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Original Investigation

Protective Effects of Quercetin on Hepatic Ischemia-Reperfusion Injury

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Abstract

Introduction: The present study was designed to evaluate the effects of quercetin on hepatic ischemia-reperfusion (IR) injury in rats caused by Pringle maneuver.

Methods: Overall, 24 5- to 6-month-old female Wistar Albino rats weighing 200-250 mg were included in the present study. In the sham group, laparotomy was performed 15 min after anesthesia induction. Ischemia was not created. In the other groups, the hepatic pedicle (portal vein, hepatic artery, and bile duct) was explored, and ischemia was created with an atraumatic microvascular clamp. Concurrently, the exposed abdomen was covered with warm gauze soaked in saline. In the study group, quercetin was intraperitoneally injected prior to laparotomy and IR. Liver tissue samples from the left lobe were analyzed under a light microscope for liver damage. For the evaluation of hepatic IR injury and assessment of the effect of quercetin on antioxidant systems, total oxidant status (TOS), and total antioxidant status (TAS) levels were measured. Oxidative stress index (OSI) was calculated

Results: Aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase values of the control group were significantly higher than those of the sham and the study groups ($p<0.001$). Although the mean TAS, TOS, and OSI values of the study group were found to be lower than those of the control group, the difference was not statistically significant ($p>0.05$). Significantly less vacuolization and sinusoidal dilatation were observed in the study group than in the control group ($p<0.05$). For necrosis and apoptosis, there was no significant difference between the control and the study groups ($p>0.05$).

Conclusion: On consideration of all findings, quercetin, which hosts many molecules within its structure with a potential utility, such as an antiinflammatory agent, antioxidant, and antiaggregant, has an effect that may be protective against hepatic IR injury.

Keywords: Quercetin, ischemia-reperfusion, liver injury, Pringle maneuver, rat model, antioxidant

Introduction

Ischemia-reperfusion (IR) injury is an important cause of liver damage in liver transplantation or liver resections with Pringle maneuver and is the main cause of liver dysfunction (1). Unfortunately, the mechanism of damage is not exactly known, but an inflammatory and oxidative process is accused in a complex pathophysiology. Many molecules are studied in animal experiments to reduce hepatic damage, but improvements are not sufficient.

Quercetin is a flavonoid found mostly in fruits and vegetables. Epidemiological studies with quercetin have started in the 1990s. The molecule is found in a wide variety of plants such as apples, onions, berries, and red grapes. Quercetin has antioxidative, antiinflammatory, antiaggregatory, and anticarcinogenic effects (2).

The present study was designed to evaluate the effects of quercetin on the hepatic IR injury of rats formed by Pringle maneuver.

Methods

Experimental design and animals

The present study was performed according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council Institute for Laboratory Animal Research, Washington, US, National Academies Press, 1996), and the animal protocols were approved by the Animal Ethics Committee of Erciyes University. Biochemical analysis was conducted at Kayseri Training and Research Hospital Biochemistry Laboratory, and pathological examinations were evaluated at Erciyes University Department of Pathology.

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Overall, 24 5- to 6-month-old female Wistar Albino rats weighing 200-250 mg were used in the present study. The rats were kept in standard plastic cages and were fed with standard rat chow and water and were subjected to 12-h light and 12-h dark cycles. Room temperature was 24±2˚C.

Preparation and experimental groups

In the present study, rats were randomly categorized into three groups. In the laparotomy procedure, the anterior abdominal wall was shaved 15 min after drug administration and then a 3-4 cm midline laparotomy incision was made. Concurrently, the exposed abdomen was covered with warm gauze soaked in saline. In the sham group, laparotomy was performed 15 min after anesthesia induction, but ischemia was not created. In the other groups, the hepatic pedicle (portal vein, hepatic artery, and bile duct) was explored, and ischemia was created with an atraumatic microvascular clamp (Vasculostatt-Scanlan, St. Louis, USA). In the study group, subjects were administered appropriate quercetin dose intraperitoneally after general anesthesia prior to ischemia application.

