Research Article

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Effect of protocatechuic acid against renal ischemia reperfusion damage on extracellular matrix integrity and related signal pathways

Renal iskemi reperfüzyon hasarına karşı protokatekuik asit'in ekstrasellüler matriks bütünlüğü ve ilişkili sinyal ileti yolakları üzerine etkisi

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Abstract

Objective: In this study, possible protective effects of protocatechuic acid (PCA) against experimentally-induced acute renal ischemia/reperfusion (I/R) damage in rats, on matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and the associated signal transduction pathways were investigated.

Methods: A total of 3–4 month-old, 200–250 g *Sprague Dawley* rats were divided into groups of five (n=7). A right kidney nephrectomy surgery was conducted to all groups under anesthesia. Rats were administered polyethylene glycol 1 h prior to ischemia (Group I, II) and PCA (Group III, IV, V) intraperitoneally. Forty five minutes before the ischemia during 24 h reperfusion on all rats except those in Group I. At the end of the experiment, blood urea nitrogen (BUN), creatinine values and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) enzyme levels were investigated in blood serum. MMP-2 and MMP-9 gene expression levels were determined by RT-PCR, and p38 and p-p38 protein expression levels Western blotting method. Renal tissue was examined histologically and immunohistochemically.

Results: It is assumed that 80 and 120 mg/kg of PCA might have a protective effect against oxidative stress damage caused by renal I/R.

Conclusion: In our study, PCA has been shown to modulate the increased expression of MMP-2 and MMP-9 mRNA along with increased oxidative stress during renal I/R, as well as oxidative damage-induced p38 protein expression. It was determined that particularly 120 mg kg^{-1} PCA reduced the renal I/R injury at a rate of 35–45%.

Keywords: ischemia/reperfusion; kidney; MMP-2; MMP-9; protocatechuic acid; p38 MAPK.

Öz

Amaç: Bu çalışmada sıçanlarda deneysel olarak oluşturulan renal iskemi/reperfüzyon (I/R) hasarına karşı protokatekuik asit (PCA)'in, hücre dışı matriks regülasyonunda önemli rolü olan matriks metalloproteinaz-2 (MMP-2), matriks metalloproteinaz-9 (MMP-9) ve ilişkili sinyal ileti yolakları üzerine olası koruyucu etkileri araştırıldı.

Materyal ve Metod: Toplam 35 adet 3–4 aylık 200–250 gr ağırlığında *Spraque-Dawley* cinsi erkek sıçanlar beş gruba ayrıldı (n=7). Anestezi altında tüm gruplara sağ böbrek

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nefroktomisi gerçekleştirildi. Sıçanlara intraperitonal olarak 15 gün boyunca günde bir kez olmak üzere polietilen glikol (Grup I, II), ve protokatekuik asit (Grup III, IV) verildi. Grup I hariç tüm gruptaki sıçanlara 45 dakika iskemi 24 saat reperfüzyon işlemi uygulandı. Deney sonunda serumda Kan Üre Nitrojeni (BUN), Kreatinin değerleri ve Süperoksit Dismutaz (SOD), Katalaz (CAT) ve Glutatyon peroksidaz (GPx) enzim seviyelerine bakıldı. MMP-2 ve MMP-9 gen ekspresyon seviyeleri RT-PCR ile p38 ve p-p38 protein ekspresyon seviyeleri Western Blot yöntemi ile belirlendi. Renal doku histolojik ve immünohistokimyasal olarak da incelendi.

Sonuçlar: 80 ve 120 mg/kg PCA'nın böbrek İ/R ile oluŞan oksidatif stres hasarı üzerine koruyucu etkisinin olabileceği düşünülmektedir.

Sonuç: çalışmamızda, PCA'nın MMP-2 ve MMP-9 mRNA'nın artan ekspresyonunu, renal I/R sırasında artmış oksidatif stresin yanı sıra oksidatif hasara bağlı p38 protein ekspresyonunu modüle ettiği gösterilmiştir. Özellikle 120 mg kg^{-1} PCA'nın renal I/R hasarını % 35–45 oranında azalttığı belirlenmiştir.

Anahtar Kelimeler: Böbrek; İskemi/reperfüzyon; Protokatekuik asit; MMP-2; MMP-9; p38 MAPK.

