Iloprost, a prostacyclin (PGI_2) analogue, reduces liver injury in hepatic ischemia–reperfusion in rats[1]

Iloprost, um análogo da prostaciclina (PGI_2), reduz danos da isquemia/reperfusão hepática em ratos

Ercan GedikI, Sadullah Girgin[I], , Basra Deniz Obay[II], Hayrettin Ozturk[III], Hulya Ozturk[IV], Huseyin Buyukbayram[V]

[I] Assistant Professor, Dicle University, Medical School, Department of General Surgery, Diyarbakir, Turkey.
[II] Assistant Professor, Dicle University, Medical School, Department of Physiology, Diyarbakir, Turkey.
[III] Associate Professor, Abant Izzet Baysal University, Medical School, Department of Pediatric Surgery, Bolu, Turkey.
[IV] Assistant Professor, 4Duzce University, Medical School, Department of Pediatric Surgery, Bolu, Turkey.
[V] Associate Professor, Dicle University, Medical School, Department of Pathology, Diyarbakir, Turkey.

ABSTRACT
Purpose: To evaluate the effects of iloprost a prostacyclin analogue on the hepatic IR injury in rats. **Methods**: Forty male Sprague-Dawley rats (250-300 g) were divided into four groups each containing 10 rats;—(1) controls: data from unmanipulated animals; (2) sham group: rats subjected to the surgical procedure, except for liver I/R, and given saline; (3) I/R group: rats that underwent liver ischemia for 45 min followed by reperfusion for 45 min; (4) I–R/ Iloprost group: rats pretreated with iloprost (10 µg kg^{-1}, i.v). Liver tissues were taken to determine SOD, CAT, GSH, and MDA levels and for biochemical and histological evaluation. **Results**: The plasma ALT and AST levels were increased in group 3 than in group 4. MDA values and the liver injury score decreased, while the SOD, CAT, and GSH values increased in group 4 compared to group 3. In group 3, hepatocytes were swollen with marked vacuolization. In group 4, there were regular sinusoidal structures with normal morphology without any signs of congestion. **Conclusion**: We demonstrated hepatoprotective effects of iloprost against severe ischemia and reperfusion injury in rat liver.
Key words: Liver. Ischemia. Reperfusion. Iloprost. Rats.

RESUMO
Objetivo: Avaliar os efeitos do iloprost, um análogo da prostaciclina nos danos causados ao fígado de ratos pela lesão de IR. **Métodos**: Quarenta ratos machos Sprague-Dawley (250-300 g) foram distribuídos em quatro grupos de dez; - (1) grupo de controle: dados de animais não manipulados; (2) grupo "sham": ratos que sofreram intervenção cirúrgica sem I/R, aos quais foram administrados solução salina; (3) grupo I/R; animais que foram submetidos à isquemia por 45 minutos seguida de reperfusão por 45 minutos; (4) grupo I – R/Iloprost: ratos previamente tratados com Iloprost (10µ kg^{-1}, i.v). Tecidos hepáticos foram retirados para determinar os níveis de SOD, CAT, GSH, e MDA e para avaliação bioquímica e histológica. **Resultados**: Os níveis de plasma ALT e AST aumentaram no grupo 3 mais do que no grupo 4. Os valores de MDA e o índice de lesões hepáticas diminuíram, enquanto os valores de SOD, CAT e GSH aumentaram no grupo 4, em comparação com o grupo3. No grupo 3, os hepatócitos se apresentaram edemaciados, e vacuolizados. No grupo 4, havia estruturas sinusoidais regulares, apresentando morfologia normal, sem sinais de congestão. **Conclusão**: Demonstramos os efeitos hepato-protetores do Iloprost contra a isquemia grave e o dano de reperfusão no fígado de ratos.
Descritores: Fígado. Isquemia. Reperfusão. Iloprosta. Ratos.

[1]Research performed at Medical School, Department of General Surgery, Dicle University, Diyarbakir, Turkey.