Histopathological study

Liver tissue samples from the left lobe of the liver were placed in containers with 10% formalin. Samples were embedded in paraffin blocks, and sections were taken in size of 4-5 µm. Samples were stained with hematoxylin-eosin. The preparations were examined by an expert histopathologist, who was blinded to the preparations, under a light microscope at a magnification of 50-100× (Table 1).

Biochemical evaluation

Blood samples were collected into tubes for biochemical analysis to obtain serum and plasma. They were centrifuged at 3000 rpm for 10 min and then the serum and plasma samples were placed in Eppendorf tubes. Samples were held at -20˚C until measurements are made. For the evaluation of hepatic IR injury and the assessment of the effect of quercetin on antioxidant systems, total oxidant status (TOS) and total antioxidant status (TAS) levels were measured. Oxidative stress index (OSI) was calculated. In addition, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were measured. Serum AST, ALT, and LDH levels were measured with colorimetric method using ready kits. For TOS level analysis, total oxidant status kit (Rel Assay Diagnostics) and Olympus AU 400 biochemistry autoanalyzer were used. Units were expressed in terms of micromole hydrogen peroxide equivalent per liter (µmol H_2O_2 eqv/L). For TAS analysis, total antioxidant status commercial kit (Rel Assay Diagnostics) and Olympus AU 400 biochemistry autoanalyzer were used. TAS results were expressed as µmol Trolox eqv/L. While calculating the value that indicates the balance of the organism's oxidant/antioxidant capacity, OSI was calculated by the formula:

OSI=100×TOS (µmol H_2O_2 eqv/L)/TAS (µmol Trolox eqv/L).

Statistical Analysis

Biochemical and histopathological values were expressed as mean±SEM. For histopathological values, average (minimum and maximum) values were used. Data were analyzed using SPSS (Statistical Package for Social Sciences) version 15.0 (SPSS Inc.; Chicago, IL, USA) and Sigma Stat 3.5 statistical package program. Among the groups, one-way analysis of variance or Kruskal-Wallis tests was used. From the multiple comparison tests, Tukey's, Dunn-Sidak, and Tamhane's T2 were used. A $p<0.05$ was considered statistically significant.

Table 1. Scoring system for ischemia-reperfusion injury of the liver

Liver Injury Histopathological Properties	Score	Description
Cytoplasmic vacuolization	0	None
	1	Rarely seen
	2	Scattered in some lobules
	3	Scattered in most lobules
	4	Widespread
Sinusoidal dilatation	0	None
	1	Rarely seen
	2	Frequent perivenular
	3	Frequent perivenular midzonal
	4	Frequent panlobular
Cell necrosis	0	None
	1	1-2 Apoptotic cells
	2	≥3 Apoptotic cells
	3	1-2 Focal necrosis area
	4	≥3 Focal necrosis area

Results

AST, ALT, and LDH levels

The mean serum AST levels were 141.4±28.2 IU/L in the sham group, 1166.6±275 IU/L in the control group, and 710.9±95 IU/L in the study group. The AST values of the control group were significantly higher than those of the sham and the study groups ($p<0.001$).

The mean serum ALT levels were found to be 63.6±964.4 IU/L in the sham group, 964.4±136.8 IU/L in the control group, and 475.6±114.7 IU/L in the study group. The ALT values of the control group were significantly higher than those of the sham group and the study group ($p<0.001$).

The mean serum LDH levels were observed to be 1368.0±316.7 IU/L in the sham group, 6576.9±1981.2 IU/L in the control group, and 3324.3±2617.4 IU/L in the study group. The LDH values of the control group were significantly higher than those of the sham group and the study group ($p<0.001$) (Table 2).

Total Antioxidant Status, Total Oxidant Status and Oxidative Stress Index

In the sham group, TAS, TOS, and OSI values were found to be significantly lower than those in the control and the study groups ($p<0.001$). Although the mean TAS, TOS, and OSI values of the control group were higher than those of the study group, these differences were not statistically significant ($p>0.05$) (Table 3).

