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Research Article

The Effect of Taxifolin in the Treatment of Renal Damage Induced by Ischemia Reperfusion in Rats

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Abstract

Objectives: Renal ischemia-reperfusion damage can develop following the conditions such as shock and trauma. Cell function disorder and even necrosis can be seen during ischemia. For this reason, it is inevitable to perform the reperfusion process as soon as possible. Taxifolin is a flavanone that is found in onions, thistle and fir bark. The purpose of study is to examinethe effect of taxifolin on renal damage induced by ischemia reperfusion in rats biochemically and histopathologically.

Methods: A total of 18 albino wistar strain male rats were used in the experiment. The animals were divided into three group: Renal ischemia reperfusion group. The group which was given 50 mg/kg taxifolin after renal ischemia reperfusion. The group which was applied sham operation.

Results: Compared with malondialdehyde levels, total glutathione levels, COX-1 and COX-2 activities of the groups: There was a statistically significant difference between the group receiving renal ischemia reperfusion and the group receiving taxifolin after renal ischemia reperfusion. In the group treated with taxifolin, renal damage observed in the renal ischemia group was found to be greatly healed histopathologically.

Conclusion: It has been concluded that the use of taxifolin may be useful in correcting renal ischemia reperfusion injury.

Keywords: Taxifolin, renal damage, ischemia reperfusion.

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Renal ischemia-reperfusion (RIR) damage can develop following the conditions such as shock, trauma and kidney transplantation.[1] In addition, kidney ischemia is carried out as a precaution against bleeding during various urological vascular surgical interventions and ischemia is terminated by reperfusion after surgery.[2] Cell function disorder and even necrosis can be seen during ischemia.[3] For this reason, it is inevitable to perform the reperfusion process as soon as possible. However, reperfusion process being performed after renal ischemia may increase cell damage occurred with ischemia and renal dysfunction further.[4]

There are a number of scientific studies to enlighten the pathogenesis of ischemia-reperfusion (I/R) damage. As is known, since molecular oxygen (O_2) is not sufficiently present in the tissue with ischemia, xanthine oxidase (XO) cannot participate in hypoxanthine metabolism and accumulates excessively. For this reason, hypoxanthine cannot be metabolized by XO and free oxygen radicals (FOR) cannot be produced unless there is reperfusion. However, excessive accumulation of XO during ischemia with the provision of O^2 in the reperfusion leads to conversion of hypoxanthine to xanthine, uncontrolled formation of FORs and reduction of antioxidants.[5, 6] These FORs, which are known as reperfusion mediators, cause cell membrane lipids to oxidize and allow toxic products such as malondialdehyde (MDA) to form from lipids.[7] Antioxidant parameters such as total glutathione (tGSH) decrease as oxidant parameters in renal I/R injury. In addition, oxidant antioxidants vary in

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favor of oxidant.[8] COX-1 is a structural enzyme responsible for cytoprotective activity in tissues.[9] COX-2 is an enzyme that plays an important role in the production of proinflammatory prostaglandins and in the induction of inflammation.[10] The I/R process results in a decrease in COX-1 activity and an increase in COX-2 activity.[9]

One of the major components of I/R damage is shown as the activation of proinflammatory polymorphic nuclear leucocytes (PNL).[11] In I/R incident, it has been reported that FORs are released from active PNLs.[12] Activated PNLs also release myeloperoxidase (MPO) enzyme; the MPO enzyme is a specific enzyme for PNLs and is considered to be a marker of reperfusion damage.[13] Literature reveals that oxidative stress and inflammation are important components of I/R pathogenesis. It also suggests that antioxidant and anti-inflammatory activities may be useful for the treatment of I/R damage.