Introduction

Liver injuries, liver tumor resection, hemorrhagic shock with fluid resuscitation, and liver transplantation are responsible for liver injury caused by ischemia/reperfusion (I/R). Various mechanisms have been proposed to explain the mechanisms of ischemia-reperfusion (IR) injury. The implicated factors include free oxygen radicals, leukocyte migration and activation, microcirculatory abnormalities, sinusoidal endothelial cell damage, activation of the coagulation cascade, Kupffer cell activation due to the release of inflammatory cytokines, and proteolytic enzymes[1-5].

Data obtained by several researchers indicate that the generation of oxygen-derived free radicals is probably the most important factor involved[6-8]. Upon reperfusion endothelial cells produce reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide in larger amounts while nitric oxide (NO) synthesis decreases significantly[9]. During the process of I/R injury, then, inflammatory reactions are activated, resulting in the formation of inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1,-8, platelet-activating factor and arachidonic acid metabolites[10].

Several enzymes and drugs have been used to prevent such injury in humans and animals. However, the role of iloprost in hepatic I/R injury is unclear. Iloprost is the long-acting stable analogue of prostaglandin I_2 PGI_2[11]. PGI_2 is one of the major cyclooxygenase products of endothelial cells. It inhibits platelet aggregation, leukocyte activation, chemotaxis, and superoxide anion production; is known to be a potent vasodilator[12] and has proven to be effective in attenuating the changes in microvascular permeability, which is the final result of I/R[13]. According to these properties, this study examined the effect of iloprost during liver ischemia/reperfusion-induced oxidative stress in rats.

Methods

Forty male Sprague–Dawley rats weighing 250–300 g were used in the study. All of the experimental protocols were performed according to the guidelines for the ethical treatment of experimental animals.

Animals and experimental protocol

The rats were housed individually in cages, and allowed free access to standard rat chow and water before and after the experiments. The animal rooms were windowless and under controlled temperature (22 ± 2ºC) and lighting conditions. The animals were fasted overnight before the experiments, but were given free access to water. They were anesthetized using 100 mg kg^{-1} ketamine and 20 mg kg^{-1} xylazine body weight, i.p. The right femoral vein was cannulated to administer drugs and saline.

The animals were randomized into four groups (n=10, each)—(1) controls: unmanipulated animals, rats not subjected to any surgical procedure or liver manipulation; (2) sham group: rats subjected to the surgical procedures described below, except for liver I/R, and administered saline vehicle and maintained under anesthesia for an equivalent duration (i.e., 45 min and 45 min); (3) I/R group: rats subjected to the surgical procedures described below that underwent liver ischemia for 45 min followed by reperfusion for 45 min (n=10); (4) I–R/Iloprost group: rats that received iloprost (10 µg kg^{-1}, i.v.; Ilomedin®, Schering, Berlin, Germany) in 1ml of 0.9% NaCl solution over a period of 3 min from the tail vein 10 min before the removal of vascular microclamp.

Liver ischemia/reperfusion

As described previously[14], the ligament attachments connecting the liver, diaphragm, abdominal wall, and neighboring organs were divided. After the organ was isolated carefully, the liver hilus was exposed to find the common hepatic artery and portal vein. A vascular microclamp was used to interrupt the blood supply to three-quarters of the liver for 45 min, and this was followed by 45 min of reperfusion. Other rats were subjected to a sham operation (sham-operated), which was identical to the surgical procedure used for the I/R group rats without clamping; the rats were kept under anesthesia for the same length of time. At the end of the experiments, the rats were killed with an overdose of sodium pentobarbital.

Measuring serum liver enzymes

The abdominal aorta was punctured and 5 ml of blood was taken and put into heparinized tubes. Plasma was separated by centrifugation (3000 rpm for 10 min at room temperature) for biochemical studies. The activities of alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury), and aspartate aminotransferase (AST, a nonspecific marker for hepatic injury) in plasma were determined in units per liter using standard auto-analyzer methods on an Abbott Aeroset (Abbott Laboratories, Abbott Park, IL, USA). Just before the rats were sacrificed, the livers were removed for histopathological evaluation.