Introduction

Ischemia/reperfusion (I/R) damage is one of the main causes of acute kidney damage, and can develop in conditions such as hemorrhagic shock, partial nephrectomy, kidney transplant, and urological surgical procedures, causing organ loss due to temporary interruption of blood flow to the kidneys [1, 2]. The mediators released by polymorph nuclear leukocyte (PMNL) cells that reside in tissues during ischemia and the reactive oxygen species (ROS) are responsible for the damage that occur as a result of I/R [3]. Matrix metalloproteinase (MMP) is zinc and calcium-dependent neutral endopeptidase playing important roles in the regulation of cell-matrix composition that is responsible for the degradation of the extracellular matrix. MMPs play important roles in tissue development and differentiation, in angiogenesis and cell migration [4–6]. MMPs are divided into six groups according to their structure and substrate specificities, and especially gelatinases (MMP-2 and MMP-9) in this group play an important role for Type IV collagen degradation, which is the main component of the basement membrane [7]. In the I/R process, *in vivo* and *in vitro* I/R models have been shown to increase ROSs and proinflammatory cytokines, especially after reperfusion, and to induce MMP-2 and MMP-9 from MMPs, and cause vascular damage with basal membrane degradation. Increased ROS production leads to the activation of the p38 mitogen, which is involved in the gene regulation of MMPs, particularly MMP-2 and MMP-9, and to the mitogen activated protein kinase (MAPK) signals [8]. MMPs are basically regulated by three MAPK pathways, p38 MAPK, c-Jun N terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) [9]. This pathway is activated specifically p38 MAPK by the ROS. With the activation of this pathway, it has been shown in many studies that MMP-2 and MMP-9 are up-regulated [10, 11].

Against the potential damages of the ROS, many cell-protective enzymes are employed, and the radical damage is limited with antioxidant agents. Phenolic compounds constitute a class of secondary metabolites produced in plants [12]. PCA (3,4-dihydroxybenzoic acid) is a simple natural phenolic compound found commonly in the nature [13, 14]. It was determined in current studies that PCA has several biological activities like anti-oxidant, anti-atherosclerosis [15], anti-inflammatory, anti-carcinogenic [16]. In addition, *in vitro* gene expression and *in vivo* metastasis models showed that it prevents the cell migration and also the metastasis with its feature that reduces the matrix deterioration [17]. The effects of PCA on MMPs and on renal I/R-induced p38 MAPK signal pathway have not been clarified completely. In this study, the effect of PCA on MMP-2 and MMP-9 gene expression and the mechanism of action on the p38 MAPK signal path were investigated.

Materials and methods

The whole experimental study was conducted with the permission numbered 615/2017 issued by Eskisehir Osmangazi University, Animal Experiments Local Ethics Committee.

Animals

3–4 month-old *Sprague Dawley* male albino rats weighing 200–250 g were used in the experimental study. All animals were obtained from Eskisehir Osmangazi University, Experimental Animal Production Laboratories (TICAM). The rats were allowed a week before the experiment to be acclimatized to laboratory conditions.

Experimental protocol

Rats were divided randomly into five groups (n=7). Group I (Sham), Group II (I/R + Polyethylene glycol), Group III (I/R + 40 mg/kg PCA), Group IV (I/R + 80 mg/kg PCA), and Group V (I/R + 120 mg/kg PCA) were marked, respectively. The PCA applied in the experiment was obtained from Sigma-Aldrich, and was prepared by injection with polyethylene glycol of 33% in a volume of 2 mL/kg. Right kidney nephrotomy was performed to all groups under xylazine and ketamine anesthesia. After the nephroctomy, recovery time was given for

15 days. After 15 days for recovery, animals in Group I and Group II were given polyethylene glycol 1 h before ischemia. Animals in Group III, IV and V were given PCA 1 h before ischemia. In the kidneys of animals in Group II, III, IV and V, the left renal artery and vein were isolated and blood flow was stopped for 45 min with the help of an atraumatic vascular clamp. After 45 min ischemia, the clamp was removed and reperfusion was performed for 24 h [18]. At the end of reperfusion process, the rats were sacrificed all removing blood from the hearts under anesthesia. The blood samples were examined to find out blood urea nitrogen (BUN) and creatinine values to assess the renal function.

Biochemical analyses

The blood samples were examined to find out BUN and creatinine values to assess the renal function. BUN (catalog number 04460715190, Roche Diagnostics) and creatinine (catalog number 04810716190, Roche Diagnostics) levels were examined by using Roche/Hitachi Cobas integra 400 plus Auto analyzer and commercial kits. The results were expressed as mg/dL. The superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) measurement in serum was made with Cayman-brand SOD (catalog number 706002, Cayman Chemical, Ann Arbor, Michigan, USA). CAT (catalog number 707002,Cayman Chemical, Ann Arbor, Michigan, USA). and GPx (catalog number 703102, Cayman Chemical, Ann Arbor, Michigan, USA) measurement kit Absorbance reading was carried out with ChemWell 2910-brand ELISA reader device (Awareness Technology, Inc. Martin Hwy. Palm City, the USA). The results were given as nmol/min/mL.

