sample using the Agilent RapidFire High-throughput Mass Spectrometry systems. Generic SPE conditions were developed for all CYP450 isoforms. The analytes were desalted using a C_4 column with water: formic acid: TFA (100%:0.09%:0.01%) and eluted with acetonitrile: formic acid: TFA (100%:0.09%:0.01%). MS conditions for each compound were optimized individually for the Agilent QqQ, while generic MS conditions were used for all compounds on the QTOF. Strong correlations between the IC_{50} values across all isoforms were seen on both of the Agilent RapidFire High-throughput Mass Spectrometry systems, as well as with values in the literature using traditional LC/MS/MS methods (corresponding IC_{50} values were within 2.0-fold of each other). These results show that for P450 inhibition assays, a significant increase in efficiency and throughput (>20 fold) compared to LC/MS/MS can be achieved with an analysis capacity of >350 samples per hour and no loss of integrity in the IC_{50} results.

P145 - UNI- AND BI-MOLECULE BINDING SYSTEM FOR THE PREDICTION OF CYP2D6-MEDIATED REACTION

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CYP2D6 has broadened substrate specificities, and mediates the oxid/reduction of small molecules such as naphthalene and of large molecules such as emetine. Early observations of the involvement of CYP2D6 on the

metabolism of basic drugs led to proposal of a possible role of a basic nitrogen group in substrates for the interaction with protein residues at the metabolic site. Thereafter, several predicting procedures, such as ligand-based models and enzyme structure-based models, have been developed to estimate CYP2D6-mediated metabolism. These studies reported good predictability for the evaluation of substrate specificity. The prediction accuracy of ligand-based models, however, depends on the training set compounds, while that of structure-based models might be affected by the quality of the crystal structure of the enzyme. Ligand-bound crystals may not always reveal the environment at the moment of oxid/reduction of substrates. In addition, no structural interpretations are provided for especially negative results. A reliable tool providing clear interpretation for the judgment is necessary. We have recently shown the advantages of flat template systems for the simulated estimation and verification of metabolism data on human CYP2E1 (Yamazoe Y., Drug Metab Rev 2011;), CYP2B6 (Koyama et al., DMPK 2011) and CYP4A11 (Yamaura et al., DMPK 2011). In the present study, we have constructed a unique template system applicable for both small and large size substrates to estimate and understand the substrate specificity of CYP2D6, and evaluated the validity. Using the system developed, CYP2D6 substrates and poor substrates that were not employed to construct the template system were used to evaluate the accuracy of the estimation of the template system. Among 54 reactions of CYP2D6 substrates, CYP2D6 was judged to mediate 50 (93%) reactions with this template method. The sites of the oxidation were also concordant with reported data. Of the 61 substrates with poor or lack of CYP2D6-mediated reactions, 60 (98%) reactions were judged as unlikely to be mediated by CYP2D6.

P146 - IN SILICO PREDICTION OF METABOLISM: A REVIEW OF OXIDATIVE N-DEALKYLATION BIOTRANSFORMATIONS

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Drugs containing secondary or tertiary amines commonly undergo metabolic reactions such as nitrone formation, lactam formation or oxidative N-dealkylation. Recent work on a knowledge-based expert system has focused on the refinement of existing biotransformations and the development of new ones in order to capture such knowledge more accurately. The formation of lactams is a well-studied route for aza-alicyclic compounds, for example substrates such as indeloxazine and meptazinol; however, literature evidence suggested that lactams, cyclic thioamides and cyclic amidines do not undergo such biotransformations. Oxidative N-dealkylation, including ring opening type reactions, are also an important metabolic route in mammalian xenobiotic metabolism and the reaction is of wide scope for secondary and tertiary amine substrates. Structure metabolism relationships were investigated for the following three types of substrate containing the following features: i) A nitrogen atom at a fusion or a bridgehead position (e.g. ezlopitant), ii) Aromatic heterocycles attached to an alicyclic methine carbon atom (e.g. tofimilast), iii) 2-Arylpyrollidines (nicotine) and 2-arylpiperidines (CP-122721). The occurrence of oxidative N-dealkylation or ring opening biotransformations for such compounds was found to be either quantitatively very low, or in some cases not observed. Knowledge collected in the course of this work has allowed new biotransformations to be written and existing ones to be refined. Transition state data has also been used to corroborate this work. A comparison between the new and a previous version of our knowledge-based expert system was carried out using reference compounds mentioned in this paper. The results showed an increased in predictive performance and selectivity for oxidative N-dealkylation reactions.

P147 - ROLES OF CYTOCHROME P450 (CYP) 2C8 AND CYP3A4 IN IMATINIB METABOLISM – IMPLICATIONS OF AUTOINHIBITION OF CYP3A4

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Background. Although CYP3A4 has been reported to be the main enzyme in imatinib metabolism[1], strong CYP3A4 inhibitors have had little effect on imatinib pharmacokinetics in vivo[2,3]. Recent data indicating that imatinib is a potent mechanism-based inhibitor of CYP3A4 in vitro[4], suggests that imatinib can affect its own CYP3A4-mediated metabolism, reducing the importance of this enzyme in its pharmacokinetics. Methods. Based on in vitro metabolism data from recombinant CYP and human liver microsomal incubations, we constructed an in silico model for imatinib within the physiologically based pharmacokinetic (PBPK) simulator Simcyp. The final model for imatinib included first-order oral absorption, intestinal metabolism, a PBPK distribution model, and renal, hepatic and additional

elimination for both unchanged imatinib and the main active metabolite, N-desmethylimatinib. Simulations were carried out aiming to evaluate the clinical importance of different CYP enzymes, in particular those of CYP2C8 and CYP3A4, in imatinib pharmacokinetics after a single dose versus multiple doses of imatinib. Results. The simulated imatinib mean peak concentration (C_{max}) of 1600 ng/ml, mean time to reach C_{max} of 2.5 h, and area under the plasma concentration-time curve $(AUC)_{0-96}$ of 27000 ng·h/ml, after a single dose of 400 mg imatinib, corresponded well with reference data[5]. According to the model, inactivation of CYP3A4 by imatinib progressed in a dose- and time-dependent manner, consequently affecting imatinib pharmacokinetics. On treatment day 1 with imatinib 400 mg once daily, the fractions metabolized by hepatic CYP2C8 and CYP3A4 were predicted to average 46% and 51%, respectively. After multiple doses at steady state, the importance of CYP2C8 was predicted to increase so that approximately 63% or 72% of imatinib was metabolized by CYP2C8 and 34% or 24% by CYP3A4 with 400 mg once or twice daily, respectively. Following a single 400 mg dose of imatinib, gemfibrozil (a strong CYP2C8 inhibitor; 600 mg twice daily), itraconazole (CYP3A4 inhibitor; 100 mg twice daily), and a combination of gemfibrozil and itraconazole were predicted to increase imatinib $AUC_{0-\infty}$ 1.9-, 1.4-, or 3.1-fold, respectively. However, when imatinib was at steady state, the $AUC_{0-\infty}$ increases were predicted to average 3.2- , 1.3-, or 4.9- fold, respectively. Conclusions. Although clinical studies are needed to confirm the present in vitro-in silico findings, it is likely that imatinib markedly affects its own CYP3A4-mediated metabolism via autoinhibition, assigning a key role for CYP2C8 in its elimination. Thus, during multiple dosing, imatinib may be sensitive to inhibition by CYP2C8 inhibitors such as gemfibrozil and trimethoprim.

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P148 - USE OF IN SILICO MODELLING TO SUPPORT SELECTION AND DEVELOPMENT OF PROTEIN-STABILISING DRUGS

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Certain endogenous proteins can acquire, through age, disease, injury or heredity, a tendency to misfold, leading to a conformational change in the normal 3-dimensional structure of the molecule. The consequences of such misfolding can include loss of normal protein function and/or an increased tendency of the misfolded protein to bind to itself forming aggregates. The body is generally incapable of clearing these aggregated proteins to any great extent, resulting in accumulation and interference with the normal capacity of the affected tissues. There are many diseases associated with protein misfolding and aggregation including Alzheimer's, Parkinson's, and cystic fibrosis; decreased protein function due to misfolding is also associated with some metabolic diseases. One growing area of research into prevention of such diseases is to stabilise, or promote, normal folding by introducing a drug molecule which binds specifically to the target protein. In silico modelling can be used to support design, selection and development of new drug molecules with optimal properties for stabilising proteins. The examples shown in this poster describe how mechanistic PD as well as PBPK models can be used to investigate: drug-protein binding characteristics (reversibility, on and off rates, multiple binding sites, cooperativity), how blood concentrations of drug can be linked to exposure at the target site, and consideration of alternative dosing regimens when a drug binds to the target protein in the active site.

P149 - THE IMPORTANCE OF PH CONTROL FOR ACCURATE ASSESSMENT OF IN VITRO PROTEIN BINDING

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The measurement of plasma protein binding is generally considered a routine in vitro study to provide information on drug binding which can be incorporated into further processing of both in vitro and in vivo data. However, recent publications have highlighted the complexities of the methodology and indicate that temperature and pH need to be carefully controlled to provide meaningful data [1]. One major factor is ensuring that the pH remains at a physiological level (pH 7.4 ± 0.1) throughout the incubation (typically ≥6 hours) to prevent any conformational changes to plasma proteins which may alter the extent of protein binding. In this study, the pH was monitored after incubating mouse, rat, rabbit, dog and human plasma in the presence and absence of 5% CO_2 at 37°C for up to 6 hours in a RED device (ThermoFisher Scientific, Waltham, MA). The affect of pH on the protein binding of three well characterized compounds, warfarin, testosterone and caffeine was also assessed. In the absence of CO_2, the pH of rat, dog, rabbit and human plasma increased to greater than pH 7.9 after a 6 hour incubation, whilst the pH in human plasma reached a maximum of pH 8.4. Conversely, the pH of mouse plasma remained constant over the 6 hour incubation (maximum of pH 7.6). In the presence of CO_2, the pH was controlled for all species within the range of pH 7.4 ± 0.1, with the exception of mouse plasma where a decrease to pH 7.12 was observed after 6 hours. The free fraction was determined in all species at 6 hours in the presence and absence of CO_2. For caffeine, the binding was consistent between the two conditions in all species. However, for warfarin and testosterone, a difference in the free fraction was observed in rat, rabbit and human plasma where an increase in the free fraction was observed in the presence of CO_2, (>1.3-fold). The greatest difference was observed in rat plasma, where a 2-fold increase in the free fraction was observed for both compounds at 6 hours in the presence of CO_2. In summary, these data indicated that there was a species difference in pH control such that the pH of mouse plasma was maintained over the 6 hour incubation without additional pH control. For the other species investigated, however, incubations in the presence of CO_2 were required to maintain the pH at physiological levels. The current study confirmed the importance of controlling pH during the plasma protein binding incubations to obtain an accurate assessment of the free fraction of compounds in vitro.

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P150 - HIGH CONTENT ANALYSIS ON PHYSIOLOGICALLY RELEVANT 3D IN VITRO MODEL FOR ASSESSMENT OF DRUG INDUCED LIVER INJURY

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HepG2 cells are used widely for studying liver toxicity of xenobiotics. In conventional 2D culture these cells have low expression of metabolic enzymes and drug transporters, which will compromise the validity of safety assessment using these cells. Cell-cell and cell-extra cellular matrix (ECM) interactions are essential for maintaining tissue specific functions. Here we describe a three-dimensional in vitro model which uses ECM proteins as scaffolds for HepG2 cell growth. In this model, robust morphological and functional differentiation is induced, with a strong induction of metabolic enzymes and transporters. Unlike primary hepatocytes in 2D culture, the metabolic competence of HepG2 cells in 3D cultures could be retained for up to 4 weeks, making chronic exposures feasible. The assay we have developed is implemented in a 384 well format for low cost and increased throughput. We combine conventional toxicity readouts with automated high content imaging to measure expression of fluorescent stress reporters, such as Srxn1(Sulfiredoxin-1) activation, combined with other fluorescent labels for cell toxicity. This assay represents a novel and more physiologically relevant method for predicting drug-induced liver injury

P151 - HEPATIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS CULTURED IN A PERFUSED TREE-DIMENSIONAL BIOREACTOR

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Drug induced liver injury (DILI) occurs frequently and is hard to predict. DILI is also the most common reason of attrition in preclinical studies, especially in the late phase of development or even when the drug has entered the market. Due to major species differences regarding drug metabolism and toxicity, animal experiments are usually not predictive for human DILI. Hence, there is a high demand for innovative human-predictive in vitro test. Human primary hepatocytes (HPHs) are considered to be one of the most important cell types for early drug discovery and toxicity studies. However, the use of HPH has several limitations: the sometimes low availability of fresh liver samples, high batch-to batch variability of the isolated hepatocytes due to inter-individual differences between donors, and instable expression levels of liver-specific genes such as cytochromes P450. Human embryonic stem cells (hESC) are pluripotent and have the capability of self-renewal which could make them an important cell source in basic research, drug development, and pharmacological toxicity screening. Several studies report on directed hESC differentiation to functional hepatocyte-like cells but most studies have been done in 2D cultures. The results are encouraging but so far no one has been able to produce stem cell-derived hepatocytes that are fully mature. The 3D bioreactor technology used in this study is a dynamic system, with decentralized medium and gas perfusion, mimicking the blood flow in vivo. Undifferentiated cells (UD), definitive endoderm (DE), hepatic progenitors (PRO) and hepatocyte-like cells (HEP) were cultured in either 3D or 2D systems and the results compared. Results from the global hierarchical clustering showed that transcripts clustered tightly together group wise for either HPH, UD, DE, PRO, or HEP. With a whole genome gene array analysis, using a combined criteria of t-test with p-value <0.05 and fold change >2, 102 up-regulated and 63 down-regulated genes were identified in 3D compared to 2D. 10 significantly up-regulated KEGG-pathways, highly related to liver functions, were identified in the 3D bioreactor differentiated cells when compared to a 2D control culture. Additionally, during 3D differentiation 19 KEGG pathways, associated with apoptosis and cell proliferation, were down-regulated,. The gene expression levels (qRT-PCR) of a large number of hepatic genes, phase I and II enzymes, and transporters were generally increased in the 3D bioreactor cultures compared to their 2D control. Immunocytochemistry data provided further evidence that the stem cells differentiated towards functional hepatocytes, showing A1AT and CYP3A4 production and express the BCRP transporter at similar patterns as seen in human liver. Moreover vWF (marker sinusoidal endothelial cells) and CK19 (marker for bile duct epithelium) staining indicates that the stem cells in the bioreactor differentiated to different hepatic cell types, although, not in fully organized structures comparable to human liver. In conclusion, our results suggest that the functionality of our differentiated hepatocyte-like cells is improved in 3D culture, and that this dynamic 3D technology could be a useful tool for derivation of functional hepatocyte-like cells.

P152 - DIFFERENTIATION INTO FUNCTIONAL ENTEROCYTE-LIKE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Small intestine plays a key role in first-pass effects of oral administrated drugs, since it is known that a number of drug transporters and metabolic enzymes are expressed. There is no appreciate in vitro evaluation model, although it is important to estimate drug absorption and metabolism in a small intestine. Human colon carcinoma cell lines, such as Caco-2 cells, are currently used in drug membrane permeability study. But these cell lines have characteristics originally different from enterocytes, it is difficult to appropriately estimate drug absorption and metabolism in a small intestine. Induced pluripotent stem (iPS) cells have been expected for not only regenerative medicine but also drug development because iPS cells have high proliferative ability and multipotency. We therefore performed differentiation into enterocyte-like cells from human iPS cells to with the aim of constructing intestinal pharmacokinetic evaluation system. Human iPS cells were differentiated into endoderm by activin A (100 ng/ml), and then intestinal stem cell-like cells by fibroblast growth factor (FGF) 2 (250 ng/ml). Finally, the intestinal stem cell-like cells were differentiated into enterocyte-like cells by epidermal growth factor (EGF, 20 ng/ml). After differentiation, the total RNA was extracted, and expression levels of intestinal stem cell and enterocyte markers

was measured by real-time RT-PCR analysis. We also conducted immunofluorescent straining of sucrase-isomaltase protein and uptake study of β-Ala-Lys-AMCA. By treatment of FGF2, mRNA expression of CDX2, intestinal transcription factor, and LGR5, intestinal stem cell marker, were observed. Enterocyte-like cells, subsequently differentiated by EGF, were expressed sucrase-isomaltase, intestinal specific marker, cytochrome P450 (CYP) 3A4 and solute carrier family 15, member 1 (SLC15A1), oligopeptide transporter. These mRNA expressions were increased by reducing serum concentration. Dipeptidyl peptidase-4 (DPP4) and SLC46A1, folate transporter, were expressed similar to adult small intestine. Moreover, uptake of β-Ala-Lys-AMCA, fluorescent-labeled substrate of oligopeptide transporter, was observed in differentiated cells, which was expressed sucrase-isomaltase protein. The present study demonstrated that human iPS cells could be differentiated into functional enterocyte-like cells. Therefore, it is suggested that human iPS cell-derived enterocytes may be useful for small intestinal pharmacokinetic study.

P153 - VALIDATION OF AN IN VITRO MODEL FOR SIMULTANEOUS EVALUATION OF CYP450 INHIBITION AND KINETIC SOLUBILITY

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Compound solubility, a well-known source of variability for in vitro profiling, has become a more relevant issue since the hydrophobicity of drug candidates has begun to increase. Precipitation of a compound during an analytical procedure can result in the misinterpretation of results. Compounds with poor aqueous solubility might precipitate during in vitroassays and result in lower concentration than was anticipated. In a CYP450 inhibition assay, compounds with poor solubility might show artificially low inhibition, which could cause compounds with potential drug–drug interaction toxicity to be overlooked. Therefore, screening of solubility early in drug discovery is of great importance. In this study we present the validation of a high throughput screening in vitromodel for simultaneous evaluation of CYP450 inhibition, using fluorescent probes and aqueous solubility determined by turbidimetric measurements. For this purpose, we selected a set of commercial compounds and drug discovery compounds for IC50 and solubility limits determination. The data obtained showed very similar results to those attained in separate experiments and with published values. This methodology offers an efficient, robust way to determine the cytochrome P450 inhibition profile and turbidimetric solubility limits in the same experiment for a large number of compounds. It also represents a useful tool to alert us to low solubility compounds in early stages of the drug discovery process which provides a better understanding of their inhibition data.

P154 - HIGH-THROUGHPUT TEST SYSTEM FOR STEM CELL RENEWAL AND DIFFERENTIATION

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Testing chemicals for reproductive toxicity remains a big challenge. Ethical issues and high costs related to in vivo testing, as well as necessity for high throughput platforms are calling for alternative in vitro test systems. Since there is no single in vitro system available that represents every step in the overall process of mammalian reproduction, the use of combinations of in vitrotests are now being used to cover more aspects of developmental toxicity at the same time. In this study we use mouse embryonic stem cells for reproductive toxicity testing. We explored the possibility to use self-renewal and pluripotency as alternative endpoints in toxicity testing in these cells. These endpoint are also relevant in the context of ageing and cancer. The capacity of mouse embryonic stem cells (ESCs) for self-renewal and pluripotency was measured by Green Florescent Protein (GFP) expression under control of the endogenous Nanog promoter, phenotyping of cell cultures, staining for alkaline phosphatase, and by RT-qPCR of differentiation markers. Further, we developed a siRNA based gene silencing method in this system to investigate mechanisms of toxicity in more detail and to shed light on the role of epigenetic modifiers on cellular differentiation and pluripotency. We tested compounds from the following biological activity classes: genotoxins, oxidative stress inducers, inhibitors of epigenetic modification, Wnt and Shh pathway (ant)agonists, non-genotoxic carcinogens and various suspected teratogens. Three days exposure with our testing system proved relevant for demonstrating whether loss of pluripotency and self renewal is included in the effect of a test compound. We showed that genotoxins as well as oxidative stress inducers do not cause loss of pluripotency. Instead, other toxic compounds such as Cyclosporin A and Colchicine, several suspected teratogens and Wnt signaling activators induced loss of

pluripotency. Interestingly, several inhibitors of epigenetic modification pathways prevented loss of pluripotency of the ES cells, but sensitized the ESCs for loss of pluripotency during coexposure with compounds that induce loss of pluripotency. In conclusion, we developed a high-throughput system for measuring effects on ESC self-renewal and pluripotency and a set of tools that can be used to investigate mechanisms of developmental toxicity related effects in detail.

P155 - TRANSFER OF A BILE TRANSPORT ANALYSIS METHOD FROM 2D TO A 3D MULTI-COMPARTMENT BIOREACTOR SYSTEM

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Primary human hepatocytes are considered as the gold standard in metabolism studies within predictive drug safety and toxicity studies, since excretion via bile of drug metabolites is a major determinant of the pharmacokinetics and clearance concerning the influence due to possible entero hepatic circuit. Therefore our aim was the development of predictive model reflecting biliary secretion processes. The three-dimensional culture of primary human hepatocytes in a perfused system enables physiological cell-cell contacts and cell-cell communication between hepatocytes, exchange of paracrine and autocrine signals, and continuous supply of oxygen/nutrients as well as metabolite removal. Therefore such systems allow a better preservation of metabolic functions and reflect the in vivo situation much closer than 2D cultures In previous studies, we developed a down-scaled 3D multi-compartment bioreactor technology for in vitro tests on hepatotoxicity. Long-term stability of metabolic activities, including CYP enzymes, was shown, both using primary human liver cells and the hepatic cell line HepaRG. Tissue-like cell reorganization and in vivo-like expression of hepatic transporters was detected in primary human liver cell bioreactors.[1],[2] The dynamics of bile release in 2D cultures of primary human hepatocytes and HepaRG cells was investigated with life cell imaging microscopy using 5(6)-carboxy-2,7-dichlorofluorescein (CDF) as a model substance for biliary transport. Based on a method established for determination of bile secretion in a 2D sandwich-culture configuration of rat hepatocytes [3] the needed Ca^{2+} modulation for the determination of bile-secreted metabolites was transferred in our 3D culture bioreactor test system. The time dependant opening of the tight junctions in the 2D culture showed a release of CDF between 20 minutes and 5 hours after application of the substance. Consequently a time frame of 20 minutes was chosen for the transfer of the method to the bioreactor system. Additionally the modulation of Ca^{2+} free buffer revealed that the complete Ca^{2+} depletion was possible in the complex 3D culture system. Measurement of LDH release as a marker for membrane integrity indicated no cell damage due to the treatment. Therefore the 3D multi-compartment bioreactor could provide a suitable model for studies on biliary transport in 3D cultures of hepatic cells.

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P156 - HEPATIC IRON LOADING IN ACUTE ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY DECREASED HEPCIDIN SYNTHESIS

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Acetaminophen (APAP) is accountable for most cases of drug-induced liver injury, which is principally mediated by oxidative stress. Recent studies have suggested a role for iron in potentiating APAP-induced liver injury, although its regulatory mechanisms are not completely understood (Kon et al., 2010; Moon et al., 2010). To unravel the role of iron regulating pathways in APAP-induced liver injury, male FVB mice were treated with a single intraperitoneal dose of APAP (0-350 mg/kg bw). Liver tissue and plasma were collected 24 h after APAP administration. Liver histology and plasma alanine aminotransferase (ALT) measurements were used to assess liver injury. Total hepatic iron concentration, as determined by the ferrozine assay, and oxidative stress measured using the thiobarbituric acid reactive substances assay were significantly increased in mice with severe APAP-induced liver injury (plasma ALT >5000 U/L). Furthermore, plasma concentration and hepatic mRNA expression of hepcidin, a key regulating hormone in iron homeostasis, was significantly reduced in mice with severe APAP-induced liver injury. QPCR analysis and Western blotting techniques showed that hepcidin transcription was reduced by multiple hepcidin-regulating factors, including the bone morphogenetic protein / small mother against decapentaplegic (BMP/SMAD) pathway, CCAAT/enhancer-binding protein α (C/EBPα) and possibly also via erythropoietin (EPO). APAP-induced down-regulation of hepcidin is probably mediated via induction of hypoxia-inducible factor 1α (HIF-1α) by oxidative stress. Western blot analysis showed increased HIF-1α protein in liver homogenates of mice with severe APAP-induced liver injury. HIF-1α can block BMP/SMAD activity by cleavage of hemojuvelin, the cofactor of the BMP receptor, but can also affect C/EBPα and EPO transcription. In conclusion, acute APAP-induced liver injury leads to activation of HIF-1α, which results in a down-regulation of hepcidin expression through a BMP/SMAD signaling pathway and through C/EBPα inhibition. Eventually, this leads to hepatic iron loading associated with APAP cytotoxicity.

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P157 - AN IMAGING-BASED RNA-INTERFERENCE SCREEN IDENTIFIES MODULATORS OF TNF-MEDIATED NF-KB SIGNALING IN DRUG-INDUCED LIVER INJURY

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Tumor necrosis factor-α (TNFα) is a cytokine that can induce hepatocyte proliferation and liver regeneration, but it also influences cell death. TNFα has been implicated in drug-induced liver injuries (DILIs). TNFα binding to its receptor the transcription factor NF-κB is activated. The protective and proliferative response to TNFα depends on the timely nuclear translocation of NF-κB to activate its target genes. The frequency and amplitude of NF-κB oscillation in liver cells can be negatively influenced by drugs that cause cell stress, thereby promoting pro-apoptotic responses. In order to identify kinases and (de)-ubiquitinases that are critically involved in regulating the NF-κB oscillatory response to TNFα, we performed a functional genomics screen. We used HepG2 cells expressing GFP-tagged p65/RelA (NF-κB subunit) in 96-well plates under control and drug-treatment (diclofenac) conditions and followed the GFP-p65 oscillation of ~200 cells per well for 6 hr using automated confocal laser scanning microscopy. The cell-specific NF-κB responses within a knockdown population were mapped by automated ImageJ-based image analysis. This work firstly uncovered distinct cell behaviors and population dynamics in the context of hepatotoxicant treatment in association with increased sensitivity towards apoptosis. We selected 115 hits out of the 1571 genes screened for validation. 29 hits were confirmed as high confidence modulators of the TNF signaling-induced NF-κB response. Pathway analysis of these hits indicates that the activity of the NF-κB response is linked to various stress responses, which, upon drug toxicity conditions, modulates the cytoprotective role of the NF-κB response.

P158 - EVALUATION OF REACTIVITY OF ACYLGLUCURONIDES IN EARLY STAGE DRUG DISCOVERY

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Purpose: Drugs containing the carboxylic functional group can be metabolized to form acylglucuronides. Acylglucuronides are kinds of reactive metabolites and have been considered to be a cause of idiosyncratic drug toxicity when these forms are unstable. In previous studies[1,2], it is obvious that the half-lives of these acylglucuronides in phosphate buffer are useful to classify the safety of these drugs, in terms of their idiosyncractic drug toxicity, into 3 categories; "safe", "warning" and "withdrawn". However, it is difficult to discontinue the clinical development of New Chemical Entities (NCE) on the grounds of the instability of their acylglucuronides in late stage drug development. In this study, we have established an optimized method to evaluate the stability of these acylglucuronides in the early stages of drug discovery. **Methods:** We evaluated half-lives of 8 drugs (3 safe drugs: Telmisartan, Gemfibrozil and Flufenamic acid; 5 warning drugs: Diclofenac, Furosemide, Ibuprofen Probenecid and Tolmetin). At first, we incubated these drugs in a human microsomes reaction mixture. After stopping glucuronidation by adding organic solvent containing 1% formic acid, we detected the acylglucuronide in the supernatant by LC-MS/MS. After confirming the acylglucuronide production, we added supernatants to phosphate buffer to evaluate the degradation half-lives of these acylglucuronides formed in the microsomes reaction mixture. **Results:** All 3 safe drugs indicated 10.6 hours or longer half-lives, on the other hand, the half-lives of all 5 warning drugs were 4.0 hours or shorter. Although we didn't use any authentic acylglucuronide standard, we have obtained half-lives of acylglucuronides in phosphate buffer similar with previous studies and were able to distinguish between "safe" and "warning" drugs. **Conclusions:** We have been able to categorize these 8 compounds into "safe" and "warning" zones according to their half-lives. As it is possible to evaluate the half-lives of NCEs even without any authentic acylglucuronide standard, our method is very suitable in early stage drug discovery.

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P159 - EVALUATION OF CHOLESTATIC COMPOUNDS INDUCED BILE ACIDS DEPEPNDENT LIVER INJURY IN SANDWICH CULTURED HEPATOCYTES

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BackgroundBile salt export pump (BSEP, ABCB11) is a membrane protein that is localized in the cholesterol-rich canalicular membrane of hepatocytes. Its function is to eliminate unconjugated and conjugated bile acids/salts from hepatocyte into the bile. In humans, there is no compensatory mechanism for the loss of this transporter. BSEP inhibitor drugs produce similar bile salt retention that may lead to severe cholestasis and liver damage. Drug-induced liver injury (DILI) is a relevant clinical issue, in severe cases ending in liver transplantation. Therefore, measurement of BSEP inhibition and liver injury by candidate drugs has high importance in drug discovery and development. Although several methods are suitable to detect BSEP-drug interactions, they have limitations. In this study, we used sandwich cultured hepatocytes (SCH) which can evaluate vectorial transport and the metabolism process to construct a quantitative evaluation system for BSEP-mediated bile acids dependent DILI. **Methods** SCH (male Sprague-Dawley rat) were exposed to about 30 hepatotoxic drugs (Cyclosporin A, Troglitazone, etc.) with or without some components bile acids. After 24 hours, we measured LDH release into medium. BEI (biliary excretion index) of ^{3}H-taurocholate (1 μM) was used as an idex of Bsep-mediated vectorial transport of bile acids. LC_{50} values of the cell toxicity were determined in each test compounds. In addition, IC_{50} values of taurocholic acid transport were obtained from membrane vesicles assay or biliary excretion index (BEI) in rat SCHs to compare the correlation of LC_{50} values and IC_{50}values. **Results** Test drugs did not show toxicity when SCH were exposed solely to the drugs. However, almost all of cholestatic drugs (Cyclosporin A, Troglitazone, Glibeclamide, etc.) which were used in this study showed the toxicity in the presence of bile acids, although non-cholestatic drugs (eg. Diclofenac) did not show toxicity in the presence of bile acids. Furthermore,in dose response study using SCH, most of Bsep inhibitors decreased BEI oftaurocholate and increase the bile acids dependent hepatotoxicity in dose dependent manner. Furthermore, LC_{50} values showed good, overall correlation with $IC_{50,\ vesicle}$ and $IC_{50,\ BEI}$ values (correlation

coefficient (r^2) = 0.4746 and 0.7318, respectively). However, a much higher LC_{50} (94.9 µM) vs. $IC_{50,\ vesicle}$ (2.8 µM) value was observed for the choleastic drug, Glibenclamide, and co-treatment with 1-aminobenzotriazole (a non-selective cytochrome P450 inhibitor) decreased the LC_{50} from 94.9 to 48.9 µM. **Discussion and Conclusion** These results suggest that the potency of BSEP/Bsep inhibitors and the extent of BSEP/Bsep dysfunction determine the severity of bile acids-dependent hepatotoxicity. Moreover, drug metabolism must be considered in the assessment of bile acids-dependent toxicity.

P160 - ISOPROSTANES AS BIOMARKERS OF OXIDATIVE STRESS IN PRIMARY HEPATOCYTES

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The assessment of the risk for idiosyncratic hepatotoxicity is essential for the development of new drugs because of potential severe type B adverse drug reactions. As the occurrence is of low frequency (<1/5000), detection is unlikely in standard preclinical and early clinical trials. One mechanism leading to such events is the formation of reactive metabolites which can covalently bind to liver proteins (CVB). This can be tested in vitro using microsomal or hepatocyte metabolic systems. However, the correlation between the amount of CVB and clinical outcome remains vague and additional cellular mechanisms leading to toxicity are discussed, warranting the need for translational markers. One group of biomarkers of oxidative stress as one potential cause are the isoprostanes, prostaglandin isomers that derive from radical-catalyzed arachidonic acid oxidation. Especially F_2-isoprostanes have been investigated in the past and can also be determined in vitro. The aim of this work was to establish a robust and sensitive method for the LC-MS/MS analysis of isoprostane markers in primary hepatocytes in order to identify compounds prone to induction of cellular oxidative stress. Freshly prepared rat hepatocytes were treated in suspension in 96-well plates with the positive controls trichlorobromomethane ($CBrCl_3$) and ferric nitrilotriacetate (Fe(III)NTA) for up to three hours at a concentration of 100 and 50 µM, respectively. Troglitazone, clozapine and other drugs associated with cases of idiosyncratic hepatotoxicity were incubated at 100 µM. A system generating hydrogen peroxide in situ was added to attenuate oxidative stress and simulate inflammatory situations within the cell system. For analysis the supernatant was measured by a selective HPLC column switching method coupled to tandem mass spectrometry in negative ion mode which had been established and qualified for the detection of above markers by use of stable labeled internal standards. The well-established isoprostane isomers prostaglandin D_2, prostaglandin PE_2 and 5-iso prostaglandin F_{2a}-VI showed significant increase upon treatment. Two isoprostane isoforms that were previously not described as biomarkers for oxidative stress were identified showing much higher response upon treatment in the biological system as compared to previously described isomers. One isomer identified as 15(R) prostaglandin D_2 responded with a 14 fold increase of peak area as compared to the negative control in cells treated with $CBrCl_3$. A treatment with Fe(III)NTA led to a 37 fold increase already after 1 h of incubation. Furthermore the treatment of samples with troglitazone significantly affected the cellular stress reaction. The presented method displays a sensitive and specific tool for the investigation of oxidative stress biomarkers in vitro and allows for establishing dose response for a set of benchmark drugs.

P161 - IDIOSYNCRATIC DRUG TOXICITY CAN NOT BE TRIGGERED BY BINDING OF THE DRUGS TO HUMAN LEUKOCYTE ANTIGEN (HLA) MOLECULES?

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Introduction: Severe toxicity is caused by several marketed drugs such as Amoxicillin, Lapatinib, Lumiracoxib and Nevirapine, which are associated with the HLA alleles DRB1*1501, DRB1*0701, DRB1*1501 and DRB1*0101, respectively. [1), 2), 3), 4)]In this study, we investigated the binding affinity of these small molecule drugs with the respective HLA alleles. Methods/Results: The binding affinity of the drugs with the binding groove of each HLA allele was predicted by molecular modeling docking study and the HLA binding study (ELISA) was also performed at EpiVax, Inc. (Providence, RI, USA). According to the results, the docking study did not predict any small molecules to bind any of the HLA alleles studied either in the binding groove or outside the binding groove. Also, the results of HLA binding study supported the predictions that none of the small molecules are bound to HLA. Conclusion: Idiosyncratic drug toxicity related drugs, Amoxicillin, Lapatinib, Lumiracoxib and Nevirapine showed no affinitiy with the binding

grooves of the HLA alleles DRB1*1501, DRB1*0701, DRB1*1501 and DRB1*0101, respectively, as predicted by the docking analysis. The findings in this study indicate that IDT may not be triggered by simple binding of the drugs to HLA molecules. References: 1) Hautekeete ML et al. (1999) HLA association of amoxicillin-clavulanate-induced hepatitis. Gastroenterology 117:1181-6. 2) Spraggs CF et al. (2011) HLA-DQA1*02:01 is a major risk factor for lapatinib-induced hepatotoxicity in women with advanced breast cancer. J Clin Oncol 29:667-673. 3) Singer JB et al. (2010) A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. Nat Genet 42:711–714. 4) Martin AM et al. (2005) Predisposition to nevirapine hypersensitivity associated with HLA-DRB1*0101 and abrogated by low CD4 T-cell counts. AIDS 19:97-9.

P162 - PRECISION-CUT MOUSE LIVER SLICES AS AN EX VIVO MODEL TO STUDY THE MECHANISM OF INFLAMMATORY STRESS-RELATED IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY

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Idiosyncratic drug reactions (IDRs) can be defined as adverse drug reactions that occur in a small minority of the patients taking clinically-relevant doses and do not involve the known pharmacological effects of the drug. IDR related to hepatotoxicity or idiosyncratic drug-induced liver injury (IDILI) has been the top reason for withdrawing drugs from the market or for black box warnings. One of the mechanistic hypotheses regarding IDILI suggests that IDILI may arise from the interaction of a reactive metabolite of a drug with a mild inflammatory episode that renders the liver more sensitive to injury resulting in increased toxicity (inflammatory stress hypothesis). With the aim to find biomarkers that can detect IDILI ex vivo, we used mouse precision-cut liver slices (PCLS) to study the interaction of an inflammatory reaction and reactive-metabolite-producing drugs. PCLS retain the normal tissue architecture of an intact liver with all its cell types in their natural environment. Mouse PCLS were incubated for 24h with IDILI-related drugs ketoconazole (KC), clozapine (CZ), diclofenac (DF), carbamazepine (CBZ) and troglitazone (TGZ), or the non-toxic comparator drugs voriconazole (VC) and olanzapine (OZ) at NOAEL concentration or slightly above it in the absence or presence of lipopolysaccharide (LPS), an inflammation inducer. Cell viability (ATP, LDH leakage, histomorphology), reduced glutathione (GSH) level, nitric oxide (NO) production, and cytokine and chemokine release were assessed. LPS exacerbated the toxicity of KC and CZ but not of VC and OZ (the non-toxic comparator drugs in the same drug class as KC and CZ respectively). However, other IDILI-related drugs (DF, CBZ and TGZ) did not cause synergistic toxicity with LPS after 24h incubation, suggesting different mechanisms of action of IDILI for these drugs. LPS treatment alone did not reduce GSH levels in PCLS, yet together with KC or CZ but not with the other drugs, LPS appeared to further lower the GSH level when compared to the treatment with these drugs alone. LPS significantly increased NO, cytokine (IL-1β, IL-6, IFN-γ, TNF-α) and chemokine (CCL3, CCL5, G-CSF, GM-CSF) release into the media of PCLS, while the treatment with the drugs alone did not cause any substantial increase. All 7 drugs drastically reduced the LPS-induced NO production, while they caused different effects on the LPS-induced cytokine and chemokine release in PCLS. Interestingly, only KC and CZ increased the LPS-induced GM-CSF release. Therefore, a correlation between synergistic toxicity and upregulated GM-CSF release in LPS+KC and LPS+CZ treatment groups was found. Based on these results, GM-CSF can be a potential biomarker to detect IDILI related to the inflammatory stress hypothesis and PCLS appear to be a promising ex vivo model to further unravel the mechanism of inflammatory stress-related IDILI.

P163 - RECONSTITUTION OF CLOZAPINE METABOLISM IN YEAST SHOWS CYTOCHROME P450 DEPENDENT INCREASE IN TOXICITY AND PROTECTION BY HUMAN GLUTATHIONE S-TRANSFERASE P1-1

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Clozapine, an often-prescribed antipsychotic drug, has been implicated in severe adverse drug reactions (ADR) including hepatotoxicity and idiosyncratic agranulocytosis. Formation of reactive clozapine intermediates by cytochrome P450 (CYPs) has been proposed as a possible explanation for these ADR. Moreover, a possible protective role for human glutathione S-transferases (GSTs), known to be polymorphic, was recently shown using purified enzymes [1]. We aim to investigate the interplay between CYP bioactivation, GST detoxification and transport of clozapine in a reconstituted, cellular context using yeast. The yeast S.cerevisiae is a model eukaryote popular for its genetic accessibility, genome-wide screens, cost-effectiveness and rapid growth. However, wildtype yeast lacks CYP-genes responsible in humans for bioactivation of drugs. We generated yeast expressing a bacterial CYP BM3 mutant, engineered to mimic the drug-metabolizing potential of human CYPs, either alone or combined with different human

GSTs. We show a bioactivation-dependent growth inhibition upon clozapine exposure. Co-expression of GSTP1-1 protected against P450-dependent growth inhibition, whereas similar expression levels of GSTA1-1 and GSTM1-1 did not. Also formation of reactive oxygen species (ROS) was observed upon clozapine treatment, which was increased by CYP bioactivation. Interestingly clozapine-induced ROS formation was not related to mitochondrial respiration, unlike we previously reported for diclofenac [2]. Alternative sources of ROS are currently being studied, as well as the effect of GSTP1-1 co-expression. Using GFP-reporter constructs, we show a clear induction of the multidrug transporter PDR5 by clozapine. Other reporter constructs are being tested. The suitability of yeast as a model system for CYP and GST interplay is discussed.

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P164 - THE ROLE OF METABOLISM IN DICLOFENAC-INDUCED INTESTINAL TOXICITY IN HUMAN EX VIVO

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The use of Diclofenac (DCF: 2-(2,6-dichloranilino) phenyl acetic acid), a non-steroidal anti-inflammatory drug is associated with severe gastro-intestinal side-effects. In vivo rat studies suggest that reactive metabolites of DCF, produced by the liver, play an important role in the intestinal toxicity; whether DCF itself induces direct toxicity is not clear. In this study, human precision-cut intestinal slices (PCIS), prepared from human jejunum were used as an ex vivo model to investigate the mechanism of DCF-induced intestinal toxicity. PCIS from 12 human individuals were incubated with a concentration range of DCF (0-600µM). After 5 h of incubation, DCF (≥400 µM) caused a significant decrease in ATP content and morphological damage. 4'and 5-Hydroxyl DCF and DCF acylglucuronide were detected as major metabolites in human PCIS, but with a large variation among individuals. Toxicity of 400µM DCF already became apparent after one hour of incubation and the metabolite formation rate was also strongly decreased at this time-point compared with that at the non-toxic doses, indicating enterocyte injury. Drug-protein adducts were detected by immunohistochemical staining. To investigate the role of metabolism in the toxicity, the PCIS were incubated with DCF in the presence of a non-toxic concentration of the CYP inhibitor cimetidine (5mM) or the UGT inhibitor borneol (0.5mM). Both inhibitors effectively decreased the metabolites formation but did not reduce the toxicity of DCF. In conclusion, using PCIS as an ex vivo model we show that DCF induces toxicity to the human intestine directly, but the detected hydroxyl or acylglucuronide metabolites are not responsible for the toxicity.