Histopathological study

The livers were divided into two pieces. One was placed in 10% formalin solution immediately, left overnight, and then embedded in paraffin blocks. The blocks were cut in 4-µm sections and stained with hematoxylin–eosin, using standard protocols. The severity of hepatic injury in the sections was evaluated using a point-counting method on an ordinal scale as follows: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting of cytoplasmic vacuolation and focal nuclear pyknosis; grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; and grade 3, severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration[15].

Biochemical analyses

The other piece was washed in ice-cold 0.9% saline solution, weighed, and stored at -70°C. Tissue homogenates were prepared as 1.0 g 10 ml^{-1} in 250 mM sucrose, 1 mM EDTA, 1 mM DL-dithiothreitol, and 15 mM Tris HCl (pH 7.4), using an all-glass Potter Elvehjem homogenizer (Selecta, Barcelona, Spain). Each homogenate was centrifuged for 20 min at 800 x g. The resulting supernatant fraction was used to determine enzyme activities. The protein concentrations in the supernatant were determined using the Bradford method[16].

Malondialdehyde determination

Liver MDA levels were determined using the method of Wasowicz *et al.*[17] based on the reaction of MDA with thiobarbituric acid at 95 to 100ºC. Fluorescence intensity was measured in the upper n-butanol phase using fluorescence spectrophotometry (F-4010; Hitachi, Tokyo, Japan) adjusted for excitation at 525 nm and emission at 547 nm. The arbitrary values obtained were compared with a series of standard solutions (1,1,3,3-tetramethoxypropane). The results are given in nanomoles per milligram of wet tissue (nmol mg wet tissue[1]).

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) determination

SOD activity was measured using the xanthine–oxidase–cytochrome c method, as described by McCord and Fridovich[18]. The final concentrations in the cuvettes were 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 10 mM cytochrome *c*, 50 mM

xanthine, 50 or 2 mM cyanide, 1 U catalase, and 0.05–0.1 mg of tissue. The reaction was initiated by adding 1 U xanthine–oxidase. The inhibition of xanthine–oxidase was followed spectrophotometrically at 550 nm. One unit of SOD activity was defined as the amount of enzyme that produced 50% inhibition of the control rate of cytochrome c reduction.

CAT activity was assayed according to the method of Beers and Sizer[19]. The final concentrations in the cuvettes were 500 mM potassium phosphate (pH 7), 100 mM H2O2, and 0.05–0.1 mg of tissue. The decrease in the absorbance at 240 nm after adding the substrate was followed spectrophotometrically.

GSH activity was assayed using a coupled enzyme system in which oxidized glutathione (GSSG) reduction was coupled to NADPH oxidation by glutathione reductase[20]. The assay mixture contained 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM NaN_3, 1 mM reduced glutathione, 0.2 mM NADPH, 1 U glutathione reductase, and tissue (0.05–0.2 mg). After a 5-min preincubation (20–25°C), the reaction was initiated by adding 0.25 mM H_2O_2. The decrease in the absorbance at 340 nm was followed spectrophotometrically.

Protein assays

The protein content of the homogenates was determined using the procedure of Lowry *et al.*[21].

Statistical analysis

Data were entered and analyzed on an IBM-compatible personal computer using SPSS version 9.0. All values were expressed as the mean ± SE. The significance of the data obtained was evaluated using analysis of variance (ANOVA). Differences between means were analyzed using the post-ANOVA test (Tukey's *b*); *p*-values less than 0.05 were considered significant.

Results

The ALT and AST levels were increased significantly in groups 3 and 4 in comparison with groups 1 and 2 (p=0.05 in all cases). However, the ALT and AST levels were decreased significantly in group 4 compared to group 3 (p<0.05) (Figure 1).