RNA isolation and cDNA synthesis

50 mg kidney tissue was extracted with Trizol (Ambion, Cat. No: 12183018A) for total RNA isolation. Then, isolation was performed according to the instructions recommended by the manufacturer, using the PureLink RNA mini kit (Ambion, Cat. No: 12183018A). After the purity of the isolated RNAs were measured by spectrophotometer (260/280 nm = 1.8–2.0) the samples were stored at −80 °C. The complementary DNA (cDNA) was obtained from the RNA using a high-capacity cDNA kit (catalog No. 4368814, Applied Biosystems, Carlsbad, CA, the USA)

RT-PCR Assay

The GoTaq qPCR Master Mix kit (Cat. No. A6001/2) was used for RT-PCR and gene expression RT-PCR experiments were performed according to the manufacturer's instructions. The DNA was amplified in a reaction mixture containing the following pairs of primers: MMP-2, 5′-GATCTGCAAGCAAGACATTGTCTT-3′ (forward) and 5′-GCCAAATAAACCGATCCTTGAA-3′ (reverse); MMP-9, 5′-GTAACCCTGGTCACCCGACTT-3′ (forward) and 5′-ATACGTTCCGGCTGATCAG-3′ (reverse); β-actin, which was used for internal standard, 5′-GCACCACACCTTCTACAATG-3′ (forward) and 5′-TGCTTGCTGATCCACATCTG-3′ (reverse). The amount of mRNA for each gene was normalized β-actin.The results were calculated according to the $2^{-\Delta\Delta}$Ct formula with the Ct (Cycle threshold) method [19].

Western blot analysis

Amersham bioscience's extracellular fluid (ECF) detection system (catalog No. RPN2232, Pittsburgh, PA, the USA) was used for Western Blotting according to the manufacturer's instructions. Kidney homogenate samples containing 50 µg of total protein were analyzed by 10% SDS-PAGE. The total protein amount of the obtained homogenate was measured with the Qubit 2.0 fluorometer quantitates device. The proteins were then transferred to polyvinylidene difluoride (PVDF) membranes at 70 V for 3 h. Nonspecific binding of antibodies was blocked in phosphate buffered saline (PBS) with 5% fat dry milk. Then, it was incubated overnight at 4 °C with primary antibody against p38 MAPK (Cell Signaling Technology, catalog no. 9212) diluted 1:1,000. The blots were then extensively washed with TBS-T and incubated with a 1:25,000 dilutions of antirabbit, alkaline, phosphatase-conjugated, secondary antibodies for 1 h. The bands were visualized using an enhanced chemiluminescence (ECL) reagent (Thermo Fisher, Rockford, IL). Finally, the blots were developed with ECL Plus western blotting substrate for 3–5 min.

MMP-2 and MMP-9 immunohistochemistry

Tissue samples for MMP-2 and MMP-9 immunohistochemistry were deparaffinized and treated with a solution of (20 mL H_2O_2 + 180 mL distilled water) for 10 min. Then it was washed with distilled water for 3 min. The prepared citrate buffer was boiled in the microwave for 15 min. The sections were then incubated at 4 °C for 18 h in a humid room with rat-specific anti-MMP-2 antibody 1:100 (Bioss, Woburn, MA) and anti-MMP-9 antibody 1:100 (Bioss). Then, the sections were incubated with biotinylated IgG that was prepared in line with the instructions of the manufacturer (Invitrogen) and then with streptavidin for 30 min. Finally, the sections were treated with 3.3′-diaminobenzidin (DAB), counter-stained with Mayer hematoxylin and were examined underlight microscope.

Histopathological analyses

Tissue samples taken for histological examination were placed into 10% neutral formaldehyde solution. After routine tissue processing stage, they were painted by using hematoxylin and eosin (H&E) Method. All tissue sections were histologically examined with CH40 model Olympus light microscope; and their images were taken with 3.2.0 model spot insight digital camera and spot advanced 4.0.6 software.

Statistical analyses

The results of the experiments were evaluated using "SPSS 20.0 for Windows" software and "One Way Annova-Tukey" tests. The average values of the groups were compared by using the Whitney U test and Kruskal–Wallis test for MMP-2 and MMP-9 gene expression analyses. After all statistical procedures, the differences between the experimental groups that appeared as numerical values (p) were considered significant at the significance level of $p<0.05$.

