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Protective effects of methylprednisolone in kidney: aortic occlusion-reperfusion model in rats

Serkan Seçici[1], Kadir Kaan Özsin[2], M. Özgür Özyiğit[3], Ömer Arda[3], Yasemin Üstündağ[4]

[1]Department of Pediatric Cardiovascular Surgery, Medicana Bursa Hospital, Bursa, Turkey; [2]Department of Cardiovascular Surgery, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey; [3]Department of Pathology, Uludag University, Faculty of Veterinary Medicine, Bursa, Turkey; [4]Department of Clinical Biochemistry, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey

ABSTRACT

Objectives: Ischemia/reperfusion (I/R) injury is commonly seen in cardiovascular surgery, activates inflammation and causes renal damage. In this experimental study, we aimed to assess the effects of different doses (5 and 30 mg/kg) of methylprednisolone (MP), which has anti-inflammatory effect, on renal ischemia/reperfusion (I/R) injury.

Methods: Thirty-two male Wistar albino rats were randomly divided into four groups (n = 8). The sham group underwent midline laparotomy and dissection of the abdominal aorta without occlusion while the I/R group underwent suprarenal aortic ischemia for 45 minutes followed by 180 minutes of reperfusion. In the 5 mg/kg MP and 30 mg/kg MP groups, MP was administered intraperitoneally. At the end of the experiment, blood samples were obtained, and kidneys were extracted.

Results: Pretreatment with methylprednisolone did not influence serum BUN and creatinine levels. Serum TNF-α levels and ischemia-modified albumin levels were significantly lower in the MP groups compared to the I/R group ($p < 0.05$). Histological examination demonstrated severe injury in the I/R group and treatment with MP attenuated the severity. The difference was significant in doses of 30 mg/kg MP.

Conclusions: This results of the model of renal I/R injury presented in this work reveal the anti-inflammatory and the protective effects of MP in cases of renal I/R.

Keywords: Ischemia-reperfusion injury, kidney, methylprednisolone, inflammation

Ischemia-reperfusion (I/R)-induced acute renal injury is a significant complication and one of the most important causes of morbidity and mortality in cardiovascular surgery [1]. Lack of oxygen caused by ischemia leads to a shift to anaerobic glycolysis, depletion of ATP, increased intracellular calcium, and formation of reactive oxygen species (ROS), resulting in cell apoptosis and necrosis [2]. While restoring the blood flow can save the affected tissue, it also activates inflammatory mediators, leading to leukocyte activation and leukocyte endothelial adhesion, which may in turn cause multi-organ dysfunction and death [3, 4].

Glucocorticoids have been used for their anti-inflammatory and immunosuppressive effects [5]. Methylprednisolone (MP) is a synthetic glucocorticoid with many systemic effects, the most important of which include anti-inflammatory, immunosuppressive,

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Address for correspondence: *Serkan Seçici, MD., Bursa Medicana Hospital, Department of Pediatric Cardiovascular Surgery, Odunluk Mah., İzmir Yolu Cad., No: 41, 16110 Nilüfer, Bursa, Turkey. E-mail: serkansecici@hotmail.com, Phone: +90 224 970 01 01*

and catabolic effects in protein metabolism. It was previously demonstrated that steroid administration in cases of renal I/R injury protected the morphology of tubular epithelium while reducing leukocyte infiltration and plasma interleukin-6 and serum creatinine concentrations in kidney tissues [6, 7].

Considering the data reported in the literature to date, we aimed to investigate the protective effects of different doses of MP against renal I/R injury in this experimental study.

METHODS

This study was approved by the Local Experimental Animal Ethics Committee of Uludag University with the number of 2018-04/07. Thirty-two male Wistar albino rats weighting 200-250 gr were employed for the study. All rats were permitted free access to standard chow and drinking water. They were maintained on a 12:12-hour light/dark cycle at a temperature of 22 ± 2°C. The included rats were randomly assigned to one of four groups with each group comprising eight animals. The rats were anesthetized intraperitoneal with 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey). Additional ketamine HCL was administered if needed. In Group 1 (Sham group), a midline laparotomy incision was made, and the intestines were wrapped in a warm wet sterile gauze. Blood samples were taken from the right ventricle, and the kidneys were extracted. In Group 2 (I/R group), the aorta was clamped at the level of the superior mesenteric artery for 45 minutes. After achieving reperfusion for 3 hours, blood samples were taken from the right ventricle, and kidneys were extracted. Before ischemia was induced in Group 3 (5 mg MP group) and Group 4 (30 mg MP group), 5 mg/kg MP and 30 mg/kg MP were injected intraperitoneally, respectively. Following 45 minutes of ischemia, 3 hours of reperfusion was achieved in both groups and then blood samples were taken from the right ventricle and kidneys were extracted.