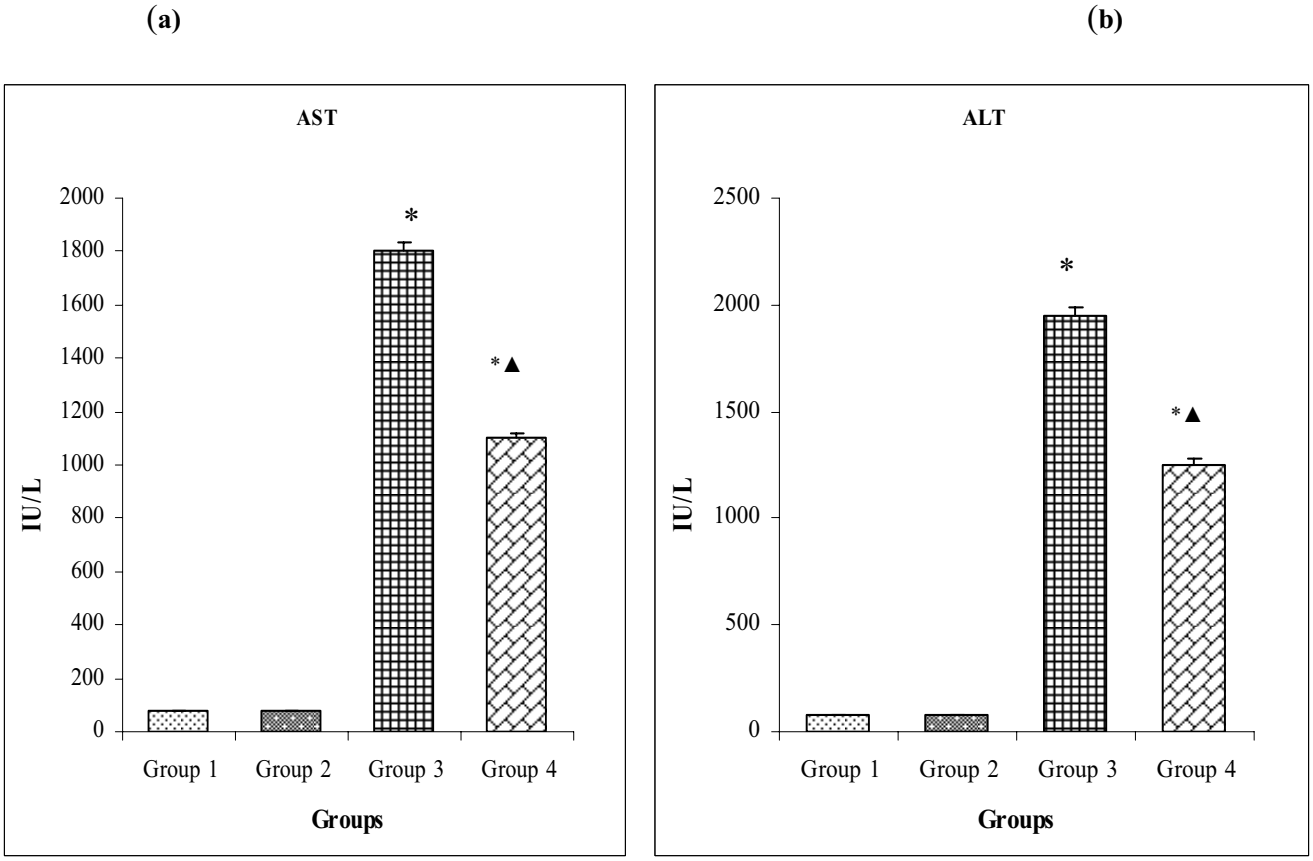

FIGURE 1 - Effects of liver ischemia/reperfusion and iloprost on liver function. The AST (a) and ALT (b) values in the iloprost group were significantly lower than in group 3. *p<0.05compared with group 1 and 2; ▲ p<0.05 compared with group 3. The values are the mean ± SE. Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase

The MDA, SOD, CAT, and GSH-Px values for the different groups are shown in Figure 2. In group 3, MDA significantly increased compared to groups 1, 2, and 4 (p<0.05 in all cases). In addition, SOD, CAT, and GSH were decreased significantly in group 3 compared to groups 1, 2, and 4 (p<0.05 in all cases).

