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Research Article

The role of prostaglandin E(1) on the model of acute ischemic reperfusion-induced renal injury in rats

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Abstract

Prostaglandin E(1) is a natural prostaglandin that has various pharmacological effects. It has been shown that prostaglandin E(1) has a protective action on some organs of rats treated with renal ischemia/reperfusion. Our aim was to investigate the role of prostaglandin E(1) in rats with renal ischemia-reperfusion-induced acute renal injury. Histological, immunohistochemical, and biochemical analyses were performed. Sprague Dawley male rats were divided into four groups in this study. The first group was given physiological saline only. Second group was administered prostaglandin E(1) (20 µg/kg) only. Third group was treated with ischemia-reperfusion. Fourth group was administered prostaglandin E(1) (20 µg/kg) and applied ischemia-reperfusion. All the rats were sacrificed after the reperfusion period. Dissected kidney tissue was used for histological examination and biochemical analysis. The kidneys of the experimental group with ischemia-reperfusion model have shown histopathologic changes as an increase in proliferating cell nuclear antigen (PCNA) and caspase 3 immunoreactivity, a significant decrease in reduced glutathione and DNA levels, and a significant increase in lipid peroxidation levels. In contrast, the administration of prostaglandin E(1) reversed these effects on kidneys of the animals applied ischemia-reperfusion model. As a summary, the histological, immunohistochemical, and biochemical evaluations have revealed that prostaglandin E(1) ameliorated renal damage of rats with renal ischemia/reperfusion.

Keywords: Acute ischemia-reperfusion, apoptosis, proliferation, prostaglandin E(1), rat.

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Sıçanlarda akut iskemi-reperfüzyon ile oluşan böbrek hasarı modelinde prostaglandin E(1)'in rolü

Özet

Prostaglandin E(1) birçok farmakolojik etkiye sahip olan doğal bir prostaglandindir. Prostaglandin'in renal iskemi-reperfüzyon uygulanan sıçanların bazı organları üzerinde koruyucu bir etkiye sahip olduğu gösterilmiştir. Bizim amacımız renal iskemi-reperfüzyon ile uyarılan akut renal hasarın araştırıldığı sıçanlarda prostaglandin E(1)'in rolünü araştırmaktı. Histolojik, immunohistokimyasal ve biyokimyasal olarak analizler gerçekleştirildi. Bu araştırmada, Sprague Dawley erkek sıçanlar dört gruba bölündü. Birinci gruba sadece fizyolojik tuzlu su verildi. İkinci gruba sadece prostaglandin E(1) (20 µg/kg) verildi. Üçüncü gruba, iskemi-reperfüzyon uygulandı. Dördüncü gruba, prostaglandin E(1) (20 µg/kg) verildi ve iskemi-reperfüzyon uygulandı. Bütün sıçanlar reperfüzyon periyodundan sonra sakrifiye edildi. Parçalara ayrılan böbrek dokusu histolojik inceleme ve biyokimyasal parametreler için kullanıldı. İskemi-reperfüzyon modeli uygulanan deney grubunun böbrekleri çoğalan hücre nukleus antijeni (PCNA) ve kaspaz 3 immunreaktivitesinde bir artış, indirgenmiş glutatyon ve DNA seviyelerinde belirgin bir azalma, lipid peroksidasyon seviyelerinde belirgin bir artış göstermiştir. Buna zıt olarak, prostaglandin E(1)'in verilmesi, iskemi-reperfüzyon modeli uygulanan hayvanların böbrekleri üzerindeki bu etkileri tersine çevirdi. Özet olarak, histolojik, immunohistokimyasal ve biyokimyasal değerlendirmeler, prostaglandin E(1)'in renal iskemi/reperfüzyon uygulanan sıçanların böbrek hasarını iyileştirdiğini ortaya koymuştur.

Anahtar Kelimeler: Akut iskemi-reperfüzyon, apoptoz, proliferasyon, prostaglandin E(1), sıçan.