Taxifolin (3.3', 4', 5.7-pentahydroxiflavanon) which we will try its protective effect against kidney I/R damage is a flavanone that is found in onions, thistle, French maritime and Douglas fir bark. Taxifolin's antioxidant and anti-inflammatory activities have been proven.[14–16] This information suggests that taxifolin may be effective and helpful in preventing kidney I/R damage. In literature, there have been no studies encountered investigating the effect of taxifolin on I/R-induced renal damage. For this reason, the purpose of our study is to examinethe effect of taxifolin on renal damage induced by I/R in rats biochemically and histopathologically.

Methods

Animals

A total of 18 albino wistar strain male rats with the weight ranging between 285-296 grams were used in the experiment. All of the rats were obtained from Atatürk University Medical Experimental Application and Research Center. Ethical approval to conduct the study was obtained from local Animal Experimentation Ethics Committee of Erzurum Atatürk University (File number: 5/123-27.04.2018). Animals were hosted and fed in groups at room temperature (22° C) under appropriate conditions before the experiment.

Chemicals

Taxifolin Evas used in the experiment were supplied from Russia and thiopental sodium used in the experiment was supplied from İ.E ULAGAY (Turkey).

Animal Groups

The animals were divided into three groups; renal ischemia reperfusion (RIR), the ones which were given 50 mg/kg taxifolin+renal ischemia reperfusion (TIR) and the ones which were applied sham operation (SG).

Application of Anesthesia

The surgical procedures on rats were carried out under sterile conditions; anesthesia was provided by giving 25 mg/kg of intraperitoneal (ip) thiopental sodium and administering xylazine by inhalation at appropriate intervals. After the injection of thiopental sodium, the rats were kept waiting for the appropriate surgery period to occur. The period when the animals are motionless in the supine position is considered to be an appropriate period of anesthesia for surgical intervention.[17]

Surgical and Pharmacological Procedures

One hour before thiopental sodium anesthesia, a dose of 50 mg/kg of taxifolin (n-6) was applied to the TIR animal group and distilled water as solvent was given to the RIR (n-6) and SG (n-6) rat groups with the same method. During the anesthesia period, the unilateral left kidneys of all rats were reached by opening a dorsal section. Then, ischemia was formed for one hour by placing clips to the renal arteries and venous veins coming to the left kidneys of TIR and RIR groups Without any operations being carried out to the left kidney of the SG group, the opened region was closed with a suture. At the end of this period, reperfusion was administered to TIR and RIR rat groups for six hours. After this period, TIR and RIR groups were killed with high dosage of anesthesia and their kidneys were removed. Biochemical and histopathological evaluations were performed on the removed kidneys. Biochemical and histopathologic results obtained from TIR and SG groups were compared with the results obtained from RIR group.

Biochemical Processes

MDA measurements were made based on the method used by Ohkawa et al.[18] tGSH analysis was performed according to the method described by Sedlak J et al.[19] Measurements of COX-1 and COX-2 activities were measured according to the method of Kulmacz RJ et al.[20]

Histopathological evaluation

Histopathologic analysis was performed of the paraffin embedded tissue sections obtained from rats. Four micron sections prepared from paraffin blocks were stained with hematoxylin and eosin staining. The preparations were evaluated by histopathologic examination.

Statistical Analysis

Descriptive statistics for MDA, tGSH, COX-1 and COX-2 were shown in bar charts (Fig. 1). For comparison of 3 or more groups Kruskal Wallis test was used. For Kruskal Wallis test, Dunn's test was used as a post-hoc test. The statistical level of significance for all tests was considered to

be 0.05. Statistical analysis was performed using the IBM SPSS ver. 19 package program (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.).

Results

Biochemical Findings

MDA and tGSH analysis results

As shown in graphic, I/R process led to an increase in MDA production in kidney tissue. The difference between MDA values of taxifolin and healthy groups was not statistically significant (p=0.279). When TIR and SG groups compared to RIR group, statistically significant differences were determined (respectively, p=0.0.037, p<0.001).

tGSH value of the kidney tissue of RIR group which had I/R process applied was found to be different from taxifolin and healthy groups. The difference between tGSH values of taxifolin and healthy groups was not statistically significant (p=0.697). When TIR and SG groups compared to RIR group, statistically significant differences were determined (respectively, p=0.0.020, p<0.001).