Blood samples were centrifuged at 4000 rpm to separate the serum, and serum samples were stored at -80 °C. Levels of blood urine nitrogen (BUN; mg/dL) and creatinine (mg/dL) were determined by spectrophotometric analysis using commercially available assay kits (Abbott Diagnostics, Abbott Park, IL, USA). Serum TNF-α concentrations were assayed using a commercial ELISA kit and measured at 540 nm using a Readwell Touch ELISA plate analyzer (Robonik Pvt. Ltd., Mumbai, India). Malondialdehyde (MDA) concentration was assayed using a spectrophotometric method [8]. Ischemia-modified albumin (IMA) was studied using the spectrophotometric method suggested by Bar-Or *et al*. [9], and results are reported in absorbance units (ABSU).

Histopathological Examinations

Paraffin sections from fixed kidneys (5 µm in thickness) were cut and stained using a standard protocol for hematoxylin and eosin. Renal slides from each group were also scored. Severe tubular lysis, loss of brush border, and/or sloughed debris in the tubular lumen space were considered as damage of tubules. Tubular damage was graded as follows: 0 = no damage; 1 = 0-25% damaged tubules; 2 = 25-50% damaged tubules; 3 = 50-75% damaged tubules; 4 = > 75% damaged tubules.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0 for Windows (IBM Corp., Armonk, NY, USA). Results were given as mean ± standard error of the mean (SEM). Multiple group comparisons were performed using ANOVA with Bonferroni post-tests. Values of $p < 0.05$ were considered significant.

RESULTS

Histopathological examination revealed severe injury in the I/R group (Fig 1). Treatment with 30 mg of MP significantly attenuated the severity of the injury ($p < 0.001$). Histological damage scores were lower in the 5 mg MP group than in the I/R group; however, this difference was not significant (Fig. 2).

Serum creatinine levels were significantly lower in the 5 mg MP ($p = 0.002$) and 30 mg MP ($p = 0.02$) groups compared to the I/R group ($p < 0.05$) (Fig. 3). Serum urea levels were significantly lower in the 30 mg MP group ($p < 0.001$) compared to the I/R group ($p < 0.05$) (Fig. 3). There was no significant difference between the I/R group and 5 mg MP group.

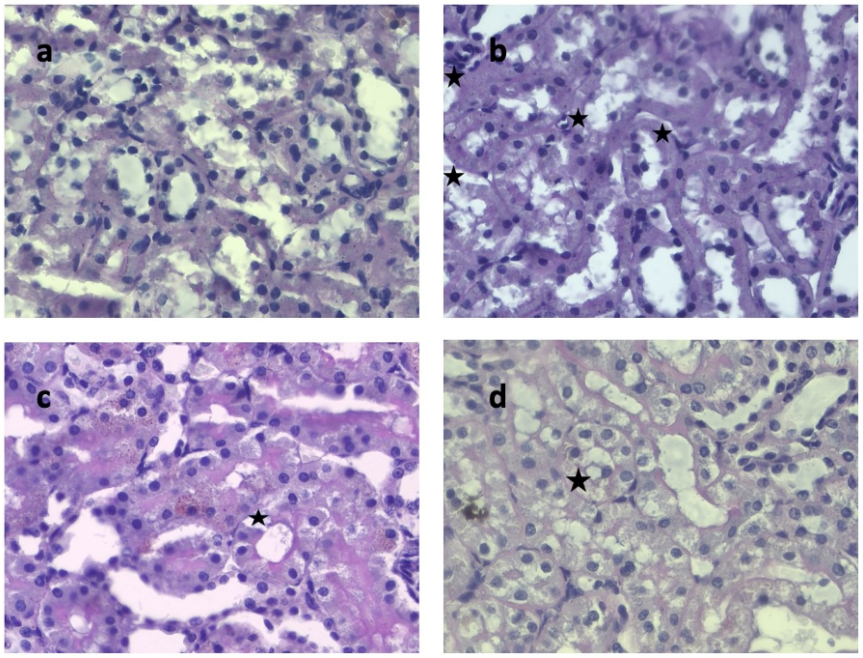

Fig. 1. Kidney sections prepared for hematoxylin and eosin staining to evaluate histological injury. (a) Sham Group, (b) I/R Group, (c) 5 mg/kg MP Group, (d) 30 mg/kg MP Group. Asterisk indicates vacuolar degenerations.