Introduction

Ischemia-reperfusion results with decreased blood flow in the kidney and is followed by reperfusion. It causes the loss of cellular function by oxidative stress, resulting in the reactive oxygen species (ROS) production, alterations in mitochondrial oxidative phosphorylation, ATP depletion, and in intracellular calcium increase (Maulik et al. 1998; Hauet et al. 2001; Sener et al. 2002; Sung et al. 2002). The generation of ROS is an important cellular injury mechanism in ischemic and reperfused tissues, causing oxidative damage to cellular macromolecules (Sehirli et al. 2003). Prostaglandin E series are vasodilators directly affect the vascular smooth muscle (Tobimatsu et al. 1985). Prostaglandin E(1) is involved in the upkeep of blood flow, distribution of blood within the kidneys, and excretion of electrolytes and water (Numajiri et al. 1994; Koch et al. 2000). It is known that prostaglandin E(1) and lithium combination exerts a neuroprotective effect on cerebral ischemia (Sheng et al. 2011). Our previous studies have shown that prostaglandin E(1) has a protective effect on renal ischemia/reperfusion-induced gastric and lung damage (Gezginci-Oktayoglu et al. 2016; Oztay et al. 2016).

Apoptosis is a form of programmed cellular death that observed in kidney ischemia-reperfusion injury, and is believed to be an important mechanism of renal dysfunction in acute renal failure (Jo et al. 2001). In experimental models of acute renal failure, cellular death is partly mediated by apoptosis. Apoptosis seems to be induced by oxidative stress developed during the reperfusion (Kunduzova et al. 2003). PCNA is also known as cyclin and it assists DNA polymerase. PCNA plays an essential role in the regulation of DNA synthesis and cellular proliferation. Tubular cell regeneration depends on PCNA expression using immunohistochemical staining (Nony and Schnellmann 2003; Wang et al. 2003).

Ischemia-reperfusion injury causes cellular injury, and is associated with lipid peroxidation. The injury associated with ischemia-reperfusion shows elevation of free radicals, and increase of lipid peroxidation after reperfusion (Nakajima et al. 1996; Weight et al. 1996; Seth et al. 2000).

The role of reactive oxygen species in this injury is demonstrated by detecting the oxidation products of target molecule (lipid peroxidation and protein oxidation), and by determining the consumption of histoid antioxidants such as glutathione (GSH) (McCord 1985; McDougal 1988). Oxidative stress affects GSH levels as an antioxidant and malondialdehyde (MDA) levels as an index of lipid peroxidation (Seth et al. 2000).

Our study aimed to examine the role of prostaglandin E(1) to see if it could prevent acute renal injury after ischemia-reperfusion injury by utilizing histologic, immunohistochemical, and biochemical methods.

Materials and methods

Animals and experimental design

The study was carried out in accordance with the guidelines of Animal Care and Use Committee of Istanbul University. Sprague Dawley male rats, with 200 to 300 g weight, were rendered free to access food and water. The rats were fasted overnight prior to the study. The rats were anesthetized with 0.75 mg/kg chlorpromazine and 100 mg/kg ketamine by intraperitoneal injection during all surgical procedures. The animals were selected randomly and arranged as four groups. Group 1 given physiological saline. Group 2 administered prostaglandin E(1) (20 µg/kg) only. Group 3 applied ischemia-reperfusion model. Group 4 received prostaglandin E(1) (20 µg/kg) and applied ischemia-reperfusion model. Prostaglandin E(1) was given twice, the first one being 30 minutes before the ischemia and the second one just before the reperfusion.

Renal ischemia reperfusion model

An abdominal incision was performed under anesthesia, followed by right nephrectomy. Left renal artery and vein were isolated after nephrectomy, and renal pedicle was occluded for an hour to induce ischemia. The clamps were removed and the renal blood flow was reestablished. During reperfusion, the abdomen was closed. The rats were sacrificed after one hour of reperfusion.

Light microscopical analysis

Renal tissue samples were fixed in Bouin's fixative and embedded in parafin. 5-µm sections were stained with Masson's triple dyes and periodic acid-Schiff and examined under Olympus CX41 light microscope.