P165 - THE INFLUENCE OF POLYVINYLPYRROLIDONE (PVP) ON THE TOXICITY OF CRYOPROTECTIVE AGENTS IN DIFFERENT LIVER CELL TYPES AND ON VITRIFICATION OUTCOME IN PRECISION-CUT LIVER AND KIDNEY SLICES

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Cryopreservation of precision-cut tissue slices would strongly improve their application possibilities in ADME-tox studies. Low toxic mixtures of cryoprotectant agents (CPAs) are needed for cryopreservation of cells, and tissues by vitrification. Many of these mixtures contain polyvinylpyrrolidone (PVP), a non-permeable macromolecule, but the effect of this agent on the viability of the tissue is unknown. We investigated the effect of adding or replacing PVP on the viability of precision cut liver and kidney slices before and after vitrification with special emphasis on the susceptibility of the different non-parenchymal cells (NPC) in the liver to PVP toxicity. **Methods:** PVP in the commercially available CPA M22 was replaced by a polymer that is one of the components of the PVP-free CPA Sol Z; the resulting solution was called CPR, and the polymer in Sol Z was replaced by PVP (called Sol ZP). The viability of PCLS was studied after loading the slices with one of the four solutions and after vitrification. Differential scanning calorimetry showed that adding or omitting PVP did not change the ability to prevent ice-crystal formation during vitrification of the CPAs. **Results**: ATP levels of PCLS treated with the PVP free Sol CPR were significantly higher than that of PCLS treated with M22 ((98% versus 78% compared to untreated controls), whereas ATP levels of PCLS treated with Sol ZP (85%) were comparable to that of PCLS treated with Sol Z (89%). HE staining of PCLS treated with the CPAs indicated that the viability of hepatocytes after treatment was comparable to that of control slices for all

four solutions. However, non-parenchymal cells (NPC) in the sinusoidal space of PCLS showed consistent changes in the morphology. Immunohistological staining for different NPC (stellate, Kupffer and endothelial cells) suggested that the viability of NPC cells was not improved by the new CPAs. After vitrification, the ATP level of PCLS treated with Sol CPR was 61 % and with M22 was 43% compared to slices exposed to the CPAs but not vitrified. This suggests that the removal of PVP from M22 has some protective effect on PCLS at both CPA loading/unloading and vitrification. Moreover, kidney slices treated with Sol CPR also showed higher viability after vitrification than slices that were treated with M22. **Conclusion:** PVP has no protective effect on the viability of liver and kidney slices after vitrification although it has been widely used for this purpose. We showed for the first time that damage due to CPA toxicity does not only affect the hepatocytes but also the NPC. Sol CPR is a promising CPA for the vitrification-based cryopreservation of PCLS, but further optimization of the vitrification technique is required to improve the viability of the slices after vitrification and re-warming.

P166 - THE MUSCLE CELL LINE RD IS MORE SENSITIVE TO STATIN-MEDIATED DISRUPTION OF PROTEIN PRENYLATION THAN THE LIVER CELL LINE HUH7: IMPLICATIONS FOR STATIN-MEDIATED TOXICITY

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Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis, and are widely used to treat hypercholesterolemia, a risk factor for cardiovascular disease. A common adverse effect associated with statin use is muscular aches, which can rarely progress to the potentially fatal rhabdomyolysis. The mechanism underlying this toxicity is still not fully understood, but secondary effects of HMGCR inhibition, such as disruption of protein prenylation, have been suggested as a likely mechanism. We have compared the effects of statins on both cell viability and protein prenylation in human liver and skeletal muscle cell lines, hypothesising that statins would preferentially disrupt prenylation within the muscle cells. Initially, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assays were carried out, in order to assess metabolic and cellular viability after cells were exposed to different statin concentrations. We observed significant reductions in cell viability for both cell lines, on treatment with simvastatin, cerivastatin and pravastatin in a dose-dependent manner. The largest reductions in cell viability were observed with the most lipophilic statins, simvastatin and cerivastatin, whereas significant loss of cell viability was only observed with very high concentrations of the hydrophilic pravastatin (1000µM and greater). Higher cell toxicity was observed for all statins in RD muscle cells compared to Huh7 liver cells. Subsequently, western blot analysis was used to examine the prenylation status of the geranylgeranylated protein Rap1A in total protein extracts from cells treated with a range of simvastatin and cerivastatin concentrations. Unprenylated Rap1A was observed in both cell lines, in a dose-dependent manner, following treatment with simvastatin and cerivastatin. This prenylation deficiency was a specific effect resulting from statin inhibition of HMGCR since co-treatment with mevalonate, a downstream intermediate in the metabolic pathway, rescued the phenotype. Both cell lines were more sensitive to cerivastatin than simvastatin, with a 10-fold difference in dose required to produce detectable levels of unprenylated Rap1A. In addition, statin treatment resulted in disruption of Rap1A prenylation at a 10-fold lower concentration in muscle than in liver cells, both for simvastatin (1µM and 10µM respectively) and cerivastatin (0.1µM and 1µM respectively). Together these data confirm that RD muscle cells, which are significantly more sensitive to statin treatment, do show preferential disruption of protein prenylation when compared to liver cells. This supports a mechanism whereby disruption in the intermediates in the cholesterol synthesis pathway, including the isoprenoids used in protein prenylation, is the primary cause of statin-induced muscle toxicity.

P167 - ABACAVIR METABOLISM IN ANTIGEN PRESENTING CELLS GENERATES A FUNCTIONAL ANTIGEN FOR T-CELLS

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The incidence of hypersensitivity reactions to abacavir [{(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl} methanol] has been effectively reduced by the introduction of genetic screening for HLA-B*5701[1]. The isolation of drug-specific $CD8^+$ T-cells from both hypersensitive patients and healthy volunteers provides evidence of an immune pathogenesis[2]**.** The mechanisms of T-cell activation are currently unclear. The

hepatic formation of a protein-reactive aldehyde metabolite has been suggested in vitro[3], however this is yet to be confirmed in patients. We sought to test the hypothesis that abacavir metabolism in antigen presenting cells (APCs) generates a functional antigen that stimulates T-cells from volunteers carrying the HLA risk allele. Abacavir-specific T-cell clones were generated by serial dilution from volunteers expressing HLA-B*5701 and other HLA molecules. Clones were then characterised in terms of their phenotype, function and mechanisms of antigen presentation. Analogues of abacavir were synthesised and used to probe the chemical restriction of the MHC-TCR interaction. The hepatic and immune cell metabolism was assessed by mass spectrometry. Abacavir-specific clones were generated from all volunteers expressing HLA-B*5701. These clones proliferated and secreted cytokines (IFN-g, IL-13) and effector molecules (FasL, GrzB and perforin). APCs pulsed with abacavir for 16h stimulated 10/19 T-cell clones. This response was blocked by fixation of the APCs with glutaraldehyde prior to adding the drug. Pulsing the APCs for 1h was not sufficient to stimulate T-cells. As described previously, abacavir was metabolised to 3 isomeric carboxylic acids in human liver cytosol[3]. The oxidation of abacavir in APCs was also detected, providing a direct link between metabolism and the T-cell response. An alternative enatiomer of abacavir (1R,4S conformation) was metabolised to the corresponding carboxylic acids but did not stimulate T-cell clones. In conclusion, chemical restriction exists at the HLA-TCR interface and metabolism is key to the activation of several abacavir-specific T-cell clones.

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P168 - CHARACTERIZATION OF PROTEIN ADDUCTS OF P450-DEPENDENT REACTIVE INTERMEDIATES OF DICLOFENAC BY MASS SPECTROMETRY

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Diclofenac (DF) is a non-steroidal anti-inflammatory drug that is widely used for rheumatoid arthritis. In a very small number of treated patients DF is causing severe idiosyncratic drug-induced liver injury (DILI). It is unknown what is the combination of factors responsible for making patients susceptible to DILI observed with DF. It is currently thought that covalent protein binding by chemically reactive metabolites is a critical event in the onset of many idiosyncratic drug toxicities. In this regard, it is important to develop analytical methodologies to assess the ability of drugs to form protein adducts and to characterize the selectivities towards individual amino acids. It is known that DF can be bioactivated by glucuronidation, by forming reactive acylglucuronides, and by P450-dependent pathways. The P450-dependent covalent binding is considered to be associated with direct cytotoxicity of DF. Two quinone-imines derived from 4'-hydroxydiclofenac (4'-OH-DF) and 5-hydroxydiclofenac (5-OH-DF) have been described by GSH-conjugation. Additionally, a quinone-methide resulting from decarboxylation of DF has been proposed. Immunochemical methods cannot discriminate between these different P450-dependent bioactivation pathways. The aim of this research was to characterize the protein adducts of these different P450-dependent reactive metabolites of DF by mass spectrometry. To this end, incubations were performed with DF itself and its monohydroxylated metabolites 4'-OH-DF and 5-OH-DF in presence of the model target protein hGST P1-1. Multiple protein adducts were formed in presence of DF itself. A number of these adducts could be attributed to the 5-OH-DF pathway by comparison with the adducts formed in incubations of 5-OH-DF itself. In addition, adducts were identified which could not be attributed to the 4'-OH-DF and 5-OH-DF metabolites. Therefore, a novel bioactivation pathway appears to be involved in formation of protein adducts of DF.

P169 - DRUG-INDUCED GENOME INSTABILITY: TRANSLATION FROM YEAST TO HUMAN

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Genomic instability is implicated in various pathological disorders and in humans it is often associated with premature aging, neurological diseases, predisposition to various types of cancer and with inherited diseases. Also, drug-induced DNA-damage can lead to chromosomal instability and aneuploidy. Paracetamol is a common over-the-counter drug, which can cause severe hepatotoxicity when overdosed. At high concentrations, the drug is also toxic in the yeast Saccharomyces cerevisiae. Three paracetamol-tolerant yeast strains were identified in a genome-wide loss-of-function screen in collaboration with Dr. Fred van Leeuwen (AvL-NKI, Amsterdam). Deletion of any of these genes, which are the members of DNA damage response (DDR), results in paracetamol resistance. We focus initially on two of the identified genes, which encode E2 ubiquitin-conjugating enzymes. We will study the role of these genes in maintaining the genomic stability in yeast as well as their orthologs in humans. In order to analyze possible drug-induced genomic instability, we would like to determine if these strains undergo genetic adaptations in order to become resistant to paracetamol. It is known that yeast cells can confer resistance for a specific drug by becoming aneuploidy so we hypothesize that yeast might become aneuploidy under influence of paracetamol. In a recent publication (Chen et al. Nature 2012) it was shown that yeast became resistant to a Hsp90 inhibitor, radicicol, by gaining an extra chromosome XV. We will use FACS, microscopy and deep sequencing technology to study the effects of paracetamol on yeast genomic DNA, cell morphology and chromosome segregation. Furthermore, genes have been identified, which by overexpression also confer resistance to paracetamol. One of these genes is TAT2, which is a high affinity tryptophan and tyrosine permease. Western blot analysis showed that one resistant deletion strain has increased levels of Tat2p suggesting that this might be the cause of the resistance in this strain. We will determine if the increased Tat2p levels are present in all three resistant deletion strains and we would like to determine if the two identified E2 ubiquiting-conjugating enzymes are involved in ubiquitylation of Tat2p as Tat2p is normally targeted for degradation by ubiquitylation.

P170 - TRAPPING ASSAYS AS THE SURROGATE FOR COVALENT BINDING TO PROTEINS

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The reactive metabolites are thought to covalently bind to cellular macromolecules and to be related to clinical adverse effects, including idiosyncratic drug-induced liver injury (DILI). Covalent binding (CB) assay using radio-labeled compounds is the most useful method for the direct quantification of reactive metabolite covalently bound to proteins. We reported that the combination of CB amount and daily dose can estimate the risk of idiosyncratic DILI (1). Early in the drug development process, it is generally difficult to conduct the CB assay because of limited availability of radio-labeled compounds. In this study, the trapping assay with dansyl glutathione (dGSH) was conducted to examine whether the amount of dGSH adducts correlate to the amount of CB. Twenty five compounds (12 drugs associated with DILI and 13 drugs not associated with DILI) were evaluated in both of dGSH trapping assay and CB assay. There was a good correlation between the amount of dGSH adducts and the amount of CB although some false negative compounds were observed in dGSH trapping assay. Recently, it is reported that the combination of GSH trapping assay and time-dependent inhibition (TDI) assay was useful for the prediction of the CB (2). The applicability of the combination of GSH trapping and TDI assay for above 25 compounds is also discussed.

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P171 - INHIBITION OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION BY DICLOFENAC AND ITS METABOLITES IN RAT LIVER MITOCHONDRIA

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Hypothesis: Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) widely used clinically for the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Hepatotoxicity is one of the side effects associated with the drug. Various mechanisms for the diclofenac-induced liver injury have been proposed, but have not been fully understood. The diclofenac liver toxicity in humans is idiosyncratic [1] and immunologic and metabolic idiosyncrasies have been suggested [2], [3] Thus, metabolic activation of this drug has been a focus and several reactive metabolites have been proposed as candidates to contribute to the toxicity in experimental animals and humans NSAIDs including diclofenac that have a common chemical structure show hepatocyte toxicity, and these toxic NSAIDs deplete cellular adenosine triphosphate (ATP) before the enzyme leakage. The toxic NSAIDs have been shown to be uncouplers of mitochondrial oxidative phosphorylation, which results in impairment of ATP synthesis. Diclofenac has been shown to impair the mitochondrial ATP production and studies have demonstrated that the ATP content in hepatocytes is affected earlier than other cytotoxicity markers, indicating that the compound causes mitochondrial dysfunction. A direct link between formation of reactive phase-I and phase-II metabolites and hepatotoxicity still remains to be verified. Whether drug metabolism is involved in the mitochondrial impairment observed with diclofenac has never been thoroughly investigated. In the present study, we investigated the inhibitory effects of diclofenac and its metabolites (both phase-I and Phase-II metabolites) on mitochondrial ATP production. This is the first study to compare the mitochondrial effects of diclofenac with the effects of both phase-I and Phase-II metabolites. Method: Isolated rat liver mitochondria (0.5 mg/mL) were pre-incubated/incubated in the presence of diclofenac or its metabolites with malate (2 mM) and sodium pyruvate (2 mM) as a substrate for oxidative phosphorylation. ATP production was initiated by addition of ADP (2 mM) and the reaction was stopped after 30 min. ATP was measured using luciferin/luciferase reagents. Results: The results showed that diclofenac inhibited ATP production in a concentration and time dependent manner. We have observed increased inhibitory effect, when diclofenac/its metabolites were pre-incubated in mitochondria both Phase-I and Phase-II metabolites of diclofenac inhibited the oxidative phosphorylation. Conclusions: The results provide evidence that drug metabolites of diclofenac could be involved in the hepatotoxic action of drug and provides new aspects of the investigation into the mechanism of drug toxicity.

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P172 - ROLES OF METABOLIC ACTIVATION, DNA DAMAGE, OXIDATIVE STRESS AND ACUTE CYTOTOXICITY IN THE LIVER CARCINOGENICITY OF 4-AMINOBIPHENYL IN THE MOUSE

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Chemicals of the aromatic amine structural class include environmental contaminants that both increase risk for human cancers and produce liver tumors in rodent models. These agents are thought to require bioactivation to DNA-damaging electrophiles by enzymes of drug metabolism for their carcinogenicity to be observed. We developed genetically modified mouse models to study the role of aromatic amine bioactivation in cancers caused by exposure to these chemicals. In in vivo carcinogenicity studies of mice exposed to the cigarette smoke and dye contaminant 4-aminobiphenyl (ABP), which can be metabolized by the arylamine N-acetyltransferase (NAT)

enzymes, we saw a lower incidence and multiplicity of liver tumors in male NAT-deficient Nat1/2(-/-) mice than in wild-type mice, while female mice were resistant to tumors regardless of NAT status. Liver-selective transgenic expression of human NAT2 in Nat1/2(-/-) mice restored the sensitivity of males to ABP-induced liver tumors, while NAT2 transgenic females remained tumor-resistant. However, liver tumor formation did not correlate with biomarkers of acute liver DNA damage or mutations in mice exposed to ABP either as adults or using the postnatal exposure protocol that we used to induce liver tumors, suggesting that the NAT proteins may be related to novel tumor growth promoting events that are distinct from carcinogen bioactivation leading to DNA damage. Susceptibility to liver tumors following the postnatal exposure of mice to the chemically unrelated nitrosamine diethylnitrosamine (DEN) has recently been correlated with its acute hepatotoxicity in adult mice, which is thought to trigger oxidative stress and inflammatory responses that may favour the subsequent proliferation of initiated cells. The goal of the present studies was to determine whether ABP exposure is also capable of producing either acute or chronic hepatotoxicity, oxidative stress and/or inflammation in male and female wild-type or NAT-modified mice. When we used a 2-dose postnatal ABP exposure protocol that is capable of producing liver tumors in male mice one year following their exposure, we observed no significant elevations in serum levels of the liver damage biomarker alanine aminotransferase (ALT) or the inflammatory cytokine IL-6 in male or female wild-type or Nat1/2(-/-) mice up to 48 hr after the second exposure. Interestingly, we also observed no increase in serum ALT or IL-6 levels in mice exposed to DEN when we used the postnatal tumor-inducing protocol, in contrast to the male-selective elevations in these parameters that were previously observed by others using a 4-fold higher dose of DEN in adult mice. Preliminary results also suggest that ABP and N-hydroxy-ABP are capable of producing oxidative stress in hepatocytes, measured by increased reactive oxygen species (ROS), lipid peroxidation and oxidative DNA damage either in ABP-exposed mouse hepatoma cells in culture or following the in vivo postnatal ABP exposure of mice. Studies are in progress to determine whether sex and strain differences in these parameters correlate with the previously observed differences in liver tumor incidence.

P173 - DIVERSITY OF METABOLIC AND TOXIC MECHANISMS OF CLIVORINE, A REPRESENTATIVE OTONECINE-TYPE PYRROLIZIDINE ALKALOID

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Clivorine, a representative naturally-occurring otonecine-type pyrrolizidine alkaloid (PA), was found in many Chinese medicinal herbs[1]. Its hepatotoxicity and/or carcinogenicity in experimental animals had been observed, imposing a potential risk on the clinical use of the clivorine-containing herbs in humans. From a viewpoint of metabolism, its toxic machinery was based on its metabolic activation by microsomal P450s especially CYP3A and successive covalent binding with essential cellular targets. Several studies also showed that other microsomal metabolic pathways including deacetylization and/or direct hydrolysis by esterase(s) might exist in a species- or stain-specific manner[2]. Clivorine was also suspected to release a direct toxic effect on hepatocytes, in which the mitochondria-mediated apoptosis via degradation of Bcl-xL and co-activation of caspase-3 and caspase-9 was found; clivorine also influenced the expression of p53 and p38 MAP kinase[3]. These evidences implied a strong link of the toxic outcome of clivorine with multiple metabolic and molecular toxicological mechanisms. In this study, the rat liver microsomal metabolic profile of clivorine was re-examined by HPLC coupled to ESI-IT-MS, and a role of autophagy, one of the important cell death/defense modalities, in the clivorine-induced hepatotoxicity were investigated. We found that the liver microsomal esterase(s) from the male rats may also involve in the generation of deacetylclivorine. Moreover, many other metabolites besides deacetylclivorine and those from bioactivation pathway (e.g., DHR, 7-GSH-DHR and clivoric acid) were found. Noteworthily, two new metabolites with MWs of 421 and 379 were detected and found to be derived from clivorine and its major metabolite deacetylclivorine via oxidations probably catalyzed by P450s, respectively. The MS/MS fragmentation of the metabolites showed the same diagnostic ions as that of otonecine-type PAs, suggesting the oxidations may be occurred in their necic acid moieties. This result indicated a novel microsomal metabolic pathway of clivorine. On the other hand, clivorine but not monocrotaline, a typical retronecine-type PA, could significantly induce the expression of the autophagy-related genes, e.g., Atg3, Atg7 and Atg 12 as well as other core component e.g., LC3 in huh-7 cells in a concentration-dependent manner. Conversely, the expression of Beclin-1 gene was up-regulated in the early stage of treatment but remarkably suppressed after 24 h. These evidences suggested that clivorine may promote autophagy prior to apoptosis once its exposure to hepatocytes. These new or previously un-described evidences demonstrated the diversity of the metabolic and

molecular mechanisms of clivorine in hepatotoxicity, which may be contributed to the understanding of the metabolism and toxicity of otonecine-type PAs and the corresponding medicinal herbs in vivo.

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P174 - ANDROGEN RECEPTOR ACTIVITY AFTER A SINGLE TESTOSTERONE DOSE – INDEPENDENT OF THE GENETIC POLYMORPHISM IN TESTOSTERONE GLUCURONIDATION BY UGT2B17

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Background: Clinical effects of androgen exposure are difficult to assess. Androgen receptor (AR) activity in serum and urine may be a valuable tool for clinical monitoring and diagnostics in androgen related disorders and therapy. In sports, it may serve as a complement to the urinary testosterone/epitestosterone (T/E) doping test, since this ratio is highly dependent on the genetic deletion polymorphism in the UGT2B17 enzyme (Jakobsson et al 2006, Schulze et al 2008). We investigated if AR activity is related to testosterone concentrations in urine and serum, and if it is dependent on the UGT2B17 genotype. **Materials and methods**: Healthy volunteers genotyped for UGT2B17 received 500 mg of testosterone enanthate intramuscularly. Serum and urine samples were collected prior to, and 2, 4 and 15 days after dose. Concentrations of unconjugated and conjugated testosterone and several metabolites including dihydrotestosterone (DHT) were analysed with LC/MS/MS. AR activity in buffer spiked with different androgens, in serum, or urine was determined using an androgen-responsive yeast based bioluminescence assay (Roda et al, 2011). **Results:** Concentration vs. AR response curves showed that DHT was the most potent androgen, followed by other androgens in the order of testosterone > 5α-androstane-3α,17β-diol > androsterone. Testosterone glucuronide, etiocholanolone and 5β-androstane-3α,17β-diol did not generate any measurable signal (except a weak signal at the highest concentration of testosterone glucuronide). The urinary androgenic activity increased 4-5 fold days 2 and 4 after dose ($p<0.0001$) but was back to basal activity on day 15. The corresponding serum AR activity and serum testosterone and DHT concentrations showed a similar profile. Serum concentrations of testosterone and DHT, as well as the AR activity were, however, still above baseline 15 days after dose. The increase and the activity profile were independent of the UGT2B17 deletion polymorphism. The AR activity was closely related to the concentration of unconjugated testosterone or DHT, but independent of the UGT2B17 genotype. **Discussion**: The AR assay captures the total activity originating from endogenous bioactive testosterone, as well as its active precursors and metabolites. Thus, the AR activity is not reflecting the large differences in total (conjugated and unconjugated) androgen concentrations based on the genetic polymorphism in UGT2B17. The AR bioluminescence assay may be useful in clinical monitoring of androgen treatment in different hormone related disorders. It may also be useful for detection of illicit androgen doping in sports, or in the society.

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P175 - METABOLIC PROFILES OF ISOCORYDINE — AN IMPORTANT APORPHINE ALKALOID

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Isocorydine is an important aporphine alkaloid, which is widely present in herbs. Isocorydine shows various pharmacological activities such as antiplasmodial, antiarrhythmic, vasodilative effect and nonspecific spasmolytic effect on diverse visceral smooth muscles. However, there are very limited data regarding hepatic metabolism of isocorydine. Therefore, we investigated the metabolism profiles of isocorydine in various vitro models including rat liver microsomes (RLMs), human liver microsomes (HLMs), recombinant human CYPs (rhCYPs) and HepG2 cells. In RLMs, the isocorydine metabolism was inhibited by 88% by ketoconazole, while the metabolic rate of isocorydine incubated with RLMs pretreated with dexamethasone was notably higher (6.4–fold) than the control (non-induced RLM), indicating that CYP3A2 can be involved in isocorydine oxidative metabolism. Classical CYPs' substrates were used to investigate the inhibition effect of isocorydine on the CYPs, with the results that isocorydine almost has no inhibition effect on these main CYP isoforms (Ki values: 151.0 ± 4.2 μM on CYP1A2, 190.3 ± 57.0 μM on CYP2C6, 264.0 ± 12.7 μM on CYP2C11, 182.7 ± 13.3 μM on CYP2D1, 107.4 ± 13.8 μM on CYP2E1, 400.2 ± 14.7 μM on CYP3A2). The metabolites of isocorydine were preliminarily characterized by UPLC-MS/MS including one mono-demethylation and one mono-hydroxylation metabolites. Metabolic profiles of isocorydine in human were a little different from the circumstances in rats. In HLMs, the metabolic pathways of isocorydine were studied using specific chemical inhibitors. It was found that CYP3A4 and CYP2C8 were two CYP enzyme isoforms that mediated the metabolism of isocorydine in human, and these results were confirmed by rhCYP3A4 and rhCYP2C8. It was found that isocorydine did not inhibit CYP2C19, CYP2D6, CYP2E1 and CYP3A4 as the IC50 values of isocorydine on these CYPs isoforms were all > 400 μM. However, the IC50 values of isocorydine on CYP1A2, CYP2C9 and CYP2C8 were 132.7 ± 1.1 μM, 146.8 ± 1.1 μM and 53.8 ± 1.1 μM, respectively, implying that isocorydine may have weak inhibition effect on CYP2C8. In addition, the induction effect of isocorydine on some metabolic enzyme isoforms regulated by two nuclear receptors (NRs) human pregnane X receptor (hPXR) and human constitutive androstane receptor (hCAR) was investigated by dual-luciferase reporter assay in HepG2 cells. Results revealed that isocorydine almost has no gene induction effect on CYP2B6, CYP3A4 and UGT1A1. These finds should be useful for the clinical application and further studies of isocorydine. This work was supported by National Major Projects of China (2012ZX09506001-004) and Nature Scientific Found of China (81173120).

P176 - INNOVATIVE AUTOMATION PLATFORM FOR METABOLISM IDENTIFICATION STUDIES

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Biotransformation data, metabolite structures and pathways have become more and more in the interest of health authorities. Since the introduction of the MIST guidance and the EMEA metabolite guidance M3, having early information on final structures of metabolites as well as metabolite exposures levels become more and more important. Metabolite profiling is a time consuming activity in particular when samples are used from in vivo studies, and especially the human ADME studies where only limited amounts of radiolabeled material are dosed. In addition, profiling of complex metabolic patterns by LC often requires analysis times of 90 to 120 min with off line radioactivity counting. As such, the sample profiling was the rate limiting step, as fraction plates had to be changed manually. Previously, only 2-3 samples could be analyzed per day. To automate process and increase throughput, we developed and implemented a novel automation technology by linking two technologies together: Specifically, a robotics platform (PlateCrane) was linked to an HPLC fraction collector (Gilson), which is also linked to a HPLC-MS. This " hybrid" system allows continuous changing of fraction collected plates. However, now sample output can be

increased up to at least 12 samples per day (based on 2h run time) on a 24/7 basis. This new technology drastically reduced manual 96-well plate collection and handling and significantly increased throughput within Biotransformation for preclinical and clinical ADME studies

P177 - A DRUG METABOLISM STUDY ON WHOLE BODY SECTIONS OF ANIMAL TISSUE ANALYSED BY HIGH DEFINITION MALDI IMAGING

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Mass spectrometry Imaging (MSI) is increasingly used in pharmacokinetic studies during preclinical studies. It has been recognized as a complementary technique to Whole-Body Autoradiography (WBA), which is traditionally used for approval of a drug by the Food and Drug Administration (FDA) agency. The two main advantages of MSI are cost savings compared to radio-labeling of the drug and the absolute confirmation that the drug and its possibly produced metabolites are indeed visualized. Here, we present the results from a study where two MS based imaging approaches were used to illustrate the spatial distributions of a drug and its metabolites (dosed in rat), along with untargeted analysis of endogenous molecules in a whole body section. The first experiment was carried out on a 2 h post-dose tissue section in a multiplex targeted MS/MS, approach where the drug Olanzapine (OLZ) and two known metabolites were imaged from a single tissue section. A second experiment was performed on a 6 h post-dose tissue section in an untargeted MS approach. Also in this instance, both the drug and the two main metabolites were imaged. However, here a vast amount of information is also generated by the ionisation of the endogenous species present in the whole body tissue section. The final part of the study involved the performance evaluation of isobaric species separation in the gas phase by means of ion mobility separation, which is integrated in the MALDI SYNAPT G2 HDMS instrument, for untargeted MALDI-MS imaging experiments of OLZ and its two main metabolites.

P178 - IDENTIFICATION OF AILDENAFIL METABOLITES IN RAT URINE USING LTQ/ORBITRAP HYBRID MASS SPECTROMETRY

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Aildenafil, 1-{[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4,3-d] primidin-5-yl)-4-ethoxyphenyl] sulfonyl}-cis-3, 5-dimethylpiperazine, as a pyrrolopyrimidinone analogue of sildenafil and verdenafil, is a potent inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V by degrading of cGMP in the corpus cavernosum, and its longer duration of action than sildenafil in the treatment of male erectile dysfunction has been demonstrated in clinical trials[1]. The urinary, fecal and biliary excretion assay in rat showed that aildenafil was extensively metabolized after oral administration with less than 5% of the dose recovered from urine (1.23%), feces (1.74%), and bile (0.04%) as parent drug; however, its metabolic profile is still unknown. This study was conducted to characterize the metabolites of aildenafil in rat urine after oral administration using LTQ/Orbitrap hybrid mass spectrometry. Accurate full-scan MS and MS/MS data were used to identify the metabolite structures. Metabolism of aildenafil is complex in rat urine with up to 22 different metabolites detected. The major metabolic pathways were loss of a two-carbon fragment from the piperazine ring (N,N'-deethylation) and the introduction of one oxygen atom to piperazine ring with dehydrogenation, other routes of metabolism included aliphatic side chain hydroxylation, oxidation of the piperazine ring, pyrazole N-demethylation, O-deethylation, dehydrogenation. Additional metabolites arose through combinations of these pathways above. No conjugated metabolites were observed. Results from this study firstly revealed the metabolic profiles of aildenafil and provided useful information that may act as a valuable reference for clinical pharmacology.

[1] Wang, J., et al., Liquid chromatography tandem mass spectrometry assay to determine the pharmacokinetics of aildenafil in human plasma. J Pharm Biomed Anal, 2007. 44(1): 231-235.

P179 - RAT SYSTEMIC EXPOSURE PROFILES OF THE BIOACTIVE COMPOUNDS FROM ORALLY ADMINISTRATED GUIZHI-FULING EXTRACT, A STANDARDIZED GYNECOLOGIC MULTIHERB MEDICINE

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Background and aim: Guizhi-Fuling extract has been widely used to treat gynecological disorders in clinic, such as cervical cancer, endometriosis, primary menalgia, ovarian cysts, uterine myomas, and ectopic pregnancy. Guizhi-Fuling extract is a multiherbal medicine consisting of cinnamon, herbaceous peony, moutan peony, peach, and hoelen. As far as we know, the chemical composition in Guizhi-Fuling extract has not well studied yet and data on the compound profiles in systemic exposure are scare as well. The objective of this study was to gain a full picture of chemical composition and compound profile in Guizhi-Fuling extract and rat plasma, bile and urine after oral administration of the gynecologic medicine to rats. Methods: Literature informatics-guided chemical profiling and quantification were performed by an Waters ACQUITY UPLC interfaced with an AB-Sciex API 4000 Q Trap mass spectrometer equipped with ESI source. Results: Guizhi-Fuling extract contained 93 constitutes including 26 triterpenes from hoelen, 6 constituents (3 volatile oils, 1 coumarin, and 1 carboxylic acid) from cinnamon, 5 glycosides from peach; 56 constituents of monoterpene glycoside, galloyl derivatives, or acetophenes from the species Paeonia of peony and moutan peony. The levels of these compounds ranged from trace (less than 0.001) to 162mg/g capsule. Among the constituents, 33 constituents were measured in the rat bile samples after a p.o. administration of Guizhi-Fuling to rats at the dose of 9 capsules/kg body weight. However, only 76% of these constituents also occurred in the plasma samples, and 39% of these constituents and 9 other constituents occurred in the urine samples. Among the compounds with an individual dose >10 mg/kg, only 7% (including amygdalin, paeoniflorin, oxypaeoniflorin, Isopaeoniflorin/Albiflorin R1, gallic acid, paeonol, and albiflorin) were measured in all the tested rat samples, i.e., plasma, bile and urine. Meanwhile, 43% of the constituents with a dose ranging from 1 to 10 mg/kg were found in the bile samples, among which only mandelic acid ¦Â-D-gentiobioside and ortho-oxypaeoniflorin were detected in all the rat samples. Among constituents with an individual dose 0.1¨C1 mg/kg, only prunasin was detected in all the tested biosamples. Constituents with an individual dose <0.1 mg/kg were not detected in the plasma, bile and urine samples, except for 16-O-acetylpachymic acid, dehydrotumulosic acid, poricoic acid DM, and paeonilactone B occurring in one of these biosamples. Conclusion: The chemical composition and compound profile of Guizhi-Fuling extract and the systemic exposure profile in rat plasma, bile, and urine after a p.o. administration of Guizhi-Fuling extract were elucidated. The current study enabled us to more precisely implement multicomponent PK study of the multiherbal medicne.

P180 - USE OF ACCURATE MASS SPECTROMETRY AND NMR FOR EARLY ASSESSMENT OF CROSS-SPECIES METABOLISM

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Understanding the biotransformation of a drug is a key activity at all stages of the research and development process. In vitro metabolite profiling by liquid chromatography – mass spectrometry (MS) techniques at early discovery stages is useful to evaluate interspecies differences and determine possible unfavourable metabolism pathways. The gained knowledge is useful in the subsequent preclinical development stage where species comparison and in vitro – in vivo correlations are carried out. These activities received even more attention since the publications issued by the U.S. Food and Drug Administration (FDA, 2008) and the International Conference on Harmonization (ICH, 2009) on the safety testing of drug metabolites early in the drug development process of a candidate drug.In this poster we present a case study of in vitro and in vivo metabolism investigations showing significant differences among species. Metabolite profiling on plasma samples obtained after human dosing allowed the detection of a major metabolite that was not as relevant in preliminary in vitro studies. Mass spectrometry systems capable of accurate mass measurements were used to identify the biotransformation and to suggest which moiety was modified in the molecular structure of the parent compound. Furthermore, the unambiguous identification of the metabolite was carried out by preparative high performance liquid chromatography (HPLC) isolation and purification followed by high sensitivity nuclear magnetic resonance (NMR) investigations. The combined investigations into in vitro and in vivo metabolism provided an explanation to the significantly higher systemic clearance in humans compared to the animal species. In conclusion, a reliable cross-species comparison of

HPLC-MS metabolite profiles, including those obtained after human dosing during early clinical trials can be crucial to keep up with the deadlines of drug development programs.

P181 - IN VITRO AND IN VIVO MONITORING OF DRUG METABOLISM BY UTILIZING ARTIFICIAL MUSCLES

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Vibrations with ultra-low frequency are prevalent in human bodies. The spontaneous fluctuations in the blood flow velocity of the middle cerebral artery, like arterial blood pressure, could be diffracted into three components at specific frequency range, designated as high-frequency (0.15 to 0.4Hz), low-frequency (0.04 to 0.15 Hz), and very low-frequency (0.016 to 0.04Hz) components [1]. NADPH, an obligatory requirement for cytochrome P450-dependent drug oxidation, for its levels, varies periodically in time in neutrophils, macrophages, and certain tumor cells, and it oscillates in an approximate sinusoidal pattern with a period between 3 and 4min in all of these cells [2]. Investigations by previous researchers already showed that the metabolism of neutrophils was sensitive to externally applied periodically pulsed electric fields matched in frequency to the metabolic oscillation [2]. Therefore, to develop new smart materials that respond to such oscillations with ultra-low frequency, so as to perform real-time control of drug metabolism, is particularly desirable. This presentation, based on our recent and latest research, will show the potentials of a novel artificial muscle utilizing ionic polymer-metal nano-composite to be used for this purpose. Artificial muscle with biocompatibility, composed of styrene-maleimide alternating polymer, vinylidene fluoride polymer and nano-platinum, was fabricated. Morphological observations revealed that much smaller and more uniform platinum particles, after the electroless-plating process, were formed on and within the polymer matrix. The electromechanical properties of the as-prepared artificial muscle were first investigated in deionized water and lithium chloride solution, respectively. Then it was incorporated by glycerol, and was tested in the open air. Under the stimuli with different wave forms, i.e. sinusoidal, rectangular, triangular, and saw wave signals, and at various frequencies, i.e. 0.005 to 10Hz, the artificial muscles showed excellent harmonic responses and durable actuation in wet and dry environments, respectively. Especially, they overcame the phenomenon of back relaxation at the constant electric signal excitation with zero frequency. Furthermore, the artificial muscles displayed much better electromechanical responses in lithium chloride solutions of higher concentration (3.0N). The present study on artificial muscle suggested that it was a promising candidate for the real-time monitoring of the ultra-low-frequency metabolic oscillations both in vitro and in vivo. Acknowledgements: Supported by the Korea Science and Engineering Foundation (KOSEF) NRL Program grant funded by the Korea government (MEST) (No. R0A-2008-000-20012-0), National Natural Science Foundation of China (No. 50973089), and the Fundamental Research Funds for the Central Universities (No. SWJTU11CX056). References: [1] T. B. J. Kuo, C. M. Chern, W. Y. Sheng, W. J. Wong, H. H. Hu, Frequency domain analysis of cerebral blood flow velocity and its correlation with arterial blood pressure, J. Cereb. Blood Flow Metab. 1998, 18, 311-318; [2] A. J. Rosenspire, A. L. Kindzelskii, B. J. Simon, H. R. Petty, Real-time control of neutrophil metabolism by very weak ultra-low frequency pulsed magnetic fields, Biophys. J. 2005, 88, 3334-3347. Corresponding author e-mails: junluprc@hotmail.com (J. Lu); ikoh@kaist.ac.kr (I.K. Oh).

P182 - CRYSTALLINE AND POROUS POLYMERIC STRUCTURES FOR DRUG DELIVERY: PRESSURE-CONTROLLED GROWTH BY THE INTRODUCTION OF A VISCOUS SOLVENT OR FULLERENE

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Encapsulation of drugs such as these for cancer therapy in carriers could reduce toxic side effects, which can protect organs from a toxic drug prior to reaching its target. Many materials have already been used to encapsulate drugs, for example, organic polymers, liposomes, silicas, bioceramics, and bioactive glasses [1-3]. Especially, micro-encapsulation of the drug molecules into a polymer matrix with porous structures was one of the most promising methods to locally deliver them in a controlled manner, over a long period of time. Controlled local delivery may potentially optimize local therapeutic responses, thereby reducing the incidents of peak-related side effects and

systemic toxic effects [1]. In this study, two polymers with biocompatibility, polycarbonate (PC) and poly (ether ether ketone) (PEEK), were crystallized at pressure with a piston-cylinder apparatus, and were investigated using wide-angle X-ray diffraction, differential scanning calorimetry, transmission electronic microscope and scanning electron microscopy. The results showed that unique porous structures, composed of folded-chain lamellae, were synthesized by introducing dioctyl phthalate and fullerene nano-aggregations into PC and PEEK, respectively. Micrometer-sized stereo-open crystals and dendritic crystals, with three-dimensional porous structures, were formed in the investigated PC and dioctyl phthalate system. The stereo-open crystalline structures in the blend became more open when the weight ratio of the viscous solvent was increased. Furthermore, the dendritic growth of PC was found to be accelerated with the increase of the applied temperature, pressure, crystallized time and dioctyl phthalate concentration. This finally resulted in the formation of the structures of the spatial cellular dendrites. As for the PEEK and fullerene blend system, elliptical micro-spheres of PEEK, with open dendritic structures, were induced by fullerene nano-agglomerations at pressure. Particularly, other condition being the same, and with the pressure increased, hollow micro-spheres of PEEK that present a homogeneous morphology, were observed in a PEEK-fullerene blend sample. The crystalline porous structures of both polymers can be easily exposed and isolated with the applied selective etching technique, and their large pore volume and tunable discrete pore size allow the loading and controllable release of a variety of guest molecules. With their crystal morphology and surface property precisely manipulated, the as-prepared porous polymeric structures may pursue niche advanced applications like targeted drug delivery. Acknowledgements: This work was supported by the National Natural Science Foundation of China (Grant Number 50973089), as well as the Fundamental Research Funds for the Central Universities (Grant Number SWJTU11CX056 and SWJTU11ZT10). References: [1] M. Vallet-Regi, F. Balas, D. Arcos, Mesoporous materials for drug delivery, Angew. Chem. Int. Ed. 2007, 46(40), 7548-7558; [2] M. Wang, J. L. Coffer, K. Dorraj, P. S. Hartman, A. Loni, L. T. Canham, Sustained antibacterial activity from triclosan-loaded nanostructured mesoporous silicon, Mol. Pharmaceut. 2010, 7(6), 2232-2239; [3] B. Lei, X. Chen, Y. Wang, N. Zhao, Synthesis and in vitro bioactivity of novel mesoporous hollow bioactive glass microspheres, Mater. Lett. 2009, 63, 1719-1721. Corresponding author e-mail: junluprc@hotmail.com (J. Lu).