Results

At the end of the experiments, BUN and creatinine values obtained from serum samples of all groups were comparatively shown in Table 1. It was found out that Group II had meaningful differences ($p<0.05$) compared to Group I in terms of its BUN and creatinine levels. When Group II was compared with Group III, IV and V, no significant differences were detected at the BUN levels of Group III and IV compared to Group II ($p>0.05$); however, a significant difference was detected in Group IV compared to Group II ($p<0.05$). In addition, significant differences were detected at the creatinine levels of Group IV and V compared to Group II ($p<0.05$); however, no significant differences were detected in Group III compared to Group II ($p>0.05$). As a result of the analysis, the SOD, CAT and GPx values of the serum samples of all groups are given in Table 1 in a comparative manner.

When the SOD, CAT and GPx enzyme levels were compared, it was determined that all enzyme levels were low in Group I, and high in Group II. On the other hand, it was observed that the enzyme values of Group IV and Group V had lower density than Group II. When the study groups were compared among each other, it was determined that the SOD, CAT and GPx enzyme levels of Group V were lower than those of Group III and IV at a significant level ($p<0.05$).

Both MMP-2 and MMP-9 mRNA expressions are shown in Figure 1. In the I/R Group, the MMP-2 mRNA expression increased compared to the Sham Group (1.58 fold). In the PCA Group, a decrease was detected at a small level depending on the dose when compared to the I/R Group (1.49, 1.37 and 1.47-fold, respectively). These differences were not at statistically significant levels ($p>0.05$). Also, the MMP-9 mRNA expression was significantly increased (4.66-fold) in the I/R group compared to the sham group. The PCA pretreatment caused a significant decrease in the

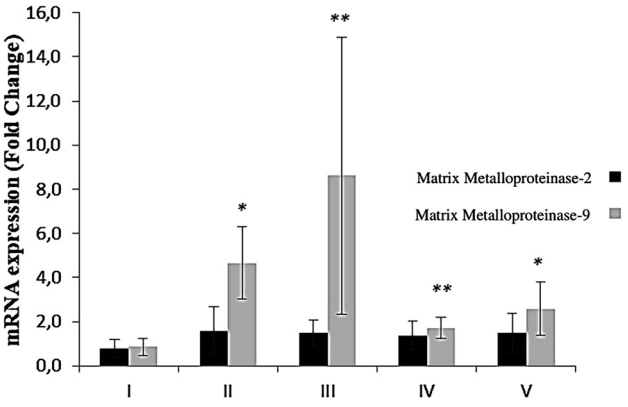

Figure 1: The matrix metalloproteinase-2 and matrix metalloproteinase-9 mRNA expression levels in the kidney tissues in the experimental groups. The data are given as means ± SD *$p<0.05$ vs. Sham; **$p<0.05$ vs. I/R.

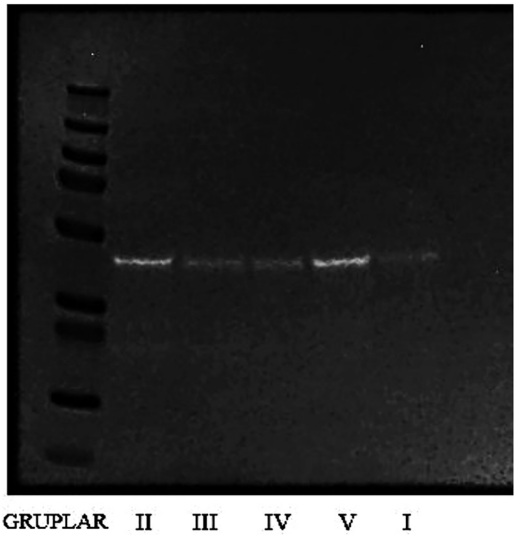

Figure 2: p38 MAPK band images of the study groups.

Table 1: The mean ± standard error (SE) of the BUN, creatinine values and SOD, CAT, GPx enzyme levels of the serum samples of the animals in the experimental group (n=7).

