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Research Article

In vitro CYTOTOXIC EFFECTS OF SOME COVID-19 DRUGS ON LUNG CANCER CELLS

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Abstract: Cancer, which is the second most common cause of death after cardiovascular diseases, is one of the most important health problems of today. Discovery of effective treatments and drugs are important in cancer treatment. The COVID-19 epidemic, which broke out in Wuhan province of China in December 2019 and is considered as a pandemic worldwide, affected millions of people. The SARS-CoV-2 virus, which causes this epidemic, affects the lungs, heart, brain, kidneys, gastrointestinal system, ovaries and testicles and various drugs are used in the treatment. In this study, we aimed to determine the cytotoxic effect of favipiravir, dornase alfa and ivermectin, which are drugs used in the treatment of COVID-19, on human lung cancer cell line (A549). Favipiravir, dornase alfa and ivermectin concentrations were prepared in doubly increasing doses (0.5-64 µg/mL). The prepared concentrations were tested on human A549 cells. After 24 hours of incubation, the cytotoxic effects of the drugs on cancer cells were detected by the MTT (3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide) method. The results were given as % viability. It was determined that favipiravir, dornase alfa and ivermectin significantly decreased the cell viability in lung cancer cell line with increasing application doses ($p<0.05$).

Özet: Kalp damar hastalıklarından sonra ikinci ölüm nedeni olan kanser, günümüzün en önemli sağlık sorunlarından biridir. Etkili tedavilerin ve yeni ilaçların keşfedilmesi kanser tedavisinde önem arz etmektedir. Aralık 2019'da Çin'in Wuhan eyaletinde patlak veren ve dünya çapında bir salgın olarak kabul edilen COVID-19 salgını milyonlarca insanı etkilemektedir. Bu salgına neden olan SARS-CoV-2 virüsü başta akciğerleri olmak üzere kalbi, beyni, böbrekleri, gastrointestinal sistemi, yumurtalık ve testisleri etkilemekte ve tedavisinde çeşitli ilaçlar kullanılmaktadır. Bu çalışmada, COVID-19 tedavisinde kullanılan ilaçlar olan favipiravir, dornaz alfa ve ivermektinin insan akciğer kanseri hücre hattı (A549) üzerindeki sitotoksik etkisinin belirlenmesi amaçlanmıştır. Çalışmada favipiravir, dornaz alfa ve ivermektin ilaçlarının konsantrasyonları iki kat artan dozlarda (0,5-64 µg/mL) hazırlandı. Hazırlanan konsantrasyonlar, insan A549 hücreleri üzerine uygulandı. 24 saatlik inkübasyondan sonra, ilaçların hücre hatları üzerindeki sitotoksik etkileri, MTT (3-(4,5-dimetiltiyazol-2-il)-difenil tetrazolyum bromür) yöntemi ile tespit edildi. Sonuçlar % canlılık olarak verildi. Artan doza bağlı olarak favipiravir, dornaz alfa ve ivermektinin akciğer kanseri hücre dizisinde hücre canlılığını önemli ölçüde azalttığı belirlendi ($p<0.05$).

Introduction

Cancer is a health problem that forms a group of diseases characterized by uncontrolled division and proliferation of cells in an organ or tissue followed by metastasis to other parts of the body (Jackson & Loeb 2001) and manifested by disruption of molecular pathways (Sivanandam *et al.* 2010, Varkaris *et al.* 2014). The complexity of molecular pathways involved in the process of carcinogenesis is one of the most important factors that makes cancer treatment difficult and slows down the development of molecular targeted therapy. In this context, it is very important to analyze the developmental stages of the cancer process properly and to apply the correct treatment for patients to regain their health.

Unfortunately, there is no definitive treatment method for cancer. In addition to classical treatment methods such as radiotherapy, chemotherapy and surgery in cancer treatment, additional targeted applications (healthy nutrition, regular physical activity, avoidance of stress and targeted therapies) are important for the success of

treatments (Huang *et al*. 2010, Nettore *et al.* 2018, Serda *et al.* 2018). Considering the complex process of cancer and different physiological characteristics of patients, the discovery of more effective drugs in cancer treatment constitute a very important research area.