P183 - COMPARATIVE METABOLISM OF QUINOCETONE IN RATS, PIGS, BROILERS AND CARPS

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Quinocetone (QCT), 3-methyl-2-quinoxalin-benzenevinylketo-N1,N4-dioxide, is an antimicrobial agent for use in animals. To reveal the disposition of QCT in the body of animals, the mass balance, metabolism and distribution of QCT in rats, pigs, broilers and carps were studied with the radioisotope labeling, radio-tracking and liquid chromatography combined with hybrid ion trap/time-of-flight mass spectrometry for the identification and determination of metabolites in the excreta and tissues of the animals. The [3H]-QCT with a chemical purity of ≥98%, a radiochemical purity of ≥99% and a specific activity of 11.25 mCi/g was gavagely administered to the animals at a single dose of 200 mg/kg feed. The total radioactivity were excreted from the treated animals at the rates of 77%~86%, 85%~92% and 95%, respectively, at 3, 7 and 14 d after dosing. The excretion ratio of QCT from feces was >65% of the dose while the ratio from the urine was 25%~27% of the dose. There were 52, 50, 28 and 51 metabolites identified in the rats, pigs, broilers and carps, respectively. The metabolites in carps were obviously less than those in terrestrial animals. The metabolic pathways of QCT in the animals were N-O group reduction, carbonyl reduction, double-bond reduction in the side chain, hydroxylation in benzene ring and methyl group, and conjugations such as sulfation, glycuronidation, methylation and acetylation. After [3H]-QCT was gavagely given to the animals at the daily dose of 100 mg/ kg feed for consecutive 7 days, the radioactivity at 6 h after the dosing could be detected in all tissues of the treated animals. The high radioactivity was found in the liver, kidney, large intestine, bile, chicken crop, fish gill and skin while less in the heart, spleen, lung, muscle, fat, skin, bladder, adrenal glands and blood. The radioactivity at 3 d after the dosing was declined to <200 μg/kg in most tissues except liver, kidney and small intestine. The radioactivity at 7 d was detectable only in the liver, kidney, bile and small intestine. There was no any radioactivity detected in all tissues at 14 d. 3-methyl-2-benzenevinylketo-quinoxaline-N4-monoxide (Q2), 3-methyl-2-benzenevinylketo-quinoxaline (Q4) and 3-methyl-2-benzeneallylalcohol-quinoxaline (Q43) were the major metabolites in the swine, Q2, Q43 and 3-methyl-2-benzeneallylalcohol-quinoxaline (Q5) were the major in the broilers, and Q2 and Q4 were the major in the carps. In conclusion, the metabolites of QCT in rats, pigs, broilers and carps were identified, and the metabolic pathways, distribution and elimination properties of QCT were elucidated in

this work, which provided with necessary information for the further studies on pharmacology, pharmacokinetics and food safety evaluation of QCT in the animals. Keywords: Quinocetone; Metabolism; Distribution

P184 - METABOLISM AND DISPOSITION OF FAMITINIB, A POTENT AND MULTITARGETED TYROSINE KINASE INHIBITOR, IN VIVO AND IN VITRO

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Famitinib, (Z)-5-(2-(diethylamino)ethyl)-2-((5-fluoro-2-oxoindolin-3-ylidene)methyl)-3-methyl-6,7-dihydro-1H-pyrrolo[3,2-c]pyridin-4(5H)-one, an oral multi-targeted tyrosine kinase inhibitor, is currently undergoing phase II clinical trials for the treatment of renal cell carcinoma, gastrointestinal stromal tumor, and nasopharyngeal carcinoma. The present study aimed to characterize the metabolism and disposition of famitinib in humans and gain an understanding of the biotransformation mechanisms using in vitro systems. Plasma, urine, and feces were collected at frequent intervals during steady state in six solid tumor patients after oral administration of famitinib (25 or 27 mg, q.d., 28 days), and the samples were analyzed by ultra-high-performance liquid chromatography/ultraviolet/quadrupole time-of-flight mass spectrometry. A total of 48 metabolites were identified in humans. Famitinib and its metabolites were eliminated primarily via fecal route. The daily fecal and urinary elimination rates were 47.4 ± 9.6% and 14.1 ± 4.7% of the dose, respectively. Unchanged famitinib was the major circulating material, followed in decreasing abundance by N-desethylfaminitib (M3), the exposure of which was less than 10% of famitinib at steady state. Metabolites detected in extreta mainly arose via N-deethylation, oxidative deamination, hydroxylation on the indolylidene, oxidative defluorination, and glucuronidation. Two cysteine conjugates of the oxidative-defluorinated metabolite 5-hydroxylfamitinib (M7) were observed in urine, feces, and plasma, implying the formation of reactive quinone-imine intermediate in vivo. In incubations of famitinib with NADPH supplemented human liver microsomes (HLMs), M3 was formed as the main metabolite, with small amounts of M7 and three indolylidene-hydroxylated metabolites. CYP3A4 was found to be the prominent enzyme in N-deethylation, and CYP1A1 and 1A2 in indolylidene hydroxylation and oxidative defluorination. Following co-incubations with GSH, two GSH conjugates of M7 were yielded through a quinone-imine intermediate. To investigate the bioactivation mechanism, M7 was incubated in NADPH- and GSH-supplemented HLMs. Unexpectedly, the conjugation was much slow in comparison with that in the famitinib incubations. This finding suggested that the quinone-imine could be oxidized directly from famitinib through epoxidation and sequential defluorination at indolylidene, and then be reduced into 5-hydroxylfamitinib or conjugated by GSH. CYP1A1 and 1A2 mediated the formation of reactive intermediate. Overall, these in vivo and in vitro findings provide insights into the biotransformation pathways of famitinib. Potential drug-drug interactions and reactive metabolite-related idiosyncratic toxicities of famitinib need to be evaluated in the future.

P185 - COMPARISON OF METABOLIC CYTOTOXICITY OF GINKGOLIC ACID (17:1) IN HEPG2 CELLS AND PRIMARY RAT HEPATOCYTES

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Ginkgolic acid (GA) is a mixture of structurally related n-alkyl phenolic acid compounds and exists in leaves, nuts especially in external seed coat of ginkgo biloba L. GA(17:1,),6-[(10Z)-Heptadecenyl]salicylic acid, accounts for about 40% of the total GAs. In vitro bioassay systems such as primary rat hepatocytes, HepG2 cells were used to determine whether the phase I metabolism could contribute to their cytotoxicity of GA(17:1). The results indicated that CYP 3A2 and CYP1A1/2 could be the main isoforms for metabolizing GA(17:1) in rat liver microsomes. The HepG2 cell culture treated with GA(17:1) for 24 h resulted in dose-dependent cytotoxicity, and the GA(17:1) IC_{50} value in HepG2 cells was 59.3±6.21μM. After the pretreatment with 25μM β-naphthoflavone (an inducer of CYP1A2 and UGT1A) or 10μM rifampin (an inducer of CYP2C9, CYP3A4 and UGT1A) both of them increased sensitivity of HepG2 cells to GA(17:1) cytotoxicity, the IC_{50} values were 40.5 ± 4.78 μM, 46.7 ± 5.43 μM, respectively. After 24 h incubation with various concentrations of GA(17:1), dose-dependent cytotoxicity was also found in primary rat hepatocytes and IC_{50} values were 76.3±9.37 μM. The toxicity of GA(17:1) decreased when Co-incubated with α-naphthoflavone (an inhibitor of CYP1A1/2) or ketoconazole (an inhibitor of CYP3A2). The IC_{50} values were 53.4 ±6.12 μM, 48.7 ± 5.63 μM for co-incubated with β-naphthoflavone and rifampin, respectively. These findings suggest that HepG2 cells were

more sensitive to the cytotoxicity of GA than primary rat hepatocytes, and CYP1A and CYP3A could metabolize GA to more toxic metabolites, the CYP1A was possibly more important than the CYP3A in this process. This work was supported by National Major Projects of China (2012ZX09506001-004,2009ZX09304-003) and Nature Scientific Found of China (81173120).

P186 - ENZYMATIC ACTIVITY AND BIOTRANSFORMATION OF ANTHELMINTIC DRUGS IN SHEEP TAPEWORM, MONIEZIA EXPANSA

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Moniezia expansa, also known as sheep tapeworm, is a helminth parasite from the group of cestodes. It affects the small intestine of ruminants (sheep, goats and cattle) throughout the world. Young animals in their first or second year of life are most often affected. Although M. expansa infections are usually asymptomatic, heavy infections may be manifested by intestinal obstruction, diarrhoea and weight reduction, thus causing severe economic losses. The administration of drugs with anthelmintic action (especially benzimidazoles) remains the most effective approach in the therapy of helminthoses. However, tapeworms can protect themselves against the toxic effect of anthelmintics and other xenobiotics by means of their biotransformation enzymes. As the defence mechanisms of parasites against the negative action of drugs has been little investigated so far, the aim of our study was to determine the activity of M. expansa reduction, oxidation and conjugation enzymes involved in the biotransformation of xenobiotic compounds and to evaluate the metabolic fate of selected anthelmintic drugs: albendazole, flubendazole and mebendazole. Both in vitro (subcellular fractions of tapeworm homogenate) and ex vivo (living parasites cultivated in medium) experiments were performed. Activities of biotransformation enzymes were assayed towards model substrates using spectrophotometric/spectrofluorimetric methods. Metabolites of the anthelmintics were identified by HPLC. In vitro experiments revealed the activity of oxidation enzymes, which play significant role in the defence against oxidative stress (catalase, superoxide dismutase, peroxidase, thioredoxin-glutathione reductase). The ability to oxidize albendazole was observed in vitro, but these results were not confirmed ex vivo. The activity of carbonyl-reducing enzymes was assayed using substrates acenaphthenol, menadion, metyrapon, 4-pyridine carboxaldehyde and daunorubicin at pH 8.5. From anthelmintics, mebendazole and flubendazole were metabolized via carbonyl reduction both in vitro and ex vivo. Regarding conjugation enzymes, a high activity of cytosolic glutathione S-transferase was observed. The activity of UDP-glucuronosyltransferase and UDP-glucosyltransferase was found in microsomes-like and mitochondria-like fractions. The obtained results indicate that M. expansa possesses enzymatic system capable of affecting the structure of administered xenobiotics, and thus protecting itself against their negative action. By this way, parasite can decrease the efficiency of anthelmintic drugs. The knowledge of drugs biotransformation and drug-metabolizing enzymes in M. expansa is important for improving the pharmacotherapy of infection caused by this tapeworm.

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P187 - REDUCTION OF SULFAMETHOXAZOLE HYDROXYLAMINE BY THE MARC-CONTAINING N-REDUCTIVE ENZYME SYSTEM

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Hypothesis: Mitochondrial amidoxime reducing component (mARC) was discovered in our lab in 2006 (1). It is the fourth human molybdenum containing enzyme. Two homologues are known, mARC-1 and mARC-2. Each homologue forms an N-reductive enzyme system together with the mitochondrial form of cytochrome b5 (CYB5B) and NADH cytochrome b5 reductase (CYB5R3) (2). This system is capable of reducing N-hydroxylated compounds (2, 3). One of these compounds is sulfamethoxazole hydroxylamine, which occurs as a metabolite in a toxification pathway of sulfamethoxazole. Sulfamethoxazole is a prototype of prohapten. Administration of high doses over a long time leads to hypersensitivity reactions (4). Additionally, cases of mild hypothyroidism have been observed (5). Aim of our study was to investigate (i) the subcellular fractions in which this reaction takes place, (ii) the components involved, and (iii) which components of the N-reductive enzyme system are necessary for reduction of sulfamethoxazole

hydroxylamine. **Methods:** Porcine liver and thyroid tissues were homogenized. From each tissue cytosolic, microsomal and mitochondrial fractions were isolated, the subcellular fractions. Presence of mARC-1, mARC-2, CYB5B and CYB5R3 in these fractions was analyzed by western blot analysis. Reduction of sulfamethoxazole hydroxylamine was investigated in vitro. Briefly, the metabolite was incubated with subcellular fractions and recombinant proteins expressed in E.coli. After stopping incubations supernatants were analyzed by HPLC. **Results:** Western blot analysis showed that only mitochondrial fractions of porcine liver and thyroid tissue contain all three components of the N-reductive enzyme system. Reduction of sulfamethoxazole hydroxylamine is an NADH dependent reaction that is enriched in mitochondria. For reaction the presence of all three components of the N-reductive enzyme system is required. In thyroid mitochondria higher reduction rates were found compared to liver mitochondria. **Conclusions:** Reduction of sulfamethoxazole hydroxylamine is located in mitochondria and is catalyzed by the mARC-containing N-reductive enzyme system. This reduction is necessary to avoid further reaction to the toxic agent nitroso sulfamethoxazole. So the mARC-containing N-reductive enzyme system plays an important role in detoxification. Dysfunction of this pathway may lead to toxification and adverse drug reactions.

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P188 - THE MOLYBDENUM ENZYME MARC: N-REDUCTIVE ACTIVITY IN DIFFERENT CELL LINES

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mARC (mitochondrial amidoxime reducing component) is a mammalian molybdenum enzyme that was discovered in our lab (1). The enzyme is located in the outer mitochondrial membrane and together with the electron transport proteins cytochrome b5 and its NADH-dependent reductase it is capable of reducing N-hydroxylated compounds such as the model substrate benzamidoxime (2, 3). Besides amidoximes, the enzyme system is also able to reduce other N-hydroxylated structures such as guanidines and sulphonamides and is thus involved in reductive drug metabolism (2). For this reason it can be employed for the activation of N-hydroxylated prodrugs using e.g. the prodrug principle of “amidoximes instead of amidines”(4). Furthermore, the mARC containing enzyme system is able to reduce N-hydroxylated DNA-bases (2) which could be a hint to its still unknown physiological function. The human genome codes for two mARC proteins, referred to as hmARC-1 and hmARC-2 (5). Our studies upon tissue distribution carried out with porcine material revealed highest expression levels for mARC in liver, thyroid and kidney (6). So far, our drug metabolism studies on N-reductive activity were carried out using porcine hepatic and extra hepatic tissue preparations including purified mitochondria, primary hepatocytes and a reconstituted recombinant enzyme system. To complete our in vitro test systems with an extra hepatic cell assay providing complete and viable cell systems we investigated different cell lines, among those kidney cell lines HEK293, RC124, HMCL and Colo357, a pancreatic cell line. Cells were cultured and incubation assays with the model substrate benzamidoxime were carried out. N-reductive activity could be found in all studied cell lines. The cell lines can therefore be used to establish a cellular in vitro test system for the reduction of N-hydroxylated structures.

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P189 - ACYLGLUCURONIDES MAY BEHAVE AS REACTIVE METABOLITES DEPENDING ON THEIR STRUCTURAL PROPERTIES

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Acylglucuronides (AGs) are known to be reactive metabolites, and this reactivity was believed to be a risk factor of drug-induced toxicities. We previously reported that the degradation rate constant (k) of AGs was a useful parameter for predicting clinical safety as an index of reactivity.[a)] The few studies conducted thus far examining structure-reactivity relationships (SRRs)[b, c)] have provided little data regarding relationships between clinical results of drugs and their reactivity, primarily because the studies were chiefly used non-drug compounds. We therefore selected 24 drugs and placed each compound into one of three categories based on the number of substituents (except for hydrogen) at the a-position of the carboxylic acid group (M, mono- substituted; D, di-substituted; F, fully-substituted), given that the steric effect of the a-position has been reported to be an important factor of k.[d)] The k values of each compound were obtained from in vitro AG biosynthesis- degradation assay,[e)] with results showing that mean values of k in each category increased in the order of "F," "D," and "M" and thereby indicating that the steric effect at a-position of carboxylic acid group does indeed influence k. However, k values were also affected by other steric and electronic factors (bulky substituents, substituent having an electron withdrawing group, hydrogen bonding, etc.). Our SRR study of k ultimately demonstrated that both steric and electronic effects around the carbonyl carbon of the carboxylic acid group is a principal factor of k, and therefore reducing the electrophilicity of the carbonyl carbon would be an effective method of reducing AG-dependent toxicities. In addition, we also investigated useful predictors of k (chemical shift of carbonyl carbon in ^{13}C-NMR and calculated parameters) without synthesis of new compounds to aid medicinal chemists in their drug design.

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P190 - DETOXIFIXCATION OF N-HYDROXYLATED BASE ANALOGUES BY THE MARC-CONTAINING ENZYME SYSTEM

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The newly discovered “mitochondrial Amidoxime Reducing Component” mARC is the fourth molybdenum containing protein in mammalians (1). All hitherto analyzed mammalian genomes harbor two mARC proteins (mARC1/mARC2). Together with its electron transport proteins the mARC enzyme works as part of a three component enzyme system. In addition to the Moco-binding mARC domain this composition consists of a flavin-containing NADH cytochrome b_5

reductase domain and a heme–containing cytochrome b_5 domain and works as an N-reductive enzyme system (1). mARC plays a major role in reductive drug and xenobiotic metabolism as it is able to reduce a variety of N-hydroxylated forms of amidines, guanidines and aromatic amines (2). Furthermore, an importance of the mARC-mediated N-reductive pathway for lipid synthesis was described recently (3). Similar structures can be found in N-hydroxylated base analogues, which can be produced as metabolites from the normal cellular metabolism or originate from chemical and physical factors (4,5). The N-hydroxylated base analogues could be converted into the desoxynucleoside triphosphates. Incorporated in the DNA these structures induce mutations due to their ambivalent coding capacity (6). We demonstrate that mARC reduces toxic and mutagenic N-hydroxylated base analogues to their corresponding non-mutagenic derivatives. Thus the reduction would serve as a detoxification process. A similar detoxification pathway was described in E. coli (7). Subcellular fractions from different porcine tissues and a reconstituted recombinant human enzyme assay were used to investigate the reduction of N-hydroxylated purine and pyrimidine nucleobases and nucleosides. The detoxification of N-hydroxylated base analogues seems to be a hint on the physiological function of mARC.

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P191 - MARC-DEPENDENT ENZYMATIC N-REDUCTION OF OXIMES TO IMINES

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The mitochondrial Amidoxime Reducing Component (mARC) is the fourth mammalian molybdenum-containing enzyme (1). It is localized in the outer mitochondrial membrane where it plays an important role in the oxygen insensitive reduction (2). Together with the electron transport proteins cytochrome b_5 and cytochrome b_5 reductase electrons are transmitted to the Moco-binding domain of mARC and reduction of a number of N-hydroxylated compounds such as amidines, guanidines, amidinohydrazones, aromatic amines, and N-hydroxylated nucleosides and nucleobases takes place (2, 3). Thus mARC contributes both to the activation of prodrugs and to the metabolism of xenobiotics (4). Oximes play an important role in drug development. In many drugs and bioactive substances oximes and their derivatives form relevant structural elements (5). We studied the reduction of oximes to imines and the involvement of mARC in this biotransformation. For this purpose in vitro biotransformation studies were performed with the isolated (E)- and (Z)-isoforms of the ketoxime 2,4,6-trimethylacetophenone oxime as a model substrate. As already shown in former studies with porcine liver subfractions stable 2,4,6-trimethylacetophenone imine was formed during incubation which could be quantified by HPLC analysis (6). When using different cell organelles mitochondria was the best enzyme source. High conversion rates were also detected during incubations with mARC-1 and mARC-2 using the recombinant human enzyme system together with cytochrome b_5 and its reductase. Differences could be observed in the reduction rates of the (E)- and (Z)-form. In conclusion we demonstrate that oximes can be easily reduced to imines by mARC. This has to be considered when developing oximes as drug candidates. Their reduction could mean a fast loss of activity.

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P192 - HUMAN ADME STUDIES: A POST-MIST ANALYSIS

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Purpose: Mass balance studies using radiolabelled compounds in laboratory animals and man are an essential component of the drug development process. The recent FDA and ICH guidelines addressing the safety of drug metabolites (commonly referred to as Metabolites in Safety Testing (MIST)) has placed the emphasis upon obtaining comparative human and animal metabolism data as early as possible in the development process. As the human ADME study occupies a key position in the safety assessment it was considered useful to review the data obtained from mass balance studies conducted by the metabolism department at Quotient and compare the results obtained pre and post guidance to see if any changes in design, conduct or mass balance were observed. **Methods:** Data were collated from human mass balance studies conducted at Quotient Bioresearch from 2002 to 2012. Each clinical study was approved by an independent review board. Radioactive doses administered were between 13 and 200 uCi with around 6 subjects being dosed per study. Radioanalysis was performed in real time with results available within 8 hours of sampling, release criteria were set at >90% or <1% in 2 consecutive 24 hour collections of excreta. **Results:** The mean recovery observed in the studies conducted pre-MIST were 90.9 ± 8.02 % whilst the mean recovery observed in studies post MIST was 92.2 ± 8.20 % There is no regulatory guidance as to what constitutes an acceptable recovery in a human mass balance study although 85% and 90% have been proposed in the literature. Recoveries of greater than 85% recovery were observed in 93% of the studies pre MIST and 92% of the studies post-MIST. Using 90% recovery as the acceptable value this was observed in 71% and 77% of the studies pre- and post-MIST respectively. **Conclusions:** No trends in radioactive dose, number of subjects or mass balance were observed in the two groups. Key factors for achieving good mass balance were: Accurate determination of the dose administered, Complete collection of the urine/fx sample, The ADME properties of the test compound.

P193 - IMPLICATIONS OF SPECIFIC METABOLISM-RELATED GENES IN NANDROLONE DISPOSITION

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Nandrolone (19-nortestosterone) is an anabolic androgenic steroid commonly abused for doping purposes. Like other androgens, it is often administrated as an ester (i.e. decanoate) to provide a depot formulation for intramuscular injections. A previous study indicated that PDE7B is involved in the hydrolysis of steroid esters.[1] Then, nandrolone is mainly metabolized in the liver into 19-norandrosterone prior to glucuronidation and excretion through urine over an extended period of time.[2] Several UGTs (i.e. UGT2B7, UGT2B15 and UGT2B17) seem to be involved in the glucuronidation of androgens.[3] However, the identity of all the enzymes involved in nandrolone metabolism together with their relative contribution and regulation remain unknown. Inhibition was assessed using human liver homogenates incubated with androgens and selective inhibitors. Human liver microsomes (n=42) were

genotyped for UGT2B15 D85Y and UGT2B7 H268Y by Taqman SNP assays. The UGT2B17 deletion polymorphism was determined by real-time PCR. Since the metabolism mainly takes place in the liver, human liver cancer HepG2 cells were cultured and exposed to androgens to determine if the transcriptional activity of the genes of interest was affected. Nandrolone decanoate appeared to be hydrolyzed by PDE7B since specific inhibitors of PDE7 inhibit about 80% of the hydrolysis in human liver microsomes. As PDE7B is active on steroid ester hydrolysis in liver, we investigated if the androgen also induces PDE7B expression in human HepG2 cells. Nandrolone decanoate increased PDE7B gene expression in HepG2 (up to 5-folds). Interestingly, free nandrolone itself induced the transcription of PDE7B similarly, whereas no effect of estradiol was observed. The glucuronidation of 19-norandrosterone was significantly higher in UGT2B15 DD than in the other genotypes ($p<0.05$). However, stratification by UGT2B7 or UGT2B17 polymorphisms did not reveal any significant difference in glucuronidation activity. Surprisingly, only UGT2B7 mRNA expression was significantly increased in HepG2 after incubation with nandrolone decanoate (1.8-folds). Our results show that PDE7B plays a crucial role on nandrolone activation by esterase catalysed hydrolysis. PDE7B mRNA expression seems to be up-regulated by androgens via the androgen receptor pathway. The UGT2B15 polymorphism (D85Y) was the only UGT genetic variation that influenced the glucuronidation activity. This could partly explain the inter-individual variation in 19-norandrosterone excretion.

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P194 - IDENTIFICATION OF APATINIB METABOLITES IN CANCER PATIENTS AND IN VITRO STUDIES OF CYTOCHROME P450 REACTION PHENOTYPING, INHIBITION AND INDUCTION

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Apatinib (N-[4-(1-cyano-cyclopentyl)phenyl]-2-(4-pyridylmethyl) amino-3-pyridine carboxamide), a selective inhibitor of endothelial growth factor receptor-2, is currently in phase II/III clinical trials in China for the treatment of solid tumors. The purpose of this study was to identify the metabolites of apatinib in cancer patients, characterize hepatic enzymes that are responsible for the metabolism of apatinib and in vitro evaluate the potential of apatinib to inhibit or induce major human P450 enzymes. UPLC/Q-TOF MS revealed 49 metabolites including 40 phase I and 9 phase II metabolites in patients; structures of nine major metabolites were confirmed by comparison with reference standards after HPLC co-chromatography, mass spectrometry, and NMR. Apatinib was extensively metabolized in humans, and its major metabolic pathways included cyclopentyl-3-hydroxylation, N-dealkylation, pyridyl-N-oxidation, di-oxidation and O-glucuronidation after cyclopentyl-3-hydroxylation. The major circulating metabolites included cis-3-hydroxy-apatinib (M1-1), trans-3-hydroxy-apatinib (M1-2), apatinib-25-N-oxide (M1-6), and cis-3-hydroxy-apatinib-O-glucuronide (M9-2). M9-2 had systemic exposure higher than that of apatinib in steady state. Incubation with human recombinant P450 isoforms and inhibition studies with selective chemical inhibitors of human P450 enzymes demonstrated that the oxidative metabolism of apatinib was mediated by multiple CYP enzymes. Taking the average abundances of CYP enzymes in human liver into account, apatinib was primarily metabolized by CYP3A4 and to a lesser extent by CYP2C9 and CYP2E1. Multiple CYP enzymes including CYP2E1, CYP2D6, CYP2C8, CYP3A4, CYP2A6 and CYP2C9 were involved in the formation of M1-1. CYP2C9 and CYP3A4 mainly mediated the formation of M1-2. CYP2D6 and CYP3A4 mainly mediated the formation of M1-6. N-Dealkylation of apatinib was predominantly mediated by CYP3A4. Incubations with recombinant human UDP-glucuronosyltransferases (UGTs) suggested that UGT2B7 were the major UGT isozyme catalyzing the O-glucuronidation of M1-1 to form M9-2. Apatinib strongly inhibited the activities of CYP3A4 (6b-hydroxytestosterone as a marker, IC_{50} = 0.83 μM) and CYP2C9 (4-hydroxytolbutamide as a marker, IC_{50} = 0.44 μM), and moderately inhibited the activity of CYP2C19 (4'-hydroxymephenytoin as a marker, IC_{50} = 2.1 μM) in human liver microsomes, while exhibited little inhibition of CYP1A2, CYP2D6 and CYP2E1. Evaluated in fresh human hepatocytes at three concentration levels from 0.2 μM to 20 μM, apatinib was demonstrated to cause induction of CYP1A2 activity (1.6 to 4.5 fold). Further in vivo studies will be conducted to evaluate the clinical drug-drug interaction.

P195 - ABSORPTION AND DISPOSITION OF MAVOGLURANT (AFQ056) IN HEALTHY VOLUNTEERS

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Mavoglurant (AFQ056) is a selective mGluR5 antagonist under development for treatment of Parkinson`s disease-associated L-Dopa-induced dyskinesia and Fragile X syndrome. In the present work, the absorption and disposition of [^{14}C]-radiolabeled mavoglurant were investigated in four healthy male volunteers after a single oral dose of 200 mg. Total radioactivity was determined in plasma, urine and feces. Mavoglurant was quantified in plasma by LC-MS/MS. Metabolite profiles were achieved in plasma and excreta by HPLC and radioactivity detection. The mavoglurant metabolite structures were elucidated by mass spectrometry, wet-chemical and enzymatic methods, NMR and comparison with reference compounds. For the metabolite profiling, the novel linked platecrane automated system was used, increasing significantly throughput. Sample analyses for this study were completed in a more efficient manner, as compared when using standard methods. **Results:** [^{14}C]mavoglurant was absorbed with a Tmax of 2.6h and an oral bioavailability of ≥ 50% .The biotransformation of mavoglurant involved two main pathways: A) hydroxylation of the tolyl-methyl group to a benzyl-alcohol metabolite (M7) and subsequently to a benzoic acid metabolite (M6); B) hydroxylation of the phenyl ring leading to a hydroxylated metabolite (M3). The elimination of mavoglurant was fast and occurred predominantly by oxidative metabolism. The subjects were mainly exposed to mavoglurant and five metabolites (M6, M15, M18, M14, M30). Drug related material was excreted mostly in feces (58.6% of dose) and urine (36.7% of dose). After 7 days, the balance of excretion was almost complete (95.3% of dose).

P196 - HUMAN CYTOCHROMES P450 1A1 AND 1A2 PLAY A DUAL ROLE IN METABOLISM OF CARCINOGENIC ARISTOLOCHIC ACID I, CATALYZING BOTH ITS REDUCTIVE ACTIVATION AND OXIDATIVE DETOXICATION: EXPERIMENTAL AND COMPUTATIONAL APPROACHES

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Aristolochic acid (AA) causes human nephropathy and urothelial cancer. Individual susceptibility to AA-induced disease might reflect individual differences in enzymes that metabolize aristolochic acid I (AAI), the major toxic component of AA. The activation pathway for AAI is nitroreduction to the cyclic nitrenium ion forming AAI-DNA adducts, 7-(deoxyadenosin-N^6-yl)aristolactam I, 7-(deoxyguanosin-N^2-yl)aristolactam I and 7-(deoxyadenosin-N^6-yl)aristolactam II, catalyzed by both cytosolic and microsomal enzymes, NAD(P)H:quinone oxidoreductase being most efficient. A detoxication metabolite, identified as 8-hydroxyaristolochic acid I (AAIa), is formed following AAI oxidative demethylation. Using two CYP1A-humanized mouse lines that carry functional human CYP1A1 and CYP1A2 genes in the absence of the mouse Cyp1a1/1a2 orthologs and hepatic, renal and lung microsomes of these mouse models, we found that human CYP1A1 and 1A2 are principally responsible for reductive activation of AAI to form AAI-DNA adducts and for AAI oxidative detoxication to AAIa, both in the intact mouse and in microsomes. Moreover, an exclusive role of human CYP1A1 and 1A2 both in AAI oxidation to AAIa and AAI reduction to the cyclic nitrenium ion forming AAI-DNA adducts was observed in human liver microsomes under the aerobic and the anaerobic conditions, respectively. A major role of human CYP1A1 and 1A2 in AAI oxidation and reduction was also proved using human recombinant CYP enzymes. The results found indicate that AAI is a ligand substrate for CYP1A1 and 1A2 at low oxygen concentrations, and is reduced instead of oxidized during the CYP-mediated reaction cycle. In contrast, under aerobic conditions AAI is a classical substrate of CYP1A1 and 1A2 in which one atom of oxygen is used to O-demethylate the methoxy group of AAI to generate AAIa. Therefore, we propose that, in addition to CYP1A1 and 1A2 expression levels, in vivo oxygen concentration in specific tissues might affect the balance between AAI nitroreduction and O-demethylation, which in turn would influence tissue-specific toxicity or carcinogenicity. Employing CYP1A1 and 1A2 homology modeling followed by docking of AAI to the CYP1A1 and 1A2 active sites, molecular mechanisms of a dual role of CYP1A enzymes in AAI metabolism might be explained. The data found in the study indicate that computational chemistry in conjunction with detailed experiments in vivo and in vitro is a very

valuable tool to explain the mechanisms of reactions that participate in AAI metabolism in humans and dictate its nephrotoxic and carcinogenic effects.

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P197 - COMPARISON OF THE KINETICS OF VERAPAMIL AND PROPRANOLOL METABOLISM BETWEEN RAT HEPATOCYTES AND LIVER MICROSOMES

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Introduction: Suspended hepatocytes have proven to be a valuable in vitro tool to assess metabolic stability of authorized drugs and new chemical entities. Careful optimization and validation of this tool is crucial if one wishes to accurately predict in vivo hepatic clearance based on in vitro incubations. Cross-validation of the optimized assay can be achieved by comparison with data generated in rat liver microsomes as a reference model for hepatic drug metabolism. Aim/goal: The aim of the present study was to implement an optimized in vitro assay for hepatic drug metabolism based on suspended rat hepatocytes and to compare the kinetics of verapamil and propranolol metabolism between hepatocytes and liver microsomes. Methods: Disappearance of compounds was assessed with suspended rat hepatocytes (freshly isolated or following resuscitation after cryopreservation) according to the half-life approach. The effects of the ratio of volume of incubation medium to well size, agitation, medium composition and glutamine content on metabolism of verapamil were investigated. In the case of rat liver microsomes the effect of glucose 6-phosphate in the incubation medium was assessed. Results: For suspended rat hepatocytes, the optimal ratio of volume of incubation medium to well size (500 µl/24-well plate well) was combined with the proper agitation speed (300 rpm) to prevent cellular sedimentation and to achieve best reproducibility. Agitation of the cell suspension during incubation resulted in a 15% increase in metabolism compared to initial conditions. Comparison of metabolic rates between Williams' E (WEM) and L-15 medium (adjusted) showed that the latter maintained a more stable pH. This may explain the better maintenance of hepatocyte viability in L-15 (98% after 120 minutes) when compared to WEM (90% after 120 minutes). An increased glutamine concentration (up to 3,6 mM) appeared to improve metabolic activity towards verapamil in cryopreserved hepatocytes. In rat liver microsomes, it was found that addition of glucose 6-phosphate can increase propranolol disappearance up to three-fold, whereas the effect on verapamil disappearance was minor. Conclusion: Fine-tuning of experimental parameters resulted in a more reliable (reproducible) suspended rat hepatocyte assay. Under the optimized conditions, comparable results on verapamil metabolism were obtained with freshly-isolated versus (in-house) cryopreserved hepatocytes. Ongoing experiments are aimed towards further investigation of differences in metabolism kinetics between suspended rat hepatocytes and liver microsomes and how this affects prediction of in vivo PK parameters.

P198 - UNDERSTANDING THE MECHANISM OF PYRIDINE OPEN-RING METABOLITES OF VISMODEGIB

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Vismodegib is a potent and selective first-in-class inhibitor of the Hedgehog signaling pathway and is currently approved for the treatment of basal cell carcinoma in the USA. Dog, rat, and clinical mass balance studies revealed the presence of several minor (<10%) pyridine open-ring metabolites [M9, M13, and M18 (structures confirmed by NMR); Qin et al DMD, 39:952–965, 2011]. Here we present a series of in vitro, in vivo, and ex vivo studies to understand where and how these open ring metabolites are formed. **Experiments and Results:** (1) In in vitro incubations with rat, dog and human liver microsomes, these metabolites were not detected. However, in cynomolgus monkeys, both M3 (monohydroxy metabolite) and M18 were formed (NADPH-dependent and inhibited by pre-treatment with ABT). (2) Under acidic conditions, M18 readily converted to M13 via hydrolysis. (3) When stable labeled vismodegib (one ^{15}N and two ^{13}C on the pyridine ring and on either side of the ^{15}N) was dosed to rats, the following metabolites were detected: M18 with all three labels retained, M13 with one only ^{13}C retained, and M9 with one ^{13}C and one ^{15}N retained. (4) We pretreated rats with antibiotics in order to understand the role of gut bacteria in the formation of these metabolites. Upon pretreatment with antibiotics, M3 in feces increased and the presence of pyridine ring open metabolites decreased by >50%. (5) When Vismodegib was studied in ex vivo rat liver

perfusion studies, M18 and M9 were formed. **Summary:** Pyridine open-ring metabolites appear to be formed by P450 enzymes in liver and by GI bacteria. Based on the stable label studies, M18 is mostly likely formed from M3. M18 could be hydrolyzed either to M13 or through cyclic intermediate to form the amide metabolite (M9).

P199 - OXIDATIVE MATABOLISM OF INSECTICIDE EPN BY HUMAN LIVER MICROSOMES AND HUMAN CYTOCHROME P450 ISOFORMS

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EPN (O-ethyl-O-4-nitrophenyl phenyl phosphonothionate) is an organophosphorous insecticide, acting as a potent inhibitor of acetylcholinesterase. This study was performed to investigate in vitro metabolism of EPN with human liver microsomes(HLMs) and characterize the specific isoforms of cytochrome P450 involved in metabolic reaction. In the presence of NADPH, EPN was bio-activated by HLMs to give its oxygen analog, EPN oxon (O-ethyl-O-4-nitrophenyl phenyl phosphonothioate) which is more toxic than its parent compound. In the absence of NADPH-generating system or with heat-denatured microsomes, the metabolite did not formed, indicating that metabolic enzymes are cytochrome P450s. In enzyme kinetic studies, Vmax(pmol/min/mg protein) of 103.71, Km(uM) of 4.75, and intrinsic clearances(Clint; Vmax / Km) of 21.81 were obtained. The selective inhibition of P450 isoforms in HLMs by chemical inhibitor did not produce significant evidences to elucidate CYP isoforms involved in the metabolism. A screen of 10 human cDNA-expressed CYP isoforms for metabolic ability with respect to the production of EPN oxon demonstrated that 2 major(CYP 3A4, CYP 2C9) and 2 minor(CYP 2C8, CYP 1A2) CYP isoforms which are responsible for EPN metabolism. The percentages of contribution of four CYP isoforms were 44.35, 39.33, 6.64, 5.13 %, respectively (total 95.45%). Enzyme kinetics of those four CYP isoforms also demonstrated that CYP 3A4 has the highest affinity to EPN.

P200 - METABOLITE IDENTIFICATION IN COMPLEX MATRICES BY STRUCTURALLY INTELLIGENT ACQUISITION AND PROCESSING

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Detection and identification of metabolites in complex biological matrices is a common problem for drug development. The combination of a high background signal and abundant matrix components makes acquisition of fragmentation data on metabolites, especially minor metabolites, difficult. The use of targeted acquisition through inclusion lists improves results but requires significant user interaction that is undesired. Utilizing structure based acquisition on a high resolution accurate mass benchtop mass spectrometer we demonstrate the ability to trigger fragmentation on even minor metabolites from in vivo human samples. An analogous, structure based processing methodology, utilizing the high resolution accurate mass data, facilitated metabolite detection and identification. In this study, time course samples of urine from a human taking loratadine were acquired and prepared for analysis by solid phase extraction to desalt the sample. Acquisition of precursor ion selected MS2 fragment scans on metabolites was triggered through a novel combined all ion fragmentation neutral loss approach. The fragmentation of loratadine was used to create a list of the top 6 observed neutral losses. These neutral losses were combined with expected neutral losses from phase II conjugates (glucuronidation, sulfation, and glutathione conjugation). Acquisition was performed using alternating full scans (70,000 resolution, FWHM @ m/z 200) and all ion fragmentation scans (70,000 resolution) on a benchtop Orbitrap™ instrument. The generation of fragments from all precursors allowed for on-the-fly detection of all neutral losses. Observation of any neutral loss triggered the automatic acquisition of a precursor isolated MS2 fragmentation scan on the parent. Since all parents and all of their subsequent fragments were scanned constantly, this approach provided constant monitoring throughout the run. The approached allowed for sensitive and selective targeted fragmentation on related metabolites in the presence of a high matrix without the need for multiple injections of time demanding inclusion lists construction. Processing of the acquired data was performed using an analogous structurally intelligent approach. Observed and predicted fragmentation of loratadine was used to search the acquired raw files and detect components based on

fragmentation. The fragments were also modified by metabolic transformation and used to improve detection and structure assignment. The structure of metabolites was determined through the fragmentation, with and without modification that was used to detect them.

P201 - DISSECTING CLOPIDOGREL AND PRASUGREL BIOACTIVATION: P450 OR PON-1?

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Clopidogrel, a widely used antithrombotic (PlavixTm), is actually a prodrug that requires a two step enzymatic conversion to an active metabolite (a N subtituted (Z) 3-carboxymethylidene 4-mercapto-piperidine). For Clopidogrel the first step is the P450-mediated mono-oxygenation of the thiophene ring, yielding a thiolactone, 2-oxo-clopidogrel (2-oxoC). Prasugrel (EfientTm) is a new derivative which upon an esterase mediated hydrolysis yields also a thiolactone, 2-oxo-prasugrel (2-oxoP) (1). In the second step P450 enzymes **catalyze the oxidative opening of the thiolactone ring to yield a sulfenic acid, which is subsequently reduced to a thiol**(2,3). These thiols can be trapped by NEM in situ during the incubations as shown below for 2-oxoP incubated with human microsomes in presence of 1 mM NEM, NADPH and ascorbic acid. Recent evidence suggested that the thiol metabolite was produced by **paraoxonase-1 (PON-1)-catalyzed hydrolysis (4).** To clarify which of these two pathways generates the active thiol metabolite of clopidogrel, we have investigated the metabolism of 2-oxo-C and 2-oxoP by human liver microsomes and serum. The thiol metabolite of 2-oxoC and 2-oxoP can exist in five isomeric forms, two cis diastereomers, two trans diastereomers, and one endo isomer resulting from double bond migration. One of the cis isomer has the highest biological activity. Incubation with liver microsomes in presence of NADPH leads to the formation of the two cis diastereomers as major products and the endo isomer as a minor product (4A). Incubation in the absence of NADPH or the reducing agent only leads to the formation of the endo thiol isomer. Similar results were obtained for prasugrel P or 2-oxoP incubated with human liver microsomes. This was demonstrated using MS detection of the thiols, or derivatized thiols with 3-methoxyphenacyl bromide or NEM at the end of the incubation. Similarly the thiols were trapped by NEM in situ during the incubations as shown below for 2-oxoP incubated with human microsomes in presence of 1 mM NEM, NADPH and ascorbic acid. The reducing agent could be replaced by dithiothreitol, GSH, NACysteine, Mercaptoethanol or TCEP. Recombinant human PON-1 was able to make the endo-thiol from oxo-ticlopidine, oxo-clopidogrel and oxo-prasugrel, with Km similar to those of microsomes. CONCLUSION : The **active cis diastereomers formation require the action of P450** enzymes **followed by reduction**, whereas **PON-1-mediated hydrolysis** only yields **the inactive endo isomer**. Thus correct identification of the clopidogrel metabolic pathway requires an analytical system that correctly identifies the individual isomeric products (5,6). The results are important, because correct identification of the enzymes involved in clopidogrel bioactivation allows physicians to identify patients who may respond poorly to clopidogrel therapy.

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P202 - EZETIMIBE AS A BIOMARKER FOR LIVER DIRECTED UGT1A1 GENE THERAPY FOR CRIGLER- NAJJAR TYPE I

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Crigler–Najjar syndrome type I (CN-I), severe unconjugated hyperbilirubinemia, is a rare disease caused by the lack of functional UGT1A1 enzyme. UGT1A1 is the only UDP-glucuronosyltransferase (UGT) enzyme responsible of bilirubin glucuronidation and its function is essential for preventing the accumulation of unconjugated bilirubin to neurotoxic levels. If left untreated, CN-I is a lethal syndrome and currently the only option for treatment (besides liver

transplantation) is an arduous phototherapy. Our aim is to develop AAV mediated gene therapy treatment for CN-I patients and, as a required step, to find a suitable biomarker for following the efficacy of the therapy and compare it to the current phototherapy treatment. Since unconjugated bilirubin levels is not a suitable biomarker in this case, we have tested whether several good substrates for hepatic UGT1A1 could serve as suitable biomarkers. The selected substrates were the steroid hormones Estradiol (formation of estradiol-3-glucuronide) and Ethinylestradiol, as well as the cholesterol lowering drug Ezetimibe. These compounds were tested in Gunn rats, a relevant animal model for CN-I and the development of treatments to it, which were infected with modified AAV that carry the human UGT1A1 gene. Based on our in vitro studies, Ezetimibe appeared most suitable, mainly because its glucuronidation rate in untreated control Gunn rat liver microsomes was low. Subsequently Ezetimibe glucuronidation was studied in vivo and Ezetimibe glucuronidation was found to be increased in AAV-treated Gunn rats, in comparison to untreated rats. This indicates that Ezetimibe may be a suitable biomarker for restored UGT1A1 activity and, after additional studies, a useful tool to demonstrate the efficacy of AAV mediated liver directed gene therapy in human CN-I patients.