Group	BUN, (mg/dL)	CRE, (mg/dL)	SOD, (nmol/dk/mL)	CAT, (nmol/dk/mL)	GPx, (nmol/dk/mL)
I	18.75 ± 0.63	0.46 ± 0.01	1.19 ± 0.28	68.71 ± 52.97	280.71 ± 89.45
II	132.60 ± 1.39^{ae}	3.98 ± 0.10^{ade}	2.28 ± 0.28^{ace}	428.43 ± 179.76^{ae}	337.14 ± 108.37
III	110.71 ± 6.45^{a}	3.06 ± 0.24^{a}	1.71 ± 0.37^{ab}	368.86 ± 133.77^{a}	435.28 ± 214.06^{ae}
IV	118.50 ± 5.65^{a}	2.90 ± 0.17^{ab}	1.98 ± 0.19^{ae}	347.86 ± 54.95^{a}	293.28 ± 147.47
V	109.55 ± 8.80^{ab}	2.41 ± 0.44^{ab}	1.63 ± 0.24^{abd}	278.71 ± 84.40^{ab}	258.14 ± 84.26^{c}

Different ^{a}: from Group I; ^{b}: Group II; ^{c}: Group III; ^{d}: Group IV and ^{e}: Group V; $p<0.05$. (BUN %cv: 3.36 r^2: 0.999, CRE %cv: 1.85 r^2: 0.999, SOD %cv: 23.52 r^2: 0.995, CAT %cv: 77.09 r^2: 0.995, GPx %cv: 31.86 r^2: 0.998). BUN, blood urea nitrogen; CRE, creatinine; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; % CV, coefficient of variation.

MMP-9 mRNA expression especially in Group IV and Group V in the I/R Group (1.71 and 2.57-fold, respectively). This increase being decreased with PCA was determined to be at a significant level ($p<0.05$). (Figure 1).

The p38 protein levels were measured according to the intensity of the signals they emitted in the Kodag Gel Logic 1500 model imaging device, and are given in Figure 2. It was determined that P38 protein was at the highest level in Group II. When Group II was compared with Group I (9.058 mm^2), P38 levels were significantly higher in Group II (25.965 mm^2). It was determined that this increase decreased at a significant level with PCA. A 2.5-fold decrease was observed in Group V (12.684 mm^2) compared to Group II. It was determined that the protein amount of Group IV (15.159 mm^2) was close to that of Group I (Figure 2). When the protein amounts were compared, a blurry band was seen in all groups; and since the band area was not clear, the protein amounts could not be measured.

The anti-MMP-2 and anti-MMP-9 immuno-positive cells in the renal tubules are shown in Figure 3. The MMP-2 expression was found to be higher at a significant level in the I/R Group compared to the Sham Group. In addition, it increased in the MMP-9 I/R Group compared to the Sham Group. With pre-treatment, PCA decreased the MMP-2 and MMP-9 expressions at a significant level compared to the I/R Group.

Histological results obtained in the study are shown in Figure 4. After the examinations with the light microscope, it was observed that kidney sections of Sham group were in normal appearance (Figure 4A). The following results were observed in the kidney sections of the I/R Group; deformation in the tubule cells because of I/R damage, degeneration in the glomerular structure, erythrocyte extravasation, cell debris in the tubule lumen (Figure 4B), tubular dilatation, inflammation and intense fluid accumulation in the tubules (Figure 4C). In the 40 mg/kg PCA Group, slight decreases were detected in the degenerations that were observed in the tubule cells and in the cortex and in the erythrocyte extravasation compared to the I/R Group, and the fluid accumulation continued in tubule cells (Figure 4D). The brush border in the tubules in the 80 mg/kg PCA Group, tubular atrophy, tubular dilatation, vacuolization, protein substance accumulation in tubules and cell debris to the tubule lumen were observed to be less (Figure 4E). When the kidney sections of 120 mg/kg PCA Group were analyzed, it was determined that the damage in the I/R group was decreased at a great deal, tubule cells and glomerular structures were close to those of the Control Group, and intratubular fluid accumulation and bleedings among tubules were at minimum level (Figure 4F).

Discussion

Renal I/R damage is one of the common causes of acute renal failure, and constitutes a complex pathophysiological process that leads to increased vascular permeability, tissue necrosis, cell damage, and finally, cell death [20]. MMPs are enzymes that are responsible for degradation of extracellular matrix (ECM); they are involved in the pathogenesis of I/R injury. In recent years, it has been shown that MMPs especially gelatinase (MMP-2 and MMP-9) have important roles in the pathophysiology of the I/R damage in many organs and in many tissues like central nervous system, the heart, liver, and the lungs [21]. One of the methods that are employed for determining the renal function after kidney I/R damage as a marker of renal function is determining the serum BUN and creatinine levels. In our study, the BUN and creatinine values in the group with I/R damage increase when compared to the Sham Group. This is an indicator showing that I/R damage affect kidney functions negatively. The BUN and creatinine values of the treatment groups IV and V were found to be lower compared to Group II (especially in Group V). This suggests that PCA might have a protective effect.