Immunohistochemical staining

Streptavidin-Biotin-Peroxidase technique was applied for PCNA and caspase 3. Kidney sections with 4 µm thickness were fixed with Bouin's solution and mounted on poly-L-lysine-coated slides. Sections were rendered free of paraffin with xylene and rehydrated by a reverse series of ethanol. Then the sections were heated in a microwave oven (10 minutes, at 700 W, contains citrate buffer, pH 6) for antigen retrieval. The sections were cooled and rinsed with phosphate buffered saline (PBS). The tissue was incubated with 0.3% Triton X-100 (10 minutes) and then rinsed with PBS. It followed with washing steps and blocking of endogenous peroxidases with 3% hydrogen peroxide (10 minutes). Non-immune serum was used for blocking unspecific binding sites (10 minutes). The sections were then incubated with a monoclonal antibody to PCNA (1:50, Mouse monoclonal antibody, NeoMarkers) and caspase 3 (1:50, Rabbit Pab, NeoMarkers) for 30 minutes. Then biotinylated anti-mouse immunoglobulin was applied (15 minutes) and streptavidin peroxidase was applied (15 minutes) at room temperature, and AEC substrate (3-amino-9-ethylcarbazole) was used to visualize PCNA and caspase 3-positive cells. As positive control for caspase 3 staining, normal female rat breast tissue was used and as positive control for PCNA staining, tissue sections from the small intestinal tissue were used.

Cell counting

A light microscope (Olymphus CX41) was used for visualization and calculation of immunopositive cells at 10 high-power random fields (per field, the area was 0.0506 mm^2, magnification = 400-fold). PCNA labeling index (proliferation index) and caspase 3 labeling index (apoptotic index) were utilized to express the ratio of positively-stained tubular cells to total tubular cells within the field.

Biochemical analyses

Renal tissue samples taken from sacrificed rats after overnight fasting were stored at –20 °C. Renal lipid peroxidation (LPO), GSH and protein levels, and DNA concentration were measured spectrophotometrically in these samples. The tissues were homogenized in cold 0.9 % serum physiologic employing a glass homogenizer to prepare a 10 % (w/v) homogenizate. After the homogenates were centrifuged, the clear supernatants were used for GSH, LPO, DNA, and protein analyses. Renal GSH levels were determined according to the method of Beutler using Ellman's reagent (Beutler 1979). The results were given as μmol GSH/g tissue. By using the method developed by Ledwozyw and coworkers (Ledwozyw et al. 1986), LPO levels were determined with malondialdehyde and thus the LPO was assayed. The results were expressed as nanomols of MDA per gram of tissue. Plummer's diphenylamine method was applied to the supernatants to find the DNA content (Plummer 1978). The protein content of the tissue samples was determined using Lowry's method (Lowry et al. 1951).

Statistical analyses

Microscopic results were analyzed with one-way ANOVA followed by Kruskall-Wallis, Scheffe, and Student's t test, and they were used to compare the control group to all experimental groups, and SPSS Version 10 was used. For biochemical results, unpaired t-test and ANOVA variance analysis were run from NCSS statistical computer package. The p values which are less than 0.05 were considered to be statistically significant.

Results

Light microscopical results

The degenerative alterations such as necrosis, desquamated nuclei and cytoplasmic debris in the widened lumens, disruption in the integrity and shortening in brush border, vacuolization in proximal tubular cells were observed in the experimental group which were applied ischemic reperfusion compared to the control group (Fig. 1A, B, C). In addition to these observations, we have also recorded desquamated nuclei in the widened lumens and the ruptures at the epithelium in distal tubules of this group. At some sections, glomerular atrophy was observed. In the renal tissues of animals of experimental group, we have also detected mononuclear cell infiltration, vacuolization, haemorrhage and hyperemia (Fig. 1C). Figure 1D shows how the treatment of prostaglandin E(1) alleviated the degenerative changes in comparison to the group applied ischemic reperfusion. Compared to the controls, PAS-positive reaction was reduced in proximal tubular cells and glomeruli in the experimental group. This reaction was increased in experimental group applied ischemia-reperfusion and given prostaglandin E(1) compared to ischemia-reperfusion group.

