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Full Length Research Paper

The effect of iloprost on renal ischemia/reperfusion injury in an experimental rat model

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In this study, the effect of iloprost on renal ischemia-reperfusion injury was investigated using an experimental rat model. Adult male Wistar rats subjected to 60 min of unilateral warm ischemia were allocated into sham (n = 4), control (n = 7) and iloprost (n = 7) groups. Iloprost was administered intravenously via the right external jugular vein at a dose of 2 ng/kg/min. Infusion started the same time as we clamped the artery of the left kidney and continued for about an hour in the early phase of reperfusion. Blood samples for biochemical parameters were drawn just before and 72 h after clamping the renal artery. Histopathological examinations of the kidney specimens were performed to detect probable changes due to reperfusion injury. Mean blood urea nitrogen and creatinine levels after 72 h were lower in the iloprost group than the control group, but the difference between these two groups was not significant. Severe (grade 2 or 3) acute tubular necrosis was detected in 86% of rats in the control group; the ratio was 57% in the iloprost group. But the difference between these two groups was not significant. Although, these findings suggest that iloprost treated rats have less injury, iloprost does not reduce ischemia-reperfusion injury in a rat model when only given during the ischemic and early reperfusion period.

Key words: Iloprost, ischemia-reperfusion injury, renal, kidney.

INTRODUCTION

Despite the advances in medical care, acute renal failure (ARF) remains as an important problem that leads to morbidity and mortality. This complication is seen in 5% of the hospitalized patients and prolongs the length of hospital stay, requires special care (such as dialysis) and causes high financial cost (Legrand et al., 2008).

The agents investigated in ischemia-reperfusion injury studies, include edaravone, aminoguanidine, ascorbic acid, stobadine, N-acetyl cysteine, taurine, monoclonal antibodies against homocysteine, polyenylphosphatidyl-choline, oxytocin, resveratrol, osajin, leflunomide, trimetazidine, zofenopril, inhalation of carbon monoxide, statins, spironolactone, nicotine and hirudin (Chatterjee, 2007). New pharmacological treatment modalities are still needed for ischemic ARF, and it seems that this field would remain a subject of intensive research.

In the current experimental study, we investigated the effects of iloprost (ILO) against renal ischemia-reperfusion (I/R) injury in a rat model and compared the effects with the control groups. The primary hypothesis was that ILO could provide protection against renal I/R injury in rats which did not receive pretreatment with ILO.

MATERIALS AND METHODS

Eighteen adult male albino Wistar rats weighing between 250 and 300 g were used in the present study. This study was carried out after obtaining approval from the Ethics Committee of DokuzEylul University, Faculty of Medicine and was funded by the authors. Rats were divided into three groups. The ILO group (n = 7), the kidneys of which were perfused during 60 min of cross-clamping. The control group (n = 7)

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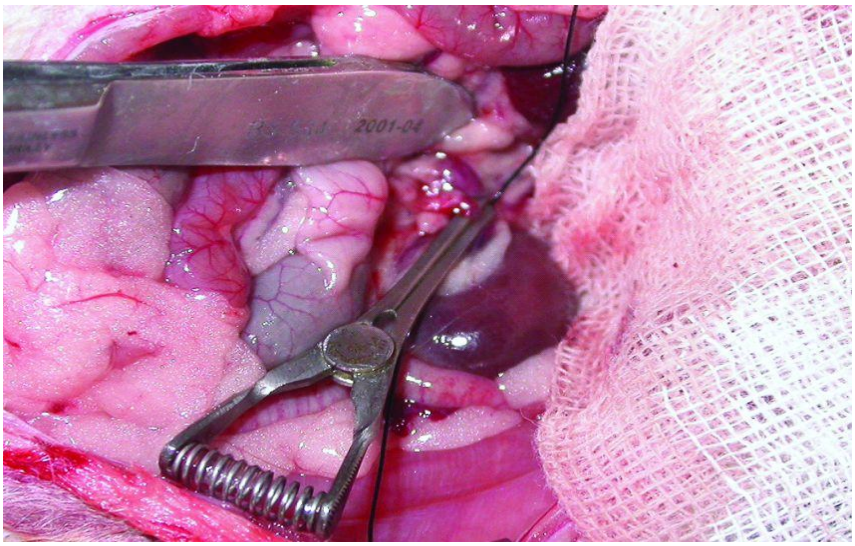

Figure 1. Clamped renal artery.