The COVID-19 epidemic, which appeared in Wuhan province of China in December 2019 and was admited a global pandemic in March 2020, is considered a global threat to public health. COVID-19 patients are either asymptomatic or have the disease with clinical course ranging from mild to severe pneumonia, respiratory failure and sometimes death. In addition to comprehensive public health preventions to deal with this disease, an unprecedented global effort is under way to identify effective drugs for treatment. As a result of understanding the virology of SARS-CoV-2, current and effective pharmacological treatments against COVID-19 are being researched (Potì *et al.* 2020). Favipiravir, dornase alfa and ivermectin are among the potential therapeutic agents used in the treatment of COVID-19.

Favipiravir is an antiviral drug approved in Japan (Joshi *et al.* 2021). Favipiravir triphosphate is a purine analog that is a competitive inhibitor of RNA-dependent RNA polymerase (Coomes & Haghbayan 2020). It has been used in many countries to treat new viral infections, including Ebola and Lassa. As an antiviral drug, Favipravir is authorized for use in the treatment of COVID-19 in many countries, including Japan, Russia and India, under emergency provisions (Nagakrishna & Thawani 2020).

Dornase alfa, known as the recombinant form of the human DNase I enzyme, is a drug that has been used for years to reduce the severity of infections in respiratory diseases and improve lung function in patients with cystic fibrosis. It is well known that pneumonia related with COVID-19 progresses to severe acute respiratory syndrome and even multiple organ failure. The highly viscous mucus structure observed in cystic fibrosis was reported to be very similar to that in COVID-19 (Okur *et al.* 2020). Dornase alfa was found to exert anti-viral effect against coronavirus in Madin-Darbybovine kidney cell line (MDBK) and green monkey kidney cell line (Vero) without cytotoxicity on healthy peripheral blood mononuclear cells (Okur *et al.* 2020).

The potential of ivermectin to reduce transmission of mosquito-induced malaria is being evaluated by various studies worldwide. Ivermectin is reported to inhibit the *in vitro* replication of some positive, single-stranded RNA viruses, such as Zika virus, yellow fever virus, dengue virus (DNV) etc. Recently, ivermectin has been reported to strongly inhibit the replication of SARS-CoV-2 virus *in vitro* (Chaccour *et al.* 2020).

This study was carried out to have a preunderstanding for how can be affected lung cells *in vitro* when a person both COVID-19 and lung cancer if use these drugs, also to determine the cytotoxic effects of favipiravir, dornase alfa and ivermectin on A549 cells. Moreover, since lack of information about cytotoxic effects of COVID-19 drugs on lung cancer cells in literature, this study was performed. However, experimental studies and analyses in this study should be supported with clinical applications and in vivo experiments.

Materials and Methods

Cell lines and culture conditions

The study was carried out at Bartın University Central Research Laboratory, Anticancer Research Laboratory. A549 cell line (ATCC) was used as the cell type in the study. Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, USA; prepared by adding 10% Fetal Bovine Serum (FBS), 0.1 mg/mL streptomycin and 100 U/mL penicillin) was used to feed A549 cells. Cells were cultured in 75 cm^2 culture flasks (TPP; Switzerland) and the cultures were incubated in a humidified incubator (37°C, 5% CO_2; N-biotech, Korea). The medium was changed every 3-4 days and the cell passages were made when the cells reached 80-90% confluence.

Test drugs

0.5, 1, 2, 4, 8, 16, 32 and 64 µg/mL concentrations of favipiravir (Santa Cruz Inc.), dornase alfa (Genentech) and ivermectin (Santa Cruz Inc.) were prepared in DMEM (for A549 cells).

MTT assay

The effects of favipiravir, dornase alfa and ivermectin on A549 cell viability were determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide) method. Confluent cells were scraped from the bottom of the flasks with Trypsin-EDTA and then counted under the microscope. The cells were seeded in the 96-well plates at a density of 15×10^3 cells per well. The seeded cells were incubated at 37°C in an incubator with CO_2 for 24 h. The media were changed following the incubation, different concentrations of favipiravir, dornase alfa and ivermectin were added to the wells in which the cells were seeded, and the incubation was done for 24 h (Koran *et al.* 2017). Subsequently, the media in the wells were withdrawn, then MTT solution (0.5 mg/mL) prepared in sterile Phosphate-Buffered Saline (PBS) was added to each well, and the plates were incubated for 3 h. Subsequently, the solution in the wells was withdrawn and incubation was stopped by adding 100 µL dimethyl sulfoxide to each well. Optical densities of the cells in microplates were determined by a spectrophotometer (Thermo, Multiscango) at 570 nm wavelength (Mosmann 1983).