Serum TNF-α levels were significantly lower in the sham ($p < 0.001$), 5 mg MP ($p = 0.006$), and 30 mg MP ($p < 0.001$) groups compared to the I/R group ($p < 0.05$) (Table 1) (Fig. 4a). There was no significant difference between the sham group and 30 mg MP group ($p = 0.9$). However, serum TNF-α levels were significantly higher in the 5 mg MP group compared to the sham ($p < 0.001$) and 30 mg MP ($p = 0.02$) groups ($p < 0.05$). Although serum MDA levels were higher in the I/R group (Fig. 4b), there was no significant difference between the groups.

Serum troponin I (TnI) levels were higher in the I/R ($p < 0.001$), 5 mg MP ($p < 0.001$), and 30 mg MP ($p = 0.001$) groups compared to the sham group ($p < 0.05$) (Fig. 4c). Although there was no statistically significant difference, serum TnI levels were found to be lower in the 30 mg MP group compared to the I/R ($p = 1$) and 5 mg MP ($p = 0.62$) groups.

Serum IMA levels were higher in the I/R group compared to the sham ($p = 0.02$), 5 mg MP ($p =$

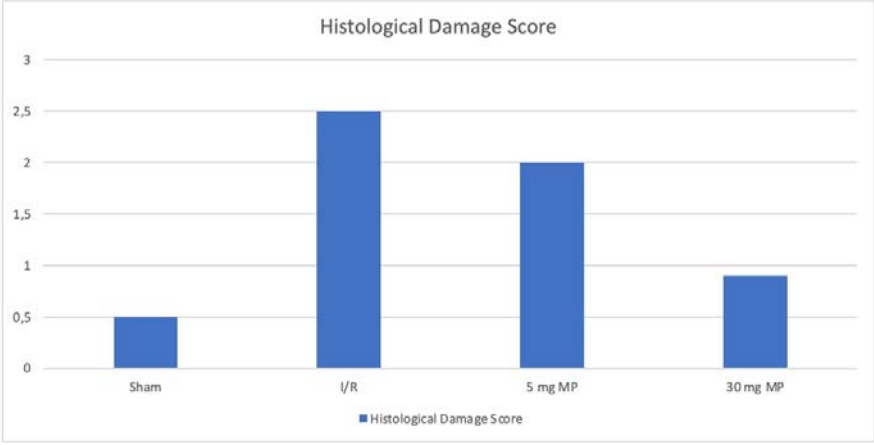

Fig. 2. Histological damage scores. All values are shown as mean ± standard error of mean (n = 8).

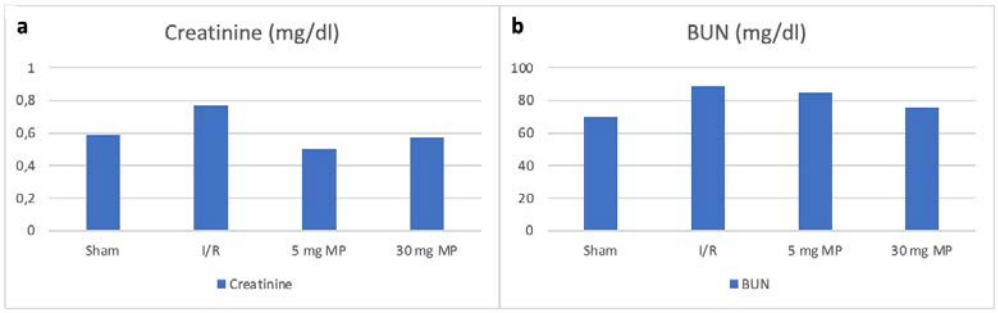

Fig. 3. Serum creatinine and BUN levels. All values are shown as mean ± standard error of mean (n = 8).

0.015), and 30 mg MP ($p = 0.011$) groups (Table 1) (Fig. 4d). Serum IMA levels were lower in the sham group than in the 5 mg MP and 30 mg MP groups, but this difference was not significant ($p = 1$ and $p = 1$, respectively).

DISCUSSION

The results of this study revealed that MP attenuated acute renal injury in a dose-dependent manner. Lower plasma TNF-α, MDA, and IMA levels were found in the MP groups. Moreover, MP administration attenuated the renal histological injury associated with I/R.