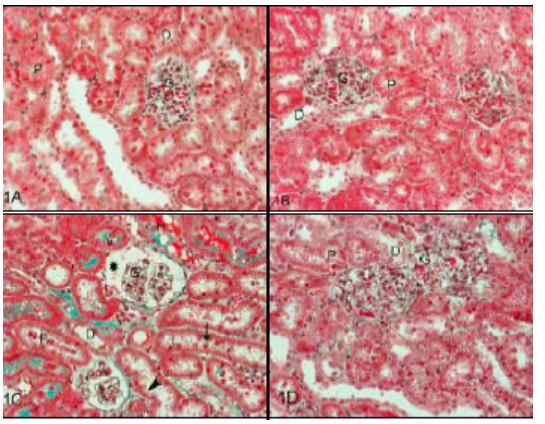

Figure 1. The kidney tissue of control rat given physiologic salt solution **(A)**, control rat given prostaglandin E(1) **(B)**, the rat with renal ischemia reperfusion: haemorrhage (H), vacuolization (V), picnotic nuclei (➡) and cytoplasmic debris in the widened lumens (L) of the proximal tubules, the shortening and rupturing at the brush border (➤), decreased at the glomeruli mass (*) **(C)**, the rat with renal ischemia reperfusion and given prostaglandin E(1) **(D)**, proximal (P) and distal (D) tubules and glomeruli (G). Mason (270X).

Immunohistochemical results

Caspase 3 immunoreactivity was observed in cytoplasm of distal tubular cells and in nucleus of some distal tubular cells in the experimental

group applied ischemic reperfusion. The stained cells for caspase 3 were minimal and similar in the kidneys of both controls (Fig. 2A, B). In the experimental group applied renal ischemic reperfusion compared to the control group, caspase positive cells were increased (Fig. 2A, B, C) ($p<0.0001$). Administration of prostaglandin E (1) caused a significant decrease in caspase 3 positive cells in the experimental group applied renal ischemic reperfusion (Fig. 2D) ($p<0.0001$). Caspase 3 immunoreactivity results were demonstrated in the whole group (Fig. 3).

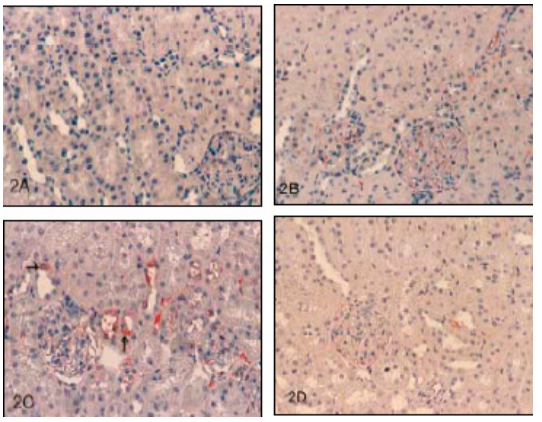

Figure 2. Immunohistochemical staining of caspase 3 in kidney tissues of control rat given physiologic salt solution **(A)**, control rat given prostaglandin E(1) **(B)**, the rat with renal ischemia reperfusion **(C)**, the rat with renal ischemia reperfusion and given prostaglandin E(1) **(D)**, arrow shows caspase 3 positive cell. 270X.

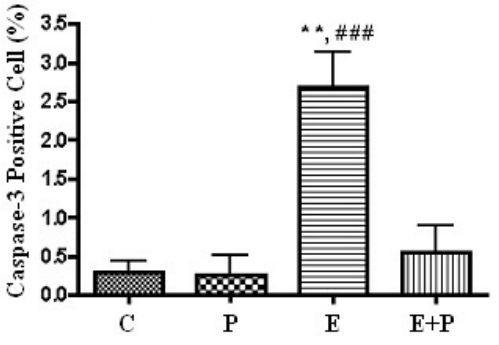

Figure 3. Caspase 3 immunoreactivity index. C=Control group (n=3) (0.28%), P=Control group administration of prostaglandin E(1) (n=3) (0.24%), E=Experimental group applied ischemia reperfusion (n=5) (2.67%), E+P=Experimental group applied ischemia reperfusion and administration of prostaglandin E(1) (n=5) (0.54%). $p<0.05$ statistically significant. *$p<0.001$ (compared with the control group), $^{\#}p<0.0001$ (compared with applied ischemia reperfusion and administration of prostaglandin E(1).