Table 1. Postoperative 72 h blood urea nitrogen and creatinine values of the study groups.

Parameter	Iloprost group (mean ± SD)	Control group (mean ± SD)	P
BUN (mg/dl)	127.5 ± 70.3	181.9 ± 108.2	0.383
Cr (mg/dl)	3.7 ± 1.7	5.0 ± 3.1	0.456

BUN: Blood urea nitrogen; Cr: creatinine.

was treated with saline. The third group was the sham group (n = 4).

Anesthesia was induced by intramuscular ketamine (75 mg/kg); then, it continued at a fractionally dose of 25 mg/kg during the intervention to allow spontaneous respiration. Noendotracheal intubation was required. The room temperature was kept between 28 and 30°C. The right external jugular vein was cannulated with a 20 gauge catheter for venous line and 1 ml blood was obtained following midline laparotomy. Heparin (2 mg/kg) was given via the venous line. The right renal artery, vein and ureter were doubly ligated. Renal ischemia was induced by clamping the left renal artery for 60 min using an arterial bulldog clamp (Figure 1). ILO (Ilomedin®, Schering Co., Germany) was given by continuous intravenous infusion at an infusion rate of 2 ng/kg/min. Rats in the control group received a continuous intravenous infusion of saline. The clamp was removed after 60 min and then restoration of blood flow was visually confirmed. After the removal of the clamp, infusion continued for another 60 min, and abdominal layers were closed. Bupivacaine (diluted 1:10 with saline) was injected into the suture line for post-op analgesia and then the rats were taken to their cages. After 72 h, animals were sacrificed with high-dose ether inhalation. Blood sample was drawn from the inferior vena cava and left nephrectomy was performed. Kidneys were divided from pole to cortex in two layers to avoid autolysis and were fixed in 10% formalin. Routine hematoxylin and eosin (H&E) staining of tissue sections and periodic acid-Schiff (PAS) staining were performed. Tissue sections were examined by an expert pathologist, who was blinded to the study. Renal injury was scored semiquantitatively according to the presence of hydropic degeneration, tubular necrosis, regenerative atrophy, interstitial fibrosis, loss of supranuclear cytoplasm and loss of brush border as; grade 0 as normal, grade 1 as mild (focal), grade 2 as moderate (multifocal) and grade 3 as severe (diffuse) histopathological changes.

Biochemical parameters [blood urea nitrogen (BUN) and creatinine (Cr)] were measured before arterial clamping and 72 h after the operation. The values of BUN and Cr were determined by an automated analyzer (Roche-Hitachi Modular D-P Autoanalyzer, Mannheim, Germany). Statistical analysis was performed using the Statistical Package for the Social Sciences software (version 12.0; SPSS Inc. Chicago, IL, USA). The groups were compared by using the Mann-Whitney U test, Wilcoxon Signed Ranks test and Student's t-test. All values were presented as mean ± standard deviation (SD). A P value <0.05 was considered statistically significant.

RESULTS

There were no significant differences between the groups with respect to preoperative BUN and Cr values (P = 0.775 and 0.189, respectively). The lack of a significant difference between preoperative and postoperative values of BUN and Cr (P = 0.432 and 0.761, respectively) in the sham group indicated that operative procedures, except for clamping, had no influence on outcomes.

Although, postoperative 72 h BUN and Cr levels were lower in the ILO group as compared to the control group, the difference was not significant. The results are as shown in Tables 1 and 2.

The results of histopathological grading are summarized in Table 2. Additionally, Figure 2 shows normal renal morphology, whereas Figure 3 shows extensive necrosis and cast formations. Severe acute

Table 2. Results of histopathological evaluation.

ATN grade	Iloprost group	Control group
	n (%)	n (%)
0: normal	1 (14.2)	0 (0)
1: mild	2 (28.4)	1 (14.2)
2: moderate	3 (42.8)	3 (42.8)
3: severe	1 (14.2)	3 (42.8)

ATN: Acute tubular necrosis.