During abdominal aortic surgery, the clamping of the aorta and the subsequent restoration of blood flow causes I/R injury, which affects not only mesenteric tissues but also remote organs [10]. Tissue hypoxia, energy production through anaerobic metabolism, lactic acid accumulation, and ROS production are the main mechanisms of I/R injury processes [11]. An acute inflammatory response occurs with the activation of neutrophils due to I/R damage [12]. The excessive production of ROS and cytokine release cause distant organ injury and apoptosis via lipid peroxidation in cell membranes with oxidative damage to DNA and proteins [1, 13].

TNF-α and IL-1β induce chemokines that play an important role in the recruitment process of leukocytes. Therefore, they are important parts of the inflammatory reaction induced by acute kidney injury [14]. As a result of renal inflammation, endothelial cell activation, vascular disruption, and increased vascular permeability occur. This facilitates leukocyte recruitment in the renal parenchyma [15]. It has been demonstrated that steroid administration reduces I/R injury in many organs [16, 17]. In a study investigating the effect of steroids on renal damage, Kumar *et al*. [5] subjected rats to bilateral renal ischemia for 60 min, followed by reperfusion for 2 or 24 h. They found that a single dose of 3 mg/kg of dexamethasone 30 min before ischemia, or at the onset of reperfusion ameliorated biochemical and histological acute kidney injury [5]. In the present study, histopathological examination revealed that while vacuolar degenerations occurred in the renal tubules after ischemia-reperfusion, degeneration decreased in methylprednisolone administered groups. Moreover, it was observed that vacuo-

Table 1. Plasma TNF-∝, MDA, TnI and IMA levels in groups

	TNF-α (pg/dl)	MDA (mmol/L)	TnI (ng/dl)	IMA (ABSU)
Sham	139.6 ± 22.53	0.49 ± 0.35	214.5 ± 142.4	0.255 ± 0.031
I/R	264.6 ± 36.69	1.03 ± 0.59	774 ± 221.3	0.464 ± 0.093
5 mg MP	209.9 ± 21.01*	0.90 ± 0.59	666.1 ± 188.5	0.297 ± 0.172*
30 mg MP	147.9 ± 35.17**	0.71 ± 0.52	615.9 ± 190.7	0.290 ± 0.041**

Values are expressed as the mean ± standard deviation. I/R = Ischemia/Reperfusion, MP = Methylprednisolone, TNF-α = Tumor necrosis factor-alpha, MDA = Malondialdehyde, TnI = Troponin I, IMA = Ischemia modified albumin.

* $p < 0.05$ (I/R vs 5 mg MP), ** $p < 0.05$ (I/R vs 30 mg MP).

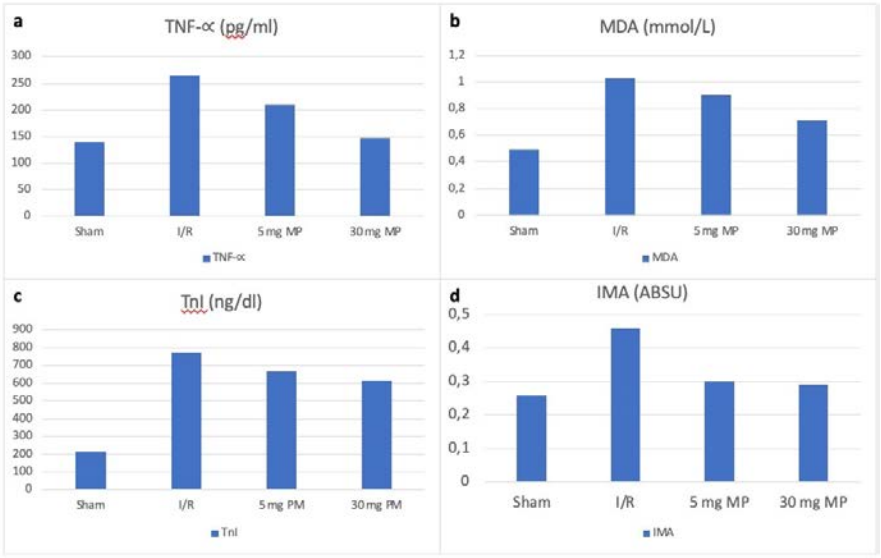

Fig. 4. Inflammatory markers in plasma: Effects of methylprednisolone plasma levels of (a) TNF-∝, (b) MDA, (c) TnI, (d) IMA in rats. All values are shown as mean ± standard error of mean (n = 8).

lar degenerations decreased with increasing dose. This was interpreted as metilprednisolone dose-dependently (30 vs. 5 mg/kg) reduced I/R injury (Figs. 1 and 2).

