Eur J Med Res (2006) 11: 295-299

Effects of Iloprost and Pentoxifylline on Renal Ischemia-reperfusion in Rabbit Model

B. Emrecan[1], E. Tulukoglu[1], S. Bozok[1], M.Kestelli[1], G. Onem[2], A. Küpelioglu[3], S. Yagdi[4], A. Gürbüz[1]

[1]Ataturk Training and Research Hospital, Department of Cardiovascular Surgery, Izmir,
[2]Pamukkale University, Department of Cardiovascular Surgery, Denizli,
[3]Dokuz Eylül University, Department of Pathology, Izmir,
[4]Ataturk Training and Research Hospital, Department of Orthopedics and Traumatology, Izmir, Turkey

Abstract
Objective: In ischemia-reperfusion, Iloprost decreases neutrophil activation and aggregation besides inhibition of oxygen-free radical production. Pentoxifylline (Ptx) attenuates reperfusion-associated membrane injury and tissue edema, suppresses leukocyte adhesion and improves hindlimb blood flow during the reperfusion period. The primary hypothesis in this study was that Iloprost could present better protection than pentoxyfillin on renal ischemia-reperfusion in rabbit model.
Materials and Methods: Forty rabbits were grouped into four. Iloprost was continuously infused starting half an hour before the reperfusion after 2 hours ischemia and during the 4 hours reperfusion period in Group 1 whereas the Group 2 was treated with pentoxyfillin. Group 3 was the control group which didn't receive any medication. Forth group was sham group. Renal tissues were histologically and biochemically evaluated.
Results: The histologic scores were obtained according to presence of tubuler necrosis and atrophy, regenerative atypia, hydropic degeneration (Group 1 vs Group 3; $p<0.001$, Group 2 vs Group 3; $p = 0.001$, Group 1 vs Group 2; $p = 0.331$). Malondialdehyde levels of the medicated groups were 109 ± 11 nmol/gr tissue in Group 1, 119 ± 15 nmol / gr tissue in Group 2 and 132 ± 14 nmol / gr tissue in Group 3 (Group 1 vs Group 2; $p = 0.130$, Group 1 vs Group 3, $p = 0.002$, Group 2 vs Group 3; $p = 0.045$). Malondialdehyde levels and histologic scores of all of the groups were significantly different from the sham group.
Conclusion: Iloprost and pentoxyfillin reduced renal ischemia-reperfusion injury in rabbit model. There was not a significant difference between these two medications.

Key words: Iloprost; pentoxyfillin; ischemia-reperfusion injury; rabbit kidney

Introduction

There are limited numbers of reports concerning the effects of Pentoxifyllin (Ptx) and iloprost (IL), a stable prostacyclin analog on ischemia-reperfusion injury. The effects of these agents on ischemia-reperfusion injury have been studied on the skeletal muscles, liver, myocardium and spinal cord. IL was shown to decrease neutrophil activation and aggregation besides inhibition of oxygen-free radical production and release of lysosomal enzymes [1, 2]. Pentoxifylline (Ptx) was also proven to attenuate reperfusion-associated membrane injury and tissue edema, to suppress leukocyte adhesion and to improve hindlimb blood flow during the reperfusion period [3]. It was also reported to improve ischemia-reperfusion injury by attenuating neutrophil sequestration, production of reactive oxygen species, and platelet activation [4].

These two agents were studied separately in different studies. In this experimental study, we investigated the effect of IL and Ptx on renal ischemia-reperfusion injury in a rabbit model. The primary hypothesis was that IL could present a better protection than Ptx on histopathologic and biochemical basis.

Materials and Methods

Forty New Zealand white rabbits of either sex weighing 2–2.5 kg were included into the study. The rabbits were groupped into four in a way that 10 rabbits were in each group. Animals in Group 1 were treated with IL during the last 30 minutes of ischemia and during the reperfusion period after the ischemic period. Animals in Group 2 were treated with Ptx during this period. Group 3 was the control group which was not medicated during this period. The forth group was the sham group. The experiment was done according to the ethics of Pamukkale University Medical School, Denizli, Turkey. The study was funded by the authors.