In a study conducted by Ozturk et al. [22] the effect of carvacrol, which was supposed to have antioxidant effect, on renal I/R injury was investigated. Two hours before the reperfusion, carvacrol was injected to the rats as 75 mg/kg intraperitoneally. Following the reperfusion, it was determined that the serum BUN and creatinine enzyme values increased in the I/R Group, and decreased in the carvacrol Group. The obtained data show parallelism with the results of our study. In similar studies, it was shown that serum BUN and creatinine values increased depending on time in the damage caused by I/R, when compared to control groups, and decreased in treatment groups [23–27]. It is a defense mechanism against the oxidative damage following I/R in the organism, and the most important of these mechanisms is the antioxidant enzyme system. In our study, PCA decreased SOD and CAT enzyme levels at a significant level during renal I/R; however, GPx did not decrease enzyme levels at a significant level. In our study, when the SOD, CAT and GPx enzyme levels were considered, all enzyme levels were at the highest level in Group II in general. This may be because of the increase in ROS production in the kidney tissue in the animals that were included in the I/R Group. The level of antioxidant enzyme being low is the indication of being exposed to ROS less. The decreases in the treatment groups made us consider that this was due to the antioxidant properties of PCA. When the literature was reviewed, it was detected that the

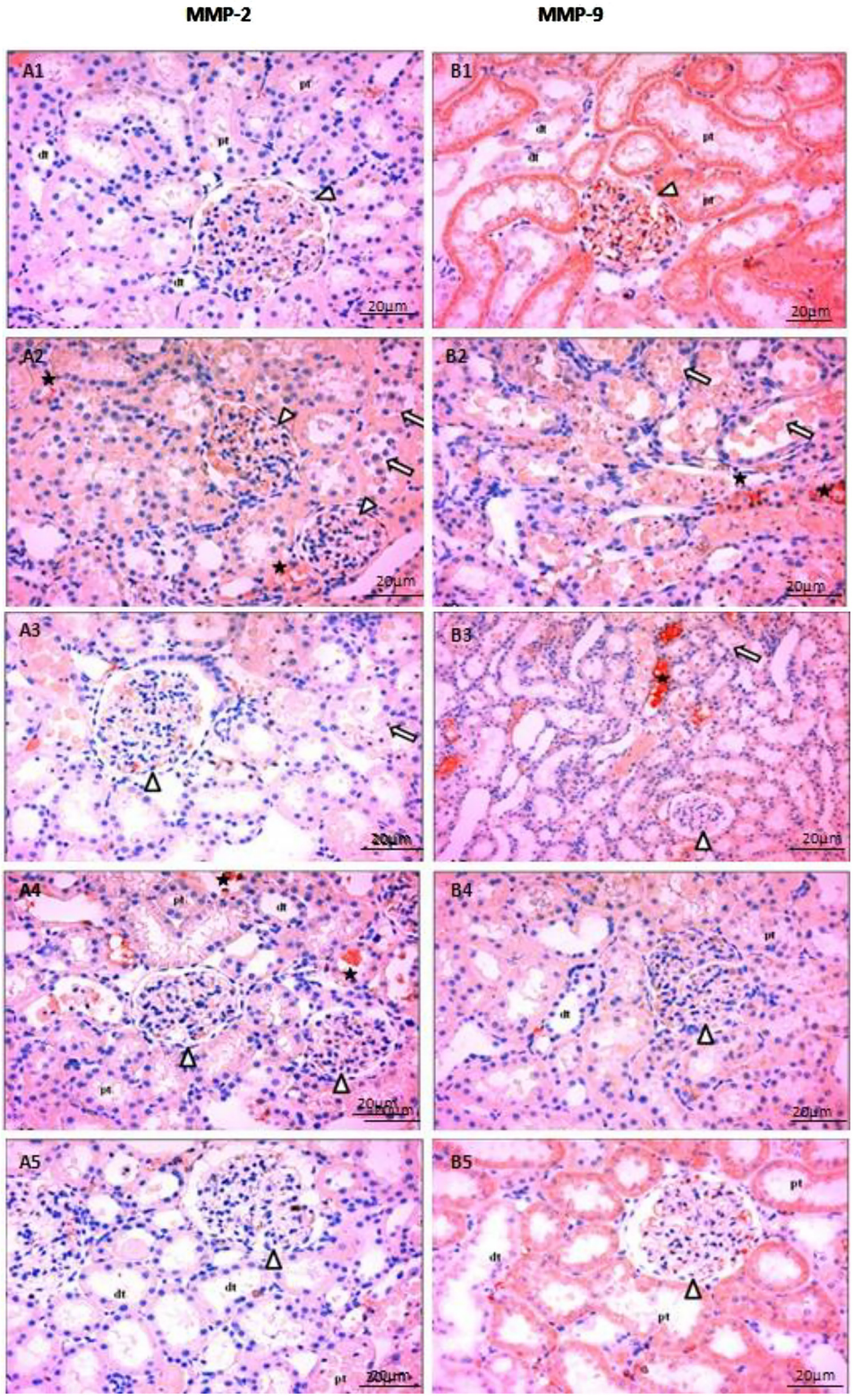