Histopathological examination

During histopathological examination, in all groups, cytoplasmic vacuolization, sinusoidal dilatation, apoptosis, and cell necrosis were evaluated. In the sham group, normal histopathological appearance was detected despite minimal sinusoidal dilatation and cytoplasmic vacuolization presence. Significantly less vacuolization and sinusoidal dilatation were observed in the study group compared with the control group ($p<0.05$). Necrosis and apoptosis also showed a similar pattern in the study group, but they were not statistically different than in the control group and the sham group ($p>0.05$) (Table 4).

Discussion

Reduced blood flow or complete cessation to any organ and a following reperfusion process leads to an acute inflammatory response. Inflammatory response results in significant cell damage and causes malfunction. This biological process is defined as IR injury (3). One of the organs in the human body wherein the said damage occurs most frequently is the liver. After hemorrhagic shock, liver transplantation, and liver resection, various degrees of hepatic IR injury are observed (3-5).

Following IR injury, with increasing blood flow, tissue oxygenation rises, and reactive oxygen species (ROS), such as superoxide anion, peroxynitrite, H_2O_2, and hydroxyl radical are produced (6). When hepatocytes face ROS, which are critical mediators in the pathogenesis of many liver diseases, the expression of various genes, synthesis of cytokines, chemokines, and cell adhesion molecules, permeability of cell and cell organelle, and protein and DNA oxidation are increased (7). These events result in the formation of oxidative stress, and oxidative stress causes cell death by creating irreversible damage or malfunction.

Increase in cell membrane permeability occurs as a result of membrane lipid peroxidation formed by ROS. After lipid peroxidation, very powerful oxidizing agents, lipid peroxide radicals, and lipid hydroperoxides are released (8). These free radicals damage cell membrane. Final products, such as nitrotyrosine, formed after protein oxidation, were high in various degrees in serum and tissue samples. Therefore, the resulting end products of this oxidation are often used as an indicator of protein oxidation (9, 10). Hepatocyte damage that occurs as a result of varying degrees of all these effects or death of hepatocytes manifests itself as liver dysfunction.

One of the most important complications of liver surgery is intraoperative bleeding which affects the perioperative conditioning of the patient. To avoid this problem, several vascular occlusion techniques have been used. In the retrospective study by Nakajima et al. (11), they investigated surgical techniques used to control bleeding in liver resection, and Bilt et al. (12) studied vascular clamp techniques in liver surgery. The frequency of vascular occlusion techniques was demonstrated in these studies. According to these studies, although hemihepatic vascular clamp technique is based on the selective interruption of the bloodstream to the lobe that is to be resected, Pringle maneuver, which is recommended and used frequently, is applied to the portal triad to cut off liver bloodstream (13). Gurusamy et al. (14) compared vascular the occlusion methods for elective liver resections by complication rates, and the intermittent portal triad clamping technique was found to be better than continuous clamping. In our experimental study, we used the intermittent portal triad clamping technique to create liver ischemia as well.

Free oxygen radicals have been shown to be the main cause of all these changes in liver damage. Many treatment strategies have been focused on either preventing free oxygen radical damage or its formation. In recent years, various local remedies, which have pleiotropic biological activity, have been used as a complementary or alternative therapy in the treatment of different diseases. Glantzounis et al. (15) showed that the use of antioxidant agents for this purpose was to reduce ROS production and to support endogenous antioxidants. One of these agents that we used in our study is quercetin (3,3',4',5,7-pentahydroxyflavone), which is a plant-derived flavonoid known as phytoestrogen (16). Quercetin exerts its antioxidant effect by scavenging free oxygen radicals and inhibiting lipid peroxidation and xanthine oxidase activity (17). The antiinflammatory action of quercetin is mediated through the inhibition of lipoxygenase and cyclooxygenase enzymes (18, 19).