COX-1 and COX-2 analysis results

As shown in graphic, RIR group has low COX-1 activity and has high COX-2 activity compared to taxifolin and healthy groups.The difference between COX-1 and COX-2 activities of taxifolin and healthy groups was not statistically significant (respectively; p=0.766, p<0.155).When TIR and SG groups compared to RIR group for COX-1 activity, statistically significant differences were determined (respectively, p=0.018, p=0.001). When TIR and SG groups compared to RIR group for COX-2 activity, statistically significant differences were determined (respectively, p=0.006, p=0.001).

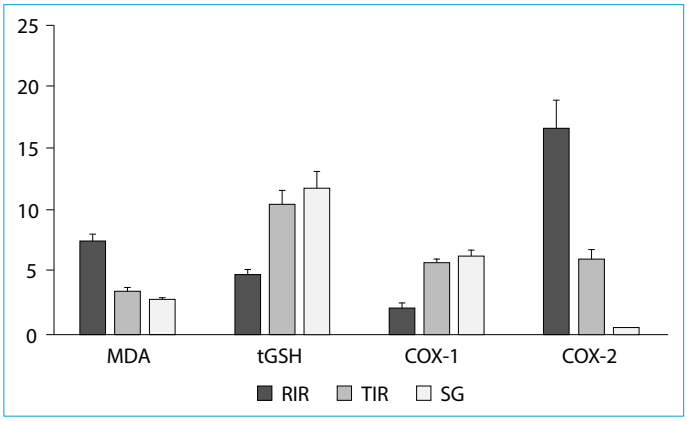

Figure 1. Comparisons of groups in terms of MDA (µmol/g protein), tGSH (nmol/g protein), COX-1 (u/g), COX-2 (u/g).

Histopathological Findings

In Figure 2, in the histopathologic examination of SG animal group's kidney tissue, normal glomerulus, Bowman capsule, proximal tubule and distal tubule structure were observed. However, in the kidney tissue of RIR group that underwent I/R process, distinct congested dilated vein structures, hemorrhagic regions and glomerular damage were observed (Fig. 3). In the kidney tissue of the TIR group treated with Taxifolin, near-normal glomerulus, Bowman capsule, proximal tubule and distal tubule structures were observed (Fig. 4).

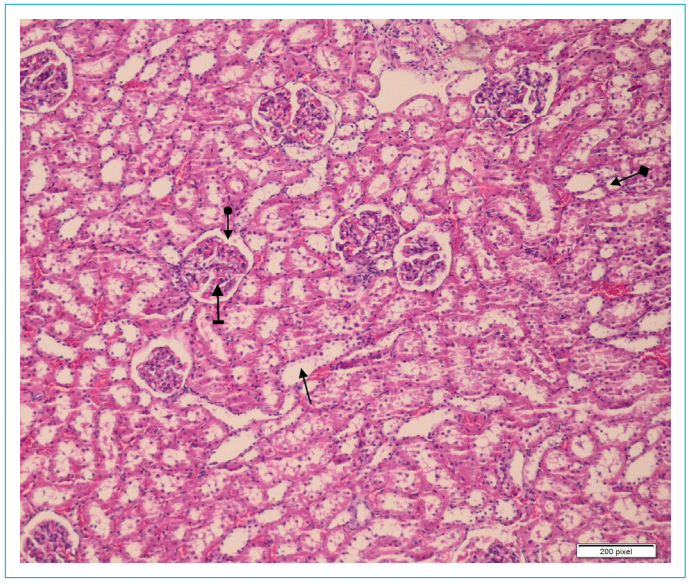

Figure 2. In the histopathologic examination of SG animal group's kidney tissue, normal glomerulus (dashed arrow), Bowman capsule (circular arrow), proximal tubule (square arrow) and distal tubule structure (straight arrow) are observed (HEX100).