The average of the absorbance values of the control wells was calculated and these values were determined as 100% cell viability. Percentages of viability values of cells were determined by proportioning the absorbance values obtained from the wells treated with favipiravir, dornase alfa and ivermectin to the control absorbance value. MTT assays were done 10 times on different days, with double repeats for each plate. According to the MTT assay results, the half maximal inhibitory concentration value (IC_{50}) was calculated using GraphPad Prism 9 (San Diego, CA, USA).

Statistical analysis

GraphPad Prism 9 package program for Windows was used in statistical analyses. One-way ANOVA was used to detect differences among the groups and multiple comparisons were analyzed with Tukey's test. Quantitative data were given as the mean with standard deviation (mean±SD) and $p<0.05$ was indicated as statistically significant.

Results

The cytotoxic effects of favipiravir and dornase alfa drugs on human lung cancer cell line (A549) are shown in Fig. 1 and Fig. 2, respectively. A decrease in cell viability was determined for favipiravir at concentrations of 2 µg/mL and above (2-64 µg/mL) and for dornase alfa at all concentrations (0-64 µg/mL), compared to the control group. These decreases in cell viability were statistically significant ($p<0.05$).

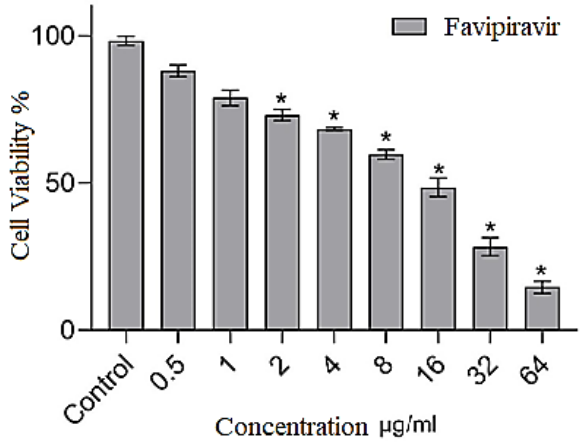

Fig. 1. % Change in viability of A549 human lung cancer cells treated with different concentrations of favipiravir for 24 hours. The data obtained are shown as mean ± SD. *p<0.05 vs control group (There are 15×10^3 cells in each well of 96 microplates).

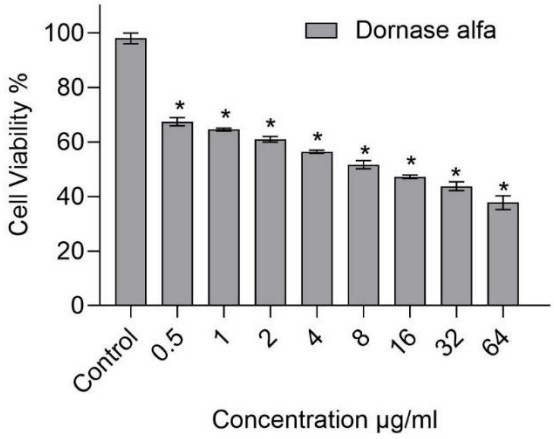

Fig. 2. % Change in viability of A549 human lung cancer cells treated with different concentrations of dornase alfa for 24 hours. The data obtained are shown as mean ± SD. *p<0.05 vs control group (There are 15×10^3 cells in each well of 96 microplates).

The effects of the ivermectin on cell viability of A549 human lung cancer cell line are shown in Fig. 3. Although a significant dose dependent decrease in viability of A549 cells treated with ivermectin was detected at concentrations of 0.5-4 µg/mL compared to control, cell viability remained almost constant between 4-64 µg/mL concentrations.

We also detected the IC_{50} values of the drugs used. While IC_{50} values of favipiravir and dornasa alfa were calculated almost close to each other, IC_{50} value of ivermectin was relatively lower than favipiravir and dornase alfa (Table 1).

