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RESEARCH ARTICLE

Quercetin protects rat skeletal muscle from ischemia reperfusion injury

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Abstract

In this study, we investigated the potential beneficial effects of quercetin on skeletal muscle ischemia reperfusion injury. Twenty-four Sprague–Dawley type rats were randomly divided into four groups. In the sham group, only gastrocnemius muscle were removed and given no quercetin. In ischemia group, all the femoral artery, vein and collaterals were occluded in the left hindlimb by applying tourniquate under general anaesthesia for three hours but reperfusion was not done. In the Quercetin + Ischemia reperfusion group, quercetin (200 mg kg^{-1} dose orally) was given during one-week reoperation and later ischemia reperfusion model was done. Finally, gastrocnemius muscle samples were removed to measure biochemical parameters. The biomarkers, MDA levels, SOD, CAT and GPx activities, were evaluated related to skeletal muscle ischemia reperfusion injury. MDA levels reduced and SOD, CAT and GPx activities increased significantly in Quercetin + Ischemia reperfusion group. Results clearly showed that Quercetin have a protective role against oxidative damage induced by ischemia reperfusion in rats.

Keywords

Antioxidant enzymes, ischemia reperfusion, quercetin, skeletal muscle

History

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Introduction

Ischemia reperfusion injury (IRI) is a case that includes various complicate processes. The physiopathology of IRI has been investigated by many researchers[1,2]. Ischemia of the extremity frequently leads to morbidity and mortality. Major reasons of acute extremity ischemia are thrombosis and embolism[3]. Additionally ischemia reperfusion (IR) can be caused by numerous events such as trauma, various orthopaedic surgery and free-flap reconstruction[4,5]. A huge number of consecutive events are initiated by free oxygen radicals. Free radicals intrinsically exist in living organisms; however, much amount of free radicals and lipid peroxides can lead to tissue damage, cell death or degenerative processes, including aspects of ageing, inflammation, neural disorders, cancer, and the circulatory system diseases by oxidise biomolecules and they are mainly responsible for oxidative stress[6–8]. Free radicals can change the structures of cellular molecules such as DNA, proteins, lipids and carbohydrates[9–12]. The effect of oxidants can be deactivated or inhibited in various ways by antioxidant compounds, scavenging and suppressing the free radicals[13–16]. Recently, natural antioxidants attracted more attention because they are accepted to function as chemopreventive and cytoprotective agents against oxidative damage[17–20]. Human body can be protected against free radicals and reactive oxygen species effect by antioxidants, moreover, antioxidants have a large biological activities spectrum[21–23]. They are usually supplemented in diets in case to inhibit the chain reactions of oxidation by averting the inception and extend steps leading for termination of the reaction, and delayed the oxidation process. Antioxidants are found in many variations such as all plants, microorganisms, fungi and even in animal tissues on earth[24–27].

Phenolic compounds are secondary plant metabolites, which have antioxidant activity[28,29]. Phenolic compounds have one or more hydroxyl groups at different positions of the aromatic ring. They occur in both free and bound forms in plant foods[30–32]. The free phenolics are mainly digested in the upper gastrointestinal tract, but the bound ones may survive upper gastrointestinal digestion and eventually be metabolized by the microflora in the colon. Available evidence indicates that the form of phenolic compounds exerts a serious impact on their antioxidant capacities. Also, phenolic compounds have a large spectrum of biological activity[33–35]. They are naturally present in almost all plant materials, including food products of plant origin. Phenolics are thought to be an integral part of both human and animal diets[35–38]. The majority of natural antioxidant compounds are phenolics, and the most important groups of natural antioxidants are flavonoids, which include flavanones, flavanols and flavanonols[39–43].

Quercetin is the most putative flavanol and found in many fruits, vegetables, leaves and grains[44,45]. It has been reported that Quercetin has some biological functions, which include anti-inflammatory, anti-ischemic effects, anti-peroxidative properties and anti-coagulation[46–48]. Also Quercetin, is a strong antioxidant

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and radical scavenger[49,50]. The IR studies of various organs suggested that Quercetin has protective effects against the oxidative stress induced IRI[51]. It has been also reported that Quercetin has the protective effect on the myocardial tissue against global IRI. In addition, a previous study has demonstrated that treatment of Quercetin is effective role against IRI in ovary model[52].

The aim of this study is to investigate the potential beneficial effects of Quercetin on skeletal muscle IRI.

Materials and methods

Quercetin and sodium carboxymethylcellulose (Na-CMC) were purchased from Sigma Chemicals, St. Louis, MO. Thiopental (Pental Sodyum; İ.E.) was supplied from Ulagay Pharmaceutical Industry Turkish Corporation, İstanbul, Turkey and was stored at 2–4 °C and protected from sunlight.

Animal preparation

The experimental protocol used in this study was approved by the Experimental Animals Local Ethics Committee, Faculty of Veterinary, Atatürk University (2014-58). Twenty-four adult Sprague–Dawley type rats weighing between 180 and 200 g were used in this experimental study. Animals were accommodated in the standard cages with a temperature-controlled room (22 ± 2 °C) with alternating photoperiod (12:12 h light:dark) and humidity ($55 \pm 5\%$) and were given water add libitum. Rats were acclimatized before the study for one week. Animals were fasted 12 h before the study, but were allowed free access to water.