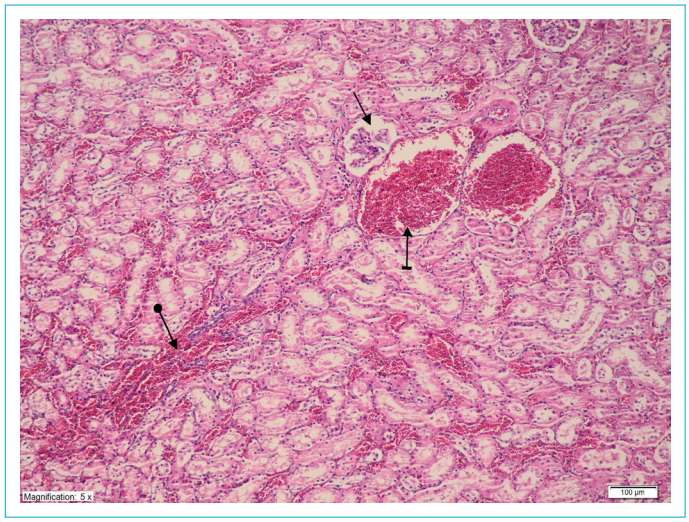

Figure 3. In the kidney tissue of RIR group which had I/R process applied, clear congested dilated vein structures (dashed arrow), hemorrhagic regions (circular arrow) and glomerular damage (straight arrow) are seen (HEX100).

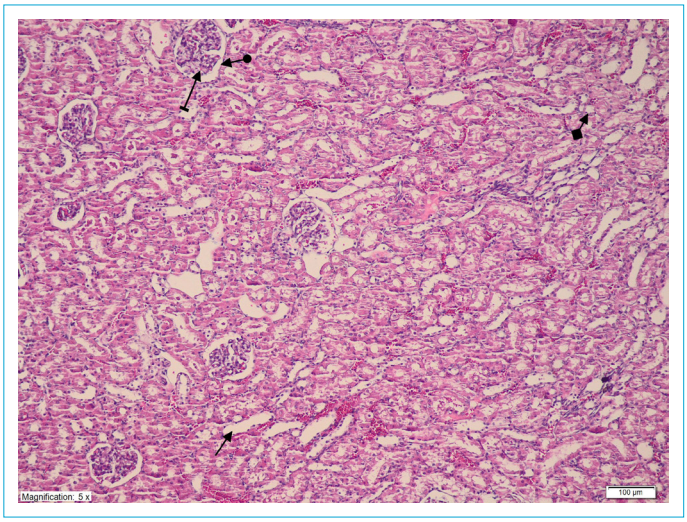

Figure 4. In the kidney tissue of TIR group treated with Taxifolin near-normal glomerulus (dashed arrow), Bowman capsule (circular arrow), proximal tubule (square arrow) and distal tubule structure (straight arrow) are observed (HEX100).

Discussion

Our biochemical test results showed that the MDA value in the kidney tissue that was applied I/R had been increased significantly compared to taxifolin and healthy group. As is known, MDA is the last product of lipid peroxidation. For this reason, MDA was used to evaluate oxidative stress.[21] Thus, an increase in MDA level in a tissue indicates that free oxygen radicals have increased MDA that is emerged as a result of lipid peroxidation is toxic and causes further destruction. As a result of this damage, the structure and function of the membrane are severely damaged and cause cell death.[22] Previous studies carried out have shown that MDA was used to assess oxidative kidney damage and that the histopathologic damage leading up to the necrosis of the kidney tissue found at high level was developed.[23] In another study, it was reported that the increase in the MDA value in kidney tissue subjected to I/R process was related to histopathological damage.[24] Recent studies have shown biochemically and histopathologically the drugs that inhibit the increase of MDA levels in renal I/R damage reduce the oxidative damage in kidney tissue.[25] Literature data suggests that it is in consistency with our experiment results.