PCNA staining was present in nucleus of proximal tubular cells. Cells demonstrating staining for PCNA were minimal and similar in the kidney of controls (Fig. 4A, B). In the experimental group applied renal ischemic reperfusion compared to the control group, PCNA positive cells were increased (Fig. 4A, B, C) ($p<0.05$). Prostaglandin E(1) caused a significant decrease in PCNA positive cells of the experimental group applied renal ischemic reperfusion (Fig. 4D) ($p<0.05$). PCNA staning results was demonstrated in all of groups (Fig. 5).

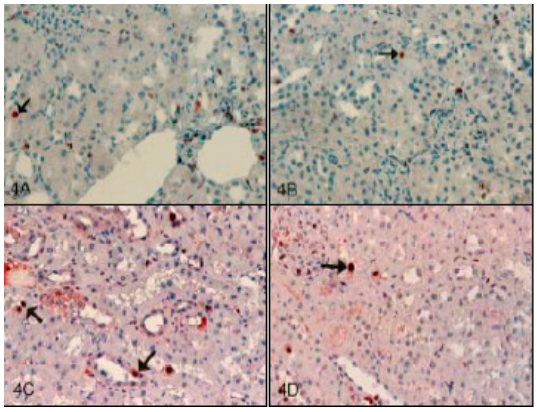

Figure 4. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in kidney tissues of control rat given physiologic salt solution **(A)**, control rat given prostaglandin E(1) **(B)**, the rat with renal ischemia reperfusion **(C)**, the rat with renal ischemia reperfusion and given prostaglandin E(1) **(D)** arrow shows PCNA positive cell. 270X

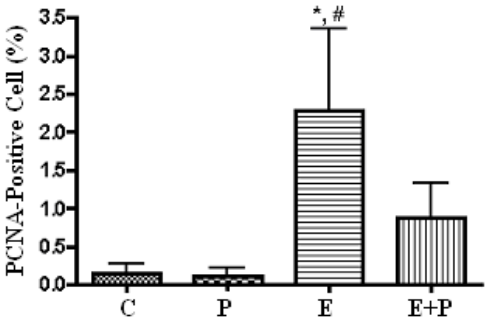

Figure 5. PCNA immunoreactivity index. C=Control group (n=3) (0.15%), P=Control group administration of prostaglandin E(1) (n=3) (0.13%), E=Experimental group applied ischemia reperfusion (n=5) (2.35%), E+P=Experimental group applied ischemia reperfusion and administration of prostaglandin E(1) (n=5) (0.89%). $p<0.05$ statistically significant. *$p<0.05$ (compared with the control group), $^{\#}p<0.05$ (compared with applied ischemia reperfusion and administration of prostaglandin E(1).

Biochemical results

Table 1 shows the levels of GSH, LPO, and DNA in kidney of control and experimental groups. The renal LPO content in ischemic reperfusion group according to the control group was significantly increased ($p<0.0001$) while it was significantly decreased with prostaglandin E(1) treatment ($p<0.001$). In the experimental group applied renal ischemic reperfusion, a decrease in renal GSH levels was detected ($p>0.05$). Administration of prostaglandin E(1) caused an elevation in kidney GSH levels in the experimental group applied renal ischemic reperfusion ($p>0.05$). Data comparison was not significant. In the experimental group applied renal ischemic reperfusion, a decrease in renal DNA content was observed ($p<0.0001$). Administration of prostaglandin E(1) caused an insignificant decrease renal DNA contents in the experimental group applied renal ischemic reperfusion ($p>0.05$).