Animal Preparation

The superficial ear veins of the rabbits were cannulated for venous line. The rabbits were sedatized by intravenous ketamine injection with a dose of 30/kg, and anesthesized by intraperitoneal pentobarbital sodium (50mg/kg), followed by intraperitoneal supplements (15mg/kg) as required. The room temperature was kept between 28-30 °C. After midline laparotromy and Heparin (400 U/kg) administration, and the left renal artery was explored and was occluded by an atraumatic bulldog clamp. The midline laparatomy incisions were closed by separate sutures thereafter. Two hours of ischemia was aimed for ischemic period. The animals in

Group 1 received continuous intravenous infusion of IL with a dose of 2 ng/kg/min half an hour before the reperfusion. The medications were started half an hour before the reperfusion in order that they would be present in the blood starting from the reperfusion period. The animals in Group 2 received intravenous bolus of Ptx with a dose of 30 mg/kg half an hour before the reperfusion, followed by continuous infusion at a rate of 0.1 mg/kg/min, throughout the rest of the ischemic period [3, 5]. Selected IL dose was the maximal dose for the human which was written in the prospectus of the drug. Control animals received only normal saline solution. After 2 hour ischemia, the clamps on the left renal arteries were removed and a 4-hour reperfusion was started. The animals in Group 1 and 2 received IL and Ptx infusions and the animals in Group 3 received saline solution infusion during the reperfusion period. After 4 hours reperfusion, left nephrectomy was done and renal biopsies were taken from the cortex of the kidneys for microscopic and biochemical evaluation. Renal tissue materials were taken from the sham group for histologic and biochemical evaluation.

Microscopic Evaluation of Ischemic Injury

Portions of the fixed renal biopsy materials were examined microscopically (Nikon model Eclipse E600W)). The specimens were fixed in 10% formalin. Paraffin blocks were cut at 5 μm and stained with hematoxylin-eosin. Microscopic renal injury was considered in the presence of tubuler necrosis and atrophy, regenerative atypia, hydropic degeneration, interstitial fibrosis, loss of supranuclear cytoplasms and brush border disappearance. Renal injury was scored semiquantatively according to these characteristics as; grade 0 as normal, grade 1 as mild (focal), grade 2 as moderate (multifocal) and grade 3 as severe (diffuse) pathologic changes (Table 1) by the same pathologist, who was blinded to the study [6].

Tissue MDA Analysis

Lipid Peroxidation Measurement was measured form the renal cortical malondialdehyde (MDA) content as Uchiama described [7]. Renal cortical tissues were homogenized by using Ultra-Turrax T25 homogenisator in 1.15 % ice cold potassium chloride solution containing 50 ml/L of Triton X-100. A 0.5mL homogenate was mixed with 3mL of 1% phosphoric acid and 1mL of 0.6% thiobarbituric acid. The mixture was heated on boiling water for 45 minutes. After addition of 4mL of n-butanol, the contents were centrifuged at 4000 gyration per minute for 10 min. The upper organic layer absorbance was measured at 532 nm spectrophotometrically. The tissue MDA levels were expressed as nmoles per gram renal tissue.

Statistical Analysis

Statistical analysis was done with SPSS 10.0 statistical software program (SPSS Inc, Chicago, IL). Continuous variables were expressed as the mean ± 1 SD. Differences between the experimental and control groups were analyzed for their statistical significance by use of the two-tailed Student t test. The p values less than 0.05 were considered to be statistically significant.

Results

Histologic evidence of reperfusion injury was the presence of tubuler necrosis and atrophy, regenerative

Table 1. Histopathological scoring.