Taxifolin, whose effect against I/R damage was examined, has been shown to prevent the increase of MDA in kidney tissue. Pathological indications such as congested dilated vein, hemorrhage edema and destruction seen in the I/R group with increased MDA value were not seen in the taxifolin group. Our findings were in consistency with the literature data mentioned above. Antioxidant activity of taxifolin has been proven in previous studies carried out. As stated above, taxifolin is a flavanone. It has been reported in literature that flavanones exhibit antioxidant activities by inhibiting enzymatic reactions responsible for the formation of lipid peroxidation and free radicals.[26]

In our study, it was also determined that I/R process suppressed the production of tGSH in kidney tissue and there was a decrease in the amount of tGSH. It has been understood that the decreased value of tGSH is compatible with histopathologic findings. There have been studies reporting that antioxidant parameters such as tGSH decrease as oxidant parameters increase in renal I/R damage. In addition, histopathological findings have been documented in studies showing that the oxidant antioxidant balance has changed in favor of oxidants[8] For this reason, antioxidant therapy is suggested to prevent tissue damage due to the increase in oxidant production.[27]

According to our results, while oxidant antioxidant balance in the kidney tissue that was subjected to I/R was found to be changed in favor of oxidants, it was understood that it was opposite in taxifolin group which was an antioxidant. The oxidative antioxidant balance, which has changed for the benefit of oxidants in I/R damage, being corrected with taxifolin, is also supported with literature data.[28]

Furthermore, I/R process caused a decrease in COX-1 activity and an increase in COX-2 activity in the kidney tissue in the study. It is known that COX-1 enzyme is a structural enzyme responsible for cytoprotective activity in tissues.[9] The COX-2 enzyme plays an important role in the production of proinflammatory prostaglandins and in the induction of inflammation.[10] Also, it is defended that COX-2 enzyme is the source of free oxygen radicals.[29] Our experimental results and literature reveal that in the pathogenesis of the I/R process, both oxidative stress and inflammatory damage occur. This confirms that the renal I/R damage is a complex pathologic process that begins with the asphyxiation of the tissues, continues with the production of free oxygen radicals and expands with inflammatory response.[30]

There have been no studies investigating the effect of taxifolin on COX-2 activity increasing during renal I/R damage. However, it has been reported that increasing COX-2 activity during brain I/R damage suppresses gene expression. Furthermore, it has been stated that taxifolin inhibits polymorphic nuclear leukocyte infiltration.[31] The literature data supports our biochemical and histopathological findings showing that taxifolin prevents I/R renal damage.

In our study, I/R process caused oxidative stress and inflammatory instances to be induced in kidney tissue. Oxidant-antioxidant balance in kidney tissue of I/R applied animals has been changed in favor of oxidants. The balance between COX-1 and COX-2 enzyme is impaired for the benefit of COX-2. Taxifolin prevented the oxidant-antioxidant balance from changing in favor of oxidants, while inhibiting

the balance between COX-1 and COX-2 from changing for the benefit of COX-2. This results to think that taxifolin protects kidney tissue against I/R damage.

Conclusion

The results support that taxifolin protects renal tissue against ischemia reperfusion injury. It has been concluded that the use of taxifolin may be useful in correcting renal ischemia reperfusion injury and that the results should be supported by research.

Disclosures

Ethics Committee Approval: This study was approved by the local Animal Experimentation Ethics Committee of Erzurum Ataturk University (Approval No.: 27.04.2018 / 5/123).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.G.B.; Design – M.G.B.; Supervision – M.G.B., H.E.; Materials – H.E.; Data collection &/or processing – M.G.B., M.G.; Analysis and/or interpretation – M.G.B., M.G.; Literature search – H.E.; Writing – M.G.B., H.E.; Critical review – M.G.B., H.E., M.G.

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