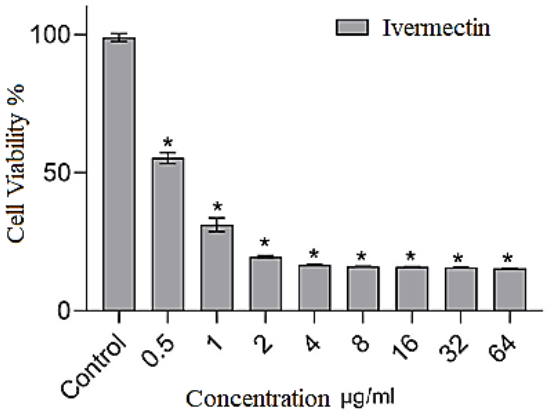

Fig. 3. % Change in viability of A549 human lung cancer cells treated with different concentrations of ivermectin for 24 hours. The data obtained are shown as mean ± SD. *p<0.05 vs control group (There are 15×10^3 cells in each well of 96 microplates).

Table 1. IC_{50} (µg/mL) values of favipiravir, dornase alfa and ivermectin calculated for A549 human lung cancer cells.

	Favipiravir	**Dornase alfa**	**Ivermectin**
Cell	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)
A549	12.55	12.13	0.306

Discussion

Studies reported that various anti-diabetic, antipsychotic, anti-malarial and antiviral drugs have antineoplastic properties against lung, prostate, colorectal, gastric, breast and overian tumors (Kaushik *et al.* 2021). In preclinical studies, it was determined that these non-neoplastic drugs trigger apoptosis by various intracellular signaling mechanisms, stop cell proliferation and exert anti-metastatic effects. Some anti-neoplastic drugs give positive results for cancer in clinical studies (Kaushik *et al.* 2020). Because of these features, it is important to define new therapeutic agents and to determine their biological activities in cancer research.

Ivermectin is a broad-spectrum antiparasitic drug (Khan *et al.* 2020) and has been reported as an anticancer agent in some cancer types due to its potential to inhibit tumor growth (Sharmeen *et al.* 2010, Melotti *et al.* 2014). Ivermectin was reported to have an anti-proliferative effect on esophageal squamous cell carcinoma (ESCC)

cells. Ivermectin also significantly inhibits ESCC cell growth, migration and invasion by blocking PAK1 signaling (Chen *et al.* 2020). We showed that ivermectin decreased A549 cell viability. However, the molecular mechanism of this effect can be elucidated by further studies.

In another study, the cytotoxic effect of ivermectin on human stomach cancer cell lines (MKN1, MKN7, MKN28, MKN45, MKN74, SH-10-TC, NUGC-3, NUGC-4, AGS, GSU and KE-39, RIKEN) was investigated for 48 hours and among the cell lines tested, it was shown that MKN1 cells were the most sensitive to ivermectin and SH-10-TC cells were also drug sensitive. In contrast, MKN7 cells and MKN28 cells were resistant to ivermectin. Therefore, MKN1 and SH-10-TC cells are defined as ivermectin sensitive and MKN7 and MKN28 cells as ivermectin resistant cells (Nambara *et al.* 2017). In our study, A549 cells did not show resistance to the test drugs we used.

Additionally, ivermectin has been reported to induce cell death in human leukemia cells (OCI-AML2, HL60, U937, KG1a) through chloride influx, membrane hyperpolarization, and increased intracellular ROS levels (Sharmeen *et al.* 2010).

As a result, we investigated, for the first time, the cytotoxic effects of favipiravir, ivermectin and dornase alfa, which are drugs used in the treatment of COVID-19, on human lung cancer cells (A549). In our study, we detected for the first time that favipiravir, dornase alfa and ivermectin decreased cell viability by showing cytotoxic effects in human lung cancer cell line. The IC_{50} values on A549 cells were 12.55, 12.13 and 0.306 µg/mL for favipiravir, dornase alfa and ivermectin, respecitevely.

In conclusion, favipiravir, dornase alfa and ivermectin can be considered to be important therapeutic agents due to their potential cytotoxic effects on A549 cells *in vitro*. With further studies, it can be revealed how these drugs affect molecular mechanisms in cancer cells and its use in cancer treatment can be investigated with *in vivo* and clinical studies.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Author Contributions: Concept: S.U.K., A.K., F.U., U.H., S.A., F.O., M.C., E.C., Desing: A.K., Execution: S.U.K., A.K., Material supplying: S.U.K., Data acquisition: F.U., U.H., S.A., F.O., M.C., E.C., Data analysis/interpretation: A.K., Writing: A.K., Critical review: S.U.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

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