	Histopathologic Score			
	0	1	2	3
Tubular necrosis	Absent	Focal	Multifocal	Diffuse
Tubular atrophy	Absent	<25%	25-50%	>50%
Regenerative atypia	Absent	Focal	Multifocal	Diffuse
Hydrophic degeneration/vacuolisation	Absent	Focal	Multifocal	Diffuse
Interstitial fibrosis	Absent	Focal	Multifocal	Diffuse
Brush border disappearance	Absent	Focal	Multifocal	Diffuse

Table 2. Histopathologic scores of the groups.

nmol/gr tissue	Grade 0	Grade 1	Grade 2	Grade 3	Mean Score
Group 1 (n = 10)	2	8			0.80 ± 0.42
Group 2 (n = 10)	1	8	1		1.00 ± 0.47
Group 3 (n = 10)		2	6	2	2.00 ± 0.66
Group 4 (n = 10)	9	1			0.10 ± 0.31

p = 0.331; Group 1vs Group 2; p< 0.001; Group 1 vs Group 3; p = 0.001; Group 2 vs Group 3; p = 0.001; Group 1 vs Group 4; p< 0.001; Group 2 vs Group 4; p< 0.001; Group 3 vs Group 4

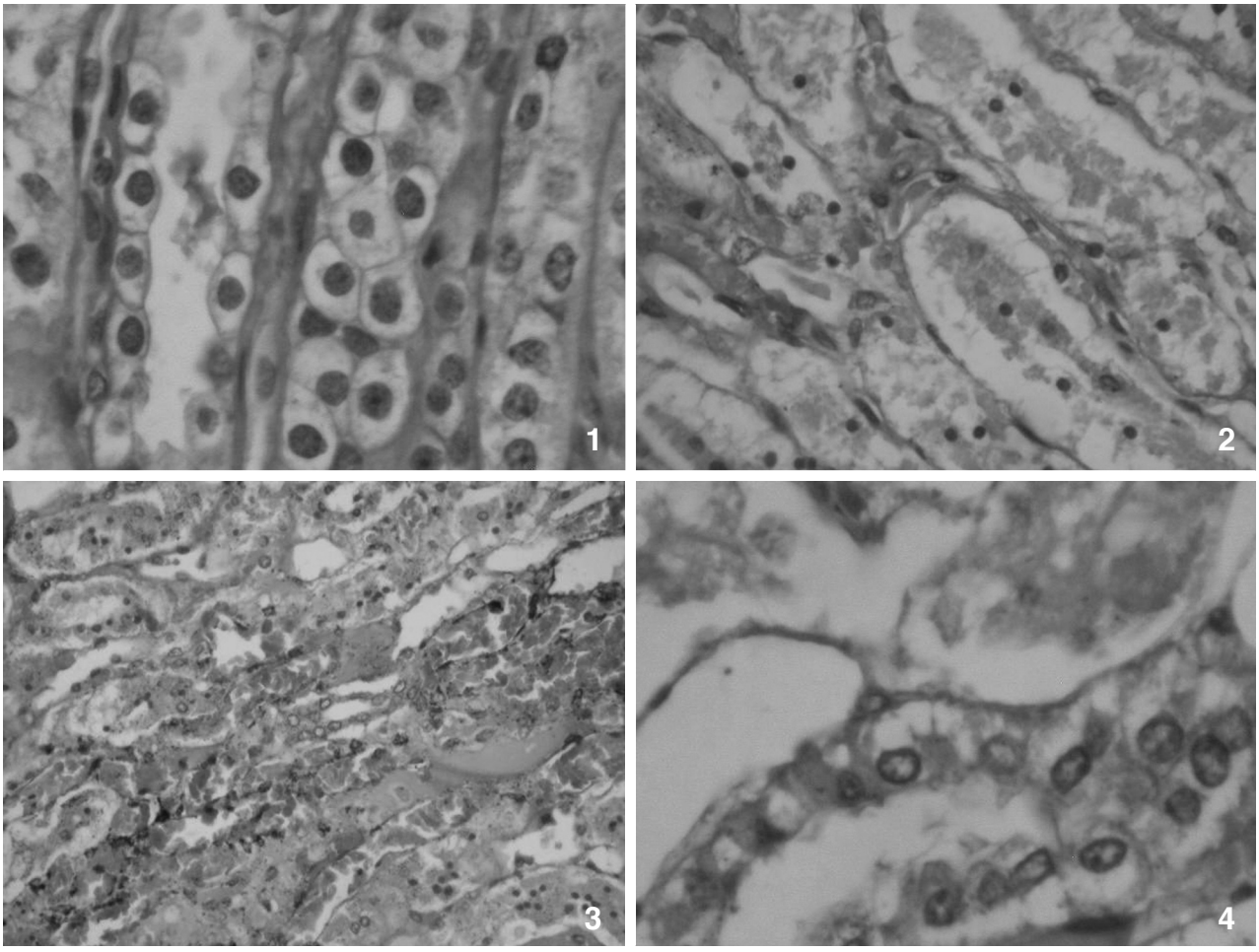

Fig. 1. Normal renal tubules. Supranuclear cytoplasms in the tubuler epithelial cells are seen to be normal. (H&E, x400).

Fig. 2. Severe, diffuse tubuler necrosis, brush border dissappearance (H&E, x200).

Fig. 3. Severe, diffuse tubuler necrosis, congestion in the interstitium (H&E, x100).

Fig. 4. Moderate multifocal loss of supranuclear cytoplasm in the tubule cells. (H&E, x400).

atypia, hydropic degeneration, interstitial fibrosis, loss of supranuclear cytoplasms and brush border disappearance. The mean histopathologic scores of Group 1 and Group 2 were significantly lower than the control group. (Group 1 vs Group 3; $p<0.001$, Group 2 vs Group 3; $p = 0.001$, Group 1 vs Group 2; $p = 0.331$). There was no significant difference between IL and Ptx groups ($p = 0.331$; NS) (Table 2). Figure 1 demostrates a normal renal histology whereas Figures 2, 3 and 4 demostrate abnormal renal morphologies.