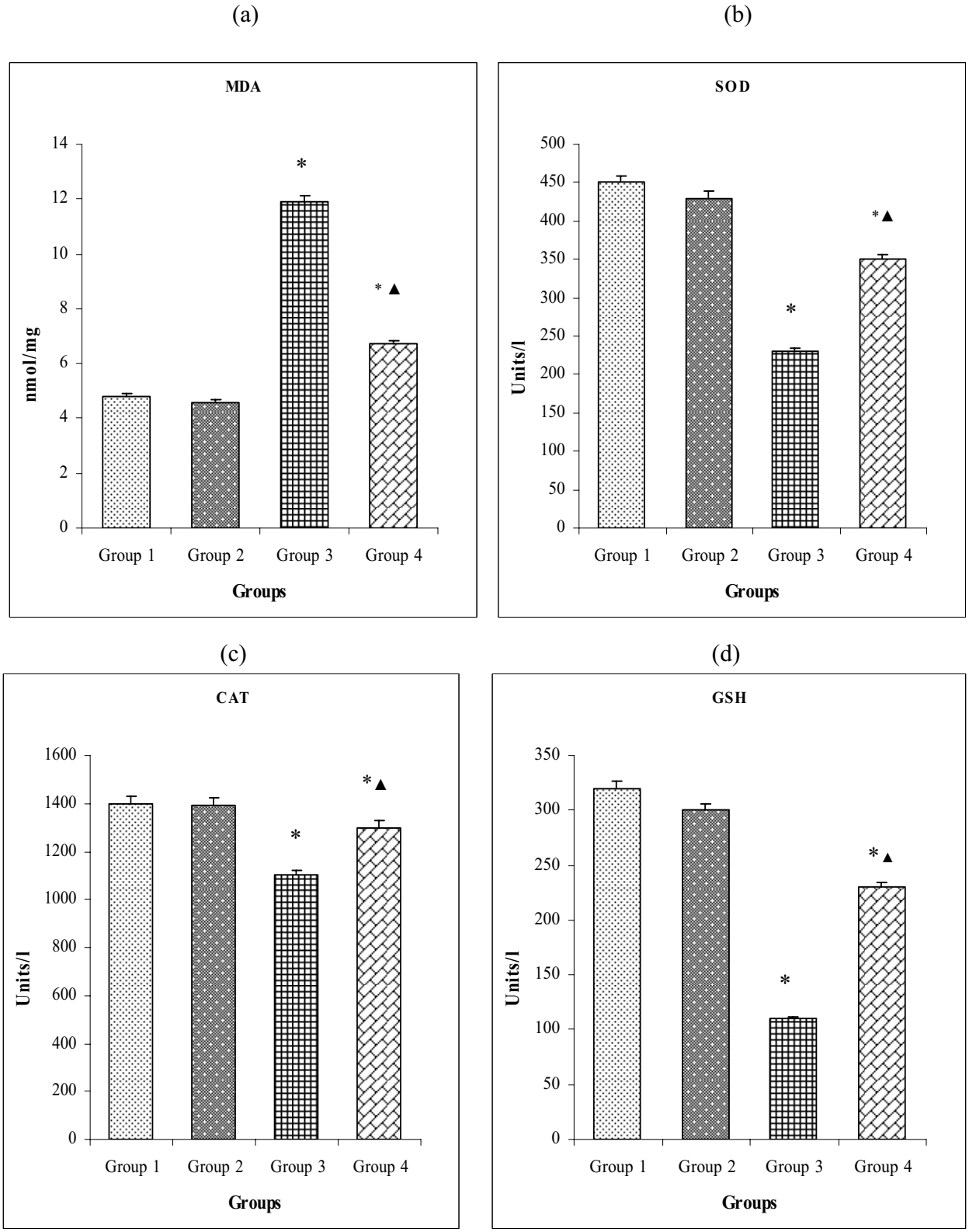

FIGURE 2 - Effects of ischemia/reperfusion and iloprost on the MDA (a), SOD (b), CAT (c), and GSH (d) levels in liver tissue. *p<0.05 compared with groups 1 and 2. ▲ p<0.05 compared with group 3. The values are the mean ± SE. MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione peroxidase

The histopathological score was 0.1 ± 0.2, 0.1 ± 0.3, 3.8 ± 0.1, and 1,3 ± 0.3 in groups 1 to 4, respectively. The histopathological score was higher in groups 3 and 4 than in groups 1 and 2 (p<0.05 in all cases). Moreover, the histopathological score was significantly lower in group 4 than in group 3 (p<0.05).