IR model and anaesthesia

All operations were performed under general anaesthesia. In brief, intraperitoneal narcosis was attained using the thiopental sodium (40–50 mg kg^{-1}). Anaesthesia was maintained with addition of thiopental sodium (10 mg kg^{-1}) intraperitoneal injections as required. Most procedures were performed with the spontaneous breathing of the animals. Unilateral hindlimb ischemia was conducted with the tourniquate model as in previous study[53]. Common femoral arteries and collateral flow were occluded tightly with rubber tourniquets, the proximal of the left extremity. Ischemia was confirmed by cyanosis and temperature drop in the extremity. Later tourniquate was released and reperfusion was initiated. Reperfusion was verified by edema, the extremity return to normal temperature, receiving pulse and the extremity colour changed to pink. All animals were sacrificed at the end of the experimental; muscle tissues were removed and washed in cold isotonic saline. Tissue samples were stored at −70 °C until malondialdehyde (MDA) levels as a lipid peroxidation marker and antioxidant enzyme activities were assayed.

Experimental groups

Totally 24 rats were randomized into four equal groups (with six rats per group) for experiments:

(1) Sham group: these rats were kept under anesthesia for 6 h but not subjected to ischemia reperfusion. Na-CMC (1%) was given 2 mL orally to ensure standardization. Later, the rats were sacrificed and gastrocnemius muscle samples were removed.
(2) Ischemia group: Na-CMC (1%) was administered prior to ischemia for one week and subjected to ischemia for 3 h.
(3) IR group: it was subjected to ischemia for 3 h and reperfusion for 3 h. Likewise, Na-CMC (1%) was performed as described in the sham and ischemia groups.
(4) Quercetin + IR group: Quercetin (200 mg/kg) was administered to this group of animals prior to IR period for one week. A solution of 200 mg/kg Quercetin was suspended into Na-CMC (1%) solution. Then, it was done with IR as described in the IR group.

Biochemical study

Tissues were stored at −70 °C until analysis and later, they were homogenized on the day they were analysed. MDA level, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were measured on the skeletal muscle samples. Tissue MDA levels, which reflect lipid peroxidation rate, were analysed[53]. The specific activity was presented as nmol g^{-1} protein. SOD activity was determined by measuring the decrease in hydrogen peroxide (H_2O_2) concentration, with a method described[53]. SOD activity was expressed as EU mg^{-1} protein. GPx activity measurement was held according to the methods disclosed by Matkovics et al.[54]. The results were given as U mg^{-1} protein. Tissue CAT activity was measured spectrophotometrically by assaying the hydrolysis of H_2O_2 at absorbance 405 nm[53]. Results were expressed as U g^{-1} protein tissue.

Statistical analysis

The data were analysed using SPSS 22.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey for multiple comparisons. $p < 0.05$ was considered as statistically significant.

Results and discussion

The results of MDA levels and antioxidant enzyme activities are summarized in Table 1. We measured the level of the MDA as an end product of lipid peroxidation. We also determined higher skeletal muscle tissue MDA levels in ischemia and IR groups according to sham group ($p < 0.001$). Treatment of Quercetin almost reduced the levels of muscle tissue MDA level of sham group ($p < 0.05$). Besides, tissue SOD activity significantly reduced in the IR group ($p < 0.01$) compared with sham group. Also, it was found that tissue SOD activity was increased by treatment of Quercetin compared to ischemia and IR groups. There was statistically significant difference in the CAT enzyme activity between Quercetin + IR and IR groups. Tissue GPx levels were significantly elevated in Quercetin + IR group according to sham group.

It was suggested that the tissue damage induced the free oxygen radical as an important statement in the physiopathology of IRI. In this way, antioxidant agents have the beneficial effects in the physiopathology of IRI as can be seen in the various studies. For this purpose, the protective effect of Quercetin on IRI in skeletal muscle was investigated in our study.

In the previous studies, accumulation of oxygen-derived free radicals has been demonstrated within a couple of minutes of reperfusion in tissues[55]. Postischemic endothelium is the main source for the free radicals[56]. It is known that the most impairing effect of free oxygen radicals is peroxidation of polyunsaturated fatty acids in the cell membrane[57–59]. Free oxygen radicals cause and start the lipid peroxidation. Oxidation of polyunsaturated fatty acids causes the formation of lipid peroxides such as the MDA and also leads to enzymatic or chemical deterioration by $O_2^{\bullet -}$ or $^{\bullet}OH$[60–62]. It has been implicated that reactive oxygen species played a role in physiopathology of much diseases[63,64]. As a conclusion, the lipid peroxidation results in cellular injury, causing structural and functional alterations in the cells[65–67]. SOD, CAT and GPx are known as endogenous antioxidants, and the first-line defence mechanism against free radical damage[58–70]. These enzymes catalyse the conversion to less reactive species of

Table 1. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) enzyme activities and malondialdehyde (MDA) levels in muscle tissue of rats with ischemia reperfusion (IR) injury. The results were suggested as mean ± SEM.