Dufour et al. (20). emphasized the sensitivity of transaminases (AST

Table 2. The comparison of AST, ALT, and LDH levels among the experimental groups of animals

Data are expressed as mean±SD from eight rats per group

	Experimental Groups		
	Sham	Control	Study
AST	141.4±28.2	1166.6±275.2	710.9±95.6
ALT	63.6±964.4	964.4±136.8	475.6±114.7
LDH	1368.0±316.7	6576.9±1981.2	3324.3±2617.4

AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase

Table 3. Comparison of TAS, TOS, and OSI. Data are expressed as mean±SD from eight rats per group

	Experimental Groups		
	Sham	Control	Study
TAS	0.96±0.11	1.33±0.08	1.22±0.18
TOS	10.98±0.55	35.2±6.3	26.1±1.7
OSI	1166.6±186.7	2657.3±503.6	2168.5±236.9

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index

Table 4. Comparison of histopathological results. Data are expressed as mean (min-max) from eight rats per group

	Experimental Groups		
	Sham	Control	Study
Sinusoidal Dilatation	0.5 (0-1)	3.5 (3-4)	1.0 (1-2)
Cytoplasmic Vacuolization	0.5 (0-1)	2.5 (2-3)	1.0 (0-2)
Necrosis, Apoptosis	0.0 (0-1)	1.0 (0-2)	0.0 (0-1)

and ALT) and LDH in monitoring hepatocyte injury. Seeto et al. (21) reported that the maximum increase was detected in liver aminotransferase levels during ischemic and toxic damage to the liver. In the same study, high aminotransferase levels were measured in 90% of the cases with ischemic or toxic liver damageand in 80% of the cases, high LDH levels were detected. In our work-up, we measured ALT, AST, and LDH levels as well which of these are the most reliable parameters indicating liver cell damage and destruction in serum. We found significant differences in the AST, ALT, and LDH levels in the sham group and the study group compared with the control group, and it gave us information about liver damage that we created in our experimental model. This assessment reveals the positive impact of quercetin on liver IR injury.

In the present study, to determine the level of oxidative stress, the TAS and TOS values were measured and OSI was calculated. Serum TAS analysis yielded relatively lower values in the study group, which was opposite of the desired result. However, serum TOS values and OSI levels were found to be higher in the control group than in the study group. In their study, Costantini et al. indicated that a marker of antioxidant capacity itself was not sufficient to make assumptions about oxidative stress and suggested an association with at least a marker of oxidative damage (22). In this context, these results were interpreted to mean that quercetin, which is known to inhibit lipid peroxidation and xanthine oxidase activity, can reduce pro-oxidant production rather than increase antioxidant capacity. Altogether, the present study demonstrated that following IR injury, oxidative/antioxidative balance shifted toward oxidative status, and higher oxidative stress was observed in the control group subjects.

Free oxygen radicals and abnormal activation of Kupffer cells play main roles in hepatic injury (23). This leads to the formation of structural and functional changes in the liver (24). In our work-up, in addition to biochemical parameters, to reveal liver damage, cytoplasmic vacuolization, sinusoidal dilatation, apoptosis, and necrosis were evaluated. Quercetin has been shown to reduce sinusoidal dilatation and cytoplasmic vacuolization significantly, necrosis and apoptosis moderately, and quercetin has been found to reduce histopathological damage. In the light of these results, we may mention the moderately positive effect of quercetin on the acute phase state of hepatic IR injury.

Conclusion

When considering all findings, quercetin, which hosts many molecules within its structure with a potential utility, such as an antiinflammatory agent, antioxidant, and antiaggregant, may have an effect that is protective against hepatic IR injury. However, especially to recommend it as a stand-alone or preoperative nutritional support for the preparation of surgery, broader comparative studies are needed.

Ethics Committee Approval: The ethics committee approval was received for this study from Erciyes University Animal Ethics Committee (Approval Date: 15.01.2014/Approval No: 14/017).

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

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