In histologic examination of the liver tissues with hematoxylin and eosin staining, we demonstrated that no morphological damage was observed in any rat in groups 1 or 2 (Figures 3A, B). In group 3, the hepatocytes were swollen with marked vacuolization and congestion was noted in the enlarged sinusoids (Figure 3C). In group 4, regular sinusoidal structures were noted with normal morphology and no signs of congestion (Figure 3D).

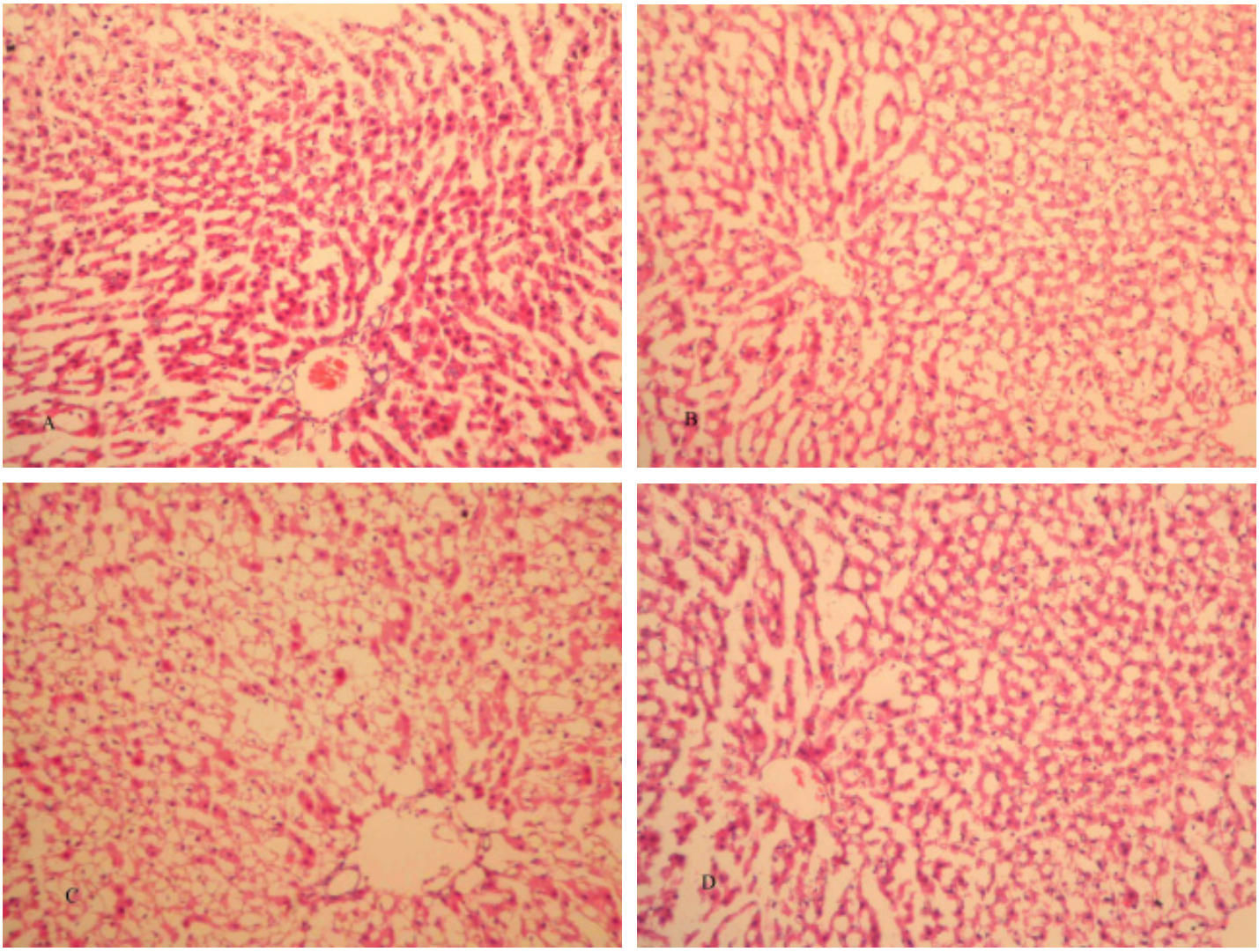

FIGURE 3 - (A, B) In groups 1 and 2, there were normal liver parenchyma with regular morphology. H&E X 200. (C) In group 3, the hepatocytes are swollen with marked vacuolization and congestion in the sinusoids H&E X 200. (D) In group 4, the hepatocytes and sinusoids show normal morphology, reflecting a well preserved liver parenchyma. H&E X 200.