	MDA (nmol g^{-1})	SOD (EU mg^{-1})	CAT (U g^{-1})	GPx (U mg^{-1})
Sham[1,*]	26.23 ± 0.39^{a}	21.29 ± 1.73^{a}	243.52 ± 13.68	64.52 ± 3.68^{a}
Ischemia[2,*]	$55.72 \pm 3.14^{a,b}$	20.55 ± 1.54^{b}	231.59 ± 11.10	45.89 ± 1.22^{a}
IR[3,*]	$61.04 \pm 1.97^{a,c}$	$13.65 \pm 0.64^{a,b,c}$	216.77 ± 9.89^{a}	$40.00 \pm 1.60^{a,b}$
Quecetin + IR[4,*]	$38.82 \pm 2.55^{a,b,c}$	23.26 ± 2.32^{c}	267.49 ± 6.20^{a}	$53.30 \pm 3.13^{a,b}$

*$n = 6$.
p Values: [a]MDA: 1–3 $p < 0.001$, 1–4 $p < 0.05$; [b]MDA: 2–4 $p < 0.001$; [c]MDA: 3,4 $p < 0.001$.
[a]SOD: 1–3 $p < 0.05$; [b]SOD: 2–3 $p < 0.05$; [c]SOD: 3–4 $p < 0.05$.
[a]CAT: 3–4 $p < 0.05$; [a]GPx: 1–2,3 $p < 0.001$, 1–4 $p < 0.05$.
[b]GPx: 3–4 $p < 0.05$.

the derived-oxygen radicals[71–74]. SOD is an important part of the antioxidant defence for the organisms. When the extreme $O_2^{\bullet-}$ occurs SOD reduces the amount of superoxide radicals ($O_2^{\bullet-}$) by converting to oxygen (O_2) and hydrogen peroxide (H_2O_2)[75–77]. CAT is primary enzymatic defence against H_2O_2 generation[78,79]. GPx protects the tissues against free radicals by limiting lipid peroxidation. Furthermore, measurement of changes in antioxidant enzyme activity can give an idea about the amount of ROS, indirectly[80]. Now, we are in a position to say that the measurement of antioxidant enzymes is important in biological samples.

Quercetin is a natural flavonoid and can be found in fruits, seeds and vegetables. It has a strong antioxidant capacity and specifically protective activity against oxidative and free radical damage in the tissues. These antioxidant features of flavonoids are significantly due to their free radical scavenging and hydrogen-donating properties[81]. Moreover, protective effect of Quercetin in several organs has been shown in the experimental studies including ovary[52] small intestine and heart[82]. Recently, the neuroprotective and antioxidant effect of Quercetin has been demonstrated. In addition, Quercetin has protected the neurons and effectively inhibited xanthine oxidase activity against oxidative injury in primary cultures rat cortical cells[83]. Whereas, as can be seen in a number of IR studies, it has been demonstrated that tissue MDA levels increased in skeletal muscle IR injury[84]. This effect has suppressed with the supplementation of free radical scavengers. Liu et al. have suggested that Quercetin has protective effects against IR damage by decreasing MDA level in heart[81]. In renal IR study, we have reported that the MDA level, as a biomarker of lipid peroxidation, increased in the IR group while it decreased in the higher dose Quercetin treated group. We have found that tissue MDA levels were increased in IR group compared with sham group. But MDA levels were decreased in the quercetin treated group. Quercetin treatment attenuated the amount of lipid peroxidation significantly.

The antioxidant enzymes such as SOD, CAT and GSH have a significant role on the antioxidative defence system. The antioxidant enzyme status has been evaluated after IR in skeletal muscle[85]. In a previous study, it has been demonstrated that quercetin has protective effects against IR damage by increasing SOD, CAT, GPx activities in heart[82]. Also, in another study, the increase of the SOD activity has been reported after quercetin treatment[86,89]. Protective effect of quercetin on experimental chronic cadmium nephrotoxicity on rats has been evaluated and increase of the SOD activity by the quercetin has been detected[87]. In the previous study, it has been noted that the activities of SOD and CAT have been increased by the quercetin[88]. Our findings are generally in the same direction with the results of previous studies. We identified the increasing of tissue SOD and CAT activity in quercetin treated group compared with sham group. SOD, CAT and GPx enzyme activities increased in skeletal muscle tissue. The real mechanism of quercetin on enzyme activities is not exactly known. But, we think that quercetin does this by increasing the levels of antioxidant enzyme and reducing the formation of free radical or sweeping of free radical.

In conclusion, in this study, it has been demonstrated that quercetin treatment might protect skeletal muscle from IRI. We believe that tissue protective effect of the quercetin is through antioxidant defence system against radical damage. We underscore the necessity of human studies with quercetin.

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Declaration of interest

The authors have declared no conflict of interest.

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