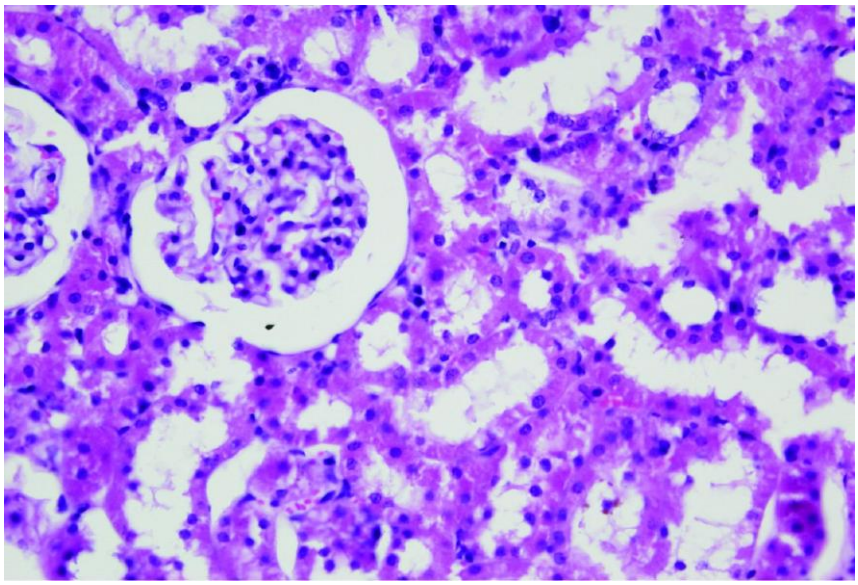

Figure 2. Normal renal morphology (H&E).

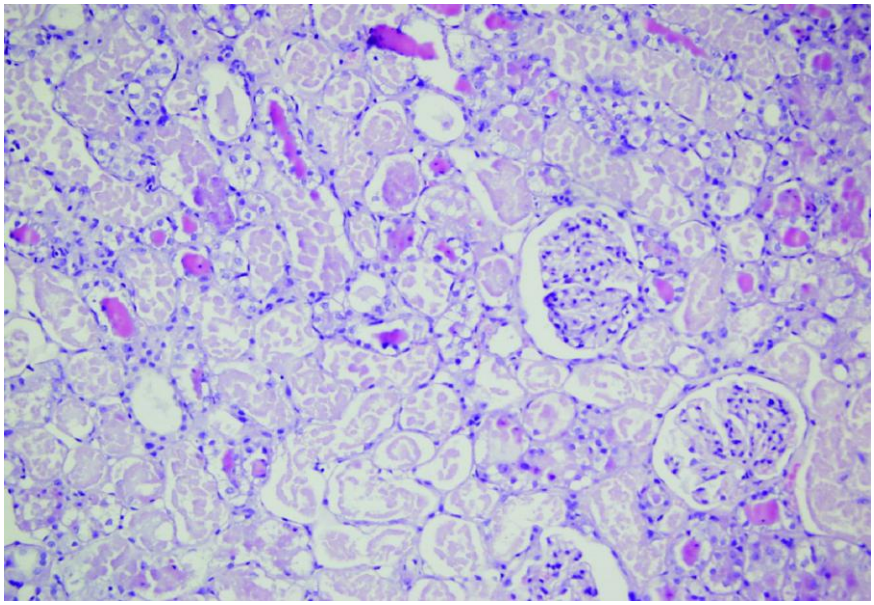

Figure 3. Extensive necrosis and cast formations (H&E).

tubular necrosis (grades 2 to 3) was detected in 86% of rats in the control group and 57% of rats in the ILO group. The median acute tubular necrosis histologic grading score was 2 in both groups, thus there was no significant difference (P = 0.209).

Furthermore, there were no significant differences with respect to the other histopathological changes due to I/R injury, such as atypical regeneration, loss of brush border, hydropic degeneration and cast formation (P = 0.805, 0.259, 0.902 and 0.805, respectively).