P203 - PRESYSTEMIC DISPOSITION OF ABIRATERONE ACETATE

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ZYTIGA® (abiraterone acetate, AA) is approved in more than 40 countries, including the United States, European Union and Canada, for treatment of patients with advanced metastatic castration resistant prostate cancer (mCRPC) who have progressed after prior docetaxel-based chemotherapy and is under investigation in Chemotherapy naïve patients with mCRPC. AA is a prodrug of abiraterone (ABT), which blocks the formation of testosterone by inhibiting CYP17A1 (CYP450c17), an enzyme also known as 17α-hydroxylase/17, 20 lyase. This enzyme is involved in the formation of DHEA and androstenedione, which may ultimately be metabolized into testosterone. In man, AA is rapidly hydrolysed to ABT. The major human circulating metabolites are sulphated ABT (ABT-S) and the N-oxide of ABT-S. Up to now, it was unresolved whether ester hydrolysis and sulphation occur presystemically. Therefore, the metabolic stability of ^{14}C-AA and -ABT was investigated in vitro in human stomach mucosa, human and dog intestinal subcellular fractions, human blood and human hepatocytes. In combination with in vitro phenotyping data, a roadmap was generated, illustrating that esterase-mediated hydrolysis of AA occurs in gastro-intestinal tissue. This hydrolysis of AA in vitro was supported in vivo by the detection of predominately ABT in blood taken from the portal vein of dogs after single oral administration of AA. Hence, not AA but ABT is the main entity being absorbed from the intestine. In vitro metabolism data demonstrated that SULT2A1-mediated sulphation of ABT also occurs at the level of the intestine. However, in vivo in dog only low amounts of ABT-S were detected in blood taken from the systemic circulation. The latter highlights a difference between man and dog, which is confirmed by in vitro comparative metabolism data in dog and human intestinal subcellular fractions and hepatocytes. Therefore, while dog is not a good model for sulphation, dog remains a representative model for ester hydrolysis. Finally, for pharmacokinetic modeling purposes in man, actual intrinsic clearance values were determined for esterase-mediated-hydrolysis of AA and SULT2A1-mediated sulphation of ABT. This information is important when extrapolating human in vitro data to a clinical situation. Overall, it can be concluded that mainly ABT is absorbed in the intestine, which is then further sulphated and oxidized to ABT-S and N-oxide ABT-S, respectively, mainly in the liver.

P204 - BIOTRANSFORMATION OF ANTHELMINTIC DRUG FLUBENDAZOLE IN PLANTS

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Benzimidazole anthelmintics are often used for treatment and prophylaxis of parasitic diseases in farm animals. Their intensive application in breeds causes massive contamination of pastures and waste waters with anthelmintics in excrements. Highly biologically active anthelmintics represent clear risk for ecosystems around farms but information about their occurrence and fates in ecosystems has been still limited. Our project was designed to study the potential of plants to uptake and biotransform anthelmintics. Reed (Phragmites australis) was chosen as

common wetland plant species , flubendazole (FLU) was chosen as common representative of benzimidazole anthelmintics. Cell suspension cultures were derived from in vitro germinated seedlings and were cultivated on nutrient RH medium supplemented by growth regulators 2,4-D (0,225 mg.dm^{-3}) a kinetin (0,2175 mg.dm^{-3}), at 24 °C, indark. FLU (10μM, pre-dissolved in DMSO) was added in medium and suspension cultures were further incubated with FLU for 48 hours. Samples of medium as well as samples of plant cells were collected at 2, 4, 8, 24 and 48h after FLU application. Prior analyses, the separated cell clusters were homogenized using Sonopuls and obtained homogenates and medium samples were undergone to liquid-liquid extraction. For identification and quantification of FLU metabolites, HPLC with mass spectrometry detection was used. The results showed that during 48 hours, concentration of FLU decreased in medium and increased in cells. After 24h, several FLU metabolites were found in cells samples. FLU with reduced carbonyl group and two glucose conjugates were the main FLU metabolites identified in cells. The results revealed that plant cells were able to uptake FLU from medium in time-dependent manner. Moreover, plant enzymes deactivated FLU via carbonyl reduction and glucose conjugation. Therefore, plants are able to partially protect ecosystems against potentially toxic effect of anthelmintics

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P205 - ADME CRITERIA AS A TOOL FOR SELECTION OF POTENTIAL ANTI-INFLAMMATORY THIAZOLES

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Tumour necrosis factor alpha (TNF-α) is a cytokine that plays a significant role in inflammation, and thus antagonists of TNF-α receptor or inhibitors of TNF-α production are proposed therapies for inflammatory diseases. In our research programme a series of novel alkoxy aryl thiazoles were synthesized, exhibiting a remarkable anti-inflammatory activity in animal models (1). Among the active derivatives prepared, four compounds with closely related chemical structures were initially chosen for in vitro ADME screening. Aqueous solubility was a major issue (≤3 μM) and Log D was also found or estimated to be higher than desirable (³3.9). Devoid of ionisable groups at physiological pH, no salts could be obtained. All of the four compounds showed high apparent permeability in MDCK cells, whereas no influence of P-gp was observed in MDR1-MDCK (2). Considered as Class 2 drugs of the BCS, an extensive metabolism might be expected. Intrinsic metabolic clearance and half life were determined in human, rat and mouse microsomes. Compounds bearing a methyl group in at the C-2 position of the thiazole ring were rapidly metabolized, whereas the aryl substituted compounds suffered virtually no metabolism. Nevertheless, those methyl substituted compounds with low intrinsic microsomal clearance, were ruled out due to their lower solubility and poor anti-inflammatory profile. For the C-2 methyl thiazole derivatives, studies of plasma protein binding and metabolic phenotyping in human supersomes were carried out. CYP2C9 was the main responsible for metabolism, and to a lesser extent, also CY2D6, 2C19 and 3A4. The high plasma protein binding found (³99%) may limit high metabolic rate though preliminary PK assays in rat carried out confirmed short half-life and low oral bioavailability. Main metabolites found in vitro and in vivo were attributed to oxidation of the parent molecule. Synthesis of new compounds was focused on potential oxidative metabolites of the lead molecule. Pharmacological activity and metabolic stability were checked for two compounds (M1 & M2) in rat, mouse, dog and human S9 fractions to take into consideration the possibility of direct glucuronidation. M1 was found to have higher in vivo inhibitory activity on TNF-α induced by LPS than the parent molecule. However, it was also rapidly cleared in S9 fractions by glucuronidation and oxidation. On the contrary, M2 showed much slower metabolism but no significant activity as TNF-α inhibitor.

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P206 - THE MITOCHONDRIAL AMIDOXIME REDUCTASE A NOVEL LIPOGENIC ENZYME SYSTEM?

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The reduction of hydroxylamines and amidoximes is not only an important concept in prodrug activation but also important for the detoxification of aromatic and heterocyclic amines. Past studies by us and others revealed that this activity is associated with both the endoplasmic reticulum and the mitochondrial fraction and high levels of amidoxime reductase activity were found in liver, kidney and adipose tissue [1]. Using recombinant and truncated enzymes it was shown that the mitochondrial amidoxime reductase system can be reconstituted using cytochrome b_5 reductase (CYB5R), cytochrome b_5 (CYB5) and molybdenum cofactor sulfurase C-terminal containing-1 and -2 (MOSC1 and MOSC2) [2, 3]. We found that the mitochondrial amidoxime reductase is exclusively localized to the outer mitochondrial membrane (OMM) isolated from rat liver where also MOSC2, CYB5R and the mitochondrial form of CYB5 (CYB5B) are localized [4]. In addition direct substrate binding to MOSC2 was demonstrated using a radiolabeled benzamidoxime analogue. Following differentiation of NIH-3T3-L1 cells into mature adipocytes, the amidoxime reductase activity was increased 20-fold and was accompanied by an increase in MOSC2 protein expression. siRNA-mediated down-regulation of MOSC2 and CYB5B, but not microsomal cytochrome b_5 (CYB5A) and CYB5R3, in differentiated adipocytes inhibited the amidoxime reductase activity further confirming their involvement. The fact that MOSC2 protein levels and reductase activity was up-regulated during adipogenesis promoted us to investigate its role in fatty acid metabolism. Indeed down-regulation of MOSC2 in mature adipocytes attenuated the intracellular triglyceride levels. The reductase activity was also found to be inhibited by free fatty acid CoA derivatives in vitro and the activity was significantly lower in livers obtained from starvation treated rats. Together these data strongly suggest a role for MOSC2 in the lipogenesis. The amidoxime reductase components MOSC2 and CYB5B were found to be present in livers and omental fat from obese patients. A more detailed lipidomic analysis of the mature adipocytes and the MOSC2 down-regulated adipocytes is underway and will give some clues to the physiological function of MOSC2 in adipocytes. In summary, we have shown using an intact cell system that MOSC2 and CYB5B are important components of the mitochondrial amidoxime reductase enzyme system. Moreover our data suggest that the endogenous role of this enzyme system appears to be linked to lipogenesis.

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P207 - THE MAMMALIAN MOLYBDOENZYME MARC – LOCALIZED IN PEROXISOMES?

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Hypothesis: The mitochondrial amidoxime reducing component "mARC" is a molybdenum-cofactor containing enzyme (1). MARC is part of a mitochondrial enzyme system together with cytochrome b5 and its reductase and this enzyme system is located on the outer mitochondrial membrane (2, 3). Mammalian genomes encode for two mARC enzymes (mARC1/mARC2) and both mARC enzymes are able to reduce N-hydroxylated compounds together with cyt b5 and b5 Red (4). Although the contribution of the N-reductive enzyme system in drug metabolism pathways is well accepted the physiological function of mARC is not fully understood. Interestingly, proteomic characterization of peroxisomes and immunocytochemically visualized Myc-tagged protein versions suggested a peroxisomal localisation of mARC2 in mammalian cells (5, 6). **Methods used:** Subcellular fractions from mammalian livers were analysed by western blot. Furthermore the N-reductive activity of these fractions was determined using the marker substrate (benzamidoxime) of the N-reductive enzyme system. **Results:** By this approach additional evidence of a peroxisomal localisation of mARC2 was provided. Furthermore it is the first time that mARC was detected in a subcellular fraction without the electron transfer proteins cytochrom b5 and its reductase. In consequence, N-reductive activity was not enriched in the peroxisomal fraction. In contrast, both mARC proteins were enriched in mitochondria, but only mARC2 was enriched in the outer mitochondrial membrane. **Conclusion:** In peroxisomes mARC seems to be involved in so far unknown redox-reactions either as a stand-alone protein or with other electron transport proteins different from cytochrom b5 and its reductase.

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P208 - UNDERSTANDING THE IMPACT OF METABOLISM BY AO AND XO ON METABOLITE SAFETY AND PK PREDICTIONS

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As our understanding of cytochrome P450-mediated metabolism grows and chemical design is able to effectively modulate P450 clearance, our attention turns to other metabolising enzymes and their potential impact on clinical efficacy and safety. Notably, aldehyde oxidase (AO) and xanthine oxidase (XO), which for some may have been considered fairly rare or unimportant metabolic routes, may be potentially important contributors to drug clearance, particularly in certain areas of chemical space, such as nitrogen-containing heterocycles which are used with increasingly regularity to inhibit kinase targets. Both these enzymes are soluble molybdenum-containing proteins, found in the cytosolic fraction of liver (and other tissue) preparations, and show a high degree of homology in their amino acid sequences; yet despite their apparent similarities, the two enzymes differ significantly in substrate (and

inhibitor) specificities. Via the incubation of precedented AO and XO substrates in a range of subcellular liver fractions from a variety of species (with and without relevant chemical inhibitors) and the analysis of the resultant metabolic profiles and intrinsic clearance values, we explore aspects of our understanding of these enzymes and their contribution to the metabolism of drugs and drug-candidates, including: the differing activities between species and strains, considering how such differences may impact the interpretation and extrapolation of in vitro screening data and pre-clinical PK data; the suitability and use of various sub-cellular liver fractions, including how in vitro data may be correlated to the in vivo scenario; the potential of in silico approaches for modelling and predicting AO/XO susceptibility, and strategies to investigate and understand the contribution and impact of AO/XO. The results presented in the poster will demonstrate the advantages and limitations of a range of in vitro approaches to assess the impact of AO and XO and predict the PK implications in both humans and toxicology species, as well as draw some conclusions around the susceptibility of various chemotypes to this type of non-P450 metabolism.

P209 - UTILITY OF DIFFERENT TEST SYSTEMS FOR DETERMINING CARBOXYLESTERASE SUBSTRATE SPECIFICITY AND INHIBITION POTENTIAL

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Carboxylesterases (CES) can hydrolyse a number of ester-containing drugs and pro-drugs. CES are mainly located in the endoplasmic reticulum of many tissues.[1] Human liver S9, cytosol or microsomes are often used for in vitro studies of CES. Plasma may also be used, however human plasma contains little or no CES, with esterase activity limited to cholinesterases, paraoxonase, or serum albumin.[2] In contrast to cytochrome P450 reaction phenotyping, esterase phenotyping is usually only utilised for mechanistic studies for specific compounds e.g. bioactivation of a series of pro-drugs. CES are expressed throughout the body but there are marked differences between the two major forms, human carboxylesterase 1 (hCE-1) and human carboxylesterase 2 (hCE-2) in terms of substrate specificity and tissue distribution. hCE-1 is the main CES in liver whilst hCE-2 is the main CES in intestine.[3] We have evaluated a number of different enzyme systems for investigating hydrolysis of drug esters. A comparison of a panel of hCE-1 and hCE-2 substrates in human liver and intestinal microsomes highlighted the limitations in these systems in determining CES substrate specificity. In contrast recombinant CES enzymes proved more useful for this purpose. A comparison of recombinant enzymes from different suppliers was also evaluated. In addition to stability studies, enzyme kinetics were established in the recombinant systems using specific probe substrates (trandolapril for hCE-1 and irinotecan for hCE-2) to develop an assay suitable for the testing of compounds as potential CES inhibitors. CES inhibitors are useful in modulating the metabolism, distribution and toxicity of compounds that undergo enzyme hydrolysis by CES.[4] Additionally, a panel of specific probe substrates and inhibitors for CES, in addition to paraoxonase, acetylcholinesterase and butyrylcholinesterase were assessed using plasma from a number of different species to determine the utility of this matrix for providing a mechanistic species-driven understanding of hydrolysis in the blood.

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P210 - PHENACETIN-INDUCED METHEMOGLOBINEMIA IS CAUSED BY METABOLIC ACTIVATION BY HUMAN ARYLACETAMIDE DEACETYLASE, CYP2E1, AND CYP1A2

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Phenacetin had been used as an analgesic antipyretic, but it was withdrawn from the market owing to the adverse reactions such as methemoglobinemia and renal failure. Phenacetin is primarily metabolized to acetaminophen

(APAP) through the deethylation and is also metabolized to p-phenetidine through the hydrolysis. It was assumed that the adverse reactions of phenacetin were induced by its hydrolysis and subsequent hydroxylation. However, the assumption has not experimentally been proven. We previously found that human arylacetamide deacetylase (AADAC) is a principal enzyme for the phenacetin hydrolysis (1). In this study, we investigated the involvement of AADAC in phenacetin-induced methemoglobinemia. One hour after administration of phenacetin (125 mg/kg, p.o.) to male C57BL/6 mice, the high methemoglobin (Met-Hb) formation (16.7 ± 2.1%) was observed. Pre-administration of tri-o-tolylphosphate (TOTP) (150 mg/kg, i.p.), an esterase inhibitor, to mice 12 hour before phenacetin administration significantly decreased the Met-Hb formation (1.2 ± 0.2%) as well as the plasma concentration of p-phenetidine. Pre-administration of TOTP did not alter the plasma concentrations of phenacetin and APAP. Thus, the in vivo study demonstrated that the hydrolysis pathway is important for phenacetin-induced methemoglobinemia. To investigate the involvement of human AADAC in the phenacetin-induced methemoglobinemia, in vitro assays were conducted. By incubating with human liver microsomes (HLM) and mouse red blood cells, the high Met-Hb formation was observed with phenacetin and p-phenetidine, but not with APAP. In addition, the high Met-Hb formation was observed by incubating with the combination of recombinant human AADAC and human CYP2E1 or CYP1A2. The Met-Hb formation by incubating phenacetin with HLM was decreased to 25.1 ± 0.7% of control by eserine, a potent AADAC inhibitor. In addition, the Met-Hb formation by incubating p-phenetidine with HLM was moderately decreased to 45.5 ± 3.0% of control by anti-CYP2E1 antibody, and was weakly decreased to 80.6 ± 7.0% of control by anti-CYP1A2 antibody. Similar results were obtained using human red blood cells. In conclusion, we demonstrated that the hydrolysis by AADAC and subsequent hydroxylation by CYP2E1 and/or CYP1A2 are responsible for phenacetin-induced methemoglobinemia in human liver.

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P211 - A PROTEIN TYROSINE KINASE INHIBITOR, GEFITINIB, INHIBITS UDP-GLUCURONOSYLTRANSFERASE 1A9 (UGT1A9) BY BINDING TO THE ALLOSTERIC SITE SHARED WITH ADENINE NUCLEOTIDES

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Inhibitors, such as Gefitinib, against epidermal growth factor receptor (EGFR)-protein tyrosine kinase have an undesired inhibitory effect on UDP-glucuronosyltransferase (UGT). This may cause a drug-drug interaction in UGT-dependent metabolism. However, the reason why Gefinitib and related compounds exhibit inhibitory effect on UGT has not been elucidated. Our recent studies have suggested that ATP is an allosteric inhibitor of UGT. Since Gefitinib is thought to bind to the ATP binding domain of the protein tyrosine kinase to exert its pharmacological activity, we examined whether Gefitinib inhibits UGT through interaction with the ATP binding site on UGT. We have suggested previously that ATP inhibits UGT in rat liver microsomes (RLM) treated with detergent. Another objective of this study was focused on a question whether the same occurs with human liver microsomes (HLM). ATP inhibited 4-metylumbelliferone (4-MU) glucuronidation when Brij-58-treated or alamethicin-treated HLM were used. Alamethicin-treated HLM exhibited lesser susceptibility than did Brij-58-treated HLM. Neither ADP nor AMP showed any inhibitory effect on whichever HLM treated with Briji-58 and alamethicin. ATP also inhibited 4-MU UGT activity of recombinant human UGT1A9 which is one of the major UGTs catalyzing 4-MU glucuronidation in HLM. As expected, Gefitinib significantly inhibited 4-MU UGT mediated by HLM and human UGT1A9. Although AMP lacks the inhibitory effect on UGT, AMP can antagonize the inhibitory effect of ATP. AMP also antagonized the Gefitinib-provoked inhibition of UGT1A9, and ATP exhibited an additive inhibitory effect, with Gefinitib, at lower concentrations. Therefore, Gefitinib is suggested to inhibit UGT1A9 by interacting with the common binding site shared with ATP and AMP. It is probably likely that ATP and AMP contents in cells largely depend on the mitochondrial function which varies in response to a change in physiological conditions. Although many more studies would be needed to understand how disease conditions affect ATP concentration within the ER, hormone metabolism and drug-drug interaction with EGFR-protein tyrosine kinase inhibitors, it is reasonable to assume that the concentration of ATP and antagonistic AMP varies dynamically in the lumen of the ER to switch on/off glucuronidation reaction.

P212 - FUNCTIONAL CHARACTERIZATION OF 50 CYP2D6 ALLELIC VARIANTS WITH REGARD TO 4-HYDROXYLATION OF N-DESMETHYLTAMOXIFEN

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N-Desmethyltamoxifen (DMT), an important primary metabolite of tamoxifen (TAM), is hydroxylated by cytochrome P450 (CYP) 2D6 to yield 4-hydroxy-N-desmethyl tamoxifen (endoxifen, EDX). Because of its high anti-estrogenic potency, EDX may play an important role in the clinical activity of TAM. A recent study has shown that plasma concentrations of EDX and the clinical outcome are dependent on the CYP2D6 genotype of patients treated with TAM. The relationship of variant CYP2D6 alleles with drug concentration in blood, drug response, and adverse drug effects and the clinical significance of these relationships can be determined by analyzing drug metabolism in patients with variant CYP2D6 alleles. However, the functional characterization of variant proteins of low-frequency alleles is difficult to study in vivo. In contrast, recombinant CYP2D6 constructed using a heterologous expression system can be used to evaluate the functional changes in the allelic variant proteins, without the need for in vivo studies. The purpose of this study was to evaluate the DMT 4-hydroxylation activity of 50 CYP2D6 allelic variants. Wild-type and variant CYP2D6 cDNAs were subcloned into the pcDNA-DEST40 mammalian expression vector. Wild-type CYP2D6.1 and its 49 allelic variants, CYP2D6.2, .7, .9, .10, .12, .14A, .14B, .17, .18, .22-.37, .39, .40, .43, .45-.55, .57, .61-.65, .70-.72, and .75 were heterologously expressed in COS-7 cells. The proteins were detected using rabbit anti-human CYP2D6 antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG. DMT 4-hydroxylation activity was determined by measuring the EDX concentration with an HPLC system equipped with a post-column in-line photoreactor placed before a fluorescence detector. Bufuralol and dextromethorphan were used as representative CYP2D6 substrates. CYP2D6.10, .12, .14A, .31, .36, .37, .40, .46, .52, .62-65, .71, .72, and .75 were catalytically inactive with regard to 4-hydroxylation of DMT. Although similar results were obtained during the enzyme activity analysis for bufuralol 1-hydroxylation and dextromethorphan O-demethylation, several CYP2D6 variants showed substrate dependence. These comprehensive findings provide useful information for further genotype-phenotype studies on interindividual differences in TAM metabolism, response, and side effects.

P213 - STRUCTURAL RATIONALIZATION OF REGIO- AND STEREO-SELECTIVE HYDROXYLATION BY DRUG-METABOLIZING CYTOCHROME P450 BM3 MUTANTS

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Results from our recent in silico studies on selective hydroxylation of biotechnologically and pharmaceutically relevant substrates by P450 BM3 mutants will be presented. In order to account for protein flexibility and plasticity, a combined molecular dynamics and docking approach was employed. Using this approach, regio- and stereo-selectivity of steroid and food-additive hydroxylation by some of our in-house BM3 mutants could be rationalized, which opens up possibilities to computationally exploit and predict additional active-site mutations with the aim of designing new mutants that show even higher selectivity in substrate conversion.

P214 - IMPACT OF CYP2B6*6 GENOTYPE ON KETAMINE HUMAN PHARMACOKINETICS AND MICROSOMAL METABOLISM

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Ketamine is a racemic NMDA antagonist drug which is being increasingly used as an adjuvant analgesic in chronic pain patients. Ketamine is partly metabolised to norketamine (active) by CYP2B6 and CYP3A4; CYP2B6 genetic polymorphisms are associated with significantly altered plasma concentrations of many CYP2B6 metabolised drugs. Ketamine's pharmacokinetics, in vitro and in vivo metabolism, and impact of CYP2B6 genetic polymorphisms in pain patients remains unknown. We aimed to investigate the influence of CYP2B6 genetic variability on ketamine pharmacokinetics in pain patients and on ketamine's in vitro human liver metabolism. Steady-state blood samples were obtained from 11 chronic cancer pain patients who had received 100 mg ketamine by continuous 24 hour subcutaneous infusion in order to provide an opioid-sparing effect. CYP2B6 altered-function alleles (*4, *5, *6, *7,

*8, *9, *13) were identified. Human liver microsomes genotyped for CYP2B6*6 were separately incubated with R- and S-ketamine and R- and S-norketamine formation kinetics quantified and linked to CYP2B6*6 genotype. A 3-fold range in racemic ketamine plasma clearance was found. In 5 wildtype CYP2B6*1/*1 patients, plasma clearance was 672±130 (mean±SD) ml/min, in 5 *1/*6 patients 541±46 ml/min and in 1 *6**/***7 patient 350 ml/min (gene-dose P=0.19). The plasma norketamine/ketamine concentration ratio was significantly higher in patients with CYP2B6*1/*1 genotype (1.1±0.2) compared to those with CYP2B6*1/*6 genotype (0.4±0.1) and *6**/***7 (0.22) [gene-dose P=0.01]. In human liver microsomes, two enzyme Michaelis-Menten kinetics were obtained and the intrinsic clearance (V_{max}/K_m) values for the high affinity-low capacity system of R- and S-ketamine to R- and S-norketamine, respectively in relation to the CYP2B6*6 genotype were: S-ketamine *1/*1 2.4±0.3 (n=4), *1/*6 0.6±0.2 (n=4) and *6**/***6, 0.2±0.04 (n=2) ml/mg protein/hr [gene-dose P=0.02]; R-ketamine *1/*1 0.8±0.04, *1/*6 0.2±0.2 and *6**/***6, 0.2±0.04 ml/mg protein/hr [gene-dose P=0.22]. These data indicate a likely impact of the loss-of-function CYP2B6*6 genetic variant on ketamine metabolic clearance to norketamine, especially for the more active S-ketamine. CYP2B6*6 genetic variability might influence the intensity and duration of response and adverse effects to ketamine when used for pain and other therapeutic areas.

P215 - AFFECTION OF VARIOUS POLYMORPHIC VARIANTS OF CYTOCHROME P450 OXIDOREDUCTASE (POR) ON DRUG METABOLIC ACTIVITY OF CYP3A4 AND CYP2B6

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Cytochrome P450 oxidoreductase (POR) is known as the sole electron donor in the metabolism of drugs by cytochrome P450 (CYP450) enzymes in human[1]. some recent studies have attempted to identify the possibility of POR as a potential rate-limiting step in CYP-mediated drug metabolism in vitro (reconstituted) systems, using N-terminal end-deleted CYPOR by bacterial system (prokaryocytes) [2-5]. However, It has been recently reported that the substantial differences were observed in the electron-transfer abilities between the full-length POR variants and N (27)-deleted form in E.coli. In order to better understand the mechanism of the activity of CYPs affected by polymorphic variants of POR and mimic the in vivo system to the extent possible, six full-length mutants of POR (e.g., Y181D, A287P, K49N, A115V, S244C and G413S) were designed and then co-expressed with CYP3A4 and CYP2B6 in the baculovirus-Sf9 insect cells to determine their kinetic parameters with their specific substrates (e.g., testosterone for CYP3A4-PORs and bupropion for CYP2B6-PORs) by HPLC. Surprisingly, both mutants, Y181D and A287P in POR completely inhibited the CYP3A4 activity with testosterone, while the catalytic activity of CYP2B6 with bupropion was reduced to approximately ~70% of wild type activity by Y181D and A287P mutations. In addition, the mutant K49N of POR increased the CLint (Vmax/Km) of CYP3A4 up to more than 31% of wild type, while it reduced the catalytic efficiency of CYP2B6 to 74% of wild type. Moreover, CLint values of CYP3A4-POR (A115V, G413S) were increased up to 36% and 65% of wide type respectively. However, there were no appreciable effects observed by the remaining two mutants of POR (i.e., A115V and G413S) on activities of CYP2B6. In conclusion, the extent to which the catalytic activities of CYP450 were altered did not only dependent on the specific POR mutations but also on the isoforms of different CYP450 redox partners. Therefore, each POR variants will require testing with each CYP superfamily member to assess their effects. Moreover, additional studies covering different other types of CYP450s are required to take either in vitro or in vivo drug clearance tests to make sure that POR-mutant patients can obtain maximum therapeutic efficiency and minimal adverse drug reactions.

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P216 - CARBOXYLESTERASE 1 POLYMORPHISM IMPAIRS OSELTAMIVIR BIOACTIVATION IN HUMANS

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Bioactivation of the antiviral agent oseltamivir to active oseltamivir carboxylate is catalyzed by carboxylesterase 1 (CES1). Our aim was to investigate the frequency distribution of the CES1 c.428G>A (p.Gly143Glu, rs121912777) single nucleotide variation (SNV) in the Finnish population and possible effects of the variation on the pharmacokinetics of oseltamivir. A total of 860 healthy white Finnish volunteers were genotyped for the CES1 c.428G>A SNV, and 22 of them participated in a pharmacokinetic study on 75 mg oseltamivir. Plasma oseltamivir and oseltamivir carboxylate concentrations were measured for up to 48 hours. The CES1 c.428A variant allele was found with a frequency of 2.2% (95% confidence interval, CI, 1.6-3.0%) in the Finnish population. Only 1 of the 860 subjects showed a homozygous variant genotype (0.05%, 95% CI, 0.02-0.7%), while 4.3% (95% CI, 3.0-5.7%) were heterozygous carriers. The mean area under the plasma concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) of oseltamivir was 18% (P=0.025) larger in subjects with the CES1 c.428GA genotype (n=9) than in those with the c.428GG genotype (n=12). There was a tendency (P=0.148) for a 9% smaller $AUC_{0-\infty}$ of oseltamivir carboxylate in subjects with the c.428GA genotype than in subjects with the c.428GG genotype, and the oseltamivir carboxylate/oseltamivir $AUC_{0-\infty}$ ratio was 23% (P=0.006) smaller in subjects with the CES1 c.428GA genotype than in those with the c.428GG genotype. Moreover, subjects with the c.428GA genotype had a 15% (P=0.011) lower C_{max} of oseltamivir carboxylate than subjects with the c.428GG genotype. In the one subject with the CES1 c.428AA genotype, oseltamivir $AUC_{0-\infty}$ was 360% larger, oseltamivir carboxylate $AUC_{0-\infty}$ 27% smaller, and the oseltamivir carboxylate/oseltamivir $AUC_{0-\infty}$ ratio was 84% smaller than in those with the c.428GG genotype. In conclusion, genetic variability in CES1 affects the pharmacokinetics of oseltamivir, indicating that CES1 plays an important role in the bioactivation of oseltamivir in vivo in humans.

P217 - NOVEL CYP2B6 POLYMORPHISMS: HOW PRECISE ARE FUNCTIONAL PREDICTIONS?

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Objective: The human cytochrome P450 2B6 is involved in the metabolism and activation of several therapeutically important drugs such as efavirenz, bupropion and cyclophosphamide. The CYP2B6 gene is highly polymorphic and 29 major alleles are known up to date, 14 and four of these showed impact on enzyme activity in vitro and in vivo, respectively (http://www.cypalleles.ki.se/cyp2b6.htm). Occurrence of CYP2B6 polymorphisms is highly variable in different ethnic groups and rare variants can have clinical impact [1]. Of broad interest, thus, is to determine the proportion of loss of function alleles in HIV patient cohorts under efavirenz treatment and to find new variants to better explain toxic side-effects. **Methods:** Rwandan HIV-1 infected study cohort samples (n=39) were sequenced in nine exons and exon/intron boundaries. New missense mutations were analyzed in silico using different prediction tools, including PolyPhen-2 and SIFT. The mutations were introduced in the reference cDNA 2B6*1 using site directed mutagenesis, sequenced, and cloned into pcDNA3 vector. The variant cDNAs were transiently expressed in COS-1 cells. Protein expression in microsomal fraction was quantified using Western-Blot. Enzyme kinetic parameters were determined for the bupropion-4-hydroxlytion and efavirenz-8-hydroxylation using LC/MS and LC/MS-MS methods, respectively. **Results:** Besides already known polymorphisms seven new exonic SNPs with amino acid changes (G110V, I114T, A279P, R487S, V183G, F213L, R253H) were identified from which the latter three were so far unknown. The residues were analyzed in silico to predict their impact on protein function and compared to the functionality of the recombinantly expressed proteins. 110V and 183G were predicted to be completely damaging consistently with all selected tools. Residue 110 is located within the substrate-recognition-site 1 and valin at position 183 is 100% conserved within species. Protein expression was 50% of 2B6.1, but bupropion- and efavirenz-hydroxylation activity was dramatically decreased. Three further variants were concordantly from most tools estimated to be benign (R253H, A279P, R487) which was in agreement with the recombinant proteins which showed normal or reduced protein expression and activity. In contrast, the predictions of two variants were inconclusive and not in agreement with the in vitro data. I114T and F213L, which were predicted to be not damaging

with most tools showed dramatically impaired enzyme function. Both residues are located in substrate-recognition sites of the enzyme. Substrate docking simulations will help to better predict sensitive positions for loss of function mutations. **Conclusions:** The characterization of novel SNPs in the CYP2B6 gene extends the knowledge about variants affecting function. Especially for the HIV therapy with efavirenz it is important to know the proportion of loss-of-function alleles in african and other populations, because these alleles are prone to lead to increased efavirenz plasma levels and thus patients are at higher risk to develop toxicity.

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P218 - THE EFFECT OF PHARMACOGENETICS ON ROSIGLITAZONE PHARMACOKINETICS AND PHARMACODYNAMICS

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Objective: The aim of this study was to examine the effect of Single Nucleotide Polymorphisms (SNP) in the genes of CYP2C8, LPIN1, PPARGCA and PPARg on rosiglitazone: i) through plasma concentration ii) response, measured as difference in glycosylated haemoglobin A1c (HbA1c) and iii) risk of developing adverse events, mainly oedema. Methods: Data used in this study were obtained from the South Danish Diabetes Study. The study was a placebo-controlled, partly blinded and multi-centre clinical trial performed in Denmark. The 371 patients with type 2 diabetes mellitus included in this trial received either insulin aspart or insulin NPH in combination with rosiglitazone, placebo or metformin for 24 months including 15 visits. There were 187 patients who received rosiglitazone and thus were included in the study. Blood samples were obtained at visits at 3, 6 and 9 months to estimate rosiglitazone through steady-state concentrations in plasma. Furthermore HbA1c was measured at 0, 6 and 24 months and relevant CYP2C8, LPIN1, PPARGCA and PPARg genotypes of the patients were determined. Oedema was defined as generalized, localized or peripheral oedema and determined as either/or by the physician. Results: The mean trough rosiglitazone steady-state concentration was 21.3 ng/ml (C.I.95% 18.8; 24.2) with observations ranging from 1 to 296 ng/ml and the median time since last dose was 14 hours. The allelic variant CYP2C8*3 (rs10509681 and rs11572080) was associated with a significantly lower trough rosiglitazone steady-state concentration and a significantly lower therapeutic response measured as the absolute difference in HbA1c in carriers of the variant compared to individuals without the variant during rosiglitazone treatment. In the LPIN1 gene, the SNP rs10192566 was correlated with a significantly lower therapeutic response to rosiglitazone at both 6 and 24 months. Patients carrying both rs10192566 in LPIN1 and the genetic variant CYP2C8*3 had a significantly lower response than individuals without any of the variants or carriers of just one SNP, and a significantly lower risk of developing oedema during rosiglitazone treatment. Furthermore, an overall significantly positive correlation between the risk of developing oedema and rosiglitazone trough steady-state concentration was discovered. Conclusion: We were able to show that the allelic variant CYP2C8*3 was connected with significantly lower trough rosiglitazone steady-state concentration and lower therapeutic response to treatment. Secondly, the relatively common, single nucleotide polymorphism rs10192566 was associated with significantly lower treatment response to rosiglitazone. 137 of 181 patients receiving rosiglitazone treatment carried the minor allele, and this underlines the importance of this SNP. Finally the carriers of both, above-mentioned SNP led to an additively lower therapeutic response, and a significantly lower risk of developing oedema during treatment.

P219 - RELEVANCE OF NON-HLA IMMUNE-RELATED GENES TO LIVER INJURY INDUCED BY FLUCLOXACILLIN AND CO-AMOXICLAV

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The antimicrobials flucloxacillin and co-amoxiclav are both associated with serious liver toxicity (drug-induced liver injury, DILI) in a small minority of patients. The current study aimed to extend associations detected previously in genome-wide association studies (GWAS) using larger numbers of flucloxacillin and co-amoxiclav DILI cases. Single nucleotide polymorphisms (SNPs) in the non-HLA immune-related genes ST6GAL1, PTPN22 and IL12RB1 were investigated. ST6GAL1 (rs10937275), which codes for a protein with a possible role in B cell differentiation, had been previously found to be a risk factor in HLA-B*5701-positive cases only in a GWAS involving 51 flucloxacillin DILI cases (1) was found as an additional risk factor in 83 flucloxacillin treated DILI subjects (P=0.00056, OR=2.63, 95% CI 1.53 – 4.52) and the risk was higher in patients already positive for HLA-B*5701 (P=0.000026, OR=3.45, 95% CI 1.95 – 6.1). The effect of ST6GAL1 genotype on co-amoxiclav DILI was also investigated but no evidence for an association was found. A SNP (rs2476601) in PTPN22, which codes for a protein involved in T-cell-receptor signalling has already been shown to be a risk factor for DILI due to co-amoxiclav in a large (n=201) study (2). Genotyping of additional co-amoxiclav cases (n= 86) confirmed this association (P=0.00017, OR=3, 95% CI 1.7 – 5.94). A significant association of PTPN22 genotype with flucloxacillin DILI (n=131) (P=0.015, OR=1.97, 95% CI 1.2– 3.4) was also seen. Genotyping of 152 co-amoxiclav DILI cases and 270 non-treated controls have confirmed the association, seen previously in an exome sequencing study (3), of the nonsynonymous SNP rs117511121 in the IL12RB1 gene with co-amoxiclav hepatoxicity (P=0.004, OR=5.2, 95% CI 1.6– 16.6). IL12RB1 is expressed on T cells and plays an essential role in interleukin 12 binding. A significant distribution difference was also detected when cases and controls for carriage of another SNP rs436857 at position -2 in IL12RB1 gene (CC + CT versus TT, P=0.028, OR=2.95). No association with IL12RB1 genotype was seen for flucloxacillin DILI. These findings indicate small contributions to DILI susceptibility for a range of SNPs in genes relevant to T and B cell responses.

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P220 - THE COST-EFFECTIVENESS OF PHARMACOGENETIC-GUIDED DOSING OF PHENPROCOUMON

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Background: Genotyping patients for polymorphisms in the CYP2C9 gene, encoding for the main metabolizing enzyme, cytochrome P450 2C9 (CYP2C9), and the VKORC1gene, encoding for the target enzyme Vitamin K epoxide reductase multiprotein complex 1 (VKORC1) helps to predict the required coumarin dose prior to treatment initiation. Objective:To examine the cost-effectiveness of pharmacogenetic-guided phenprocoumon dosing for Dutch patients with atrial fibrillation. Design: Using a decision-analytic Markov model we estimated the change in quality adjusted life years (QALYs) and incremental cost-effectiveness ratios (ICERs) for patients receiving pharmacogenetic-guided therapy versus standard care. Time spent in different INR ranges was used to estimate the risk of thromboembolic and bleeding events. Costs were determined from a third-party payer perspective and a lifetime horizon was used. Additionally, sensitivity analyses for a selection of variables were performed. Results: In the base-case scenario, the total costs for standard care were 7220 euro and total QALYs were 8.6511. The total costs for the genotyping strategy were slightly higher, 7240 euro and total QALYs were 8.6575. This meant an increase in costs of 20 euros and an increase in QALYs of 0.0064, which led to an ICER of 3125 euro per QALY gained. Our results were particularly sensitive to the cost of genotyping, the effect of genotyping on time spent within the different INR ranges and the costs of adverse events. Conclusions: Pharmacogenetic-guided dosing of phenprocoumon has the

potential to decrease the risk of bleeding and thromboembolic events and thereby increase health slightly. More importantly, genotyping may be able to achieve this in a cost-effective way. The impact of genotyping is dependent on the quality and costs of care. The effect of genotyping on time spent within the different INR ranges is currently being investigated in clinical trials.

P221 - SUBSTRATE SPECIFICITY OF CYP2C8*3

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Cytochrome P4502C8 (CYP2C8) constitutes of 7% of the total hepatic CYP content and is involved in the metabolism of the clinically important drugs such as the anti-cancer drug paclitaxel, the antimalarial drug amodiaquine and the anti-diabetes drug rosiglitazone. The CYP2C8*3 allelic variant carrying the Arg139Lys and Lys399Arg amino acid substitutions is found in Caucasians at an allele frequency of about 13 %. Lower paclitaxel clearance has been reported for paclitaxel in patients carrying the CYP2C8*3 allele compared to carriers of CYP2C8*1. Regarding to amodiaquine, lower clearance has been observed in CYP2C8*3 subjects. Interestingly, a higher clearance of the thiazolidinedione rosiglitazone has been reported for patients with the CYP2C8*3 allelic variant. There is a need to characterize the properties of the CYP2C8*3 allele since the previous in vitro data found in the literature on the enzyme kinetic s of the CYP2C8*3 enzyme in the presence of paclitaxel and rosiglitazone are scarce and controversial. In this study, we have used the well-established HEK293 FlipIn system for stable expression of the *1 and *3 variants of CYP2C8. The enzyme kinetics of 3 different drugs, paclitaxel, amodiaquine and rosiglitazone was investigated using an intact cells system. In well agreement with what has been observed in patients carrying the CYP2C8*3 variant, a higher rate of metabolism of rosiglitazone was found in HEK293 cells overexpressing CYP2C8*3 as compared to cells expressing CYP2C8*1. Regarding the substrates paclitaxel and amodiaquine, CYP2C8*3 catalyzed the metabolism at similar or lower rate than CYP2C8*1 metabolism. The present study indeed indicates that the previously described in vivo data are inherent in differential substrate binding and/or conversion of CYP2C8*3 as compared to CYP2C8*1.

P222 - STUDY OF THE INTER-INDIVIDUAL VARIABILITY OF ARTEMETHER AND DIHYDROARTEMISININ ELIMINATION: THE IMPACT OF GENETIC POLYMORPHISM IN THE CYP3A4/5, CYP2B6 AND UGT2B7 GENES

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Malaria is one of the oldest diseases know to mankind, still killing 1 million people/year. In the absence of viable vaccines, chemotherapy represents the major strategy for its treatment and control, with a number of drug administrations per year overpassing one billion doses. In the new Millennium, specific artemisinin combination therapy strategies have been successfully implemented, based on the fast acting artemisinin derivatives (artesunate, artemether), associated to longer half life quinolinic antimalarials (amodiaquine, mefloquine, lumefantrine). Of particular interest is the combination artemether-lumefantrine (Coartem®), due to its widespread administration in Africa, and in regions of Asia and Central/South America. Artemether (ATM) is mainly metabolized into dihydroartemisinin (DHA), its main active metabolite by the Phase I drug metabolizing enzymes CYP3A4/5, CYP1A2 and CYP2B6. DHA is bio-converted by Phase II enzymes, through glucuronidation by the UDP-glucuronosyltransferases isoforms, UGT1A9 and UGT2B7. Several single nucleotide polymorphisms have been identified in these enzymes, however little is know on the effect of these SNPs on the PK of this particular antimalarial drug. In this study we intend to correlate genetic polymorphisms in the genes coding for the enzymes CYP3A4/5, CYP2B6 and UGT2B7 with variability in PK parameters of the antimalarials ATM and DHA. The pharmacokinetic data was obtained from a clinical trial performed in Tanzania. Briefly, the study included 50 hospitalized patients (ages 1-10 years) with microscopically confirmed acute uncomplicated P. falciparum malaria were included. Coartem® (Novartis Pharma, Ltd., Switzerland) was administered in weight-based doses (20 mg

artemether plus 120 mg lumefantrine) at 0, 8, 24, 36, 48, and 60 h. Venous blood samples for drug concentration analyses and determination of parasitemia were obtained at 0, 2, 4, 8, 16, 24, 36, 48, 60, and 72 h following treatment initiation. Concentrations of artemether and dihydroartemisinin in plasma were measured by high-throughput liquid chromatography-tandem mass spectrometry. DNA samples from the 50 patients were analyzed for the main alleles described for CYP3A4/5, CYP2B6 and UGT2B7(www.cypalleles.ki.se). A correlation was observed between the DHA/ATM drug ration and the carriers of the CYP3A5*3 allele. As expected, the drug ratio was lower in the carriers of this snp (p=0.018). This corroborated the already described data regarding the phenotype of this SNP, and indicates that this enzyme is also involved in the ATM-DHA conversion. With this data we hope to help in the increase success of these therapies, by showing data for evidence-based decisions on future changes in dosages or administration schemes.