The MDA levels of the medicated groups were significantly lower than the levels of the control group. Mean MDA levels were 109 ± 11 nmol/gr tissue in Group 1, 119 ± 15 nmol / gr tissue in Group 2 and 132 ± 14 nmol / gr tissue in Group 3 (Group 1 vs Group 2; $p = 0.130$, Group 1 vs Group 3, $p = 0.002$, Group 2 vs Group 3; $p = 0.045$).

Mean MDA level was 33 ± 7 nmol/gr tissue in Group 4 (Group 1 vs Group 4; $p<0.001$, Group 2 vs Group 4; $p<0.001$, Group 3 vs Group 4; $p<0.001$). This showed that the ischemia reperfusion model was well formed in the animals.

Discussion

The results did not confirm the primary hypothesis that IL showed a better protection than Ptx..However, it was proven that Ptx and IL significantly reduced the ischemia-reperfusion injury on the light microscopic basis. It can be said that Ptx and IL significantly improved the ischemia-reperfusion injury in the renal tubules.

There are few reports concerning the effects of IL and Ptx on skeletal muscle ischemia-reperfusion injury. IL functions as a membrane stabilizator and decreases myocardial enzyme release. It inhibits neutrophil functions which are potential mediators of ischemia-reperfusion injury. Neutrophils may cause local injury by formation of oxygen derived free radicals and release of lysosomal enzymes. Neutrophil infiltration and activation, intracytosol calcium influx, complement activation, and generation of oxygen-free radicals are associated with reperfusion syndrome [8]. IL also decreases white blood cell aggregation and adhesion to vascular endothelium, superoxide radical production from

stimulated canine and human neutrophils, and free radical formation in myocardium subjected to ischemia-reperfusion injury [9]. IL has been shown to protect myocardium [2, 10], skeletal muscles [3, 8, 9], lungs [11], spinal cord [12, 13] in ischemia-reperfusion models. IL is cytoprotective on 60-min warm ischemia-reperfusion injury of rat kidneys [14]. Our study also revealed that IL protected the kidneys against ischemia-reperfusion.