Baker *et al*. [18] also investigated the effects of steroids on renal damage in their study, in which clamped infrarenal abdominal aorta for 150 min followed by 180 min of reperfusion in a porcine model and showed that MP reduced the reperfusion injury of kidneys at a dose of 30 mg/kg. Fontana *et al*. [6] searched effects of 5 mg/kg s.c. daily prednisolone administration on ischemia-reperfusion injury. They demonstrated that continuous prednisolone application has protective effects and prednisolone treated group had significantly reduced TNF-α levels. Gozdzik *et al*. found that combination of inhaled NO and IV steroid diminished the systemic inflammatory response in I/R injury animal model and significantly lower TNF-α and IL-10 levels in treatment group [19]. In the present study, the TNF-α levels, one of the indicators of renal damage, were examined. Treatment with both 5 and 30 mg MP significantly reduced the serum TNF-α levels when compared to the I/R group ($p < 0.05$). However, this reduction was more evident in 30 mg/kg group, and the serum TNF-α levels were significantly lower when compared to the 5 mg MP group.

MDA, the end product of lipid peroxidation, is an important indicator of I/R injury. It has been demonstrated that MDA levels are sensitive markers of the rate of lipid peroxidation [20]. Singh *et al*. [21] reported that MDA levels increased during renal I/R in rats. It was found herein that there were lower serum MDA levels in the MP-treated groups when compared to the I/R group. However, no statistically significant differences were detected. Gurer *et al*. [22] found that 30 mg/kg methylprednisolone treatment reduced MDA levels significantly. However, in this study groups were subjected to ischemia by aortic occlusion for 20 min which is shorter than our study.

In the event of I/R, structural changes occur in the albumin under the influence of acidosis and reactive oxygen radicals. Albumin has the ability to bind to some metal ions, particularly copper, nickel, and cobalt. However, this ability decreases, and the albumin cannot bind to those ions. This new form of albumin is known as ischemia-modified albumin (IMA) [23, 24]. IMA is accepted as a nonspecific biomarker in ischemic tissues [24]. Several studies showed increased IMA levels in cases of renal I/R injury parallel with the duration of ischemia [25, 26]. In the present study, the IMA levels of the groups receiving MP were significantly lower when compared to the I/R group ($p < 0.05$).

I/R injury may cause remote organ injury, including injury to the heart, liver, or intestines [27]. Yeginsu *et al*. [16] investigated the effects of different doses of MP on lung injury developing after extremity ischemia reperfusion in rats, and administered 15, 50, and 150

mg/kg MP. They found that MP reduced remote organ injury. However, the effect was in a dose dependent manner, and it was maximum at a dose of 150 mg/kg. Tai *et al*. [28] found that renal I/R injury was associated with cardiac dysfunction. TnI is a specific marker for myocardial damage. In the present study, no significant differences were detected between the I/R group and the MP treatment groups in this regard. Thus, it was not possible to demonstrate a protective effect of MP on remote cardiac injury.

In previous studies, protective effects of 5 and 30 mg/kg MP on I/R injury have been revealed [6, 18, 29]. In the current study, 5 and 30 mg/kg MP was administered. The results showed that the serum TNF-α levels, IMA levels, and histological damage score decreased in the 5 mg MP and 30 mg MP groups. Therefore, it can be concluded that MP treatment had protective effects on the suprarenal aortic I/R injury. However, the serum TNF-α levels and histological damage score were significantly lower in 30 mg/kg MP group, when compared to the 5 mg/kg MP group. These results indicated that treatment with 5 mg/kg MP may not be as beneficial as treatment with 30 mg/kg MP.

CONCLUSION

In conclusion, this experimental study has demonstrated that MP at doses of both 5 and 30 mg/kg attenuates I/R injury in the kidneys induced by suprarenal aortic I/R in rats. However, this benefit was dose dependent, and was more evident with the administration of 30 mg/kg MP.

Authors' Contribution

Study Conception: SÇ; Study Design: SÇ, KKÖ; Supervision SÇ, KKÖ, ÖA; Funding: SÇ, MÖÖ, ÖA, YÜ; Materials: SÇ, MÖÖ, ÖA, YÜ; Data Collection and/or Processing: SÇ, MÖÖ, ÖA, YÜ; Statistical Analysis and/or Data Interpretation: SÇ, MÖÖ, ÖA, YÜ; Literature Review: SÇ, KKÖ, MÖÖ; Manuscript Preparation: SÇ, KKÖ and Critical Review: SÇ, KKÖ, MÖÖ, ÖA, YÜ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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