P223 - PRELIMINARY RESULTS OF THE EFFECT OF GENETIC VARIATION IN CYP4F2 AND CYP3A4 ON THE PHENPROCOUMON AND ACENOCOUMAROL MAINTENANCE DOSE

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Background: SNPs in CYP4F2 have shown to affect the phenprocoumon and acenocoumarol dose requirements. The CYP3A4 enzyme also plays a role in the metabolism of phenprocoumon and acenocoumarol. Genetic polymorphisms in this gene might therefore influence dose requirements. **Objectives:**To investigate whether the phenprocoumon and acenocoumarol maintenance dose is influenced by genetic variations in CYP4F2 and CYP3A4. **Design:** For the analysis, the Pre-EU-PACT database was used. Dosages, genetic information, demographic information and information on concurrent medication were available for 551 phenprocoumon and 372 acenocoumarol users. The influence of genetic variations in CYP4F2 (rs2108622) and CYP3A4 (rs2740574 and rs35599367) on the phenprocoumon and acenocoumarol maintenance dose was investigated by performing ANOVA, comparing the adjusted mean maintenance dose differences between all genotype classifications. **Results:** For phenprocoumon, a significant increase in the maintenance dose of 0.12 mg/day (p=0.016) was found for patients carrying one variant CYP4F2 allele (n=185) if compared to wild type patients (n=325), and an even larger increases was found for patients carrying 2 CYP4F2 variant alleles (n=41); plus 0.25 mg/day, p=0.009. No significant effect of CYP3A4 on the phenprocoumon maintenance dose was found. For acenocoumarol, no significant effects on the maintenance dose were found for both CYP4F2 and CYP3A4. **Conclusion:** Genetic variations in CYP4F2 appear to influence the phenprocoumon maintenance dose. This effect is not seen in patients using acenocoumarol. No effect is observed for CYP3A4 genotypes on both the phenprocoumon and acenocoumarol maintenance dose.

P224 - LACK OF EFFECT OF COMMON CYP1A2 SEQUENCE VARIATIONS ON THE PHARMACOKINETICS OF TIZANIDINE

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The centrally acting muscle relaxant tizanidine is metabolised principally by cytochrome P450 (CYP) 1A2 and can be used as a probe substrate to study CYP1A2-mediated metabolism in vivo. This study aimed to characterize the possible relationship between polymorphisms in the CYP1A2 gene and the pharmacokinetics of tizanidine and caffeine. The exons, exon-intron boundaries and promoter region of CYP1A2 were re-sequenced in 96 healthy Finnish volunteers (15 male smokers, 15 female users of oral contraceptives and 66 subjects with no other medication) who had previously participated in pharmacokinetic studies with tizanidine, and in 10 control subjects. Each pharmacokinetic study subject ingested a single oral dose of 4 mg tizanidine. Plasma concentrations of tizanidine were measured up to 24 hours and a caffeine test was performed. There was a good correlation between tizanidine area under concentration–time curve (AUC) and caffeine/paraxanthine ratio (r^2 = 0.76). The AUC of tizanidine and caffeine/paraxanthine ratio were significantly higher in the users of oral contraceptives, and

significantly lower in smokers compared to the group with no concomitant medication ($P < 0.05$). In the whole population, 23 sequence variations were detected, forming 29 different haplotypes. The most frequent sequence variations were g.-163C>A, g.2159G>A and g.5347C>T, with variant allele frequencies of 65,1%, 53,8% and 54,7%,respectively. However, none of the sequence variations or haplotypes was associated with the pharmacokinetics of tizanidine or the caffeine/paraxanthine ratio. For example, the AUC of tizanidine in nonsmokers with no other medication was 4.36 ± 2.0, 4.86 ± 3.9 and 4.66 ± 3.0 ng·h/ml (mean ± SD) in subjects with the g.-163C/C (reference), g.-163C/A and g.-163A/A genotype, respectively. In conclusion, common sequence variations in the CYP1A2 gene did not explain the variability in CYP1A2 function in vivo, as measured by the CYP1A2 probe substrate tizanidine and caffeine test.

P225 - ROLE OF GENETIC POLYMORPHISM OF DNA REPAIR GENE XRCC1 IN THE MODULATION OF DNA DAMAGE IN FARMERS OCCUPATIONALLY EXPOSED TO PESTICIDES: A PRELIMINARY STUDY FROM DENIZLI, TURKEY

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Pesticides are one of the most widely used chemicals all over world. Many of them are classified as toxic and carcinogenic compounds thus representing a great threat for both environment and exposed individuals. Farmers are densely exposed to these chemicals by the way of inhalation and direct skin contact during the preparation and applications, as well as working in the pesticides treated fields. Pesticides or /and their active metabolites can bind to DNA and may cause direct or indirect DNA damage disrupting genomic integrity when repaired inadequately and ultimately leading to initiation and progression of several serious diseases including cancer. It is well known that genetic polymorphisms in DNA repair genes may affect repair capacity and modulate disease susceptibility in case of chemical exposure. In this pilot study, the potential DNA damage in 25 open field farmers from Denizli Province (Turkey) was investigated by using cytokinesis-blocked micronucleus assay in peripheral lymphocytes. A non-exposed group comprised of 23 individuals from the same area and with same demographic characteristics was studied and the data obtained from both groups was compared. Additionally, in the current work, we aimed to explore the relationship between DNA repair gene XRCC1 Arg399Gln polymorphism known for its deficient DNA repair capacity and susceptibility of increased DNA damage induced by pesticides within exposed group. Our results showed that the micronuclei (MN) frequency of farmers (9.56±0.65; range: 4-16)‰; was significantly higher than that of control group (2.65±0.28; range:0-6)‰ ($p<0.0001$). Regarding the genotypes for XRCC1 gene, it was found that individuals carrying the variant allele (Gln399) showed higher DNA damage as evidenced by higher MN frequency compared to individuals with wild type genotype ($p=0.029$). This is the first study showing an association of XRCC1 399Gln alleles with the increased DNA damage among individuals occupationally exposed to pesticides. These preliminary results reinforces that evaluation of the potential DNA damage as a result of pesticide exposure in farmers together with genetic polymorphisms of DNA repair genes as one of the susceptibility biomarkers can be an early warning signal for the detection of genotoxicity that may present serious health problems in case of long term exposures.

P226 - HOMOLOGOUS RECOMBINATION REPAIR POLYMORPHISMS AND SUSCEPTIBILITY TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Polymorphisms in DNA repair genes may influence DNA synthesis or repair capacity and therefore modify susceptibility to childhood acute lymphoblastic leukemia (ALL). In the present study, we investigated the influence of single nucleotide polymorphisms (SNPs), haplotypes and gene-gene interactions in homologous recombination (HR) repair genes with susceptibility to ALL. As the change in susceptibility conferred by a single common variant is probably small, our hypothesis was that the influence of a combination of several SNPs on susceptibility to ALL may be greater than the effect of individual SNPs in candidate genes. We also aimed to determine the influence of selected SNPs on the amount of DNA damage, detected with comet assay. In total, 121 children with ALL and 184 unrelated healthy controls of Slovenian origin, younger than 30 years, were genotyped for eight tag SNPs in three

genes, NBN, RAD51, and XRCC3, using a fluorescence-based competitive allele-specific PCR (KASPar assay). Comet assay was performed in a subgroup of 26 healthy individuals. Six SNPs, NBN Glu185Gln (rs1805794), NBN 3474A>C (rs1063054), RAD51 -98G>C (rs1801320), RAD51-61G>T (rs1801321), RAD51 1522T>G (rs12593359), and XRCC3 -316A>G (rs1799794), were in Hardy-Weinberg equilibrium in controls and their genotype frequencies were in agreement with those reported in other Caucasian populations. We did not observe significant differences in genotype distributions between ALL cases and controls for any of the investigated SNPs. However, after stratification by immunophenotype, a significantly decreased susceptibility to B-lineage ALL was observed in carriers of NBN 185Gln variant allele (OR=0.46; 95% CI=0.22-0.96; p=0.037). This SNP may influence DNA repair capacity, because we observed statistically significant association of this SNP with the amount of DNA damage, detected with comet assay (p=0.002). In haplotype analysis, RAD51 GTT haplotype significantly decreased the susceptibility to ALL (OR=0.66; 95% CI=0.47-0.93; p=0.016). This haplotype included polymorphic -61T allele that was also associated with significantly decreased amount of DNA damage on comet assay (p=0.034). We also observed significant influence of NBN Glu185Gln – RAD51 -98G>C interaction on susceptibility to ALL (OR=0.07; 95% CI=0.01-0.66; p=0.021). Our results show that polymorphisms in HR repair genes affect the amount of DNA damage and suggest that combination of SNPs influencing DNA repair may have greater effect on susceptibility to childhood ALL than individual SNPs.

P227 - EFFECT OF ABCG2 C.421C>A POLYMORPHISM ON THE PHARMACOKINETICS OF IMATINIB IN HEALTHY KOREAN SUBJECTS

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Imatinib mesylate (STI571, Gleevec) is a competitive tyrosine kinase inhibitor commonly used for treatment of chronic myeloid leukemia (CML). In vitro and in vivo evidences have shown that imatinib is a substrate of ATP-binding cassette (ABC) transporter ABCG2 (BCRP) pump expressed at the apical membrane of the small intestine and in the bile canalicular membrane. We explored the role of ABCG2 genotype (c.421C>A) in the pharmacokinetics of imatinib in healthy Korean subjects. Twenty-seven healthy individuals were enrolled according to ABCG2 genotype (12 subjects for c.421CC, 10 subject for c.421CA, and 5 subjects for c.421AA). After administration of a single dose of 400 mg imatinib, plasma concentrations of imatinib were measured up to 72 hours and its pharmacokinetic data were analyzed and compared according to ABCG2 genotype. ABCG2 c.421C>A genotype did not affect the pharmacokinetics of imatinib with a statistical significance. The mean values of imatinib AUC_{inf} (area under the time versus concentration curve from 0 to infinity) were 26838.2 ng*hr/mL for c.421CC, 30440.7 ng*hr/mL for c.421CA, and 31734.3 ng*hr/mL for c.421AA (P=0.2674). Additionally, the mean values of imatinib oral clearance were 15.6 L/hr for c.421CC, 13.6 L/hr for c.421CA, and 13.2 L/hr for c.421AA (P=0.1846). ABCG2 c.421C>A polymorphism appears to be a minor determinant of interindividual variability in imatinib pharmacokinetics.

P228 - UGT1A4*3 ENCODES SIGNIFICANTLY INCREASED GLUCURONIDATION OF OLANZAPINE IN PATIENTS AND IN RECOMBINANT METABOLIC SYSTEMS

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Olanzapine is a world-leading antipsychotic drug that displays considerable inter-patient variability in hepatic clearance. In a 2010 pilot study, we suggested that the UGT1A4 142T>G SNP might contribute to this variability (1). Therefore, a validation study was carried out in a new, larger patient population, and in recombinant metabolic systems in vitro (2). Polymorphic glucuronidation of olanzapine by UGT1A4 was investigated retrospectively in patient samples from routine therapeutic drug monitoring. Multivariate analyses revealed that patients being heterozygous or homozygous for the UGT1A4*3 allelic variant (142G), had significantly higher serum concentrations of the major metabolite olanzapine 10-N-glucuronide (+38% [p=0.011] and +246% [p<0.001], respectively). This was in line with significantly increased glucuronidation activity of olanzapine by recombinant UGT1A4.3 (Val-48)

compared to UGT1A4.1 (Leu-48) [1.3-fold difference, p<0.001] and in agreement with recently reported in vitro-data from others (3). In contrast, serum concentrations of the parent drug were not significantly influenced by UGT1A4 genotype. Thus, our findings indicate that UGT1A4-mediated metabolism is not a major contributor to inter-patient variability in olanzapine levels (2). However, for other drugs with a dominant role of UGT1A4 in hepatic clearance, such as lamotrigine, increased glucuronidation encoded by UGT1A4*3 might impact on the risk for subtherapeutic drug exposure (4).

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P229 - CYTOCHROME P450 AND ABCG2 POLYMORPHISMS IN A NON-SMALL-CELL LUNG CANCER PATIENT SUFFERING TOXICITY FROM ERLOTINIB TREATMENT – A CASE REPORT

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Erlotinib is a small molecule tyrosine kinase inhibitor that blocks epidermal growth factor receptor (EGFR) mediated intacellular signaling and is appoved for the treatment of patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC). Erlotinib exhibits considerable interindividual variability, both in pharmacokinetics, tumor response and the occurence of toxicities that may limit its use. Erlotinib is extensively metabolised by cytochromes P450 (CYP), predominantly by CYP3A4 and CYP1A1, while CYP3A5 and CYP1A2 have a minor role. Erlotinib is also a substrate of ABCB1 and ABCG2 transporters. Genetic variability in ABCG2, in particular 421C>A (Gln141Lys) polymorphism associated with low ABCG2 expression levels has been implicated in higher erlotinib accumulation in the cell and increased risk for toxicity [1]. We present a case of a NSCLC patient in which genetic testing was undertaken to explain the toxicity of erlotinib. In a 56 years old female patient with metastatic NSCLC (stage T4N0M1) tumor progression was observed 14 month after initial treatment with radiotherapy. As tumor was positive for EGFR mutation, 150 mg/day erlotinib was initiated and partial tumor response was observed after the first month of treatment. Due to several episodes of acute toxicites, mosty skin rash grade 1 and/or 2, hepatotoxicity grade 1 and diarea, the patient was switched to gefitinib, but did not tolerate it. Therefor erlotinib was reinstalled and the patient was continued on erlotinib 100 or 150 mg/day, depending on the occurence of toxicities, resulting in prolonged (>20 months) favourable tumor response. A blood sample was obtained for DNA extraction and pharmacogenetic testing. Genotyping of CYP1A1*2A (3798T>C, rs4646903), CYP3A4*1B (-392A>G, rs2740574), CYP3A5*3 (6986G>A, rs776746), ABCB1 (2677G > T/A and 3435C > T), ABCG2 34G>A (Val12Met, rs2231137) and ABCG2 421C>A (Gln141Lys, rs2231142) was performed using a fluorescence-based competitive allele-specific PCR (KASPar assay). Gentyping analysis showed that the patient was a heterozygous carrier of CYP1A1*1/*2A and ABCG2 421C/A polymorphisms, but other genotypes were normal. ABCG2 421C>A is a functional SNP leading to Gln141Lys substitution. As this variant was associated with low ABCG2 expression levels and reduced transport of erlotinib, it may result in increased intracellular levels of erlotinib. Because CYP1A1 is also expressed in skin, increased erlotinib metabolism via CYP1A1 may lead to increased production of metabolites that may be accountable for skin toxicity. Our observation is in agreement with previous reports that patients heterozygous for variant genotype had a significantly decreased erlotinib clearance and suffered more often from skin toxicities [1]. In conclusion, this case illustrates that patient-specific genetic profiles of genes related to drug disposition and biological activity can help to explain drug toxicity and predict drug reponse in NSCLC patients. However, before pharmacogenetic testing can be applied for individualized and safer erlotinib treatment in clinical practice, independant confirmation of the clinical significance of observed effects in a larger number of patients is needed.

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P230 - PHARMACOKINETICS OF THE CIME COCKTAIL AND THEIR METABOLITES AFTER A SINGLE ORAL DOSING IN HEALTHY VOLUNTEERS

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Cocktails of drugs are of high interest to predict phenotype of drug metabolism or carrier-mediated transport enzymes in an individual. We recently designed the CIME cocktail to simultaneously phenotype six CYP (1A2/2C8/2C9/2C19/2D6/3A), one phase II enzyme (UDP-glucuronosyltransferase 1A1/6/9, UGT1A1/6/9), two drug transporters (P-gp and OATP1B1) and the renal tubular reabsorption (Videau et al., 2010). The in vitro phenotyping performance of CIME cocktail has been recently demonstrated on primary human hepatocyte cultures (Prot et al., 2011). To evaluate the potential interest of the CIME cocktail for in vivo phenotyping, the aims of this study were to 1) describe the pharmacokinetics (PK) of the ten drugs combined in the CIME cocktail after a simultaneous single oral dosing in healthy volunteers and 2) to assess tolerance. Ten healthy subjects participated to this pilot, phase I, prospective, monocentric, open clinical trial. INSERM was the promoter. Subjects were orally dosed with the CIME cocktail consisting of caffeine (CYP1A2), repaglinide (CYP2C8), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A), acetaminophen (UGT1A1/6/9), digoxin (P-gp), rosuvastatine (OATP1B1) and memantine (renal tubular reabsorption). Blood was collected over 3 days (18 samples) and again on day 7. The CIME cocktail and metabolites were assayed by LC-MS/MS (Videau et al., 2010) on a XEVO TQ MS. PK parameters were calculated using the R software by mean of a non-compartmental approach. The CIME cocktail tolerance was good, with infrequent or mild reversible adverse events. All substrates and nearly all were quantified in a sufficient number of plasma samples to calculate the PK parameters (Cmax, AUC, Cl, Vd, t1/2 and metabolic ratio). No significant differences were noticed between drug given in cocktail compared to published data on drug given alone. One volunteer was phenotyped as a CYP2D6 poor metabolizer (MR of 132 vs. 0.06-1.7 in other subjects) in agreement with its mutated homozygoth genotype. Outliers in PK profiles were also observed: one volunteer with high rosuvastatin concentration (low activity of OATP1B1 and/or high absorption) and one with intermediate concentration of dextromethorphan and normal metabolic ratio (possibly related to a low activity in intestinal CYP3A). It was possible to accurately determine PK parameters for all the CIME cocktail substrates and for seven out of ten metabolites. The future step will focus on saliva and urine LC-MS/MS analysis, calculation of phenotyping index, and further compartmental human population pharmacokinetic modeling.

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P231 - PHARMACOKINETICS OF TAURINE-MAGNESIUM COORDINATION COMPOUND IN RATS

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Background: Taurine magnesium is a new compound which combined taurine and magnesium ions together. There are two kinds of structure form: taurine magnesium salt (TMS) and taurine magnesium coordination compound (TMC). TMC can be synthesized in our lab. At present, pharmacology study shows that TMC can effectively treat hypertension, arrhythmia, eclampsia and other diseases. And TMC on myocardial ischemia and reperfusion injury has protective effect. So TM has great research and development value. Although pharmacodynamic research of TMC has made progress, pharmacokinetic studies are lack. Pharmacokinetics is needed not only in drug clinical research, but also in the early stage of drug screening. Objective: To establish the determination method of TMC in the blood plasma and the tissue, and analysis the concentration-time procedure and tissue distribution of TMC. Methods: TMC was administrated in three doses (40 mg/kg, 80 mg/kg, 160 mg/kg) tail-intravenously in Wistar rats.

Plasma and tissues samples were collected respectively before and after administration 1,3,5,10,15,20,30,45 min,1,1.5 and 2 h. Acetonitrile was used for protein deposition and 2,4-dinitrofluorobenzen(DNFB)was used for pre-column derivatization. The plasma concentration of TMC was determined by HPLC(reversed-phase Venusil C18 column; 0.05 mol/L CH3COONa(pH6.0)and CH3CN-H2O(50:50, v/v)as mobile phase; detection at 360 nm)to draw the concentration-time curve and calculate the pharmacokinetics parameters. Results: The concentration-time profile of TMC in dose of 40 mg/kg, 80 mg/kg and 160 mg/kg were shown to fit one-compartment nonlinear pharmacokinetic model. The pharmacokinetics parameters: T1/2 were 20.61+/-3.53,37.36+/-7.30 and 77.55+/-5.44 min($P<0.01$), C0 were 317.28+/-11.84 ,477.63+/-48.35 and 818.02+/-43.11 ug /ml, CLs were 0.004+/-0.001,0.004+/-0.0005 and 0.002+/-0.0002 ug/ml/min, and AUC(0-t) were 10132.0+/-247.5,18305.5+/-1664.4 and 59508.6+/-3759.4ug/ml/min respectively;MRT(0-t) wree 28.579+/-1.05,42.549+/-1.24 and 49.701+/-0.86 min respectively. TMC could be detected in the main tissue(heart, liver, brain, kidney)quickly after iv in dose of 80 mg/kg. At 10 min after administration, the top tissue content was found in heart, and subsequently in liver and kidney, the lowest content was detected in brain. Conclusion: HPLC method to determine the TMC in rat plasma and tissue is established successfully. The pharmacokinetics behavior in single dose of 40 mg/kg,80 mg/kg and 160 mg/kg fit one-compartment nonlinear model. TMC in dose of 80 mg/kg is distributed wildly in tissues, the highest in heart and the lowest in brain.

P232 - TRITIUM LABELLING AND LOW LEVEL RADIOACTIVITY COUNTING IN PK ASSESSMENT OF PROTEIN THERAPEUTICS

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Purpose: The number of potential protein therapeutic agents in preclinical and clinical development continues to increase, and methods to provide a proper evaluation of the pharmacokinetics of these compounds is therefore becoming more critical. Current methodologies available to determine concentration in body fluids are generally of the bioassay or immunoassay types. Bioassays provide good sensitivity, but are non-specific and often difficult to perform, resulting in highly variable results. Immunoassays, while relatively specific and easy to do, may not distinguish bioactive from non-active forms of the compound. The aim of the current work was to investigate the use of a simple radiolabelled tag coupled with phosphor plate detection to follow proteins in vivo. **Methods:** Four proteins, human IgG, an antibody fragment, hirudin (used in blood coagulation disorders) and human albumin, were chosen for comparative purposes. Proteins were labeled using [^{3}H]-N-Succinimidyl Propionate, purified using a size exclusion column and their purity checked by HPLC. Groups of mice were dosed intravenously with [^{3}H]-protein and serial blood samples taken for determination of radioactive concentrations in plasma. Analysis was performed by spotting plasma onto FTA DMPK cards from Whatman. Samples were dried and radioactive content determined by placing the cards against imaging plates (FujiFilm) for 168 hours. Radioactive standards were prepared to provide a standard curve for quantification. Imaging plates were analysed using a FujiFilm 5100 analyser. **Results:** The mean plasma half-life of [^{3}H]-NSP labeled proteins ranged from around 10 minutes (hirudin) to around 300 hours for the human IgG. **Conclusions:** The lower blood volume required by the blood card method meany that serial samples could be taken from small rodents and therefore fewer animals were required. The processing of these samples is rapid and once the cards are dried, they can be stored at ambient temperature and re-analysed if required. The phosphor plate detection method shows promise as a detection method with a lower limit of detection than conventional liquid scintillation counting.

P233 - ENHANCEMENT OF TAURINE ON THE MYOCARDIAL PROTECTIVE EFFECTS OF GRADUAL REPERFUSION

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Objective To observe the enhancement of taurine on the myocardial protective effects of gradual reperfusion in isolated rat hearts. **Methods** Isolated myocardial ischemia-reperfusion model was replicated by Langendorff perfused rat heart. Sprague-Dawley rats were randomly divided into seven groups: Normal group (Nor), ischemia-reperfusion group(I/R), gradual reperfusion group (GR), taurine of lower concentration group(20 mmol·L^{-1},T20), taurine of higher concentration group(40 mmol·L^{-1},T40), gradual reperfusion combined with taurine of lower concentration group (GT20) and gradual reperfusion combined with taurine of higher concentration group (GT40). The cardiac function was recorded. The activity of LDH in coronary effluent and myocardial Caspase 3 were detected. The myocardial infarct size and cardiocyte apoptotic index(AI) were measured with TTC and TUNEL staining method

at the end of reperfusion in each group. **Results** Compared with I/R group, cardiac function was improved significantly, the myocardial infarct size, the activity of LDH and myocardial Caspase 3, and the apoptotic index were reduced remarkably in GR, T20, T40, GT20, GT40. The protective effects of gradual reperfusion combined with taurine on I/R injury were more potent than applying gradual reperfusion or taurine alone, and the protective effects of GT40 were the most obvious. **Conclusion** Gradual reperfusion and taurine can reduce Ischemia-reperfusion injury in isolated rat hearts. Anti-apoptosis may be one of the mechanisms of the myocardial protective effects. The myocardial protective effects of gradual reperfusion were enhanced by taurine, especially taurine of higher concentration.

P234 - PHARMACOKINETICS OF RECOMBINANT HUMAN THYMOSIN Á1 (RHTÁ1) IN RATS

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Background: Thymosin ¦Á 1 (T¦Á1) is a 28-aminoacid peptide with an acetylated N-terminus. T¦Á1 stimulates T-cell proliferation and maturation, modulate cytokines and chemokines production and block steroid-induced apoptosis of thymocytes. T¦Á1 is used for the treatment of several diseases, including chronic hepatitis B and C, acquired immunodeficiency syndrome (AIDS), depressed response to vaccination, and cancer. So far, T¦Á1 used in clinic is synthesized by solid-phase peptide synthesis. Recombinant human thymosin¦Á1 (rhT¦Á1), investigated in this paper, is produced by the genetic engineering techniques.The purpose of this studies was to characterize the pharmacokinetics, tissue distribution, and excretion profiles of rhT¦Á1 following a single subcutaneous (s.c.) administration to rats. **Methods:** Based on characteristic of biotech drugs, radioactive isotope trace assay, were established and employed. The rhT¦Á1 was labeled with ^{125}I and the pharmacokinetics of ^{125}I-rhT¦Á1 was investigated by two assays: the total radioactivity assay (RA method) and the radioactivity assay after precipitation with trichloroacetic acid (TCA-RA method). **Results:** The rats were randomly assigned into three groups in which the rats were given subcutaneously rhT¦Á1 at doses of 150, 300 and 600 μg/kg, respectively. The maximum serum rhT¦Á1 concentrations (C_{max}) and the area under the concentration-time curve (AUC) increased with the dose increment. The serum elimination half-life ($t_{1/2b}$) and systemic clearance (CL) did not display markedly dose-dependence and were relatively consistent in the range of 5.4-7.1 h and 79-88 mL/kg/h using TCA-RA method, respectively. In the rats with 300 μg/kg rhT¦Á1 (s.c.), the levels were the highest in the kidneys at all time points tested (0.25, 1, 4, and 12 h), and the lowest in the brain and muscle tissues. The tissue distribution pattern of radioactivity measured by RA and TCA-RA was similar. The accumulative excretion percentages of ^{125}I-rhT¦Á1 from urine and feces reached 82.4 % and 7.6 % of the administered radioactivity within 120 h, and 4.4 % from bile within 24 h, respectively. **Conclusions:** rhT¦Á1 has linear pharmacokinetic properties within the therapeutic dose range. The rhT¦Á1 has character of targeting special tissue distribution (the highest level of the drug was found in kidneys), which may reflect that urinary excretion is the dominant route of elimination via the kidneys in rats following s.c. administration of rhT¦Á1.

P235 - CONSTRUCTION OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS EXPLAINING DRUG-DRUG INTERACTIONS VIA THE INHIBITION OF ORGANIC ANION TRANSPORTING POLYPEPTIDES (OATPS) IN THE LIVER

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[Background and Purpose] OATP-mediated drug-drug interactions (DDI) have been suggested in several clinical studies. Based on a static model, the extent of DDI is estimated by calculating 1+Iu/Ki value, where Iu represents a maximum (constant) unbound concentration of an inhibitor at the inlet to the liver. On the other hand, a physiologically-based pharmacokinetic (PBPK) model, a dynamic model in which 1+Iu/Ki value is changed over time depending on the concentration of an inhibitor, provides us with a time profile of the plasma concentration of a victim drug. The purpose of this study was to construct a rational PBPK model in order to explain clinically observed DDI due to the inhibition of OATPs, which mediate the hepatic uptake of various anionic drugs. [Methods and Results] At first, time profiles of the plasma concentration of statins, typical OATP substrates, with or without co-administration of OATP inhibitors such as cyclosporine A and rifampicin were collected from previous reports. In each DDI case, the decrease in the hepatic overall intrinsic clearance (CLint,all) of a substrate was estimated based

on the clearance concept. Since CLint,all was a hybrid parameter governed by the hepatic uptake clearance, efflux clearance and intrinsic clearance (metabolism and biliary excretion), we optimized these clearance values as well as other PK parameters (absorption rate constant, absorption lag time and distribution volume) in a simple PBPK model, which consists of a central compartment, hepatic extracellular and intracellular compartments, by fitting to the plasma concentration-time profile. Finally, considering a time-dependent change in the concentration of inhibitors and subsequent 1+Iu/Ki values, we calculated the in vivo Ki values for OATPs by fitting. [Discussion] We are now further attempting to compare thus obtained in vivo Ki values with in vitro Ki values obtained from the uptake experiments using hepatocytes or OATP-expressing cell systems.

P236 - IMPACT OF IN VITRO SYSTEM, CHEMICAL MIXTURES, AND STEREOCHEMISTRY ON THE INTRINSIC CLEARANCE OF 1,2,4-TRIAZOLE FUNGICIDES IN HUMAN AND RAT

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The exposure of humans and ecologically important species to environmental chemicals typically occurs at unknown concentrations and for uncertain durations. Exposure becomes an internal dose when the chemical crosses the body barrier. Characterizing internal dose is important for reconstructing exposure events and predicting undesirable effects. Chemical clearance, which depends on the intrinsic ability of organs to metabolize or eliminate the chemical from the body, can significantly affect internal dose. Clearance provides an understanding of a chemical's metabolic stability and the contribution of metabolism to total clearance in vivo. In vitro methods are frequently used to measure intrinsic clearance and can be used to rank chemicals based on their metabolic stability. In conjunction with toxicity information, this rank-order approach can be used to prioritize the testing of chemicals for which there is little exposure or effects data. As a first step in evaluating this prioritization process, we examined the clearance of twenty, 1,2,4-triazole agricultural fungicides in purified human CYP3A4 and rat and human cryopreserved hepatocytes and hepatic microsomes. Agricultural and pharmaceutical 1,2,4-triazole fungicides are potent cytochrome P450 modulators that can disrupt mammalian steroid biosynthesis and promote tumor formation in rodents. Michaelis-Menten kinetics (V_{max} and K_M) and the in vitro substrate depletion $T_{1/2}$ method were used to measure intrinsic clearance. We observed good agreement between the two approaches; clearance values spanned several orders of magnitude for each system, and the relative order of fungicide clearance was similar between the in vitro systems and between the species. In general, fungicides containing straight alkyl chains were cleared quickly while fluorine-containing compounds exhibited very slow clearance or were unreactive. Least-squares linear regression models were developed to extrapolate clearance between the different in vitro systems and species. We also developed quantitative structure activity relationships for each in vitro system to predict fungicide clearance based on molecular descriptors. To approximate chemical mixture exposures, we measured the clearance for 100 binary, fungicide mixtures. In general, the compound having the higher individual clearance value was less impacted in the mixture studies. Nearly all 1,2,4-triazole agricultural fungicides are chiral and sold as specific mixtures of stereoisomers. To that end, we measured the clearance of the combined and separate stereoisomers (up to four) for several chiral fungicides. In all cases, the clearance for the individual stereoisomers varied up to an order of magnitude. The slower clearing stereoisomers in the commercial mixture appeared to inhibit the clearance of the more quickly cleared stereoisomers. This difference in stereoisomer clearance could have significant consequences in assessing risk due to major differences in stereoisomer toxicities. Future efforts include utilizing a ligand-based pharmacophore approach to understand clearance differences among the 1,2,4-triazole fungicides and to refine single fungicide clearance models. We are also examining our clearance data and models in the context of available toxicity information, as well as measuring other pharmacokinetic parameters that may impact chemical clearance such as transport and protein binding.

[SM1]Future efforts should probably be all together

P237 - EVALUATING DOSING REGIMENS OF A HEPATITIS C NS3 INHIBITOR USING PK/PD MODELING

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ACH-0141625, currently in development for treatment of HCV infection, is a potent inhibitor of HCV NS3 protease suited for once daily oral dosing. The aim of this modeling exercise is to evaluate several candidate dosing regimens on their impact on PK and viral load. A pharmacokinetic model was built in NONMEM v6 using data from a randomized, double-blind, placebo controlled dose escalating phase 1 study on both healthy volunteers and hepatitis C infected subjects. Absorption was described with a time-dependent first order absorption rate. An increase in bioavailability for subsequent intakes was implemented, as well as a food effect. Subsequently, individual PK curves were linked to anti-viral activity using a viral dynamics model as described by Neumann. Due to a prolonged anti-viral effect after the end of treatment, efficacy was related to concentrations in an effect compartment. Simulations were performed to assist in choosing a next dosing level, to evaluate the necessity of a loading dose and to assess the anti-viral effect for several different dosing schemes. A VPC confirmed that the PK model described the data well. Bioavailability for multiple dosing increased 3-fold for fasted intakes and 6-fold for fed intakes. Simulations showed that a loading dose can be used for reaching near steady state after one intake, but that the effect on viral inhibition is negligible. Estimates for EC50 and EC90 were obtained by linking anti-viral efficacy to individual PK curves. The effect-site equilibrium delay rate constant implied that steady state at the site of action will be reached in about 3 days. Simulation showed that all patients receiving 200 mg QD fed, 200 mg QD fed with an accidental fasted intake at steady state or 200 mg QD fasted would reach trough concentrations at the site of action above the EC50. Corresponding fractions of patients reaching trough concentrations above EC90 are 100%, 99.8% and 98.4%, respectively. Viral load was described well using a viral dynamics model as described by Neumann but with the effect linked to an effect compartment. As fluctuations in concentration at the site of action are small, it shows that average plasma concentrations are more relevant than plasma C0h.

P238 - THE UTILISATION OF QUANTITATIVE AUTORADIOGRAPHY TO INVESTIGATE THE PENETRATION OF UNGUAL TREATMENT

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Drug penetration through the nail is a major challenge and a key requisite for efficacy of local treatment for diseases such as psoriasis or onychomycosis. A method of treatment for nail disorders that involves local application of drugs to previously drilled nails is under investigation. This method improves first the penetration and so may reduce treatment duration and then may improve patient compliance. This study was conducted to evaluate the extent of diffusion at the nail bed of a radiolabelled drug topically applied on drilled nails to determine the optimal number of holes needed to cover the nail bed with the drug treatment. This study was conducted using a quantitative whole body autoradiography methodology (QWBA). This appears to be the first application of QWBA for such type of investigation. Four frozen human cadaver fingertips with nails were used. Each fingernail contained two series of three holes, one set at 2 mm apart and the other set at 4 mm apart. Three of the nails received an application of the radiolabelled test item during one hour; one received an application of a non-radiolabelled test item. Each sample was frozen onto a support using adapted QWBA procedures. Sections were then taken through each fingernail onto tape and were analysed using QWBA methods to determine the diffusion of radioactivity at the nail bed level from each site of administration. A 3-dimensional graph of the concentration of radioactivity was then created to visualize the diffusion through the nail matrix. The data showed that there was a diffusion of the radiolabelled drug at the nail bed level around the holes. The extent of diffusion around each holes could be measured. In this study, QWBA was effective to investigate the penetration through the nail of radiolabelled drug topically applied. Furthermore, QWBA proved its versatility in the investigation of distribution in unusual samples for a “non-standard” purpose.

P239 - THE EFFECT OF PROBIOTIC ESCHERICHIA COLI NISSLE 1917 ON THE PHARMACOKINETICS OF AMIODARONE IN THE MALE RAT

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Microorganisms Escherichia coli Nissle 1917 (serotype O6:K5:H1) as a probiotic have beneficial effects on the gastrointestinal tract [1]. The aim of this study was to find whether E. coli Nissle 1917 influences pharmacokinetics of concomitantly taken pharmacotherapeutic agent. An antiarrhythmic drug amiodarone was used in this study. The probiotic strain E. coli Nissle 1917 was administered to thirty male Wistar rats for seven days and the saline solution was administered to another thirty experimental animals for seven days (as a control group) as well. The eighth day, the amiodarone hydrochloride was applied to all sixty rats. Then, after 1, 2, 3, 4, 5.5, 7, 9, 14, 22 and 30 hours, the blood samples were taken from rat abdominal aorta; afterwards, the plasma level of amiodarone and of its metabolite N-desethylamiodarone was measured by HPLC-UV. The results show that the plasma level of amiodarone is higher after application of E. coli Nissle 1917 to rats. Apparently, the application of E. coli Nissle 1917 causes increased bioavailability of amiodarone from the gastrointestinal tract. The level of metabolite is also increased after E. coli Nissle 1917 administration which is in line with increased activity of the liver CYP2C enzymes found in our earlier study [2] (the CYP2C enzymes are known to take part in deethylation of amiodarone in the rat and human [3]). Taken together, the probiotic E. coli Nissle 1917 (serotype O6:K5:H1) administration may exhibit its beneficial effect through better bioavailability of the drug.

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P240 - IMPROVING PRECLINICAL PREDICTABILITY OF DRUG EFFECTS: COMBINING PHARMACOKINETICS AND PHARMACODYNAMICS OF STATINS IN THE APOE*3LEIDEN MOUSE MODEL

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Preclinical efficacy studies of novel drug candidates should demonstrate the disease-modifying potency of those candidates and facilitate compound prioritizing based on their predicted relative potency in man. However, translation from mouse to man proves to be difficult due to either differences in efficacy and/or pharmacokinetics. Humanized mouse models are being developed to improve such prediction. For example, ApoE*3Leiden (E3L) transgenic mice develop a human-like lipoprotein profile upon feeding a high-cholesterol diet (with elevated plasma levels of VLDL and LDL) and represent an established model for studying the differential effects of cholesterol-lowering drugs (e.g. statins, fibrates, niacin) and for cholesterol-induced atherosclerosis. The aim of the current study was to map the therapeutic predictability of the cholesterol-lowering effect of statin treatment in this E3L mouse model and to explore whether combining pharmacokinetic with pharmacodynamic data (PKPD relation) could increase the predictability of this pre-clinical model. Statins are widely prescribed cholesterol-lowering drugs with proven efficacy in humans that act by inhibiting 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase, a rate-limiting enzyme of the cholesterol synthesis that is mainly expressed in the liver. First, based on 90 head-to-head randomized control trials, we determined the efficacy of 5 currently marketed statins (atorvastatin, simvastatin, lovastatin, pravastatin and rosuvastatin) in patients with hypercholesterolemia (LDL ≥ 160 mg/dl). Next, a complete preclinical PKPD dataset for these 5 statins was determined. To this use, E3L mice were fed a high-cholesterol diet

containing 1% cholesterol for 4 weeks leading to 5.3-fold elevated plasma total cholesterol (TC) levels, followed by a drug intervention period of 6 weeks. During this period 10 groups of mice received 2 different dosages of the 5 individual statins which were added to the diet. In addition, plasma and tissue levels of the statins were determined 30, 60, 90, and 120 minutes after the mice received 10 mg/kg of the drug by oral gavage. We found that the efficacy of the different statins to lower plasma TC levels in E3L mice did not correlate with the efficacy of these statins in patients with hypercholesterolemia (R^2 = 0.11, p = 0.572). However, interestingly, by taking into account the effective hepatic concentrations of the different statins in E3L mice, the correlation between statin efficacy in E3L mice and humans markedly increased (R^2 = 0.88, p = 0.018), improving the translational value of this E3L mouse model. In conclusion, in this demonstrator study we provide evidence that a targeted combination of pharmacokinetics with pharmacodynamics (therapeutic efficacy of the drugs) in case of statin treatment in E3L mice does substantially increase the clinical therapeutic predictability of these drugs. We hypothesize that a similar strategy could be used to increase the clinical predictability of drug treatment of other classes of drugs in various pre-clinical (disease) models.

P241 - COMPARATIVE PENETRATION OF VARIOUS NATURALLY OCCURRING COMPOUNDS THROUGH BLOOD BRAIN BARRIER AND GASTROINTESTINAL BARRIER

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Background and aim: Blood brain barrier (BBB) and gastrointestinal barrier (GIB) are the two major physiological barriers preventing xenobiotics entering into the body. For central nervous system (CNS)-targeting agents, it is desirable to penetrate through BBB. However, for agents not targeting CNS, excessive exposure in brain will cause unexpected toxicity. Therefore, it is very important to evaluate their efficacy and safety. In the current study, an integrated 3 "in" method (in silico, in vitro and in vivo) was employed for the investigation of physicochemical properties, apparent permeation, brain exposure levels and the fraction absorbed from the gastrointestinal tract of naturally occurring compounds, to study the comparative penetration of these compounds through BBB and GIB. **Methods:** In silico physicochemical properties of tested compounds: Physicochemical properties of the tested compounds were predicted using different commercial available computational models such as Pallas, ACD software. Compound brain exposure levels: Drug brain exposure levels were examined by in vivo microdialysis technique. A microdialysis probe was implanted in the striatum of rat brain, the dialysates were collected at 10 min intervals till 2 h post dosages. Rat blood-to-plasma drug concentration ratio: Fresh rat blood were spiked with drugs at three concentrations and incubated at 37°C. At four different time periods, plasma were obtained and drug concentrations were measured. The fraction of compounds absorbed: The fraction of compounds absorbed were calculated from formula: $F_a=F/[1-(CL_{total}-CL_{renal})/Q_H]$, with the precondition that the possible minimum quantity of drug eliminated from organs other than liver and kidney (A_{min}/D) is less than zero. In vitro membrane permeability: In vitro Caco-2 monolayer cell model was used to investigate the apparent permeability (P_{app}) of drugs tested, in addition, The efflux ratio of tested drugs on Caco-2 cells were also examined to check if transporter-mediated permeation exists. Drug concentration measurement: Concentrations of drugs in various biofluids were measured by a generic liquid chromatography/tandem mass spectrometry-based method. **Results:** Partitioning constant (K_p) value of tested drugs were obtained by comparing the free drug exposure levels in brain to those of plasma. Rat blood-to-plasma drug concentration ratio were investigated ranging from 0.49 to 1.43. The fraction of drugs absorbed were calculated ranging from 0.47 to 100. In vitro membrane apparent permeability were observed ranging from 0.11 to 295×10^{-6} $cm\cdot s^{-1}$. The efflux ratio of tested drugs were examined <3, which indicated that these drugs were not substrates of the efflux transporters on Caco-2 cells. **Conclusion:** Differences and relations were observed on BBB and GIB. This study may be useful for the efficacy and safety evaluation of naturally occurring compounds.