Ptx pretreatment was shown to prevent renal cell injuries. This effect was suggested to be due to the prevention apoptosis [15]. PTX inhibits generation of leukocyte-derived reactive oxygen species in exercise [16]. Ptx was suggested to prevent hypoxia-related changes in renal function of transplanted kidneys. This was concluded to be due to stimulation of renal prostaglandin synthesis, as well as interaction at the level of the adenosine receptors [17]. The data of the current study also supported this suggestion because both Ptx and IL protected renal cells against ischemia-reperfusion. In another study, Ptx was suggested to exert a protective effect against ischemic acute renal failure by inhibiting the production of tumor necrosis factor-alpha.in rabbits [3].

MDA is an indicator for lipid peroxidation which is trigerred by free oygen radicals. MDA is liberated as an end product from hydroxyperoxide distruction. For this reason, it has been used as an indicator of lipid peroxidation due to ischemia-reperfusion injury [18-21]. Free oxygen radicals have been shown to be formed in the first few minutes when tissues were reperfused [22]. Lipid peroxidation reaches to a peak four hours after reperfusion. Leucocyte accumulation occurs a few hours after the beginning of ischemia [23]. MDA is formed as an end product of conjugated diens and hydroxyperoxides which is trigerred by free oxygen radicals, and it was used as an indicator of ischemia-reperfusion injury MDA [24-26],. Therefore, 2-hour ischemia and 4-hour reperfusion seems to be sufficient for the histologic and biochemical analysis. Lower MDA levels in the recent study demonstrated that lipid peroxidation was avoided in the the IL and Ptx treated groups. There was not a significant difference between the IL and Ptx treated groups concerning lipid peroxidation.

In the present study, we evaluated the ischemia-reperfusion injury on histologic and lipid peroxidation basis. IL and Ptx were proven to reduce ischemia-reperfusion injury in rabbit kidneys microscopically and biochemically. The use of a model of unilateral ischemia does not allow functional assessment so we can only speculate whether the observed protective effect at the tissue level also could translate into preservation of renal function after ischemia. Therefore, conclusion on protective effects of IL and Ptx renal ischemia-reperfusion injury needs further comprehensive studies.