DISCUSSION

Iloprost (ILO) is a long-acting prostacyclin analogue and a potent vasodilator Wilhelm and Grundmann, 2004). As an antiaggregant, ILO is much more potent than acetylsalicylic acid, which is being widely used all over the world. Thus, it prevents thrombus formation both in arteries and in the microcirculation, and increases capillary perfusion by dilating both arterioles and venules (Müller et al., 1987). It inhibits the release of harmful vasoconstrictor and cytotoxic agents, such as thromboxane A2 (TXA2) and 5-hydroxytryptamine (5-HT) (Menys and Davies, 1983). Many studies have shown that adhesion and aggregation of neutrophils play a key role in the cascade of events that lead to I/R injury (Sehirli et al., 2003). ILO influences neutrophil function and inhibits adhesion and aggregation (Simpson et al., 1997). There are various studies investigating the different effects of prostacyclin and ILO on I/R injury in various tissues (Rowland et al., 1999). Lefer et al. (1978) showed that prostacyclin reduced neutrophil adhesion and preserved myocardial tissue in acute myocardial infarction. Although, Casey et al. (1980) failed to show a statistically significant effect of prostacyclin on I/R injury in dogs, they observed its beneficial effects at the tissue level. Langkopf et al. (1986) obtained good results in five pigs, the kidneys of which they infused with ILO at a dose of 0.5 ng/kg/min for 15 min and then performed transplantation after a warm ischemic period of 45 min; they did not observe renal failure due to I/R injury. ILO has been shown to decrease neutrophil activation and aggregation in addition to inhibition of reactive oxygen species production and release of lysosomal enzymes (Döşlüoğlu et al., 1993). A study investigating the effects of ILO on I/R injury in rat liver showed that ILO was beneficial in preventing injury (Okboy et al., 1992). It has been reported that endothelin-1 (ET-1) plays a negative role in pulmonary I/R injury and that ILO shows its beneficial effects by inhibiting ET-1 release (Kawashima et al., 2003). Pretreatment with ILO before the onset of ischemia and continuation into the reperfusion phase was thought to decrease the muscle infarct size and the rise in vascular permeability (Belkin et al., 1990).

In the present study, renal functions of the subjects, which underwent reperfusion after a warm ischemic period of 60 min, were evaluated based on serum BUN and Cr levels, as well as histopathological changes. Accordingly, it was investigated whether ILO therapy, which was started simultaneously with ischemia and continued for about an hour in the early phase of reperfusion, had beneficial effects on I/R injury. In the present study, different from other renal I/R experiments, instead of the renal pedicle, we selectively clamped the renal artery to create renal ischemia. This has remarkably increased the difficulty of surgical procedure, but cessation of arterial blood flow was verified both macroscopically (the color of the kidney became pale as soon as the clamp was placed) and histologically. In the present study, ILO was infused at a dose of 2 ng/kg/min after being diluted in 0.9% sodium chloride; this is the maximum dose used in humans in conditions requiring ILO therapy, such as pulmonary hypertension and non-healing wounds due to atherosclerosis. ILO infusion was started simultaneously with clamping. Therefore, peak serum level of ILO was provided at the time of removal of the clamp, that is, when reperfusion began. Infusion was continued during the first hour of reperfusion, which is the early phase that the harmful effects of reperfusion are mostly seen. In the control group, ILO was diluted in the same volume of 0.9% sodium chloride. Thus, possible hydration effect was made equal for both groups. For biochemical analysis, before arterial clamping and 72 hours after the operation, blood samples, as small as possible (1 ml), were obtained in order not to alter hemodynamic variables and cause hypovolemia. This volume was compensated with the infusions applied to give medications. Infection was not noted in any of the subjects in the study groups.

Although, postoperative 72 h BUN and Cr levels were lower in the group treated with ILO as compared to the control group, the difference was not significant. Histopathologically, the rate of severe acute tubular necrosis (grades 2 to 3) was 86% in the control group and 57% in the ILO group. There were no significant differences between the two groups with respect to median acute tubular necrosis score. Furthermore, no significant differences were found between the groups with respect to histopathological changes related to I/R injury, such as regenerative atypia, loss of brush border, hydropic degeneration and cast formation.

Conclusion

The results of the present study demonstrated that the ILO-treated group was less prone to injury, although, the difference was not significant. The small number of the subjects (limited by the local ethics committee) was a limitation to the study and might be associated with lack of significant differences.

In order to prevent renal failure due to I/R injury and consequently to decrease morbidity and mortality, there is need for much further comprehensive studies on therapeutic agents tested.

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