P242 - MICRORNA REGULATION BY CONSTITUTIVE ANDROSTANE RECEPTOR IN HUMAN HEPATOCYTES

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Background: MicroRNAs (miRNA) are small non-coding RNA molecules which regulate genes at the post-transcriptional level. They are believed to regulate about 50% of all human genes and to play important roles in nearly all cellular processes. Several ADME genes including CYP3A4, CYP2E1, HNF4a and others have been shown to be regulated by miRNAs. To understand the concept behind this regulation, it would be interesting to know more

about the regulation of miRNA gene expression. Since miRNA genes contain similar promoters like protein coding genes, we assume that the nuclear receptors are good candidates as regulators of miRNA gene expression in the liver. **Methods:** Here we used lentiviral knock-down of CAR in human hepatocytes in combination with a prototypical agonist for comparison. Alterations in the mRNA expression profiles of CAR and its target genes were determined by quantitative real-time PCR using a self-designed taqman low-density array covering 45 ADME genes. Changes on protein levels of CYP2B6, as a prototypical target gene of CAR were analysed by Western blotting to prove knock-down or activation efficiency of CAR. The influence of these perturbances on miRNA expression was analyzed using taqman low-density arrays. **Results**: Knock-down of CAR with a validated shRNA in three independent donors resulted in a 50% down-regulation of CAR and about 45% of CYP2B6, CYP3A4 and other target genes of CAR. In addition, several miRNAs (e.g. miR-7a, miR-7f, miR-29b, miR-141, miR-15b and miR-101) were down-regulated by more than 40%, but up-regulation of others, for example miR-223, miR-454, miR-155 and miR-146a, was also observed. These results were confirmed by CAR agonist treatment in two independent donors. Knock-down of CAR and simultaneous agonist treatment resulted in lower effects than activation itself, providing additional prove of concept. To investigate the potential impact of those miRNAs regulated by CAR on gene expression, in silico analyses were implemented. **Conclusions:**This study identified several miRNAs that are directly or indirectly regulated by CAR in primary human hepatocytes. Interestingly, miRNA expression is influenced by CAR in both directions. In spite of the fact that human hepatocytes have a high interindividual variability, our results were robust. Therefore this method could be useful to investigate additional nuclear receptors or other regulators of miRNAs in human hepatocytes.

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P243 - IMPACT OF PKA SIGNALLING ON HUMAN CAR AND PXR TRANSCRIPTIONAL ACTIVITY

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Objective: The expression of genes involved in absorption, distribution, metabolism and excretion (ADME genes) is inducibly regulated by the xenosensors CAR and PXR, nuclear receptors that are activated upon the binding of specific ligands. Recent reports have shown that PXR and CAR undergo different post-translational modifications, which can influence their transcriptional activity. For example, phosphorylation of PXR by protein kinase A (PKA) leads to a decreased expression of human CYP3A4. The hormone glucagon, which triggers the activation of PKA, is released under fasting conditions to mobilize energy within the liver and other tissues. We investigated whether this type of regulation also applies to other ADME genes. **Methods:** Primary human hepatocytes were treated for 24h with rifampicin, CITCO and/or 8-Bromoadenosine 3',5'-cyclic monophosphate (8-Bromo-cAMP) to investigate changes in their mRNA expression-profiles by high-throughput quantitative real-time PCR on a Fluidigm platform. Luciferase reporter gene assays using promoter-constructs of CYP3A4, CYP2B6 and MDR1 in the presence or absence of co-transfected hPXR or hCAR expression plasmids were performed in HepG2 cells treated with the PXR or CAR agonists rifampicin and CITCO in combination with or without the PKA activator, 8-Bromo-cAMP. Changes in the promoter-activities were determined after 48h of treatment by measuring the relative luciferase activity. **Results:** We showed that activation of PKA down-regulates not only rifampicin-induced CYP3A4 promoter activity, but also decreases promoter activity of CYP2B6 and MDR1 in a PXR-dependent manner. Additionally we revealed that the transactivation capacity of CAR on the promoters of CYP3A4, CYP2B6 and MDR1 was down-regulated by PKA activation in the same way. To investigate the effect of PKA on the expression of a broader range of ADME genes we treated primary human hepatocytes with prototypical agonist for CAR and PXR (CITCO and rifampicin) together with or without the PKA activator 8-Bromo-cAMP. Treatment with CITCO and rifampicin led to an increase in expression of various ADME genes, whereas if PKA was activated, this increase was reduced or abolished in many cases. **Conclusion:** Our results show that increased expression of a large set of ADME genes by the CAR and PXR agonists CITCO and rifampicin is reduced by simultaneous activation of PKA by 8-Bromo-cAMP. These data suggest that the drug detoxification/ADME system of the liver is impaired in response to fasting conditions due to inactivation of PXR and CAR by PKA-mediated phosphorylation.

This study was supported by the German Federal Ministry of Education and Research (Virtual Liver grant 0315755) and the Robert Bosch Foundation, Stuttgart, Germany.

P244 - LOW STRESS WEEKENDS PROMOTE ADAPTATION TO STRESSFUL WEEKS: NUCLEAR RECEPTORS COORDINATION OF BIOLOGICAL RESPONSES TO STRESS

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The body is exposed to a wide range of external chemicals, both deliberately (e.g. medicines) and accidently (e.g. environmental contaminants). To account for this, a fundamental and essential property of evolvable biological systems is robustness. This allows system to conserve thier functionalities against a backdrop of internal/external perturbations and uncertainties. A classic example of such a system is the network of metabolic processes that are involved in both homeostasis of endogenous chemical and the response to external chemical challenge. Proteins involved in this network include active transport pumps, plus Phase I and Phase II metabolic enzymes, which together act to response to chemical flux through active efflux and metabolism respectively. The expression of many drug metabolising enzymes and drug transport proteins are under control of members of the nuclear receptor superfamily 1. These ligand-activated transcription factors include members who predominantly regulate endogenous functions, including the glucocorticoid receptor (GR), the progesterone receptor (PR) and the androgen receptor (AR), and those that act as sensors for external chemical challenge, such as the pregnane X-receptor (PXR) and the constitutive androstane receptor (CAR). However, it is becoming increasingly clear that these nuclear receptors do not function in isolation, but contribute towards a complex regulatory signal network, which allows the control of overlapping target gene sets and the refinement of biological response(s) to individual chemical challenges. In this study, we describe the interactions of the stress hormone cortisol with its two nuclear receptor targets, the high affinity glucocorticoid receptor (GR) and the low affinity pregnane X-receptor (PXR) using a mathematical model based on realistic kinetic parameters. We demonstrate the importance of regulatory loops within this network, in terms of both pharmacodynamic and pharmacokinetic responses. In addition, we demonstrate the network response following cortisol challenge; both to a single peak in cortisol concentration, reminiscent of a single stress event, and a repeated cortisol challenge, reminiscent of repeat stress events with differing frequencies and time frames. We reveal that the network is robust towards low frequency perturbations, shows adaptation at moderate stress frequencies, but shifts to an altered steady state at high frequency stimulation. The latter might be viewed as a predisposing factor towards stress-induced pathologies. Hence, we expand upon the current paradigms of stress-induced disruption of endogenous chemical homeostasis, demonstrating the role in nuclear receptors in mitigating such stressors

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P245 - REGULATION OF MATRIX METALLOPROTEINASES BY FXR IN METASTATIC BREAST CANCER

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Metastasis is associated with poor prognosis in breast cancer. Thus it is crucial to understand the molecular mechanisms of tumour cell invasion and metastasis in order to identify therapeutic targets. Metastasis requires degradation of the extracellular matrix (ECM), permitting cancer cells to invade and spread to distant sites. Matrix metalloproteinases (MMPs) are essential for degrading the proteins of the ECM. In particular, MMP-2 and -9 expression correlate with poor breast cancer prognosis. MMP activity is regulated by the tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 inhibits MMP-9 whereas TIMP-2 inhibits MMP-2. Studies have shown that the nuclear receptor Farnesoid X receptor (FXR) is involved in MMP and TIMP regulation in hepatic and vascular tissues and is highly expressed in breast cancer[1-3]. So the aim of this project was to investigate whether FXR is a novel regulator of MMP-2 and -9 in breast cancer cells. Initially the cell viability of breast cancer cell lines MCF7 (Estrogen

receptor positive) and MDA-MB-468 (Estrogen receptor negative) after treatment with FXR agonists chenodeoxycholic acid (CDCA) and 3-[2-[2-Chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4 isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid, known as GW4064 were measured. FXR ligands decreased the cell viability in both cell lines and the effects were more significant in MDA-MB-468. The mRNA and protein levels of MMP-2 and -9 within both cell lines did not change after FXR activation, when measured by real time PCR and Western blotting. Interestingly, when the activity of the MMP-2 and -9 enzymes secreted into the culture media was measured using a fluorescent substrate; GW4064 increased MMP activity in a concentration dependant manner, whereas CDCA had no effect. To determine whether this was due to FXR regulation of TIMPs, their mRNA expression was measured by real time PCR. There was no effect of FXR ligands on TIMP-1 and -2 mRNA levels. In conclusion, FXR activation by GW4064 increases secreted MMP-2 and -9 activity in breast cancer cells but this effect is not via transcriptional regulation of the MMPs or their inhibitory factors TIMP-1 or -2.

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P246 - NUCLEAR RECEPTOR INTERACTIONS WITH ISOXAZOLYL PENICILLINS

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The β-lactamase resistant penicillin, flucloxacillin, is a common cause of idiosyncratic drug-induced liver injury (DILI). Flucloxacillin belongs to a family of semi-synthetic isoxazolyl penicillins that include oxacillin, cloxacillin and dicloxacillin. In contrast to flucloxacillin, reports of liver injury caused by the other isoxazolyl penicillins are extremely rare. Previous work has shown that flucloxacillin is an activator of the pregnane X-receptor (PXR) and inducer of CYP3A4 [1]. Further studies on human hepatocytes using sandwich culture have shown induction of CYP2B6 in addition to CYP3A4. In view of the effect of flucloxacillin on CYP2B6, the possibility that flucloxacillin is also a constitutive androstane receptor (CAR) agonist was investigated. CAR shares an overlap in some substrate specificities and transcriptional targets with PXR. Reporter gene studies were performed to establish whether flucloxacillin also interacts with CAR. A CYP2B6 reporter construct containing two distal response elements to which activated CAR binds was transiently transfected into Caco-2 cells along with a human CAR expression vector. Cells were treated for 24 hours with flucloxacillin over a concentration range of 100 µM to 1 mM, with the known hCAR activator CITCO as a positive control. Compared to the untreated control, flucloxacillin had no effect on CAR activation at any of the described concentrations suggesting that it is unlikely that flucloxacillin is a CAR substrate. Three tag SNP's for the main haplotypes in the CAR (NR1I3) gene were also genotyped in a number of flucloxacillin-DILI case samples and controls from flucloxacillin-prescribed healthy individuals. No significant differences in genotype frequency were found between cases and controls. Reporter gene studies were performed to compare PXR activation by flucloxacillin to that of cloxacillin and dicloxacillin. A luciferase construct for CYP3A4 activation was transfected into HepG2 cells which were treated with flucloxacillin, cloxacillin and dicloxacillin at concentrations of 500 µM to 2 mM for 72 hours. Cloxacillin and dicloxacillin were found to be more potent PXR activators than flucloxacillin. Comparative studies on CYP3A4 induction in LS180 cells by the isoxazolyl penicillins are in progress. We postulate that this greater PXR activation may affect drug metabolism and clearance and be a factor in differences seen in hepatotoxicity between the drugs.

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P247 - A

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It is difficult to deliver biopharmaceutical agents, such as peptides, to the brain because these macromolecule drugs cannot cross the blood-brain barrier (BBB), which is formed by brain microvascular endothelial cells (BMEC). To solve the problem, the drug delivery strategy which uses receptor-mediated transcytosis involved with an internalizing receptor expressed on the surface of BMEC has been developed. This strategy requires fusion of macromolecule drugs and a peptide or a peptidomimetic monoclonal antibody against an internalizing receptor, such as lipoprotein receptor-related protein 1 (LRP1). Combination of receptor and the fusion macromolecule drugs triggers their transport across the BBB, therefore the receptor functions in BMEC have key roles in the delivery macromolecule drugs to the brain. To evaluate macromolecule drug permeability across the BBB, an in vitro BBB model with BMEC expressed various receptor genes can be a useful tool. We previously established a novel conditionally immortalized human brain microvascular endothelial cell line, termed HBMEC/ciβ, possessing the representative BBB functions of BMEC. However, usefulness of HBMEC/ciβ as an evaluation tool for the BBB penetration of macromolecule drugs. Therefore, the purpose of this study was to assess receptor genes expressions in HBMEC/ciβ, as an initial part of our ongoing effort to develop evaluation tool for the BBB penetration of macromolecule drugs. HBMEC/ciβ was cultured with a modified EGM-2 medium on collagenated culture dishes and passaged every 3-4 days at approximately 90% confluence. Expression of various receptors in HBMEC/ciβ was examined by reverse transcription-polymerase chain reaction as well as by western blotting analysis. The results showed that HBMEC/ciβ expressed mRNAs of LRP1, transferrin receptor (TfR), low density lipoprotein receptor (LDLR), transmembrane protein 30 A and insulin receptor at the levels similar to those in primary human BMEC. The results also showed that LRP1, TfR and LDLR proteins were expressed in HBMEC/ciβ. In conclusion, our results showed that HBMEC/ciβ expresses various receptor genes mRNA and protein. Currently we are examining the functional analysis of various receptors. In line with the results presented here, further experiments will offer useful tools to evaluate the BBB penetration of macromolecule drugs.

P248 - ACTIVATION OF PREGNANE X RECEPTOR BY ANTIMALARIAL DRUGS AND DRUG METABOLITES: IDENTIFICATION OF CARBOXYMEFLOQUINE AS PXR AGONIST

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State-of-the-art treatment of malaria is relying on the combination of two or more antimalarial drugs. However, combination therapies may increase the risk of pharmacokinetic drug-drug interactions. Thus, we here investigate the capacity of antimalarial drugs for induction of drug metabolism and transport by activation of the xenosensor pregnane X receptor (PXR, NR1I2). 21 selected antimalarial drugs and 11 major drug metabolites were screened for ligand-binding to PXR using a cellular PXR-coactivator interaction assay. Compounds identified as agonists of the receptor were further characterized by the use of additional cellular mammalian two-hybrid assays, which are based on mimicking intramolecular conformational changes in the ligand binding domain (assembly assay) and on different protein-protein interactions of PXR. Furthermore, RNA interference was deployed to demonstrate PXR-dependency of activation of promoter reporter genes and endogenous target genes. Primary human hepatocytes were used to determine the induction of hepatic gene expression. The screen of antimalarial drugs and metabolites for PXR ligand activation newly identified carboxymefloquine, the major and pharmacologically inactive metabolite of mefloquine as a putative PXR agonist ligand with EC_{50} value of approx. 30 µM. Induction of the assembly of the PXR ligand binding domain, recruitment of coactivators and release of corepressor SMRT further confirmed ligand binding of carboxymefloquine to PXR. The metabolite induced the PXR-dependent activation of the CYP3A4 enhancer/promoter reporter gene in HepG2-PXR cells and of the endogenous target genes ABCB1, CYP2B6, CYP2C8 in intestinal LS174T cells and primary human hepatocytes. In contrast to LS174T cells, carboxymefloquine did not induce CYP3A4 expression in primary human hepatocytes. Carboxymefloquine further demonstrated to be a specific ligand of PXR, not activating other nuclear receptors. Besides artemisinin and its derivatives arteether and artemether (1), only carboxymefloquine among all antimalarial drugs and metabolites, which have been tested, induced drug metabolism via agonist binding to PXR in cellular assays, suggesting that PXR activation may not be a general problem in antimalarial therapy. However, further in vitro analysis is clearly required to substantiate our

findings, as many antimalarials proved to be cytotoxic at doses exceeding 10 µM. On the other hand, activation of PXR by carboxymefloquine may be of clinical relevance, given that its plasma levels come up to 15 µM (2), thereby suggesting that intrahepatic levels may be sufficiently high to activate PXR in vivo.

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P249 - EXAMINATION OF TRANSCYTOTIC RECEPTOR GENES EXPRESSION IN A NEWLY-DEVELOPED CONDITIONALLY IMMORTALIZED HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELL LINE

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Delivery of biopharmaceutical agents, such as peptidomimetics, into the brain has been a challenge because these agents cannot readily cross the blood-brain barrier (BBB), which is formed by brain microvascular endothelial cells (BMEC). To overcome this inaccessibility of the agents to the brain, the strategy utilizing receptor-mediated transcytosis occurred at BMEC has been focused on. In this strategy, biopharmaceutical agents are designed to contain a binding motif to a transcytotic receptor that is expressed on the surface of BMEC, such as transferrin receptor (TfR). Then binding of the agents to the receptor triggers transport of the receptor-agent complex across the cells (transcytosis). It is considered likely that in vitro BBB models are useful tools for evaluation various types of the agents and the receptors in order to refine the strategy. Therefore, we previously established a new conditionally immortalized human brain microvascular endothelial cell line (HBMEC/ciβ). However, it is yet unknown whether HBMEC/ciβ expresses a series of receptors that are required for investigation of receptor-mediated transcytosis of biopharmaceutical agents. Therefore, the purpose of this study was to clarify the expression profile of transcytotic receptor genes in HBMEC/ciβ. HBMEC/ciβ was cultured with a modified EGM-2 medium on collagenated culture dishes and passaged every 3-4 days at approximately 90% confluence. Expression of several receptor genes in HBMEC/ciβ was examined by reverse transcription-polymerase chain reaction as well as by western blotting analysis. The results showed that HBMEC/ciβ expressed mRNAs of lipoprotein receptor-related protein 1 (LRP1), TfR, low density lipoprotein receptor (LDLR), transmembrane protein 30 A and insulin receptor at the levels similar to those in primary human BMEC. The results also showed expression of LRP1, TfR and LDLR proteins in HBMEC/ciβ. Therefore, we concluded that HBMEC/ciβ is a promising cell line to investigate receptor-mediated transcytosis of biopharmaceutical agents and to develop the sophisticated strategy by which the agents are efficiently delivered into the brain. In order to obtain further evidence for usefulness of HBMEC/ciβ in such studies, we are currently examining the transcytotic functions of the receptors identified in the cells.

P250 - CYP46A1 – 24S-HYDROXYCHOLESTEROL – LXR AXIS AFFECTS MEMBRANE TARGETING OF SGTPASES IN NEURONAL CELLS

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The liver X receptors (LXRs), are nuclear receptors that control the expression of genes involved in cholesterol metabolism and lipoprotein remodelling, and have recently been considered drug targets in neurodegenerative disorders with altered cholesterol metabolism, such as Alzheimer´s disease. The CYP46A1 gene codes for the cholesterol 24-hydroxylase, a cytochrome P450 that is specifically expressed in neurons, and plays an essential role on cholesterol turnover in the brain, since it is responsible for the conversion of cholesterol to 24S-hydroxycholesterol (24OHC) - an endogenous LXR ligand – which is the major pathway for brain cholesterol elimination. Interestingly, the alteration in cholesterol turnover observed in Cyp46a1 knock-out mice reduces the synthesis of isoprenoids, which are required for learning and for synaptic plasticity. However, it is not known if alterations in CYP46A1 expression, or in the activation of LXRs, can significantly affect the amount of isoprenylated proteins, namely the isoprenylation of small guanosine triphosphate-binding proteins (sGTPases). Abnormal activity

of sGTPases has been associated with Alzheimer's disease, since they affect synaptic function and amyloid-β peptide production and toxicity. In this study, we have transfected human neuroblastoma SH-SY5Y cells and primary cultures of rat cortical and hippocampal neurons with an expression vector for the human CYP46A1 or incubated these cells with 24OHC and the LXR synthetic agonist TO901317. Western blot analysis of total cell protein extracts and cytosolic and membrane protein fractions revealed that CYP46A1 overexpression leads to an overall increase in the levels of different sGTPases in the membrane fraction when compared to control levels. Moreover, incubation with 24OHC and TO901317 also result in the increase in membrane content of sGTPases. Besides the membrane targeting, the total expression of different sGTPase is also affected by these treatments. Our results suggest that overexpression of CYP46A1 can efficiently lead to an increase in the targeting of sGTPases to the membrane, possibly due to an increase in sGTPases prenylation as a consequence of an increase in CHOL turnover. Interestingly, treatment with 24OHC, the CYP46A1 product, induces an overall comparable effect to CYP46A1 overexpression and to that of the LXR synthetic agonist, suggesting that increased membrane targeting of sGTPses is dependent on LXR activation. These results point to a possible important link between LXR-mediated signalling and learning and memory functions.

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P251 - EFFECTS OF A NONGENOTOXIC CARCINOGEN ON HEPATIC PROGENITOR DIFFERENTIATION

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Background and aims: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and the third leading cause of cancer death. Cytokeratin-19 (CK19), a marker specific of cholangiocytes and hepatic progenitor cells, was found to be expressed in 10% to 20% of the liver tumors. CK19-positive HCCs are a definite clinico-pathological entity more aggressive and of poorer prognosis than CK19-negative HCCs. CK19-positive HCCs are characterized by a hepatic progenitor molecular signature, suggesting that these tumours could derive from hepatic progenitor cells or from malignant hepatocytes adopting an immature liver progenitor-like phenotype during tumour progression. Our goal is to study the behaviour of normal human hepatic progenitor cells subjected to some of the etiologic agents of HCC. Methods: Non parenchymal epithelial (NPE) cell fraction containing a progenitor population was isolated from human liver specimens (Duret C et al, Stem Cells, 2007) and exposed to dioxin, a nongenotoxic environmental carcinogen, under various experimental conditions: during proliferation or differentiation, and in the absence or presence of proinflammatory cytokines (IL1b, IL6, TNFalpha) to mimic the inflammatory environment that prevails during cirrhosis. Impact on cell survival, proliferation and hepatic and biliary differentiation was assessed. Results: The presence of dioxin during cell amplification resulted in an increase of proliferation. We couldn't detect any apoptotic events. When NPE cells were exposed to dioxin during cell differentiation, we observed a marked decrease in expression of both hepatocyte (albumin, HNF4alpha, CYP3A7) and biliary cell (SGLT1, and CFTR) markers. Dioxin effect was partially reversed in the presence of CH223191, a ligand-selective antagonist of the aryl hydrocarbon receptor (AhR), or in the presence of small-hairpin RNA (shRNA) against AhR. In parallel, dioxin induced IL-1beta expression, as well as IL-6 and TNFalpha expression, creating a proinflammatory context. Furthermore, addition of recombinant cytokines partially inhibited hepatocyte differentiation. Conclusion: These data suggest that the inhibition of adult human progenitor differentiation by some nongenotoxic carcinogens is AhR-dependent and may involve a proinflammatory environment.

P252 - THE ROLE OF RESIDUES T248, Y249 AND T422 IN THE FUNCTION OF HUMAN PREGNANE X RECEPTOR

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The steroid and xenobiotic-responsive pregnane X receptor (PXR), plays a critical role in mediating drug-drug interactions in humans. One of the primary targets of PXR is cytochrome P450-3A (CYP3A) gene expression that is responsible for the metabolism of a wide variety of xenobiotics including 60% of all clinically used drugs. PXR has

recently emerged as an important regulatory protein with multiple ligand-dependent functions, but ligand activation alone cannot be sole determinant of PXR activation states. However, the regulatory mechanisms of PXR are not entirely understood, there are several indications on the role of phosphorylation in modulation of PXR transcriptional activity. Phosphorylation of PXR or a protein–protein interaction between PXR and another signaling molecule is probably responsible for the PXR-mediated repression of CYP expression. Although several kinases have been shown to directly phosphorylate this receptor, very little is known about specific site(s) of phosphorylation. In the present work we examined putative phosphorylation sites at T248, Y249 and T422. Side-directed mutagenesis was performed to generate phospho-deficient and phospho-mimetic mutants. We measured fold activation of the p3A4-luc reporter gene construct transiently transfected together with PXR plasmids to human hepatoma HepG2 cells. Next the effects of mutation on basal and inducible levels of CYP3A4 mRNA in HepG2 cells were determined. We have also investigated the role of residues in interaction with a common heterodimerization partner RXRalpha and ability to bind to the CYP3A4 promoter to reveal an importance of these residues in hPXR function. We identified that phospho-deficient mutant T248V exhibited very low basal and ligand-inducible transactivation capacity. Contrary, phospho-mimetic mutation at position T248 resulted in constitutive activation of CYP3A4 promoter independently of the presence of a ligand. Both phospho-deficient and phospho-mimetic mutant of T422 drastically diminished transactivation of the CYP3A4 promoter. Reduced potency of PXR ligand towards mutant Y249D was observed. We show that all studied hPXR mutants formed heterodimer with RXRalpha at a similar level to that observed with wt-PXR. Finally, we found that T422D and both T248V and T248D mutants substantially decreased their ability to bind to DNA in vitro compared to wt-PXR. In the current study we highlight the important role of studied hPXR residues in the function of PXR. Our data indicated that all specific sites especially the position of T248 and T422 are critical for the activity of hPXR.

P253 - USE OF HUMANIZED MOUSE MODELS IN DRUG-DRUG INTERACTION, PHARMACOKINETIC AND SAFETY TESTING OF COMPOUNDS

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Though 57 active P450 enzymes have been described in humans, only a small fraction thereof account for the vast majority of P450-mediated metabolic reactions of clinically used drugs. It was estimated that ~95% of such reactions are dependent on either CYP3A4/5, CYP2D6, CYP2C9/2C19 or CYP1A1/1A2. In order to overcome the differences which exist in the substrate specificity, regulation of expression and multiplicity of CYPs between species, significant efforts have been made over recent years in the generation of humanized mouse models for some of these key enzymes with the aim of providing animal models that better predict human drug metabolism. This presentation describes the current state-of-the-art in the field of humanized mouse models for pathways of drug metabolism and disposition and their combination into complex, multiple humanized mouse lines. For example, the use of a triple humanized model for the pregnane X receptor (PXR), constitutive androstane receptor (CAR) and CYP3A4 in ranking different PXR activators according to their potency of inducing CYP3A4 in humans and to quantitatively predict PXR/CYP3A4-mediated drug-drug interactions in the clinic will be presented. Furthermore, it will be shown how CYP2D6 drug-drug interaction and polymorphic metabolism can be modeled in humanized CYP2D6 mice. In addition to other examples, the benefit and limitations of such models in drug development will be discussed.

P254 - INTERSPECIES IN VITRO EVALUATION OF STEREOSELECTIVE PROTEIN BINDING FOR 3,4-METHYLENEDIOXYMETHAMPHETAMINE

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3,4-Methylenedioxymethamphetamine (MDMA) undergoes stereoselective metabolism in vivo. Although MDMA is ingested in racemic form, the (+)-(S)-MDMA enantiomer is preferentially metabolised. The cytochrome P450 enzymes involved in the metabolism of MDMA are stereoselective, however, it is unknown if binding of MDMA to plasma or serum proteins is similarly stereoselective. In this study, in vitro protein binding of MDMA to human plasma, rat and mouse sera is determined using a centrifugal ultrafiltration device. Assessment of stereoselectivity in protein binding was possible using a stereospecific gas chromatography – mass spectrometry assay. The results indicate that total protein binding is approximately 41 – 51% in human plasma and 38 – 50% in rat serum whereas

for mouse serum, total protein binding is much lower at 11 – 36%. Stereoselectivity in protein binding was not evident for human or rat samples. As for mice samples, (+)-(S)-MDMA was more highly bound compared to (-)-(R)-MDMA, indicating that significant stereoselectivity for protein binding occurs in mice. However, due to total protein binding of MDMA in mice being rather low, this stereoselectivity would not be a major contributor to stereoselective disposition of MDMA in this species.

P255 - INTERSPECIES VARIATION IN THE ENANTIOSELECTIVE METABOLISM OF TEGAFUR TO 5-FLUOROURACIL

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Tegafur (5-fluoro-1-(2-tetrahydrofuryl)-2, 4(1H, 3H)-pyrimidinedione; FT) is a prodrug of 5-fluorouracil (5-FU) and is used in cancer chemotherapy at a wide range of doses as a combination drug with S-1 or tegafur-uracil (UFT). FT has a chiral center and is a racemate comprising the enantiomers R-FT and S-FT, but little is known about the enantioselectivity in its metabolism. In the present study, we investigated interspecies variation in the in vitro enantioselective metabolism of FT to 5-FU by using animal liver preparations. An inhibition study with a non-selective cytochrome P450 (CYP) inhibitor, 1-aminobenzotriazole, revealed that CYP mainly contributes to the conversion of both FT enantiomers to 5-FU in rats, dogs, monkeys, and humans. Regarding species differences in the enantioselectivity of FT metabolism, while the 5-FU formation rate from R-FT was faster than that from S-FT in rats, monkeys, and humans, S-FT was a preferential substrate for dogs. Kinetic analysis with the Eadie–Hofstee plot showed that 5-FU formation from R-FT in humans and from S-FT in dogs occurs in a biphasic manner, suggesting the involvement of multiple CYP isoforms. Microsomal preparations from different human livers (N = 5) exhibited a comparative enantioselectivity in the metabolism of FT, and the interindividual variability was mainly attributed to the difference in the V_{max} value. In vivo pharmacokinetic studies following intravenous administration of FT to animals showed apparent species differences in plasma clearance between R-FT and S-FT, and such variance seemed to be entirely consistent with the differences in in vitro clearance. These results suggest that the difference in metabolic clearance between R-FT and S-FT directly influences plasma clearance, regardless of animal species. Furthermore, it is reported that the area under the plasma concentration–time curve and terminal elimination half-life value in cancer patients were lower in R-FT than in S-FT. This observation suggests that the plasma clearance of R-FT is higher than that of S-FT; this is consistent with the in vitro preferential metabolism of R-FT. The biphasic nature of the in vitro clearance of R-FT demonstrates that the relative contribution of CYP isoforms to 5-FU-formation from R-FT may vary depending on the in vivo blood concentration of FT. In conclusion, evident interspecies variations were observed in the enantioselective metabolism of FT, and the in vivo selectivity could be extrapolated from the in vitro metabolic activities in humans and animals.

P256 - ENGINEERED P450 BM3 AS BIOCATALYSTS FOR REGIO- AND STEREOSELECTIVE HYDROXYLATION

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Selective hydroxylation of non-activated C-H bond by conventional chemical synthesis still poses a challenge due to the lack of selectivity yielding a variety of side products. Therefore, enzymatic and microbial biotransformations for selective hydroxylation have gained importance over the years. Cytochrome P450 BM3 from Bacillus megaterium (CYP102A1) is a highly active monooxygenase that has been engineered both by site directed and random mutagenesis to catalyze the hydroxylation of a wide range of drug-like molecules. In this work, the ability of engineered P450 BM3 to perform regio- and stereoselective hydroxylation of ionones and larger substrates like testosterone has been studied. P450 BM3 mutants that can selectively produce individual metabolites have been identified.Thus, engineered P450 BM3s are promising tools for selective hydroxylation of industrially relevant molecules.

P257 - SENSITIZATION OF PANCREATIC CANCER CELLS TO GEMCITABINE VIA INHIBITION OF MULTIDRUG RESISTANCE PROTEIN 5 (MRP5) ACTIVITY BY CURCUMIN

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Treatment of pancreatic cancer with gemcitabine often fails due to the development of resistance during chemotherapy. Recent studies have suggested that MRP5 conferred resistance to gemcitabine by its active efflux from the cell [1, 2]. Curcumin is a phytochemical present in turmeric (Curcuma longa) which is widely used as a spice and medicinal agent in traditional Indian and Chinese medicine [3]. Our aim was to evaluate whether curcumin could reverse MRP5-mediated gemcitabine resistance in vitro. The role of MRP5 in cellular gemcitabine sensitivity was studied by comparison of cytotoxicity in parental and MRP5-silenced MiaPaCa-2 pancreatic cancer cells. MRP5 mRNA levels were determined by real-time PCR, and MRP5 protein detected by immunocytochemistry and western blotting. The cellular accumulation of a specific MRP5 fluorescent substrate 2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) was measured by flow cytometry in parental and MRP5-overexpressing HEK293 (HEK-MRP5) cells, and cell cytotoxicity determined by a 72-h CyQuant assay. In the presence of curcumin (5 and 10 μM), the cellular accumulation of BCECF increased 68 ± 9% ($p<0.01$) and 215 ± 12% ($p<0.01$) in HEK293/MRP5 cells. Curcumin had no effect on cellular accumulation of BCECF in parental HEK293 cells. The expression of MRP5 protein was significantly decreased 48-h after small interfering RNA (siRNA) treatment. Knockdown of MRP5 by siRNA increased cellular accumulation of BCECF and reversed gemcitabine resistance in MiaPaCa-2 cells. In the cytotoxicity assays, curcumin caused a concentration-dependant increase in the sensitivity to gemcitabine in HEK-MRP5 cells and in MiaPaCa-2 cells. Curcumin at tested concentrations had no effects on gemcitabine toxicity in parental HEK293 cells. Our results suggest that curcumin is an inhibitor of MRP5 and may be useful in the reversal of gemcitabine resistance in pancreatic cancer chemotherapy.

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P258 - MIRABEGRON, A POTENT AND SELECTIVE B_3-ADRENOCEPTOR AGONIST, IS A P-GLYCOPROTEIN (P-GP) SUBSTRATE: CHARACTERIZATION OF ITS EFFLUX TRANSPORT IN CACO-2 AND HUMAN P-GP EXPRESSING CELLS AND BCRP AND MRP2 EXPRESSING VESICLES

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The involvement of human P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance associated protein 2 (MRP2) in the membrane permeation of mirabegron was investigated. Also, the inhibitory effect of mirabegron on the transport of a probe P-gp substrate, vinblastine, was evaluated. Mirabegron showed asymmetrical trans-epithelial fluxes with basolateral-to-apical permeability coefficients ($P_{app,B\text{-}to\text{-}A}$) exceeding those from apical-to-basolateral direction ($P_{app,A\text{-}to\text{-}B}$) in Caco-2 cells. The $P_{app,B\text{-}to\text{-}A}$/ $P_{app,A\text{-}to\text{-}B}$ ratio (P_{app} ratio) of mirabegron was 9.0 at 1 μM and decreased to 4.1 at 250 μM. It also decreased in the presence of P-gp inhibitors, verapamil and quinidine, but not with a BCRP inhibitor, Ko143. The P_{app} ratio of mirabegron across LLC-PK1-MDR1 (P-gp-expressing) cell monolayers was higher than that across control cells. The apparent K_m value for transport of mirabegron was 294 μM. In contrast, no clear BCRP- or MRP2-mediated uptake of mirabegron was observed in BCRP- or MRP2-expressing vesicles, indicating that mirabegron is a substrate of P-gp but not of BCRP or MRP2. At 250 μM, mirabegron did not affect the $P_{app,B\text{-}to\text{-}A}$ of [^{3}H]vinblastine but significantly increased the $P_{app,A\text{-}to\text{-}B}$ (340% of control) in MDCKII-MDR1 (P-gp-expressing) cell monolayers. Mirabegron may have weak inhibitory effects on P-gp-mediated drug transport at high concentrations.

P259 - AN IN-SILICO MODEL OF ESTRADIOL METABOLISM AND TRANSPORT IN THE LIVER: DETERMINING THE RELATIVE INPUT OF ABC TRANSPORTERS

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The superfamily of ATP binding cassette transporters is made up of 7 subfamilies, which play an important role in determining the fate of a drug and its conjugates; influencing uptake, metabolism and efflux. The main class of transporters responsible for cellular efflux of drug conjugates are the ABCC subfamily, also known as the MRPs, whereas parent compounds are mainly excreted by ABCB1 (MDR1) [1]. In the liver, apical efflux transporters such as ABCC2 and ABCB1 have been highly studied; however, very little is known about the role of basolateral efflux transporters on determining drug pharmacokinetic and pharmacodynamic profiles. Importantly, in MRP2 knockout mice increased expression levels of the basolateral transporters MRP3 and MRP4 are observed in the liver and kidney, causing a shift to urinary excretion of compounds and their conjugates. We thus hypothesise that the basolateral (MRP3/MRP4) and apical (MRP2/MDR1) drug transporters are able to function as a coordinated system, producing the most efficient excretion for any given compound under differing physiological conditions. To test this hypothesis we have examined the transport of the endogenous hormone estradiol, which is a substrate for all of the above mentioned transporters. Initially, an in-silico model of estradiol transport and metabolism within the hepatocyte was generated using CellDesigner v4.2. This qualitative model was then populated with realistic kinetic parameters taken from the literature, and steady-state simulation shown to predict endogenous metabolite levels within the expected physiological bounds. Using both a metabolic control analysis approach and a series of virtual knock-out experiments we are able to demonstrate the relative input of each transporter and metabolic process in determining the fate of estradiol and its metabolites. In general, under physiological conditions metabolic processes exert the highest control coefficients on estradiol fate, presumably with transport processes occurring at non-limiting rates. However, deletion/reduction of any process, mimicking pathological conditions and or overdose, results in an increased input of transporters in determining compound fate. This data is consistent with our hypothesis and lends weight to the paradigm of an integrated drug metabolism-transport system that allows rapid adaption to chemical challenge.

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P260 - INVESTIGATION OF PHARMACOKINETICS DIFFERENCES OF 4-AMINO-3-CHLOROPHENYL HYDROGEN SULFATE, A METABOLITE OF RESATORVID, IN RATS AND DOGS

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Resatorvid (TAK-242: ethyl (6R)-6-[N-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate, CAS 243984-11-4), was discovered by Takeda Pharmaceutical Company Limited as a potential new anti-sepsis drug. When phenyl ring ^{14}C-labeled TAK-242, [phenyl ring-U-^{14}C]TAK-242, was intravenously given to rats and dogs, the concentration-time profiles in the plasma of the total ^{14}C showed extreme species differences. The extreme species differences were characterized by the elimination half-life of the total ^{14}C from the plasma between rats and dogs with the values of 5.6 and 158 hours, respectively[1)]. Because it was suggested that the elimination of 4-amino-3-chlorophenyl hydrogen sulfate, M-III of resatorvid, from plasma to urine via the renal distribution would be one of the possible factors for the extremely different ^{14}C concentration-time profiles in the plasma between rats and dogs after intravenous injection of [phenyl ring-U-^{14}C]TAK-242[1)], the pharmacokinetics of M-III in rats and dogs were investigated using radio-labeled M-III ([^{14}C]M-III). The elimination half-life of ^{14}C in the plasma of rats was about 1/30 of that of dogs after intravenous dosing of [^{14}C]M-III at 0.5 mg/kg to rats and dogs. The in vitro and in vivo plasma protein binding ratios of M-III were relatively high and the same in both the species. The intrinsic clearance (CL_{int}) of M-III in the rats was much higher than the glomerular filtration rate in rats. Furthermore, ^{14}C kidney/plasma ratio in rats was much higher than that in dogs, indicating that M-III was effectively taken up into the kidney and was excreted into the urine in rats; however in dogs, ineffective renal uptake of M-III was presumed. When [^{14}C]M-III and probenecid were simultaneously and continually infused intravenously to rats, the CL_{int} of M-III decreased with

increasing plasma concentrations of probenecid. This result suggested that kidney uptake of M-III in rats was inhibited by probenecid. It was also thought that uptake by the organic anion transport system(s) in the basolateral membrane is involved in the renal uptake of M-III in rats. The pharmacokinetic difference of M-III between rats and dogs are considered to be mainly caused by the difference in the urinary excretion via the renal distribution process.

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P261 - MULTIDRUG AND TOXIN EXTRUSION TRANSPORTER 1 (MATE1/SLC47A1) AND ORGANIC CATION TRANSPORTER 3 (OCT3/SLC22A3) EXPRESSION PROFILE AND FETUS PROTECTIVE ROLE AT DIFFERENT STAGES OF GESTATION IN RAT

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Transporter proteins in physiological barriers determine the exposure of sensitive tissues to xenobiotics. During gestation, such transporters localized in the placenta and/or fetal physiological barriers are of high importance since they are crucially responsible for fetus ontogeny. Good knowledge of all transporters involved in fetal protection, regulation of their expression, and their interactions with xenobiotics is deeply required for predicting the risk of treatment during gestation. The aim of the present study was to investigate the expression and localization of Multidrug and Toxin Extrusion Transporter 1 (Mate1/SLC47A1) and Organic Cation Transporter 3 (Oct3/SLC22A3) in the rat placenta and fetal tissues at different stages of pregnancy using qRT-PCR, western blot analysis and immunohistochemistry. Using infusion of 1-methyl-4-phenylpyridinum (MPP^+), a well-established substrate of both Mate1 and Oct3, we also studied functional changes of both transporters in the placenta at different gestation days (gds). We observed significantly higher expression of Mate1 and Oct3 mRNA in the placenta compared to the maternal kidney; their mRNA and protein levels increased towards the end of gestation. Regarding fetal tissues, the highest levels of both transporters were observed in the kidney, brain and intestine on gd 21. Immunohistochemical visualization revealed preferential localization of Oct3 on the fetal side; on the other hand, Mate1 was shown to be localized preferentially towards the maternal circulation in the placenta. Functional tests with MPP^+showed highest fetal exposure on gd 12 compared to gds 15, 18 and 21. In conclusion we suggest that throughout gestation both the placenta and fetus express Mate1 and Oct3 in a dynamic manner resulting in increased functional activity. This study provides a new insight to better understanding of functional role of Mate1 and Oct3 in the placenta and outlines possible function in fetal organs. However many questions regarding developmental expression and regulation of these transporter proteins in placenta and fetal tissues remain to be elucidated in further studies.