Table 1: LPO, GSH and DNA levels in experimental and control group animal renal tissues*

GROUP	LPO n mol MDA/ mg protein	GSH n mol GSH/ mg protein	DNA µg/g
Control (n=10)	0.42 ± 0.09	18.34 ± 5.50	64.70 ± 13.67
PGE(1) (n=10)	0.44 ± 0.11	19.58 ± 4.70	59,00 ± 14.55
Experimental (n=10)	0.74 ± 0.16	13.59 ± 5.50	36.09 ± 13.88
Experimental +PGE(1) (n=10)	0.50 ± 0.1**	16.25 ± 4.20	33.40 ± 9.82
$\mathbf{P}_{ANOVA}$	0.0001	0.05	0.0001

*mean ± SD
n: Number of animals.
$p < 0.05$ statistically significant
*$p<0.001$

Discussion

Renal ischemia reperfusion damage is experienced in renal transplantation, shock, cardiac dysfunction, bleeding, renal arterial operations, sepsis, and hydronephrosis (Weight et al. 1996; Sung et al. 2002; Ushigome et al. 2002). Structural and functional damages occur in proximal tubules by renal ischemia reperfusion damage with renal artery constriction seen in the acute stage, reduced glomerular filtration, and tubular congestion (Kribben et al. 1999; Liu 2003). Previous studies have reported that an acute tubular damage and vacuolization of epithelial cells were observed after 30-min ischemia and 1-hour reperfusion time after right nephrectomy (Oberbauer et al. 2001). A significant tubular necrosis, hyperemia, and polymorph nuclear cell infiltration were also reported after 1-hour ischemia and 72-hour reperfusion time (Thiemermann et al. 2003). We have observed glomerular and tubular damages, marked hyperemia, haemorrhage, and mononuclear cell infiltration, and necrotic sites in some individuals. The histopathologic findings occurred in the renal damage generated with ischemia reperfusion model were consistent with the existing data reported by other researchers.

It is suggested that apoptotic cellular death has a role in recuperation of inflammation and damage after renal ischemia in experimental animal models and in humans (Daemen et al. 1999; Gezginci-Oktayoğlu et al. 2016). In a study with generated ischemic acute tubular necrosis, it was reported that desquamation and apoptosis are important in recovering the original tubular structure in the recuperation stage (Shimizu and Yamanaka 1993). The observation of more apoptotic tubular cells in distal tubules than proximal ones shows that distal tubules are more likely to undergo apoptosis (Shimizu and Yamanaka 1993; Daemen et al. 1999; Gobe et al. 2000; Jo et al. 2001). We have also observed that caspase 3 immune labelling was more seen in distal tubules in renal damage generated with ischemia reperfusion. Caspase 3 immunoreactivity was observed mainly in cytoplasm and with lesser importance the nucleus (Eckle et al. 2004). We observed caspase 3 positive cells located mainly in distal tubular cytoplasm, but an immunoreactivity in the nucleus was also noted. We have found that prostaglandin E(1) treatment could attenuate the increased caspase 3 immunoreactivity in distal tubular cells of experimental group. Consistent with our other studies (Gezginci-Oktayoğlu et al. 2016), our results show that prostaglandin E(1) reduces the apoptotic cells in acute renal damage occurred with ischemia reperfusion.

A close relation was shown between apoptosis and cellular cycle occurred during regeneration (Wang et al. 2003). In our study, both caspase 3 and PCNA immunoreactivity was increased in renal damage caused by renal ischemia reperfusion model. Labelling of PCNA cyclin polypeptide with monoclonal antibodies is a very sensitive method to determine cells at S phase of the proliferation. PCNA is related to early S and later G1 phases of cellular cycle (Nony and Schnellmann 2003; Wang et al. 2003; Bonventre 2003). It was reported that PCNA positive nuclear count was increased considerably after 6th, 48th, and 72nd hours of ischemia reperfusion damage in proximal tubules (Kunduzova et al. 2000). It was also noted that PCNA immunoreactivity increases until 48 hours after the first 12-24 hours of renal ischemia (Nakajima et al. 1996). In addition, we observed that prostaglandin E1 increased PCNA positive epithelial cells in gastric damage induced by renal ischemia-reperfusion injury in rats (Gezginci-Oktayoglu et al. 2016). We also observed an increase of PCNA positive cell nuclei in the experimental group with ischemia reperfusion. It is considered that reduced cellular replication observed in the group applied with renal ischemia reperfusion and administered with prostaglandin E(1) did not necessitate cellular regeneration relevant to reduced toxic effect due to the protective feature of PGE(1).