REFERENCES

1. Fantone JC, Marasco WA, Elgas LJ, Ward PA. Stimulus specificity of prostaglandin inhibition of rabbit polymorphonuclear leukocyte lysosomal enzyme release and superoxide anion production. Am J Pathol 1984;115:9–16.
2. Simpson PJ, Mitsos SE, Ventura A, et al. Prostacyclin protects ischemic reperfused myocardium in the dog by inhibition of neutrophil activation. Am Heart J 1987;113:129–37.
3. Kishi M, Tanaka H, Seiyama A, Takaoka M, Matsuoka T, Yoshioka T, et al. Pentoxifylline attenuates reperfusion injury in skeletal muscle after partial ischemia. Am J Physiol Heart Circ Physiol 1998;274:1435-42
4. Adams JG Jr, Dhar A, Shukla SD, Silver D.. Effect of pentoxifylline on tissue injury and platelet-activating factor production during ischemia-reperfusion injury. J. Vasc. Surg. 1995;21:742-8
5. Kim YK, Yoo JH, Woo JS, Jung JS, Kim BS, Kim SY. Effect of pentoxifylline on ischemic acute renal failure in rabbits. Ren Fail. 2001;23(6):757-72
6. Onal A, Astarcioglu H, Ormen M, Atila K, Sarioglu S. The beneficial effect of L-carnitine in rat renal ischemia-reperfusion injury] Ulus Travma Derg. 2004;10(3):160-7
7. Uchiyama, M.; Mihara, M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 1978;86:271-8
8. Mohan C, Marini C, Gennaro M, Ascer E. The value and limitation of iloprost infusion in decreasing skeletal muscle necrosis. J Vasc Surg. 1992;16(2):268-73
9. Belkin M, Wright JG, Hobson RW. Iloprost infusion decreases skeletal muscle ischemia-reperfusion injury. J Vasc Surg 1990;11:77-83.
10. Fitzl G, Winkler D, Wassilev G, Welt K.The protective effect of iloprost, a PGL2 analogue, on ultratructure of ischemic and reperfused myocardial myocytes. Exp Toxicol Pathol. 1999;51(1):81-8.
11. Kawashima M, Nakamura T, Schneider S, Vollmar B, Lausberg HF, Bauer M, et al. Iloprost ameliorates post-ischemic lung reperfusion injury and maintains an appropriate pulmonary ET-1 balance. J Heart Lung Transplant. 2003;22(7):794-801.
12. Katircioglu SF, Ulus AT, Gokce P, Surucu S. Iloprost protects the spinal cord during aortic cross-clamping in a canine model. J Cardiovasc Surg. 2000;41(1):89-93
13. Silistreli E, Kabakci B, Yilmaz E, Sonmez A, Erdal C, Karabay O, et al. Early protective effects of iloprost, a stable prostacyclin analog, during spinal cord ischemia in a rabbit model. Heart Vessels. 2005;20(2):66-71.
14. Dosluoglu HH, Aktan AO, Yegen C, Okboy N, Yalcm AS, Yahn R, et al.The cytoprotective effects of verapamil and iloprost (ZK 36374) on ischemia/reperfusion injury of kidneys. Transpl Int. 1993;6(3):138-42
15. Kim YK, Choi TR, Kwon CH, Kim JH, Woo JS, Jung JS. Beneficial effect of pentoxifylline on cisplatin-induced acute renal failure in rabbits. Ren Fail. 2003;25(6):909-22.
16. Ciuffetti G, Mercuri M, Ott C, Lombardini R, Paltriccia R, Lupattelli G, et al. Use of pentoxifylline as an inhibitor of free radical generation in peripheral vascular disease. Results of a double-blind placebo-controlled study. Eur. J. Clin. Pharmacol. 1991;41; 511-5
17. Berens KL, Luke DR. Pentoxifylline in the isolated perfused rat kidney. Transplantation. 1990;49(5):876-9
18. Kihara M, Nickander KK, Low PA. The effect of aging on endoneurial blood flow, hyperemic response and oxygen-free radicals in rat sciatic nerve. Brain Res. 1991;18;562(1):1-5.
19. Veveris M, Dambrova M, Cirule H, Meirena D, Kalvinsh I, Wikberg JE. Cardioprotective effects of N-hydroxyguanidine PR5 in myocardial ischaemia and reperfusion in rats. Br J Pharmacol. 1999;128(5):1089-97.
20. Kabasakal L, Sehirli O, Cetinel S, Cikler E, Gedik N, Sener G. Protective effect of aqueous garlic extract against renal ischemia/reperfusion injury in rats. J Med Food. 2005;8(3):319-26

21. Erkasap S, Ates E. L-Arginine-enriched preservation solution decreases ischaemia/reperfusion injury in canine kidneys after long-term cold storage. Nephrol Dial Transplant. 2000;15(8):1224-7.
22. Schoenberg MH, Beger HG. Reperfusion injury after intestinal ischemia. Crit Care Med. 1993;21(9):1376-86. Review
23. Savas S, Delibas N, Savas C, Sutcu R, Cindas A. Pentoxifylline reduces biochemical markers of ischemia-reperfusion induced spinal cord injury in rabbits. Spinal Cord. 2002;40(5):224-9
24. Cetinkale O, Bilgic L, Bolayirli M, Sengul R, Ayan F, Burcak G. Involvement of neutrophils in ischemia-reperfusion injury of inguinal island skin flaps in rats. Plast Reconstr Surg. 1998;102(1):153-60
25. Kihara M, Nickander KK, Low PA. The effect of aging on endoneurial blood flow, hyperemic response and oxygen-free radicals in rat sciatic nerve. Brain Res. 1991;18;562(1):1-5
26. Veveris M, Dambrova M, Cirule H, Meirena D, Kalvinsh I, Wikberg JE. Cardioprotective effects of N-hydroxyguanidine PR5 in myocardial ischaemia and reperfusion in rats. Br J Pharmacol. 1999;128(5):1089-97

Received: February 29, 2006 / Accepted: May 11, 2006

Address for correspondence:
Bilgin Emrecan
226.sok 17/10 Hatay
35280 Izmir/Turkey
Phone: +905054889916
Fax: +902322434848
E-mail: bilginemrecan@yahoo.com