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P262 - ORGANIC ANION-TRANSPORTING POLYPEPTIDES 1A/1B CONTROL THE HEPATIC UPTAKE OF PRAVASTATIN IN MICE

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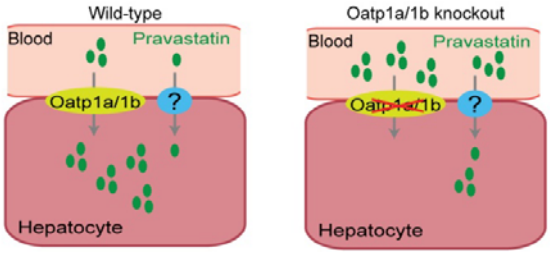
Organic anion-transporting polypeptides (OATPs) mediate the hepatic uptake of many drugs. The cholesterol-lowering drug pravastatin is a prototypical OATP1A/1B substrate. Liver uptake and plasma clearance of pravastatin and other statins are thought to be importantly mediated by OATP1B1, because activity-reducing OATP1B1 polymorphisms are associated with increased statin plasma exposure and myopathy, the main toxic side effect of statins. Clear empirical insight into the relationship between OATPs and statin oral bioavailability, plasma levels and liver exposure is lacking, however. Using Oatp1a/1b-null mice we found that intestinal absorption of pravastatin was

not affected by Oatp1a/1b absence, but systemic plasma exposure (AUC) increased 7-fold after bolus administration of 10 mg/kg oral pravastatin, and 30-fold after 5 mg/kg. This increased plasma exposure resulted from reduced hepatic uptake, as evident from lower liver AUC values, a lower hepatic extraction ratio, and 10-100 times lower liver-to-plasma concentration ratios. However, the reductions in liver exposure were far smaller (<2-fold) than the increases in plasma exposure. Reduced pravastatin liver uptake in Oatp1a/1b-null mice was more obvious shortly after intravenous administration (at 5 mg/kg), with 8-fold lower biliary pravastatin excretion. Although mice chronically exposed to pravastatin for 60 days did not evince muscular toxicity, Oatp1a/1b-null mice displayed 10-fold higher plasma concentrations and 8-fold lower liver concentrations than wild-type mice. **Conclusion:** Oatp1a/1b transporters control the hepatic, but not the intestinal uptake of pravastatin. After bolus administration, Oatp1a/1b transporters affect plasma exposure more than liver exposure, but after chronic administration their relative impact on plasma and liver exposure is similar. OATP1B polymorphisms may thus simultaneously reduce pravastatin therapeutic efficacy in the liver and increase systemic toxicity risks, thus compromising its therapeutic index in a two-edged way.



P263 - CONSTRUCTION OF DOUBLE-TRANSFECTED CELLS EXPRESSING THE HUMAN TRANSPORTER MDR1 AND CYTOCHROME P450 3A4

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MDCK-MDR1 cells stably expressing P-gp were constructed by our laboratory[1]. The cloned human CYP3A4 cDNA was subcloned to pMD19-T Vector. After getting correct sequences, it was digested by KpnI and XhoI sites, and was subcloned into pcDNA3.1/ Hypgro vector. Plasmid pcDNA3.1/ Hypgro/CYP3A4 was constructed. The transfection of plasmids into MDCK-MDR1 cells was performed using Lipofectamine™ LTX (Invitrogen). The expression of human CYP3A4 in the transfected MDCK-MDR1 monoclone cells was characterized by immunoblotting. The activity of P-gp and CYP3A4 in the double-transfected cells were confirmed by using their typical substrates determined by LC/MS. GA is a substrate of P-gp and CYP3A4, and the GA is converted to more toxic metabolite by CYP3A4[2]. MDCK-pcDNA3.1 cells were used as control cells. GA was incubated with MDCK, MDCK-MDR1 and MDCK-MDR1/CYP3A4 cells in 96-well plates at concentrations of 10,20,40,80,100 microM, respectively. Each experiment was conducted in triplicate. The values $IC_{50}$of GA in the MDCK MDCK-MDR1, MDCK- MDR1/CYP3A4 cells using MTT assay were 37.96,47.54,31.66 microM, respectively. GA, a P-gp substrate in the MDCK-MDR1 cell was less toxic than that in the MDCK cell The GA was most toxic in the MDCK-MDR1/CYP3A4 cells resulting from GA metabolite metabolized by CYP3A4. The results showed that MDCK-MDR1/CYP3A4 cells can be useful for screening or evaluating the P-gp and CYP3A4 substrates and activation by CYP3A4.This work was supported by National Major Projects of China (2012ZX09506001-004,2009ZX09304-003) and Nature Scientific Found of China (81173120).

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P264 - SPECIES DIFFERENCES OF ORGANIC ANION TRANSPORTERS INVOLVED IN THE RENAL UPTAKE OF 4-AMINO-3-CHLOROPHENYL HYDROGEN SULFATE, A METABOLITE OF RESATORVID, BETWEEN RATS AND DOGS

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Previous studies on the metabolic fate of resatorvid (TAK-242: ethyl (6R)-6-[N-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate, CAS 243984-11-4) have shown that species differences in the pharmacokinetics of 4-amino-3-chlorophenyl hydrogen sulfate (M-III), a metabolite of TAK-242, between rats and dogs are mainly attributable to the urinary excretion process[1,2)]. In the present study, the renal uptake mechanism of M-III was investigated using kidney slices from rats and dogs and Xenopus laevis oocytes expressing rat organic anion transporter 1 (rOat1; Slc22a6) and rOat3 (Slc22a8). The uptake of p-aminohippuric acid (PAH), a substrate for Oats, by kidney slices from rats and dogs increased at 37°C with initial uptake clearance of 0.398 and 0.390 mL/min/g kidney, respectively. M-III inhibited the uptake of PAH by kidney slices from both rats and dogs. The initial uptake clearance of M-III by rat kidney slices was 0.295 and 0.0114 mL/min/g kidney at 37°C and 4°C, respectively. The Eadie-Hofstee plot of M-III uptake at 37°C revealed two-component transport processes with Km values being 6.48 and 724 µmol/L for the high and low affinity component, respectively. The uptake of M-III by rat kidney slices at 37°C was inhibited by probenecid (PBC), PAH and benzylpenicillin (PCG). In contrast, in dog kidney slices, the initial uptake clearance of M-III was 8.70×10^{-3} and 9.00×10^{-3}mL/min/g kidney at 37°C and 4°C, respectively, and the uptake of M-III was not inhibited by PBC. Furthermore, rOat1- and rOat3-expressing oocytes mediated M-III uptake and the uptake was inhibited by PAH and PCG, respectively. These results suggest that rOat1 and rOat3 are responsible for the renal uptake of M-III in rats, whereas Oat(s) is unable to transport M-III in dogs. It is most likely that the difference in the substrate recognition of Oat(s) lead to the species difference in the pharmacokinetics of M-III between rats and dogs.

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P265 - DNA METHYLATION INFLUENCES THE TISSUE-SPECIFIC AND DISEASE-RELATED EXPRESSION OF THE SODIUM-TAUROCHOLATE COTRANSPORTING POLYPEPTIDE (NTCP, SLC10A1) IN HUMAN LIVER, KIDNEY AND HEPATOCELLULAR CARCINOMA

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The human sodium-taurocholate cotransporting polypeptide (NTCP, SLC10A1) is the main bile acid uptake transporter in human hepatocytes. Besides its physiological substrates it also transports xenobiotics, like chemotherapeutics coupled to bile salts, from the blood plasma across the basolateral membrane into the hepatocytes. The expression of this liver-specific transporter can be affected by diseases like hepatocellular carcinoma (HCC), where the SLC10A1 expression is decreased compared to normal liver tissue. Since epigenetic mechanisms, as DNA methylation, are known to influence gene expression, DNA methylation could contribute to the one of SLC10A1. To investigate this, the specific DNA methylation at the SLC10A1 gene in normal liver (n=100), HCC (n=15) and normal kidney samples (n=18) was measured by MALDI-TOF MS. The SLC10A1 expression of the respective samples was analysed by TaqMan quantitative real time PCR. For an in vitro approach cell culture experiments were performed. No SLC10A1 expression was detectable in the kidney samples, where the gene showed a high DNA methylation pattern of about 90 % on average. In contrast to this the normal liver samples had a reduced DNA methylation pattern in the promoter (about 55 %) and exon1 region (about 45 %). In HCC tissue the SLC10A1 expression was decreased compared to normal liver (p=0.006) and the promoter as well as the exon1

region showed a significantly higher DNA methylation. Additionally, the in vitro cell culture experiments showed a general influence of DNA methylation on the SLC10A1 expression. The expression in different tumor cell lines was increased after a DNA-demethylating treatment with 5-aza-2`-deoxycytidine, whereas the expression of luciferase reporter constructs was decreased by 45% to 65% after in vitro methylation of the upstream located SLC10A1 promoter. Considering these results, one can conclude, that DNA methylation is involved in the regulation of the SLC10A1 expression.

P266 - PREDICTION OF ATORVASTATIN ACID-LACTONE INTER-CONVERSION AND ORAL PHARMACOKINETICS FROM IN VITRO DATA USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELLING

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Previous work has shown that physiologically-based pharmacokinetic (PBPK) models for atorvastatin (acid), incorporating metabolic in vitro clearance data, resulted in considerable under-prediction of its oral clearance [1]. In addition to the acid form, atorvastatin-lactone plasma concentrations have been reported to be high; conversion to this pharmacologically inactive form is postulated to occur via an unstable atorvastatin glucuronide-intermediate. In this study, intrinsic clearance (CLu_{int}) data for CYP and UGT (+/- 1%BSA) mediated metabolism of both forms were determined in pooled human liver and intestinal microsomes. Furthermore, conversion kinetics of atorvastatin-lactone in human plasma, HLS9 and phosphate buffer (pH 7.1 and 7.4) were assessed and included within an atorvastatin PBPK model constructed in Matlab v.7.12. Atorvastatin efflux kinetics (Caco-2 cells), pH dependent inter-conversion and permeability (pH 1–7.4, relevant during intestinal transit), active uptake in human hepatocytes and intracellular binding were also included in the PBPK model in order to improve prediction of atorvastatin oral clearance. The inclusion of these various processes affecting atorvastatin pharmacokinetics was performed in a step-wise manner to assess their individual impact on the prediction success of oral atorvastatin clearance. In the case of liver, atorvastatin lactone CYP mediated CLu_{int} was >100-fold higher in comparison to the acid form (49.1µL/min/mg). UGT mediated CLu_{int} was increased in the presence of 1%BSA in human liver microsomes (6.91 vs. 2.53µL/min/mg); however, it only accounted for 12% of the total in vitro CLu_{int} of atorvastatin. Scaled metabolic intrinsic clearance (both CYP and UGT considered) of 217L/h was approximately one third of the active uptake clearance (675L/h). Inclusion of hepatic uptake into the PBPK model increased predictions of both i.v. and oral clearances due to increased unbound hepatic concentration relative to the plasma. However, in order to recover the reported i.v. clearance of atorvastatin (37.5L/h), an empirical scaling factor of 3.7 on hepatic uptake V_{max} was required. The use of metabolic data (liver and intestine) and optimised hepatic uptake clearance, without any consideration of inter-conversion or intestinal efflux, predicted 14% of the observed oral atorvastatin clearance. Further inclusion of luminal conversion of atorvastatin to atorvastatin-lactone and intestinal efflux resulted in a minor additional increase in prediction success (17% of the observed value). The high metabolic clearance of atorvastatin-lactone combined with the low rate of formation in vitro (atorvastatin UGT << CYP CLu_{int}) and a high predicted volume of distribution predicted a very low exposure of the lactone form, in contrast to observations in vivo. Therefore, it would appear that formation of atorvastatin-lactone may be facilitated by mechanisms other than UGT-metabolism or that sites other than the liver and small intestine are capable of mediating atorvastatin-lactone formation.

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P267 - MECHANISTIC MODELLING OF ATORVASTATIN UPTAKE AND ACID – LACTONE INTER-CONVERSION IN PLATED RAT AND HUMAN HEPATOCYTES

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Atorvastatin (ATV) is administered as the active hydroxyl acid which undergoes substantial conversion in vivo to the inactive lactone form. Clinical data suggest contributing role of hepatic uptake transporter OATP1B1 to ATV acid disposition; however, in vitro transporter kinetic data concerning both the mechanism of uptake and the acid-lactone inter-conversion are limited. The main objective of this study was to evaluate the uptake of ATV in plated rat

and human hepatocytes accounting for the acid-lactone inter-conversion and its impact on parameter estimates. Freshly isolated rat hepatocytes and human cryopreserved hepatocytes were plated in collagen coated 24-well plates at $0.24x10^6$ cells/well and $0.4x10^6$ cells/well, respectively. The uptake of ATV acid and lactone forms was investigated over concentration range for up to 90 minutes to allow equilibrium between intracellular and media concentrations to be reached. In order to inhibit phase I metabolism, 1mM 1-aminobenzotriazole was added to the incubations. During the experiments both acid and lactone forms were quantified using LC-MS/MS. A mechanistic two-compartment model was constructed in Matlab v.7.12 to accurately estimate the kinetic parameters describing uptake kinetics (K_m, V_{max}), passive diffusion (P_{diff}) and intracellular binding (fu_{cell}) using experimental data at 37°C, as described previously [1]. The conversion of acid to lactone form of ATV was negligible (up to 2%) in the cells and had no impact on data analysis. The uptake rate of ATV acid was 2-fold higher in rat compared to human hepatocytes, whereas uptake binding affinity constant and P_{diff} were consistent between the two species (K_m=2.74±1.34µM, P_{diff}=8.20±4.06µL/min/10^6cells and K_m=3.05µM, P_{diff}=6.00µL/min/10^6cells, for rat and human hepatocytes, respectively). No apparent active uptake was observed for the lactone form. A high $P_{diff,u}$ was determined for ATV lactone in both rat (99.9±36.3 µL/min/10^6cells) and human hepatocytes (17.1µL/min/10^6cells). In contrast to the acid, the lactone displayed considerable nonspecific binding to the experimental support (>65%). Furthermore, a high degree of conversion to the acid form was observed for the uptake experiments with the lactone. The conversion rate constant inside the cells determined in rat hepatocytes was >10-fold higher than chemical conversion rate constant obtained at pH 7.1 (corresponding to intracellular pH) [2]. In conclusion, active uptake and passive diffusion are the major processes driving hepatic distribution of ATV acid and lactone, respectively, in agreement with the differences in $logD_{7.4}$ of these forms. The current experimental design allows incorporation of acid-lactone inter-conversion in the assessment of atorvastatin hepatic uptake in vitro in a mechanistic manner. Generated parameters can be used as input parameters within physiologically-based pharmacokinetic models for in vitro-in vivo extrapolation of transporter-meditated pharmacokinetics.

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P268 - USE OF SANDWICH-CULTURED RAT HEPATOCYTES FOR THE DETERMINATION OF INTRINSIC UPTAKE AND BILIARY CLEARANCES

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The sandwich-cultured rat hepatocyte (SCRH) system represents a valuable model system with which to simultaneously study drug uptake and efflux since both sinusoidal and canalicular transport proteins have been shown to be functional in this model. In vitro intrinsic biliary clearances ($Cl_{int,biliary}$) determined in sandwich-cultured hepatocytes are reported to correlate well with observed in vivo $Cl_{biliary}$, suggesting that this is a useful approach to predict hepatobiliary excretion of drug candidates. In contrast, the predicted absolute hepatic clearance values may be underestimated when based on in vitro experiments with SCH due to the down-regulation of function and expression of uptake transporters in rat hepatocyte cultures. The purpose of this study was to assess the feasibility of using primary SCRH as a model to characterise the interplay between uptake and efflux transporters. Here we compare uptake clearance ($Cl_{int,uptake}$) in SCRH with conventional, in-house hepatocyte assays [1, 2], for 4 metabolically stable, actively transported, compounds; rosuvastatin, valsartan, fexofenadine and pravastatin. The rank order of $Cl_{int,uptake}$ values was consistent throughout all three hepatocyte models, however, $Cl_{int,uptake}$ in SCRH was significantly reduced compared to suspension and plated hepatocyte $Cl_{int,uptake}$, ranging from 0.8 – 3.4 µl/min/10^6 cells compared to plated (11.6 – 84.9 µl/min/10^6 cells) [1] and suspended hepatocytes (33.4 – 425 µl/min/10^6 cells) [2]. These results confirm that the uptake activity is considerably diminished in primary cultured rat hepatocytes, even in sandwich format. Since marked differences in activity or expression levels of uptake transporters will influence intracellular drug concentration and, in turn, hepatic clearance, $Cl_{int,biliary}$ was also examined. Comparable values to $Cl_{int,uptake}$ were found ranging from 0.4 – 1.9 µl/min/10^6 cells. Average biliary excretion index (BEI) and

predicted $Cl_{biliary}$ of rosuvastatin, valsartan, fexofenadine and pravastatin were 39%, 21%, 39%, and 37%, respectively, and 11.0, 5.5, 5.5 and 3.0 ml/min/kg, respectively. These latter values underestimate the observed in vivo behaviour but the rank order of these values are consistent with those reported in the literature for other SCRH studies. In conclusion, reduced hepatic uptake in SCRH may limit the access of drugs to canalicular efflux transport proteins, highlighting the importance of elucidating the interplay of these transport proteins for the prediction of absolute hepatic clearance.

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P269 - MULTIDRUG RESISTANCE PROTEIN 4 (MRP4) CONTROLS TUMOR GROWTH IN A HUMAN ACUTE MYELOID LEUKEMIA IN VIVO MODEL

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Less than a third of adults with acute myeloid leukemia (AML) are cured by current treatments, emphasizing the need for new approaches to therapy. Resistance to drug therapy can arise from diverse mechanisms, among them drug export from tumor cells or alterations in intracellular signaling. Both mechanisms can be mediated by blast expression of members of the multidrug resistance-associated proteins (MRPs). In particular, MRP4/ABCC4 is implicated in cAMP efflux and also confers resistance to nucleoside analogs, cytostatic drugs used in the treatment of AML (1). We have recently demonstrated in AML cell lines that MRP4 has a relevant role in the regulation of intracellular cAMP levels, leading to the inhibition of cell proliferation and promoting differentiation (2). Thus, we have postulated that the inhibition of cAMP efflux by blocking MRPs, mainly MRP4, may lead to a decrease in proliferation and tumor growth. To test this, a human AML model was established by subcutaneous injection of U937 cells into Swiss nu/nu mice. In order to investigate the effect of the regulation of cAMP levels, mice were treated with probenecid (MRP inhibitor, 50 mg/kg), rolipram (phosphodiesterase 4 inhibitor, 1.5 mg/kg), probenecid+rolipram or with vehicle for 2 weeks. Interestingly, all treatments showed an inhibition on tumor growth compared with vehicle-treated mice ($P<0.01$). A significant decrease in the mitotic index was observed with all the treatments ($P<0.001$). Moreover, treatment with rolipram+probenecid was even more effective in decreasing the mitotic index compared with rolipram or probenecid-treated mice. However, no differences in apoptosis were observed. To further investigate the specific role of MRP4 in tumor growth and proliferation, Swiss nu/nu mice were injected with U937 cells expressing short hairpin RNA (shRNA) against MRP4 (U937-shMRP4) or scrambled shRNA (U937-scramble). A third group of mice carrying U937-scramble tumor was treated with rolipram+probenecid for 2 weeks. Remarkably, blockage of MRP4 strongly reduces tumor growth and tumor size was even smaller than in the rolipram+probenecid-treated group ($P<0.05$). In addition, a decrease in the mitotic and apoptotic index was observed. In conclusion, these results show for the first time the importance of MRP4 in tumor growth in a human AML in vivo model, providing the basis for a novel promising target for leukemia therapy.

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P270 - INTERACTIONS OF RESVERATROL METHYL ANALOGUES WITH BREAST CANCER RESISTANCE PROTEIN (BCRP/ABCG2) – DIFFERENTIAL INHIBITION POTENCY AND TRANSPORT AFFINITY

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Purpose: Breast cancer resistance protein (BCRP/ABCG2) plays an important role in the disposition of many xenobiotics and is also one of the prominent efflux transporters involving in multidrug resistance (MDR). Resveratrol (3,5,4'-trihydroxystilbene) has been previously reported to be both an inhibitor and a substrate for BCRP. In this work, the interactions between several resveratrol methyl analogues (4'-methoxystilbene, 3,5-dimethoxystilbene, 3,4'-dimethoxystilbene, and 3,5,4'-trimethoxystilbene) and BCRP were studied. The aim of this study was to determine if the structural modification alters i) the BCRP-inhibition potency of resveratrol and ii) the transport affinity of resveratrol by BCRP. **Methods:** The BCRP-modulating activities of the analogues were studied in two assays. A cell-based assay quantifying the accumulation of mitoxantrone (a fluorescent BCRP substrate) with and without the treatment of the analogues was first performed in wild-type (WT) HEK293 cells and HEK293 cells overexpressing BCRP to confirm the BCRP-inhibiting effects of the analogues. The inhibition potencies of the analogues (IC_{50}) were then generated by evaluating their concentration-dependent inhibition of ^{3}H-methotrexate transport (a standard BCRP substrate) in Sf9 membrane vesicles overexpressing BCRP. The assessment of the transport affinities of the analogues by BCRP is on-going using a bi-directional transport assay in monolayer MDCKII cells overexpressing BCRP. **Results:** In the presence of 10 µM resveratrol methyl analogues, intracellular accumulation of mitoxantrone increased 1.51-1.66 fold in HEK293/BCRP cells while no increase was observed in WT HEK293 cells. The IC_{50}values of the analogues as evaluated in the membrane vesicular transport assay indicate inhibition potencies in the order of 3,5-dimethoxystilbene > resveratrol > 3,4'-dimethoxystilbene > 4'-methoxystilbene > 3,5,4'-trimethoxystilbene. **Conclusion:** Our results indicated that methylation at different positions alters the inhibition potency of resveratrol. The stronger inhibition potency of 3,5-dimethoxystilbene suggested that it may be a better MDR-reversal agent than resveratrol. Methylation at the 4' position appears to reduce the inhibition potency/affinity of resveratrol towards BCRP, which may serve as a basis for enhanced bioavailability in vivo.

P271 - THE MODULATORY EFFECTS OF NOVEL CURCUMIN ANALOGUES ON XENOBIOTIC ABC TRANSPORTERS

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The phytochemical curcumin, has previously been demonstrated to inhibit the transport activity of the major ABC transporters involved in cancer multidrug resistance (MDR), i.e., P-glycoprotein (Pgp), breast cancer resistance protein (BCRP) and multidrug resistance protein 1 (MRP1)(1). Curcumin has therefore been investigated as a potential MDR reversal agent, but rapid clearance and low oral bioavailability has limited its potential for clinical use(2). Novel analogues have been synthesised which lack the chemical moiety responsible for curcumin's rapid clearance, and which may have promise as in vivo inhibitors(3). Aim: To investigate the inhibitory activity of curcumin analogues on Pgp, BCRP and MRP1. Methods: 15 novel analogues were screened by flow cytometry using BCRP- and Pgp-transfected Madin-Darby canine kidney II cells (MDCKII), MRP1 transfected human embryonic kidney cells (HEK293), and the corresponding parental cells. Inhibition was monitored by the intracellular accumulation of fluorescent substrates (rhodamine-123 for Pgp, mitoxantrone (MXN) for BCRP, and calcein-AM for MRP1) after co-incubation with test compound. Identified inhibitors were then tested in cytotoxicity assays to confirm activity. Cells were exposed to cytotoxic ABC transporter substrates in the presence or absence of test compound, or previously reported inhibitors, and IC_{50} of growth inhibition calculated from concentration-response curves. Results: From the flow cytometry assay, A12 [(2E,6E)-2,6-Bis(2,5-dimethoxy-benzylidene)cyclohexanone] and A13 [(2E,6E)-2,6-Bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone] inhibited MXN efflux in MDCKII/BCRP cells with an IC_{50} of 1.2±0.1 µM and 4.3±0.7 µM (± SEM), respectively. A13 also inhibited calcein-AM efflux in HEK/MRP1 cells with an IC_{50} of

11.9±0.7 µM. C10 [(2E,4E)-8-Methyl-2,4-bis(3,4,5-trimethoxybenzylidene)-8-azabicyclo[3.2.1]octan-3-one], inhibited rhodamine-123 efflux in MDCKII/Pgp cells with an IC_{50} of 2.8±0.4 µM. These compounds did not significantly affect parental cells. Cytotoxicity assays confirmed the observed inhibition for A12, A13 and C10. Addition of 2 and 5 µM A12 sensitised MXN resistant MDCKII/BCRP cells, reducing MXN IC_{50} from 10.0±0.7 nM to 5.1±0.4 nM and 1.6±0.2 nM, respectively. The BCRP inhibitor Ko143 (1 µM), reduced the IC_{50} to 1.1±0.2 nM, similar to parental cells (0.9±0.2 nM). A13 also significantly reversed MXN resistance with IC_{50} of 3.4±0.5 nM and 1.9±0.3 nM after addition of 1µM and 5 µM of compound. Low concentrations of C10 (0.5 and 1.0 µM) reversed paclitaxel resistance in MDCKII/Pgp cells with a decrease in IC_{50} from 206.6±11.9 nM to 20.6±2.2 and 7.5±2.0 nM, respectively (compare 1.5±0.9 nM for positive control, 25 µM verapamil). A13 did not affect HEK/MRP1 cells at the concentrations tested (1 and 2µM) and high cytotoxicity precluded increasing concentrations >3 µM. Conclusions: Both cytotoxicity and flow cytometry demonstrated that the curcumin analogues A12, A13 and C10 are able to inhibit the transport activity of ABC transporters and may have promise as MDR reversal agents.

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P272 - ONTOGENY OF HEPATIC UPTAKE TANSPORTERS AT THE FUNCTIONAL LEVEL IN THE DEVELOPING WISTAR RAT

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From birth onward, it is known that various organ systems including those involved in xenobiotic clearance pathways are still in the developing phase. As a consequence, developmental changes in the kidney and liver might cause differences in xenobiotic response between children and adults and even between different pediatric populations. In the liver, elimination occurs in three steps: (i) cellular uptake from the systematic circulation, (ii) metabolic clearance, and (iii) biliary excretion. It has been shown that many drug metabolizing enzymes mature during the first year of life to reach adult activity levels. However, information regarding developmental regulation of hepatic drug transport is presently scarce, but some studies have demonstrated that most hepatic uptake and efflux transporters undergo ontogenic regulation and their protein expression can be detected within the first weeks after birth. The aim of the present study was to profile the functional ontogeny of drug transporters in rat liver. The activities of Ntcp (Na^+-taurocholate co-transporting polypeptide) and transporters belonging to the Oatp (Organic anion transporting polypeptide) family were assessed by measuring uptake of known substrates: ^{3}H-taurocholate (TC, for Ntcp), sodium fluorescein (NaFluo, for Oatps) in freshly isolated hepatocytes from rats aged 2, 3, 4 and 8 weeks. The results obtained show age-dependent activity of these transporters. Relative to adult values, transporter-mediated uptake clearance values in suspended hepatocytes from rats aged 2, 3, 4 weeks reached 40, 72 and 91% for TC, and 14, 12, and 18% for NaFluo, respectively. In conclusion, activities of hepatic drug uptake transporters gradually increase during development, in a transporter-specific manner, and consistent with previous reports on corresponding mRNA levels. Knowledge on the population-specific activity levels of transporters is of great help in understanding pediatric PK which will eventually benefit safe and effective drug therapy in children. Ongoing research involves functional profiling of all relevant drug transporters in rat liver.

P273 - RESVERATROL AND ITS SULFATED CONJUGATES ARE SUBSTRATES OF ORGANIC ANION TRANSPORTING POLYPEPTIDES (OATPS): IMPACT ON RESVERATROL DISPOSITION

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Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a naturally occurring polyphenolic compound found in grapes, wine and medicinal plants with a variety of biological and pharmacological activities. These effects are observed despite its extremely low bioavailability and rapid clearance from the circulation due to extensive phase II metabolism. Whether resveratrol and its metabolites can accumulate to bioactive levels in organs and tissues by active transport mechanisms is not known yet. As OATPs mediate the uptake of endogenous compounds and clinically important drugs in various tissues, thereby affecting drug disposition, we investigated the transport of resveratrol and its three major sulfate conjugates in OATP1B1, OATP1B3 and OATP2B1-expressing Chinese Hamster Ovary (CHO) cells. Cells were cultured in 12 well plates and transport assays were performed using trans-resveratrol, trans-resveratrol-3-O-sulfate, trans-resveratrol-4-O-sulfate, and trans-resveratrol-disulfate at increasing concentrations. Samples were then analyzed by HPLC-MS/MS. For inhibition studies, cells were incubated in presence and absence of the OATP substrates bromosulphthalein and rifampicin. Results revealed 4- to 6-fold higher uptake rates for resveratrol in OATP1B1, OATP1B3 and OATP12B1- transfected CHO cells within 1 min, compared to wild type cells. Net OATP-mediated accumulation rates followed Michaelis-Menten kinetics with Km and Vmax values of 79.77 µM and 1149 pmol/mg protein/min for OATP1B1, 42.94 µM and 653.5 pmol/mg protein/min for OATP1B3, and 168.2 µM and 1216 pmol/mg protein/min for OATP2B1, respectively. Resveratrol-3-O-sulfate was only transported by OATP1B3 (Km: 277.0 µM; Vmax: of 186.8 pmol/mg protein/min), while resveratrol-disulfate was equally transported by OATP1B1 Km: 44.23 µM; Vmax: 100.7 pmol/mg protein/min) and OATP1B3 (Km: 41.4 µM; Vmax: 126.8 pmol/mg protein/min). Resveratrol-4-O-sulfate had a low affinity to all three OATPs as uptake was below detection limit in the tested cell lines. Furthermore, uptake of resveratrol, resveratrol-3-O-sulfate and resveratrol-disulfate was efficiently inhibited by bromsulfphthalein and rifampicin with IC_{50} values below 1 µM. In conclusion, our results elucidate, for the first time, the relevance of OATP1B1 and OATP1B3 as transporters for resveratrol and its sulfates.

P274 - INFLUENCE OF DNA METHYLATION ON TISSUE AND TUMOR SPECIFIC EXPRESSION OF MONOCARBOXYLATE TRANSPORTER 1 (MCT1/SLC16A1) IN HUMAN LIVER AND KIDNEY TISSUE

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The monocarboxylate transporter 1 (MCT1/SLC16A1) is responsible for the H^+-coupled transport of monocarboxylates, e.g. pyruvate or lactate, across the plasma membrane. MCT mediated lactate transport fulfills major physiological functions. Whereas influx of lactate is essential for energy metabolism in tissues where it is used as respiratory fuel or for gluconeogenesis, e.g. in liver and kidney, efflux of lactate is required for pH regulation in order to avoid intracellular acidification. Due to these essential properties, MCT1 seems to be a promising target for anticancer therapy. In this work we investigated MCT1 mRNA expression in a tissue scan, covering 20 different human normal and cancer tissues and in sample sets of human normal liver tissue (n=150) and 64 pairs of normal and tumor kidney tissue by TaqMan quantitative real time PCR. Since DNA methylation is an important epigenetic mechanism for the regulation of gene expression, we examined whether MCT1 mRNA expression is influenced by DNA methylation in the promoter region of the gene SLC16A1 by use of MALDI-TOF MS. In addition, we assessed the relationship of DNA methylation and mRNA expression with non-genetic factors (e.g. age, sex) and with clinicopathological features. The tissue scan revealed that MCT1 mRNA was expressed in liver as well as in kidney tissue with a higher expression level in normal liver than in normal kidney tissue. Comparison of mRNA expression in the kidney sample set showed a highly significant increase in mRNA expression in tumor tissue compared to normal kidney tissue (P<0,0001). According to the mRNA expression levels, the investigation of the DNA methylation pattern in the respective promoter region revealed a lower percentage of methylation in liver compared to normal kidney tissue as well as a significant reduction of methylation in renal tumor tissue compared to normal kidney samples. The MCT1 mRNA expression as well as the promoter DNA methylation pattern was highly variable among individuals in

liver and in kidney tissue. Correlation of mRNA expression with non-genetic factors and clinicopathological features revealed an association of cholestasis with lower MCT1 expression in liver. The interindividual variability of the DNA methylation pattern could not be explained by non-genetic factors or clinicopathological parameters, neither in liver, nor in kidney tissue. From these results we hypothesize that DNA methylation has an influence on tissue specific expression of MCT1. In addition, we conclude that the increased MCT1 mRNA expression in kidney tumor tissue compared to normal kidney tissue is affected considerably by reduced DNA methylation in the promoter region of SLC16A1.

P275 - PET-CT IMAGING WITH [^{18}F]-GEFITINIB TO MEASURE ABCB1A/1B (P-GP) AND ABCG2 (BCRP1) MEDIATED DRUG-DRUG INTERACTIONS AT THE MURINE BLOOD-BRAIN BARRIER

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The ABC (ATP-binding cassette) transporters ABCB1 (P-glycoprotein, P-gp) and ABCG2 (Breast Cancer Resistance Protein, BCRP) are expressed at the blood-brain barrier (BBB), and are involved in limiting the brain penetration of a wide range of drugs. The broad substrate specificity of ABCB1 and ABCG2 can cause transporter-mediated drug-drug interactions (DDIs) in vivo, for example when two drugs compete for efflux by ABCB1 and/or ABCG2. This can lead to increased brain concentrations of drugs in treated patients, potentially resulting in unexpected toxicity. It is therefore important to determine whether a drug may be involved in transporter-mediated DDIs in humans. PET-CT imaging, a quantitative and non-invasive technique, is especially suited for such a purpose. It requires limited numbers of animals for preclinical studies and it can also be used to investigate drug transporter activities in humans. We therefore developed a PET-CT imaging method using the ABCB1 and ABCG2 substrate [^{18}F]-gefitinib to quantitatively measure Abcb1a/1b and Abcg2 mediated DDIs at the murine BBB. For this purpose, we set up an automated synthesis for the PET tracer [^{18}F]-gefitinib and imaged the pharmacokinetics of [^{18}F]-gefitinib (1 mg/kg) after intravenous (iv) administration to wild-type, Abcb1a/1b$^{-/-}$, Abcg2$^{-/-}$ and Abcb1a/1b;Abcg2$^{-/-}$ mice with and without iv co-administration of the ABCB1 and ABCG2 inhibitor elacridar (10 mg/kg). All PET scans were followed by a CT scan to determine the brain and heart regions for quantification and [^{18}F]-gefitinib brain and heart levels were quantified 30-60 min after [^{18}F]-gefitinib administration. To assess the quality of the method, a validation study with the same mouse strains was performed using [^{14}C]-gefitinib (1 mg/kg, iv) with and without co-administration of elacridar (10 mg/kg, iv). PET-CT imaging clearly showed that Abcb1a/1b and Abcg2 together limit the brain penetration of [^{18}F]-gefitinib. The brain/heart ratios of [^{18}F]-gefitinib were 2.1-fold increased in Abcb1a/1b;Abcg2$^{-/-}$ mice, compared to wild-type. In single knockout animals, no differences in brain accumulation of [^{18}F]-gefitinib were found, showing that both transporters can compensate for each others' functions in vivo. Furthermore, co-administration of elacridar led to significantly increased brain levels and brain/heart ratios of [^{18}F]-gefitinib in wild-type, Abcb1a;1b$^{-/-}$ and Abcg2$^{-/-}$ mice, but not in Abcb1a/1b;Abcg2$^{-/-}$ mice. This shows that the effects of elacridar were caused by inhibition of Abcb1a/1b and Abcg2 at the BBB. Highly comparable results were obtained when the brain penetration of [^{14}C]-gefitinib in the presence and absence of elacridar in wild-type, Abcb1a/1b$^{-/-}$, Abcg2$^{-/-}$ and Abcb1a/1b;Abcg2$^{-/-}$ mice was analyzed. We conclude that PET-CT imaging with [^{18}F]-gefitinib is a useful method to non-invasively assess and quantify potential Abcb1a/1b and Abcg2 mediated DDIs in vivo, using limited numbers of animals. Furthermore, this method may in the future be used to assess drug transporter activity in patients before drug treatment with ABCB1 and/or ABCG2 substrates.

P276 - TRANSPORT OF FLAVOPIRIDOL BY OATP1B1, OATP1B3 AND OATP2B1: IMPACT ON CANCER THERAPY

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Flavopiridol is a cyclin-dependent kinase inhibitor in phase II clinical development for treatment of various forms of tumors including breast cancer. Genes known from in vitro and in vivo studies to specifically affect flavopiridol disposition include UGTA1, UGT1A9, MRP2 and ABCG2. Furthermore, a very recent clinical study found an association of the organic anion transporting polypeptide (OATP) 1B1 expression with flavopiridol outcomes. In this

study, we therefore investigate whether other OATPs may also contribute to flavopiridol transport by using OATP1B1, OATP1B3 and OATP2B1- expressing Chinese Hamster Ovary (CHO) cells. Cells were cultured in 12 well plates and transport assays were performed using flavopiridol at increasing concentrations (0-800 µM). Samples were then analysed by HPLC-MS/MS. Uptake of flavopiridol into these cells shows typical Michaelis-Menten kinetics with about 4-fold higher Vmax and decreased Km values for OATP1B3 compared to OATP1B1 (Vmax: 2274.0 pmol/mg protein/min vs. 552.2 pmol/mg protein/min; Km: 147.5 µM vs. 202.3 µM). Uptake of flavopiridol into both cell lines was efficiently inhibited by rifampicin and bromsulphthalein with IC_{50} values below 5 µM. OATP2B1-transfected CHO cells, however, do not transport flavopiridol as uptake rates were in the low range of cells transfected with the empty vector. Uptake of flavopiridol by OATPs seems to be also an important determinant in breast cancer cell lines. The much higher mRNA level for OATP1B1 found in wildtype ZR-75-1 compared to OATP1B1-knock out cells correlated with higher initital uptake rates with up to 2-fold higher Vmax and slightly higher Km values for flavopiridol (Vmax: 2036.0 pmol/mg protein/min vs. 809.4 pmol/mg protein/min; Km: 108.7 µM vs. 83.86 µM). This leads to higher IC_{50} values in the cytotoxicity experiments. In conclusion, our results revealed OATP1B1 and OATP1B3 as high affinity flavopiridol transporters, supporting a major role for these polypetides in the disposition of flavopiridol during therapy.

P277 - IN VITRO AND IN SILICO SCREENING OF OATP1B1 AND OATP1B3 MODULATORS

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The organic anion transporting polypeptides OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3) are uptake transporters predominantly expressed on the basolateral membrane of human hepatocytes. The importance of these transporters has been illustrated in several recent studies showing that OATP1B inhibition or induction can be the underlying mechanism of clinically relevant drug-drug interactions. This study aimed to develop a high-throughput in vitro assay and in silico proteochemometric model to identify OATP1B modulators. In the present study, 2000 molecules were tested in vitro as potential modulators of the uptake of the OATP1B substrate, sodium fluorescein, in OATP1B1 or 1B3-transfected Chinese Hamster Ovary cells. At an equimolar substrate-inhibitor concentration of 10 µM, 241 and 141 molecules were identified as OATP1B1 and OATP1B3 inhibitors (min 50 % inhibition), respectively. Subsequently, a training set (80 % of the compound library) was randomly selected to develop a proteochemometric-based in silico model. Two models were created that predicted OATP1B inhibitors in the test group (the remaining 20 % of the compound library) with high specificity (98 %) and high sensitivity (82 %). In addition, substructures related to OATP1B1/1B3 inhibition or inactivity were identified. Our results demonstrate that the integrated in vitro and in silico approach is useful to identify and predict interactions with OATP1B1 and OATP1B3.

P278 - CRYOPRESERVED HUMAN HEPATOCYTES – OPTIMIZATION OF UPTAKE ASSAYS

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Freshly isolated or cryopreserved human hepatocytes are often used as an in vitro model to assess transporter uptake activity by the liver. Hepatocytes in suspension are incubated with known (radiolabeled) uptake substrates and a test article (TA) to assess inhibition of uptake, or with a TA alone to assess uptake potential. Cells may be separated from the assay buffer by the traditional oil spin method, after which the amount of substrate or TA present in the cells is measured. The traditional oil spin method relies on a thin tube, typically with a volume of 0.4 µl. The tube is filled with 2M NaOH and a layer of oil on top of that. Hepatocytes are incubated for a short time (30 seconds – 180 seconds) with a probe substrate and with or without TA to assess inhibition potential of a TA. Active transport of the TA may be measured in a time or dose dependant manner. The hepatocyte assay mix is loaded on top of the oil layer, and the cells are separated from the assay mix by centrifugation. The hepatocytes migrate through the oil layer into the NaOH and get lysed. Subsequently the tube is frozen and the lower part is cut off and subjected to liquid scintillation counting. In the current study the traditional oil spin separation method was

compared with a method relying on separation of hepatocytes from assay mix by means of filtration. The new method uses a filter plate to separate and wash hepatocytes. This method allows for several rinses. Scintillation cocktail is added to lyse cells for liquid scintillation counting. The advantage of the filter plate method is that it allows for higher throughput and is less laborious and difficult to handle. The assays are optimized for incubation times and wash protocols. We used tritiated substrates to measure specific transporter activities: taurocholate for NTCP, MPP+ for OCT1 and E3S as well as E2-17bG for OATPs. Results are reproducible by both methods. Our data shows that the oil spin method does not directly correlate with the filter plate method; however, the rank order is maintained. The standard deviation (n=3) is low, for all substrates with the exception of E3S. Overall separation of human hepatocytes in the suspension uptake assay by the filter plate method seems to be a feasible alternative with advantageous features over the traditional oil filtration method.