Oxidative stress in the tissue increases the LPO of cellular membrane in ischemia reperfusion damage. It was shown that membrane fluidity and cellular integrity is disrupted after LPO (Freeman and Crapo 1982; Campos et al. 1993; Seth et al. 2000; Sung et

al. 2002). Renal ischemia reperfusion increases MDA levels (Campos et al. 1993; Sener et al. 2002; Sehirli et al. 2003). It was also shown that LPO increase due to renal ischemia reperfusion reduces glomerular filtration and causes apoptosis in renal cells (Seth et al. 2000). In addition, we observed that prostaglandin E1 reduced LPO in lung damage induced by renal ischemia-reperfusion injury in rats (Oztay et al. 2016). We also noted a similar increase in MDA levels of renal tissues. It is known that there is a relation between apoptosis and LPO, which is an indicator of oxidative damage (Ueda et al. 2000). The decreases in both determined LPO level with prostaglandin E(1) administration and caspase 3 led us to consider that PGE(1) administration reduces the damage and therefore it has a protective effect on renal tissue. GSH is known to be an effective protector against the damage due to reactive oxygen intermediates and free radical reactions (Seth et al. 2000). A significant decrease in GSH levels was observed in renal damage due to experimental ischemia and ischemia reperfusion (Campos et al. 1993; Sener et al. 2002). In our study, we observed an increase in GSH amount with prostaglandin E(1) administration in the renal injury generated by reperfusion after ischemia. These results indicate the possibility of reduced renal damage due to the stimulation of GSH by prostaglandin E(1). DNA is another important macromolecule, to which the renal ischemia reperfusion damage-induced reactive oxygen radicals attack (Cuzzocrea et al. 2001; Mene et al. 2003). We noted a remarkable decrease of DNA amount as a result of renal injury due to renal ischemia reperfusion model. This injury is likely to be caused by oxidative damage. Prostaglandin E(1) administration, and possibly the recuperation from it, led to an increase of DNA amount.

It is speculated that the renal ischemic injury is controlled by the balance between thromboxan A2 and prostaglandins, and that when thromboxan A2 is inhibited with prostaglandin administration, the damage is reduced. Prostaglandin has a vasodilator effect and its exogenous application is reported to increase prostaglandin/thromboxan ratio and therefore to protect the ischemic kidney (Kaufman et al. 1987). Another study reports an application of a compound very commonly used in toxicity investigations and named as radiocontrast media to induce acute renal deficiency, whereas prostaglandin E(1) was administered as intravenous infusion. Protective effect of prostaglandin E(1) in the renal deficiency due to this compound is therefore known (Koch et al. 2000). Healing was provided with intravenous administration of 15d-prostaglandin J(2) in ischemia-induced renal deficiency in rats (Chatterjee et al. 2004). In dogs, it was suggested that intravenous prostaglandin E(1) infusion was protective on acute renal deficiency caused by ischemia (Tobimatsu et al. 1985). Ischemic reperfusion injury to the rat kidney, prostaglandin E(1) and allopurinol suggested significant protection (Gupta et al. 1998). In hepatic ischemia reperfusion injury, it is known that N-acetylcysteine and a prostaglandin E(1) analog, alprostadil shows a curative effect (Hsieh et al. 2014). We have shown that prostaglandin E(1) has a protective effect on renal ischemia/reperfusion-induced gastric and lung damage (Gezginci-Oktayoglu et al. 2016; Oztay et al. 2016). Our study was consistent with those in which prostaglandins were protective on ischemia reperfusion induced renal deficiency.

Reducing of GSH level in tissues, increasing LPO, and lowered DNA levels during ischemia reperfusion is harmonious with tissue damage and also with apoptosis and cellular reproduction. We consider that a protective effect of prostaglandin E(1) occurs against the damage due to renal ischemia reperfusion when the following findings were observed: Rats with ischemia reperfusion and prostaglandin E(1) showed recuperation in tubular injury, a decrease in LPO rate, and decreases in PCNA- and caspase 3-immunoreactive cellular count.

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