Figure 3: Immunohistochemical analysis results of MMP-2 and MMP-9 belonging to the groups. The kidneys, Malpighian corpuscle and tubular structures of the rats in the Sham Group (▷) are seen in normal histological structure together with their tubular structures. pt: proximal tubule, dt: distal tubule (HE X20) (A1, B1). Decrease in the Bowman range of the kidney cortex of the rats in the I/R Group (▷) (A2) Vascular congestion in the interstitial area (★) and breakdown in the tubule epithelial cells (⇨). (HE, X20). (A2, B2). Although normal structure is seen in the Malpighian corpuscle in the kidney cortex of the rats in the 40 mg PCA Group (▷), there is vascular congestion (▷) and tubule epithelial cell

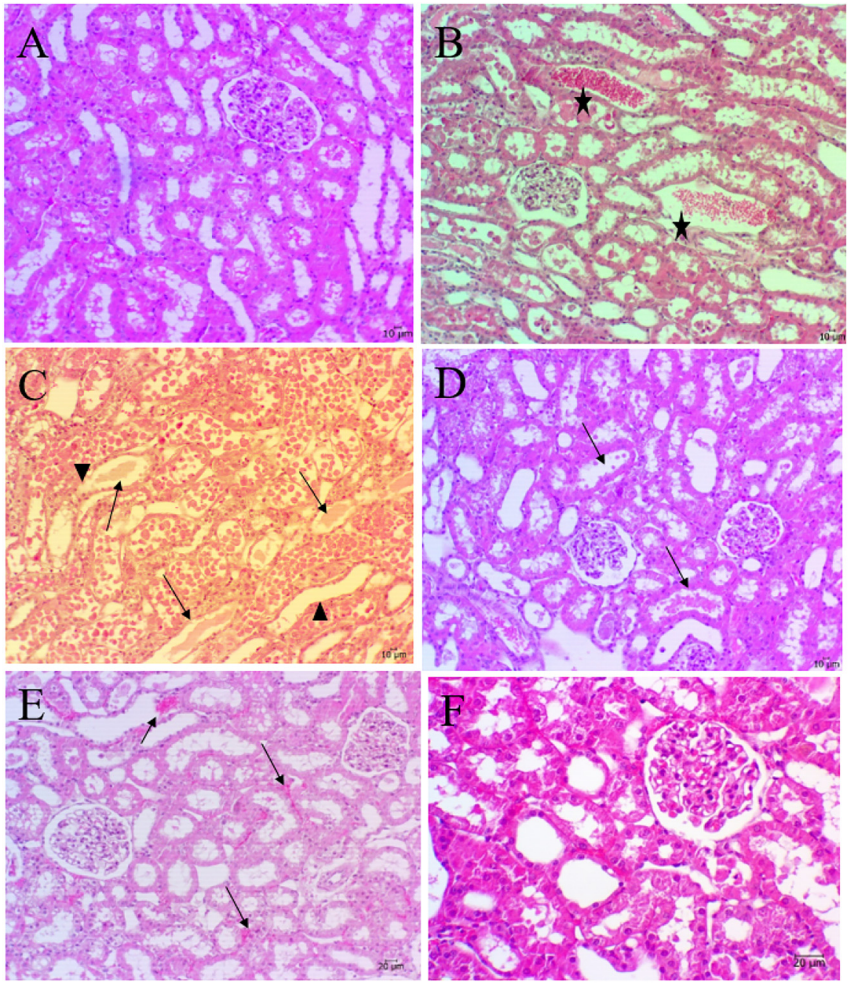

Figure 4: Examining the kidney tissues with H&E staining. A: The glomerulus, Bowman capsule and tubule cells in the kidney sections of the Sham Group; B: The erythrocyte extravasation of the kidney tissue of the I/R Group (★); C: The fluid accumulation in the tubule in the kidney tissue of the I/R Group (↗), tubular dilatation (▲); D: 40 mg/kg PCA Group: Partial decrease in the degeneration in the tubule cells (↗); E: 80 mg/kg PCA Group: fluid accumulation in the tubule lumen (↗); F: 120 mg/kg PCA Group; Close-to-normal glomerulus and tubule cells.

results of our study were similar those of previous studies that were conducted with renal I/R models [8, 28–30]. In another study that was conducted by Canbek et al. [31] after renal I/R damage that was induced with 45 min ischemia and 6 h reperfusion, it was reported that the SOD, CAT and GPx values of the I/R group were higher at a significant level; and there were decreases in the treatment group to which gallic acid was administered.