P279 - ROLE OF EFFLUX TRANSPORTERS MDR1 AND BCRP IN RIVAROXABAN DRUG DISPOSITION

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Thromboembolic events resulting from blood clotting disorders are a significant source of mortality and morbidity, often necessitating life-long oral anticoagulation (OA) therapy. Although warfarin has been the mainstay of therapy for the past 60 years, the unpredictable response and laborious anticoagulation monitoring has meant that new OAs that do not require such monitoring could potentially supplant warfarin use. Rivaroxaban is a novel factor 10a inhibitor, where hepatic CYP metabolism as well as renal clearances pathways account for its overall disposition. Importantly, the extent of renal clearance exceeds that based on glomerular filtration rate, thus, we hypothesized that the efflux drug transporters, P-glycoprotein (MDR1) and breast-cancer resistance protein (BCRP), are involved in rivaroxaban excretion and disposition. The ability of MDR1 and BCRP to mediate rivaroxaban transport in vitro was assessed in Caco-2 cells in presence or absence of a specific MDR1 (LY335979), BCRP (fumitremorgan C) inhibitor or both. To determine the in vivo relevance of MDR1 and BCRP to rivaroxaban disposition, plasma and tissue concentrations were determined in Mdr1a deficient ($Mdr1a^{def}$), $Bcrp^{-/-}$ knockout, and Mdr1a/Mdr1b/$Bcrp^{-/-}$ triple knockout (TKO) mice following oral administration. Rivaroxaban concentrations were measured by liquid chromatography tandem-mass spectrometry. A significantly greater vectorial transport of rivaroxaban was observed in the basolateral to apical direction (B-A) compared to A-B in Caco-2 cells, which was attenuated in the presence of selective inhibitors. Following oral administration of rivaroxaban in vivo, plasma concentrations did not significantly differ between wild-type and $Mdr1a^{def}$, $Bcrp^{-/-}$, or TKO mice (n=6/group). Renal accumulation was marginally higher in $Mdr1a^{def}$, $Bcrp^{-/-}$, and TKO mice compared to wild-type. Interestingly, rivaroxaban brain exposure was not increased in $Mdr1a^{def}$ or $Bcrp^{-/-}$ mice, but was significantly elevated in TKO mice. In conclusion, rivaroxaban is a shared substrate of both MDR1 and BCRP transporters. In vivo, both transporters appear to function synergistically to limit brain penetration of rivaroxaban. Further studies are required to elucidate the influence of genetic variation in MDR1/BCRP to rivaroxaban disposition and the potential impact of drug-drug interaction(s) involving these two efflux transporters.

P280 - CROSS SPECIES CHARACTERISATION OF DRUG TRANSPORTERS IN THE INTESTINE: IMPLICATIONS FOR DRUG UPTAKE

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The understanding of a drugs action in humans is essential for its safe and efficacious use. Drugs are tested both in vitro and using model species in vivo. Significant variability between these systems and human has been demonstrated; these differences must be understood to allow correct prediction of drug effects in humans. Increasingly, initial drug screening is undertaken in silico, through the development of predictive models. Such models offer increasing opportunities to make accurate predictions, but also to 'mine' archive data, increasing our knowledge of basic biological networks, and thus direct future research. Much work is currently being undertaken to increase the robustness of in silico extrapolation of oral uptake data from pre-clinical test species to humans: In particular, the inclusion of active transport systems in PBPK models has come under close scrutiny[1]. We have examined the expression of a range of drug transport proteins at the transcript and protein level, in rat, dog and human intestine. Differences in both the absolute expression of each transporter was observed between species per

region (jejunum, ileum and colon), as well as differences in the overall expression profile down the intestine. To determine how these differences in expression may influence drug uptake we have developed a deterministic model for the uptake of the tracer dye rhodamine 123[2] into the intestinal epitheilium. The model is populated a combination of archive kinetic data, de novo expression data or parameters fitted within biologically plausible bounds. Simulations with this model predict two important potential confounders in the prediction of drug uptake: Firstly, cross species differences in transporter expression may lead to significant underestimation of intestinal drug absorption in the human situation compared to the pre-clinical species rat and dog. Secondly, the interindividual expression of drug transporters within a small set of human samples (n=13), produces far less variability in the predicted uptake compared to that introduced during cross-species extrapolation.

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P281 - AN INTEGRATED APPROACH TO ASSESS RISK OF DRUG INDUCED LIVER INJURY POSED BY BSEP INHIBITION

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Inhibition of the activity of biliary efflux transporters such as the bile salt export pump (BSEP) has been implicated as one of the mechanisms by which drugs can initiate drug-induced liver injury (DILI), a major cause of drug attrition and serious ill health in humans. We have used a range of in silico, in vitro and in vivo models to investigate the potential of pharmaceuticals to interfere with BSEP function and to develop an integrated assessment of DILI risk posed by candidate pharmaceuticals applicable to the drug development process. Inhibition of human BSEP mediated uptake of [^{3}H]-taurocholate was determined in vitro in membrane vesicles. In a study of 624 drugs, physicochemical properties such as molecular weight and lipophilicity were found to be strong determinants of a drug's ability to impair BSEP function. A predictive BSEP inhibition in silico model was developed, using a range of molecule descriptors and non-linear machine learning methods, which classified BSEP inhibitors with 87% accuracy. A comparison between in vitro BSEP inhibition potencies of 85 pharmaceuticals and whether they caused DILI in human (classified into cholestatic/mixed vs. hepatocellular vs. no DILI) revealed that potency (35.3 vs 158.5 vs. 158.5 µM) and frequency (57 vs. 18 vs. 24%) of BSEP inhibition were higher for drugs that cause cholestatic/mixed DILI, indicating that BSEP inhibition is correlated with cholestatic DILI risk (1). Several BSEP inhibitors caused a dose- or exposure-dependent, transient increase in plasma bile acid levels after single oral doses to rats demonstrating functional interference with bile salt excretion in vivo: glibenclamide (5-500 mg/kg), cyclosporine A (15-40 mg/kg) and a chemokine receptor antagonist (CKA; 20-2000 mg/kg; 1-(4-Chloro-3-trifluoromethyl-benzyl)-5-hydroxy-1-H-indole-2-carboxylic acid). Immunohistochemical staining of liver sections revealed increased canalicular expression of BSEP and MRP2 after repeat oral dosing of rats with cyclosporine A (14 days) or CKA (5 days). Single oral doses of CKA also caused a marked and dose-dependent effect on biliary clearance parameters of the magnetic resonance imaging contrast agent gadoxetate in vivo, which could be quantified using a nonlinear single compartment model (2). We conclude that in silico and in vitro BSEP inhibition models enable early identification of compounds with high propensity to cause DILI in humans, providing the potential opportunity for early deselection of such compounds from development. When further progression of compounds that inhibit transporters in vitro is considered warranted, quantification of plasma bile acid levels and/or hepatic gadoxetate clearance can support in vivo hazard identification and risk assessment.

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P282 - IDENTIFICATION OF A PROLACTIN-RESPONSIVE STAT5 BINDING ELEMENT IN THE 5'-FLANKING REGION OF THE HUMAN BCRP/ABCG2 GENE

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The breast cancer resistance protein, BCRP/ABCG2, is a drug transporter that is associated with multidrug resistance in cancer cells. It is now known that BCRP is also expressed in various normal tissues including normal mammary gland. In particular, BCRP is upregulated in the lactating mammary gland where it contributes to the transfer of drugs, nutrients and toxins into breast milk. To date, it is unclear what upregulates BCRP in the lactating mammary gland. Given the importance of the hormone prolactin in lactogenesis, we sought to determine the role of prolactin in the regulation of BCRP. We found that prolactin dose-dependently induced BCRP mRNA and protein in human T-47D breast cancer epithelial cells. RNA interference-mediated knockdown or small molecule inhibition of STAT5, a key transcription factor activated in prolactin signalling, attenuated the inductive effects of prolactin on BCRP expression. In silico examination of the 10kbp 5'-flanking region of the ABCG2 gene for consensus STAT5 binding DNA sequences called GAS elements revealed a putative distal and proximal site at -4459 and -434, respectively. Using ChIP, we observed STAT5 recruitment to the proximal GAS element but not the distal GAS element after prolactin treatment, the effect of which is dependent on time, STAT5 expression and activation. Next, a series of experiments were performed using a luciferase reporter construct driven by the ABCG2 gene promoter and 5'-flanking region that contain the putative proximal GAS element and surrounding regions. Reporter activity was induced upon prolactin exposure and addition of the distal GAS element to this construct did not further enhance reporter activity. In contrast, single mutation to the putative proximal GAS element significantly reduced reporter activity. To further confirm that the proximal GAS element is functional, we created luciferase reporter constructs with various tandem repeats of the proximal GAS element. These constructs were highly responsive to prolactin treatment showing both copy number and prolactin-dose dependency. Furthermore, co-transfection with a rat Stat5a expression construct synergistically enhanced prolactin-induced reporter activity. To determine if the MAPK and PI3K pathways that are also activated in prolactin signalling modulate the prolactin-BCRP response, we systematically inhibited each pathway using small molecule inhibitors. PD98059 and U0126 inhibition of MAPK signalling, and LY294002 and wortmannin inhibition of PI3K signalling attenuated prolactin-induced BCRP expression. However, these pathway specific inhibitors did not affect STAT5 recruitment, suggesting that a complex interplay between multiple signalling pathways mediate the prolactin-BCRP response. Taken together, our results demonstrate that prolactin induces BCRP expression in T-47D cells, in part, by recruitment of STAT5 to a proximal GAS element in the 5'-flanking region of the ABCG2 gene. This project is supported by a grant and graduate scholarship from CIHR.

P283 - THE INTERACTION BETWEEN TETRAHYDROPALMATINE ENANTIOMERS AND DRUG TRANSPORTERS

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Tetrahydropalmatine (THP), with one chiral center, is one of the major constituents isolated from traditional Chinese medicine Rhizoma corydalis. It has been reported that THP possess various pharmacological actions, including analgesic, sedative-tranquilizing hypnotic, cardiac protective, antihypertensive and antioxidant actions. It is well known that ATP-binding cassette (ABC) and solute carrier (SLC) transporters play important roles in drug disposition and drug-drug interaction. Since THP is widely applied in clinical practice, it is necessary to investigate the interaction between THP and transporters. In the present study, the effect of THP on activity of P-glycoprotein (P-gp), multidrug resistance protein 1 (MRP1), breast cancer resistance protein (BCRP) and organic cation transporter 1 (OCT1) were studied in corresponding transfected cell lines. The intracellular accumulation and bidirectional transport assays suggested THP enantiomers were inhibitors of P-gp and OCT1, but not inhibitors of MRP1 and BCRP. The half

maximal inhibitory concentration (IC_{50}) values of (-)-THP and (+)-THP on rhodamine 123 efflux were 48.6 and 20.0 μM, respectively. The IC_{50} values of (-)-THP and (+)-THP were 7.56 and 13.5 μM on OCT1 substrate 1-methyl-4-phenylpyridinium uptake, 3.36 and 8.08 μM on metformin uptake, respectively. The results indicated THP enantiomers were moderate inhibitors of P-gp and strong inhibitors of OCT1. Otherwise, the intracellular accumulation and bidirectional transport of THP enantiomers showed that both THP enantiomers were not substrates of P-gp and OCT1, which suggested the inhibition caused by THP enantiomers was not competitive. The Western blot and Real-time RT-PCR assays showed that, THP enantiomers reduced the protein expression level of P-gp, but did not affect the mRNA expression level of P-gp, which suggested THP enantiomers may regulate the P-gp expression in the post-transcriptional or translational level. Since P-gp does not only play an important role in drug disposition, but also contributes a lot to multidrug resistance (MDR) in tumor cells. To examine the effect of THP enantiomers on P-gp mediated MDR, the in vitro cytotoxicity test was carried out. When treated with THP enantiomers, the P-gp mediated MDR tumor cells, K562/ADR were re-sensitized to anticancer drug doxorubicin. Co-incubated with 40 μM (-)-THP and (+)-THP, the IC_{50} values of doxorubicin in K562/ADR decreased from 8.69 μM to 4.45 and 3.98μM, respectively. The present study suggested the possible drug-drug interaction between THP enantiomers and P-gp/OCT1 substrates, which may raise the risk of adverse drug reaction or decrease the efficacy of some drugs. Meanwhile, as an analgesic and MDR reversal agent, THP may also be helpful in chemotherapy of cancer.

P284 - NON RADIO LABELED METHOD APPLIED TO OATP1B1 TRANSPORTER STUDYING

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Liver was one of the main metabolic and excretory organs for drug, so it was meaningful to research the mechanism of drug absorption and excretion in liver. Liver transporter was the functional protein expressed on the surface of hepatocyte membrane. It was one of the most important approaches for drug to pass in and out of hepatocytes. Host cell such as S2, HEK293,CHO and MDCK could be used to build cell line expressing liver transporter proteins. Evaluation research in vitro and liver metabolism mechanism for drug could be investigated with cell line expressing liver transporter proteins. Through researching the affinity relationship between drug and liver transporter, liver absorption and bile excretory for drug could be predicted. Drug transporter inhibition test could be used to explore drug-drug interaction(DDI) which may lead to side reaction. OATP1B1 is a typical representative of the organic anion transporting polypeptide families (OATPs), and it's also one of the hot spots of transporter research. OATP1B1 is expressed in liver cells selectively. The OATP1B1 substrates contain statins, repaglinide, estrone sulfate, estradiol-17-D-glucuronide, olmesartan, bilirubin, valsartan and so on[1]. OATP1B1 has a very broad coverage of the transporter substrates, and this is also the explanation why liver is one of the main organs of drug metabolism. As we know, combined medication may lead to serious mutual inhibition between drugs and lead to hepatotoxicity. So drug-drug interaction has been recognized as one of the most important researching directions. Although, radio labeled method could be used to study drug-drug interaction and non-radio labeled method for transporter studying is really difficult because lower limit of quantification of non-radio labeled method is not enough for analyzing. However, our experiments validated that chromatography/tandem mass spectrometry (LC/MS/MS) also could be used to investigate drug-drug interaction. A new LC/MS/MS method was established for OATP1B1 substrate rosuvastatin quantification. And the lower limit of quantification could reach 0.5ng/ml which was good enough for studying drug-drug interaction between target drugs and rosuvastatin. This method could be used more widely in the future for transporter function validation and transporter substrates screening.

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P285 - STUDY ON ABSORPTION AND TRANSPORT MECHANISMS OF B. SERRATA EXTRACTS

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The content of activated pharmaceutical ingredients can be absorbed in intestine of human, it is a major factor, deciding whether the medicine can play biological activity or not. Nowadays, Caco-2 and MDCK cell models are used widely to study drug intestinal absorption process in vitro experiments [1-2]. Caco-2 cell line derived from human colon adenocarcinoma by Fogh. et al in 1977 [3]. MDCK cell line is derived from Madin-Darby canine kidney, and transfected with mammalian expression vector containing human MDR1 gene to get expression of MDR1 protein. Because of the culture cycle of MDCK is short than Caco2 cells, it appears in drug absorption studies as Caco-2 cell alternative model. AKBA is extracts from B. serrate, Caco-2 cell and MDCK-MDR1 cell models were used to study the mechanisms of absorption and transport. **Methods:** The apparent permeability coefficient (P_{app}) of AKBA in Caco-2 and MDCK-MDR1 cell monolayers from apical side(AP) to basolateral side(BL) or from BL to AP was evaluated. The concentration of AKBA was measured by LC-MS/MS . **Results:** In Caco-2 cell model, P_{app} (AP-BL) was 0.79 10^{-6}cm/s; In MDCK-MDR1 cell model, P_{app} (AP-BL) was 0.26 10^{-6}cm/s;and P_{app} (BL-AP)/ P_{app} (AP-BL) in Caco-2 and MDCK-MDR1 cell monolayers all smaller than two. **Conclusion:** B. serrata extracts AKBA was not the substrate of P-gp; and it's absorption rate was low; AKBA was absorpted through active transport absorption and passive diffusion possiblely.

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P286 - ESTABLISHMENT OF NOVEL CONDITIONALLY IMMORTALIZED HUMAN ASTROCYTES: A PROMISING TOOL FOR DEVELOPMENT OF A NEW IN VITRO CO-CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

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The blood-brain barrier (BBB) restricts influx of a variety of drugs into the brain parenchyma, which makes it difficult to predict BBB permeability of drugs based on their physicochemical properties. It is believed likely that application of in vitro BBB models will provide more accurate prediction for BBB permeability of drugs in vivo. In in vitro BBB models, the principal cellular constituents are brain microvascular endothelial cells (BMEC), but astrocytes are also crucial because they release various soluble factors, including basic fibroblast growth factor (bFGF), to support BMEC's functions. Accordingly, several studies have reported that barrier property of co-cultured models with BMEC and astrocytes is higher than that of BMEC mono-culture models. Although primary human BMEC and astrocytes have been used to develop such in vitro BBB models, the cells have several limitations in their use, such as finite proliferation ability. To solve the problems, we previously established conditionally immortalized human BMEC cell line, termed HBMEC/ciβ, which shows long-term proliferation ability and representative BBB functions. In the present study, to obtain human astrocytes-derived cells useful for development of in vitro BBB models, we aimed to establish novel conditionally immortalized human astrocytes (ciASTR), possessing both high proliferation ability and functionality. ciASTR were obtained by transducing temperature-sensitive simian virus 40 large tumor-antigen gene into human primary astrocytes. ciASTR exhibited the morphology similar to that of human primary astrocytes. The cells showed high and long-term proliferation ability at 33°C. The results of reverse transcription-polymerase chain reaction showed that ciASTR expressed many mRNAs of astrocytes-enriched genes, such as bFGF, excitatory amino acid transporter 2, and glucose transporter 1. In addition, ciASTR showed equilibrative nucleoside transporter 1

mRNA expression along with sodium-independent adenosine uptake activity (150 pmol/mg protein/min), which is known to be one of representative functional markers of astrocytes. In conclusion, by cell immortalization technique, we obtained ciASTR that possess infinite proliferation ability as well as astrocytes-specific features. To obtain initial clues to their usefulness in development of the new in vitro human BBB model in collaboration with HBMEC/ciβ, we are currently investigating expression of a series of soluble factors in addition to bFGF and their effects on BBB gene expressions in HBMEC/ciβ.

P287 - LOCALIZATION AND EXPRESSION PATTERN OF ATP-BINDING CASSETTE DRUG TRANSPORTERS IN NORMAL AND DIABETIC HUMAN PLACENTA

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Human placenta plays a role of semi-permeable barrier protecting the fetus from exposure to xenobiotics, including drugs, and endogenous metabolites present in the maternal blood. Placental disposition of these compounds depends on many factors, including the expression and activity of protein transporters. ATP-binding cassette (ABC) drug transporters protect placental and fetal tissues by the efflux of exogenous compounds. Changes in placental ABC transporter expressions observed in some disorders may result in clinical implications and influence the effectiveness of pharmacotherapy during pregnancy. In this study, placentas from uncomplicated term and diabetic pregnancies were obtained immediately after vaginal or abdominal deliveries. After the delivery, sections through the central part of each placenta were made, and tissues were fixed in buffered formalin, embedded in paraffin and cut into histological slices. Localizations of ATP-binding cassette drug transporters: P-glycoprotein (Pgp, ABCB1), multidrug resistance protein 3 (MDR3, ABCB4), multidrug resistance-associated protein 1 (MRP1, ABCC1) and breast cancer resistance protein (BCRP, ABCG2) in the placental tissues, were detected immunohistochemically and their expression was quantified by densitometry (Image ProPlus program). The expression of ABC transporters was found both in the fetal and maternal part of human placenta, especially in fetal syncytiotrophoblast and capillary epithelium. Pgp and BCRP were expressed at the apical surface of syncytiotrophoblast. MDR3 and MRP1 were localized predominantly in the basolateral membranes of syncytiotrophoblast and in fetal capillary endothelium. In placentas taken from diabetic women the changes in expression and, and in smaller degree, in immunolocalization of investigated transporters were observed. These changes potentially can be connected with differences in the transport and metabolism rate of drugs, and possibilities of side effects in diabetic pregnancies, comparing to normal ones.

P288 - CLASSIFICATION OF INHIBITORS OF LIVER SPECIFIC OATPS – INFLUENCE OF PROTEIN EXPRESSION ON DRUG-DRUG INTERACTIONS

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The hepatic organic anion transporting polypeptides (OATP) 1B1, 1B3 and 2B1 influence the pharmacokinetics of drug classes like statins and angiotensin II receptor antagonists and are involved in many clinical drug-drug interactions (DDIs). Predicting potential interactions with OATPs during drug discovery is, therefore, of value. Here, we developed in vitro and in silico models for the identification and prediction of specific and general inhibitors of the OATPs. The maximal transport activity (MTA) of each transporter in the human liver was predicted from transport kinetics and protein quantification in vitro and in vivo. We used this in vitro to in vivo extrapolation to quantify the effects of a subset of inhibitors on atorvastatin uptake in vivo. Using a dataset of 225 drug-like compounds, 91 OATP inhibitors were identified, of which 38 were novel inhibitors. The in silico model identified lipophilicity and polar surface area as key molecular features of OATP inhibition. Predictions of MTA of each OATP identified OATP1B1 and OATP1B3 as the major contributors to atorvastatin uptake in vivo. The relative contributions of OATPs to overall hepatic uptake were, however, changed when inhibitors with different isoform specificities were used in the DDI predictions.

P289 - VARIABILITY IN OATP PROTEIN EXPRESSION AND INFLUENCE ON ATORVASTATIN UPTAKE AND DRUG-DRUG INTERACTIONS

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In the liver organic anion transporting polypeptides (OATP) 1B1, 1B3 and 2B1 are expressed in the sinusoidal membrane of the hepatocyte mediating uptake of drugs from the blood into the cells. These transport proteins are also known to be involved in many clinical drug-drug interactions. In general, OATP1B1 is considered as the major OATP transporter expressed in the liver and as the major OATP of clinical significance. In a previous study we have shown that there are large interindividual differences in the membrane protein expression levels of these transporters in human liver. Here we have, together with transport kinetics and protein quantification in vitro, utilized the quantitative expression levels of OATP1B1, OATP1B3 and OATP2B1 in twelve human livers for prediction of maximal transport activity (MTA) of each transporter and for each individual donor. Together with IC_{50} determinations of 15 potential OATP inhibitors, the predicted MTAs were subsequently used for extrapolation of the inhibitory effect on the OATP-mediated uptake of atorvastatin in vivo. Indeed, the results showed that both the relative and absolute contribution of each OATP transporter to atorvastatin uptake differed between the individuals, in some cases as much as 30-fold. In addition, the predictions showed that if atorvastatin mediated uptake was greatly dependent on one transporter the effects of DDIs can be further enlarged in presence of an inhibitor. These results show that even if OATP1B1 in most cases is the major atorvastatin OATP uptake transporter, for some individuals the contribution of OATP1B3 is greatly increased. Hence, the clinical significance of especially OATP1B3 but also OATP2B1 might need to be reconsidered. Further studies are needed to elucidate how these differences in protein expression levels affect the uptake of other substrates than atorvastatin and if these differences are connected to OATP polymorphisms.

P290 - TESTING COMPOUNDS RECOMMENDED BY FDA FOR P-GP AND BCRP INTERACTION USING MEMBRANE ASSAYS

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The 1st FDA Critical Path Transporter Workshop (Bethesda, October 2-3, 2008) identified two efflux transporters P-gp (ABCB1/MDR1) and BCRP (ABCG2/MXR) as the ones playing the most critical role in ADME properties of drugs. Therefore, we tested the substrates and inhibitors defined by the FDA Draft Guidance on transporters (September, 2006) for these two transporters in ATPase and vesicular transport assays. These membrane assays are high-throughput methods that correlate well with the monolayer efflux assays currently accepted as gold standard for classifying compounds as substrates, inhibitors. Seven compounds classified in the FDA list as P-gp substrates and 10 compounds classified as P-gp inhibitors were tested in the ATPase assay using both the activation and inhibition protocol. Compounds were also tested for inhibition of ^{3}H-N-methyl-quinidine (NMQ) transport in a vesicular transport assay. All compounds classified as substrates activated the ATPase. Interestingly 6 of the compounds classified as inhibitors also activated the ATPase. Some of them (e.g. verapamil, ketoconazole, itraconazole) have high passive permeability and the effect of the transporter interaction is not seen in a bidirectional MDCKIIMDR1 monolayer efflux assay.[1] Verapamil also tested in another study[2] was classified as a transported substrate. Three of the low-to-intermediate passive permeability compounds ritonavir, erythromycin, quinidine also activated the ATPase. These compounds have been shown as transported substrates in monolayer efflux studies.[1,3] Cyclosporine A is the only exception that has been classified as a transported substrate[1] but failed to activate the ATPase. The ATPase inhibition experiments were only conclusive for compounds that have not activated the ATPase. All the tested compounds inhibited the NMQ transport with a ranking of IC50s similar to what was observed in ATPase EC50s (activators) and/or IC50s (inhibitors). We have received similar correlations between ATPase and VT assay results with BCRP with the notable exception that none of the compounds listed as inhibitors by the FDA activated the BCRP ATPase. This may be attributed to the fact that all the substrates listed were of low passive permeability, therefore, could be unambiguously classified as substrates in a monolayer efflux assay. Our data show that most of the compounds currently listed as P-gp inhibitors display substrate features in various in vitro and in vivo experimental systems. Therefore, the FDA classification for this group should be reviewed/reevaluated.

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P291 - THE HUMAN ORGANIC CATION TRANSPORTER 1 GENE TRANSACTIVATION BY GLUCOCORTICOID RECEPTOR

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The human organic cation transporter 1 (OCT1, SLC22A1), one of three isoforms of membrane-potential dependent OCTs (OCT1-3, SLC22A1-3) is expressed predominantly in hepatic basolateral membrane. OCT1 transports various lipophilic organic cations and thus play important role in xenobiotic disposition and elimination, including clinically used drugs. OCT1 gene is transactivated by HNF4alpha, a key regulator of hepatic transport and metabolism of bile acids and drugs. However, little is still known about OCT1 gene regulation. By computer analysis, we identified putative glucocorticoid response elements in the OCT1 promoter. We also analyzed other transcription factors PGC1alpha and C/EBPbeta whether they are included in OCT1 gene transactivation. Methods: Effect of dexamethasone on the OCT1, PGC1alpha and C/EBPbeta mRNA expression and effects of transcription factors HNF4alpha, PGC1alpha and C/EBPbeta on OCT1 mRNA expression were studied using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) in primary human hepatocytes. Effect of glucocorticoids on OCT1 transporter activity was studied using accumulation study with 3H-MPP+ as substrate in primary human hepatocytes. Results: We found that dexamethasone up-regulated OCT1 mRNA level in primary human hepatocytes maintained in dexamethasone-free medium, whereas glucocorticoid receptor antagonist RU486 downregulated OCT1 mRNA level in primary human hepatocytes maintained in standard medium, suggesting involvement of glucocorticoid receptor in OCT1 gene regulation. On the other hand, accumulation study using 3H-MPP+ as a substrate did not show any difference in OCT1 activity in primary human hepatocytes maintained in standard medium and treated with RU486 or vehicle control. Conclusion: Our results suggest that OCT1 gene expression is transactivated by glucocorticoid receptor at mRNA level. However this hypothesis was not confirmed in 3H-MPP accumulation study, treatment with glucocorticoid receptor antagonist RU486 did not suppress OCT1 activity in primary human hepatocytes. This work was supported by GACR P303/12/6163.

P292 - DEVELOPMENT OF AN EXCIPIENT-TRANSPORTER INTERACTION ASSAY SYSTEM FOR THE HUMAN ABCB1 AND ABCG2 TRANSPORTERS IN CACO-2 MONOLAYERS

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It has been reported that excipients can interact with the major efflux transporters in the gastrointestinal tract and therefore increase the permeability of transporter substrates.[1,2,3] To detect transporter specific effects of excipients an in vitro experimental setup was designed based on the Caco-2 monolayer system, focusing on two apical efflux transporters ABCB1 (P-glycoprotein, MDR1) and ABCG2 (BCRP, MXR) which are held to play an important role on drug disposition. Model Excipients (TWEEN 80, Cremophor EL, Vitamin E TPGS) with reported data on transporter function were selected based upon literature data.[2,3,4] Initial concentrations were set to their reported Critical Micelle Forming concentrations in aqueous solutions.[4] Specific probe substrates for the two major efflux transporters (ABCB1 and ABCG2) were selected. Digoxin is a well known ABCB1 reference substrate in in vitro transport experiments. Estrone-3-sulfate is efficiently transported by ABCG2 but is not a substrate of ABCB1, therefore a suitable ABCG2 selective probe in systems like Caco-2 where both transporters are present. Specific small molecule inhibitors (PSC833 of ABCB1 and Ko143 for ABCG2) were also used to demonstrate the transporter specific inhibitory effect. Passive permeability controls with no known transporter interactions (Antipyrine and Lucifer Yellow) were applied to serve as an interim quality control and to ensure that the inactive component in detected transport values is within normal range. Na-Caprate a known permeation enhancer was applied as a positive control for paracellular transport. All Excipients were able to increase the A-B transport and decrease the B-

A transport of Digoxin, markedly reducing the efflux ratio. All Excipients were able to significantly reduce B-A permeability of Estrone-3-sulfate, but have a more modest effect on A-B permeability. Results generated however show that the excipients do not show real selectivity between ABCB1 and ABCG2 as all compounds were able to cross inhibit the other transporter as well. Transporter specificity have to be covered with using transporter specific probe substrates and small molecule inhibitors, or transfected cell lines expressing one specific transporter in future testing of excipients. The observed effect of model excipients on the permeability of passive permeability controls corresponded with previous data and expectations. This experimental setup can serve as a reliable in vitro platform for testing effect of excipients on transporters and passive permeation.

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P293 - TRANSPORT FUNCTION AND PH DEPENDANT TRANSPORT OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 2B1 TRANSCRIPTIONAL START SITE VARIANTS

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The human Organic Anion Transporting Polypeptide 2B1 (OATP2B1) is a membrane transporter that facilitates the cellular uptake of a number of endogenous compounds and drugs. OATP2B1 is expressed in several tissues including the small intestine, liver, kidney and skeletal muscle. Recently, it has been shown that differential promoter usage in tissues results in the expression of several OATP2B1 splice variants which utilize 5 distinct first exons and promoters but share common subsequent exons. These splice variations are expected to encode either a full length or truncated protein missing 22 amino acids from the N-terminus. Since little is known about OATP2B1 splice variants we investigated the transport function and pH dependent transport of the truncated OATP2B1 variant. Using adenovirus overexpression in cultured cells, we compared the transport kinetics (Vmax and Km) of the two forms of OATP2B1. Importantly, we demonstrate that the truncated variant was capable of transporting the known OATP2B1 substrates, estrone sulfate and rosuvastatin. We compared the pH dependent transport of estrone sulfate and rosuvastatin with the full length and truncated version of OATP2B1. Both the full length and the truncated OATP2B1 variant show pH dependent transport for rosuvastatin while transport of estrone sulfate is not pH dependent. These findings indicate that differential regulation of OATP2B1 splice variant expression in tissues could contribute to variation in drug response.

P294 - ENDOCRINE-DISRUPTING CHEMICALS MIGHT ACT AS MALE TOXICANTS THROUGH INTERACTION WITH THE EFFLUX PUMPS IN THE BLOOD-TESTIS BARRIER

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Endocrine-disrupting chemicals (EDCs) are exogenous agents that interfere with steroid hormone function. In males, the testis is highly susceptible for EDC-induced toxicity. Efflux transporters expressed in the blood-testis barrier might influence the exposure to chemicals. Among these are the ABC transporters, P-glycoprotein (P-gp/ABCB1), breast cancer resistance protein (BCRP/ABCG2) and multidrug resistance protein 1 (MRP1/ABCC1). Here, we hypothesized that these efflux transporters are key determinants in EDC-induced testis toxicity. The selected EDCs are widely used in the production of plastics and/or flame retardants and include bisphenol A (BPA),

tetrabromobisphenol A, bis(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). Their interaction with human P-gp, BCRP and MRP1 transport activity was tested in membrane vesicles of HEK293 cells, overexpressing the transporters. Direct transport of the EDCs was assessed by an accumulation assay, exposing transporter-overexpressing MDCKII cells with or without the presence of transporter inhibitors. Also, the effects of these EDCs on androgen production and CYP17 and CYP19 expression and activity were determined in mouse Leydig MA10 cells, mouse Sertoli TM4 cells and human H295R adrenocorticocarcinoma cells. Our data show that the EDCs decrease the production of testosterone and/or DHEA in MA10 cells, probably via interference with CYP17 and CYP19 activity, two key enzymes in sex steroidogenesis. Besides, we show that BPA, TBBPA, PFOA and PFOS inhibit P-gp, BCRP and/or MRP1 activity and that BPA is transported by BCRP, indicating that efflux transporters present in the blood-testis barrier are potential determinants in endocrine disruption.

P295 - EVALUATION OF IN VITRO TO IN VIVO CORRELATION OF TRANSPORTER INHIBITION : RANK ORDER APPROACH

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For the last years, there has been a growing interest for transporters, principally involved in drugs and xenobiotics' ADME, which would be responsible of an increasing number of drug drug interactions (DDIs). DDIs remain a key problem for drug discovery and development drugs nowadays. Indeed, transporters are proteins playing a major role in absorption, distribution and elimination of drugs in the organism. Transport of molecules is a saturable process and regarding the diversity of substrates that can be transported by the same transporter, a significant number of drug interactions can take place and lead to a modification of the disposition of the drug and/or to serious secondary effects. Although remarkable progresses have been achieved in identification and functional characterisation of transporters, prediction of interactions due to these ones remains very difficult compared to cytochrome P450s. Moreover, quantitative role of transporter inhibition is not well characterized and sometimes huge ranges of K_i or IC_{50} values were observed for the same inhibitor and transporter. So prioritizing, in vivo DDIs based on the relative ranking of inhibitory potency in vitro as it was performed in many cases for CYPs[1] appears tricky, challenging and very attractive. Furthermore, a comprehensive characterization of whether the relative in vivo inhibitory potency matches the in vitro ranking for a given transporter has not been conducted quantitatively yet. As a result, the aim of this study was to determine how well in vitro and in vivo findings correlate for drug interactions associated with transporter inhibitors focusing on gut, liver and kidney tissues where the following families of transporters are expressed OATP, OAT, OCT and MDR. To perform this study, the Aureus Transporter Database®, was used to retrieve all reported in vitro and in vivo data associated with inhibitor of transporters. In vitro data were extracted for identified transporter inhibitors. K_i or IC_{50} values from same studies when possible or same transporter systems from different studies were used. $[I]/K_i$ approach was used to rank in vitro data. The systemic plasma concentrations of inhibitors were extrapolated as the Cmax at the dose used in in vivo studies. Data from in vivo inhibition studies were collected for similar dose of the inhibitor. Finally, inhibitors were classified based on the extent of inhibition (AUC fold change) to potent, moderate and weak when possible.

[1] R. S. Obach, R. L. Walsky, K. Venkatakrishnan, J. B. Houston, and L. M. Tremaine In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: Qualitative relationships, quantitative predictions, and the rank-order approach Clin. Pharmacol. Ther. **2005**;78(6):582-92

P296 - ABCB11 DEFICIENCY INDUCES CHOLESTASIS COUPLED TO IMPAIRED B-FATTY ACID OXIDATION IN MICE

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The bile salt export pump (BSEP) is an ATP–binding cassette transporter that serves as the primary system for removing bile salts from the liver. In humans, deficiency of BSEP, which is encoded by the ABCB11 gene, causes

severe progressive cholestatic liver disease from early infancy. In previous studies of Abcb11 deficiency in mice generated on mixed genetic background, the animals did not recapitulate the human disease. We reasoned that ABCB11 deficiency may cause unique changes in hepatic metabolism that are predictive of liver injury. To test this possibility, we first determined that Abcb11-knockout (KO) C57BL/6J mice recapitulate human deficiency. Before the onset of cholestasis, Abcb11-KO mice have altered hepatic lipid metabolism coupled with reduced expression of genes important in mitochondrial fatty acid oxidation. This was associated with increased serum free-fatty acids, reduced total white adipose and marked impairment of long-chain fatty acid β-oxidation. Importantly, metabolomic analysis confirmed that Abcb11-KO mice have impaired mitochondrial fatty acid β-oxidation with the elevated fatty acid metabolites phenylpropionylglycine and phenylacetylglycine. These metabolic changes precede cholestasis, but may be of relevance to cholestatic disease progression because altered fatty acid metabolism can enhance reactive oxygen species that might exacerbate cholestatic liver damage.

P297 - DRUG TRANSPORTERS FOR LIVER TARGETING

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Liver transporters are known to be important for the hepatic distribution and clearance of drugs. We are taking advantage of liver specific transporters to develop the hepatoselective tissue distribution strategy for the treatment of Type 2 diabetes. Glucokinase is a key regulator of glucose homeostasis, and a liver selective glucokinase activator (GKA) may offer an effective glycemic control with reduced hypoglycemia risk due to activation of the enzyme at low glucose levels in the pancreas. A GKA compound was successfully designed with low passive permeability to minimize distribution into extra-hepatic tissues, and was optimized as a substrate for liver specific uptake transporter, OATP1B1 and OATP1B3. The liver selective GKA compound demonstrated >50-fold liver-to-pancreas ratio of tissue distribution in rodent and non-rodent species. The compound had a minimal metabolism in liver, and was predominantly eliminated through transporter-mediated hepatic clearance, as well as minor renal clearance as a parent compound in both human and rat. With such a clearance mechanism, it was important to define what the specific contributors to clearance are, so that appropriate clinical studies could be designed (e.g. drug interactions, genetic polymorphism, etc). In vitro transporter studies have identified that GKA compound is a good substrate of liver specific hepatic uptake transporter, hOATP1B1 and hOATP1B3, as well as a substrate of biliary efflux transporter BCRP. Based on in vitro hepatocyte studies, the transporter-mediated active uptake of GKA into liver was about 90%. The major human renal transporters, OAT1 and OAT3, were involved in the renal secretion. To predict the potential transporter-mediated DDIs with co-meds including statins and metformin, the compound was tested in the OATP1B1/1B3, OAT1/3 as well as OCT2/1 inhibition assays. The inhibition potency was very weak with IC50s much higher than the efficacious concentration. Therefore, the risk of GKA to cause transporter-mediated DDI as a perpetrator is minimal. Since the compound is liver targeting through OATP1B1/1B3, it is important to assess the DDI risk with the potent OATP inhibitors, such as CsA, Rifampin and Gemfibrozil. Based on the static model and dynamic SimCyP models, the predicted AUC changes of the compound with CsA, a most potent in vivo OATP inhibitor, will be less than 4-fold. Consequently, in vitro transporter studies and predictions supported that the transporter-mediated DDI issues for GKA compound will be unlikely. These studies have demonstrated that liver targeting through liver specific transporter is a valid approach, and in vitro transporter studies are able to help predict the propensity of compounds to cause transporter-mediated DDIs in vivo which aid in the development of clinical DDI strategies.

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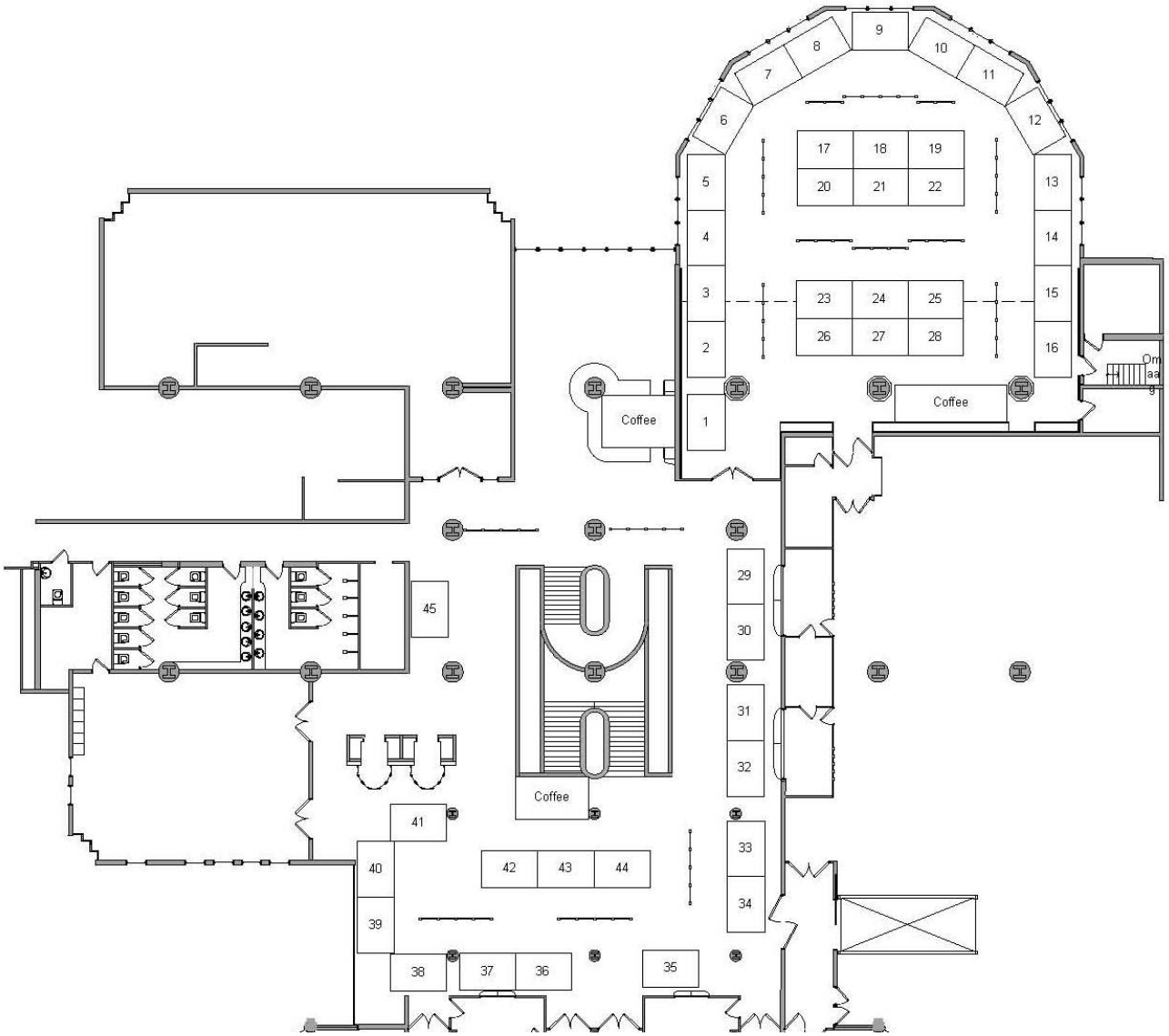
19th MDO and 12th European Regional ISSX Meeting

June 17 – 21, 2012

Noordwijk aan Zee, the Netherlands

Exhibit Hall Floor Plan



MDO ISSX

Noordwijk aan Zee The Netherlands

June 17-21, 2012

19th International Symposium on Microsomes and Drug Oxidations

12th European Regional ISSX Meeting