Findings showing that there were increases in the expression of MMP-2 and MMP-9 mRNA during I/R are consistent with the findings of previous studies [27, 32]. While it was determined that there was an increase in the mRNA expression at a significant level in PCA application, there were no significant differences in MMP-2 gene expression level. As mentioned previously, the post transcriptional regulations of MMP-9 and MMP-2 are different from each other. For this reason, the ROS and proinflammatory cytokines that are produced during I/R might induce the transcriptional expression and/or the activation of MMPs after the translation. It is known that the MAP-Kinase signal transduction pathways (p38 kinase, c-Jun N-terminal Kinase (JNK) and ERK pathway) play important roles in the regulation of the expression of MMPs. With this study, it was shown that the p38 signaling pathway can be induced by ROS [9]. For this reason, phosphorylated p38 (p-p38) and total p38 protein expressions were analyzed with the Western Blot Method to evaluate the signal transduction

breakdown (⇨) in the interstitial area (HE, X10, X20). (A3, B3). There is normal structure in the Malpighian corpuscle in the kidney cortex of the 80 mg PCA Group (▷) vascular congestion in the interstitial area (★). Decreased damage and close-to-normal tubular structures are observed in the tubular structures compared to the I/R Group. pt: proximal tubule, dt: distal tubule (HE, X10, X20). (A4, B4) normal structure is seen in the Malpighian corpuscle in the kidney cortex of the rats in the 120 mg PCA Group (▷). Although partial damage is seen in some tubules, there are decreased damage and close-to-normal tubular structures compared to the I/R Group. pt: proximal tubule, dt: distal tubule (HE, X10, X20) (A5, B5).

pathway better in our study. In the analyzes, it was determined that the p38 protein amount increased at a significant level in the I/R Group compared to the Sham Group; however, this increase decreased at a significant level with PCA. Cavdar et al. [8] examined the role of p38 MAPK signaling in the regulation of the expression and activity of MMP-2 and MMP-9 in renal I/R model in taurine rats. Taurine pretreatment decreased MMP-2 and MMP-9 mRNA expression and the I/R-induced MMP-9 activity at a significant level. The findings are similar to those that were found in our study. In addition, in our study, although the band area of the p-p38 protein expression was not measured due to low density, it was determined that the p-p38 protein expression was increased at a significant level with renal I/R in rats, which is consistent with previous reports [33–35].

When the study was evaluated in histological and immunohistochemical terms, it was determined that the glomerulus and bowman capsule of kidney tubule cells were normal in the tissue samples of Group I, and there were no fluid accumulations in and between the tubules. When Group II was considered, it was observed that there was intense tubular damage, blood build-up in the glomerulus, expansion in the bowman capsule range, vacuolization in some areas, erythrocyte extravasation, intense fluid accumulation in the tubules, tubular debris and inflammation at places. It was observed that PCA decreased the damage at 40, 80 and 120 mg/kg application doses compared to the I/R Group. However, it was also observed that 40 mg/kg PCA decreased the damage at an insignificant level, and 120 mg/kg dose was more effective. This shows that PCA might prevent the ROS effect. When previous studies were considered, it is known that the organs exposed to I/R damage show tubular dilatation, brush border loss, vacuolization, deformation in glomerular structure, PMNL infiltration, etc. [8, 27, 32, 36]. In a study that was conducted by Cavdar et al. [27] possible protective effects of alpha-lipoic acid in renal I/R damage was examined. In H&E stained sections, there were hemorrhage, tubule dilatation, erythrocyte extravasation, glomerulus, cell debris around the capillaries and peritubular areas, thyroidization and inflammatory cell infraction in the I/R Group. However, the histological appearance of tubules in the kidney was better in the animals that were treated with alpha-lipoic acid before the ischemia compared to the I/R Group. These findings on reducing the histological damage are parallel to our findings.

In the light of the histological and biochemical results obtained from this study, it is assumed that, depending on the dosage, especially 120 mg kg^{-1} PCA application has a protective effect.

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Ethical approval: Research involving animals complied with all relevant national regulations and institutional policies (Eskisehir Osmangazi University, Animal Experiments Local Ethics Committee.) for the care and use of animals. (615/2017).

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