Discussion

The present study demonstrates that iloprost, while improving liver functions, significantly decreased the I/R induced elevations of lipid and protein oxidation, and they also maintained GSH levels. Furthermore, histologic findings also support the the protective role of iloprost.

It is widely accepted that the formation of ROS in the early phase of reperfusion plays a major role in initiating and propagating oxidative stress after reperfusion in different organs, including the liver[22-24]. During ischemia, cells can not keep their membrane integrity; and this causes release of calcium and phospholipid A_2 as well as formation of polyunsaturated fatty acids and fatty acid radicals. If oxygenation is reestablished at that stage of ischemia, fatty acid radicals react with oxygen and perform the lipid peroxidation reaction. This reaction increases membrane permeability and stimulates chemotaxis of leukocytes, which can release oxygen-derived free radicals and proteolytic enzymes when activated[25-26]. Despite their important initiative function ROS are not responsible for the whole pathophysiological process by their own namely, other inflammatory mediators originating frompost-ischemic tissues, such as eicosanoids, can also, contribute significantly to the pathophysiology of I/R injury[27]. Especially PGI_2 and PGE1 induce vasodilatation, inhibit platelet and leukocyte aggre-

gation, exhibit anti-inflammatory activity such as suppression of tumor necrosing factor-α production and probably have direct cyto-protective effects[28]. Prostacyclin is a member of the prostaglandin family of lipid mediators and is the dominant cyclooxygenase metabolite of arachidonic acid in vascular endothelium[29].

Iloprost, a PGI_2 analogue, mimics the pharmacodynamic properties of this compound like potent inhibition of platelet activation and aggregation, vasodilation and yet ill-defined direct cytoprotection[30]. Kawashima *et al.*[31], in an isolated lung perfusion model, showed that iloprost ameliorates postischemic lung reperfusion injury. In a hind limb I/R induced experimental lung injury model, Koksela *et al.*[9] demonstrated that iloprost attenuates ischaemia induced remote organ reperfusion injury. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, remove oxygen free radicals[32]. MDA is the end product of lipid peroxidation and is a well-known parameter for determining the increased free radical formation in tissue[33,34]. In this study, we found that reperfusion injury produces oxidative stress in the liver, as shown by the increased MDA content and decreased SOD, CAT, and GSH activities. However, the mean SOD, CAT, and GSH levels increased in the I–R/ Iloprost group compared to the I/R group. This was probably related to the stimulation of PGI_2 expression by a PGI_2 analogue Iloprost, the blockage of ROS production, and a decrease in the consumption of the antioxidant enzyme. In addition, the MDA levels significantly decreased in the I–R/Iloprost group when compared to the I/R group. Ischemia/reperfusion is also associated with the release of enzymes, e.g., ALT, and AST, which are markers of cytolysis. In this study, the biochemical parameters were better in the I–R/ Iloprost group than in the I/R group. Histological examination of the liver revealed regular sinusoidal structures in the I–R/ Iloprost group, versus swollen, markedly vacuolized cells in the I/R group.

Conclusion

The administration of iloprost prevented hepatic malfunction, inhibited the generation of free radicals, and improved hepatic microcirculatory impairment after hepatic I/R injury. These results may have important implications for the therapeutic potential of a PGI_2 analogue iloprost in treating hepatic ischemia.

References

1. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg. 2001;181:160-6.
2. Giakoustidis D, Papageorgiou G, Iliadis S, Kontos N, Kostopoulou E, Papachrestou A, Tsantilas D, Spyridis C, Takoudas D, Botsoglou N, Dimitriadou A, Giakoustidis E.Intramuscular administration of very high dose of alpha-tocopherol protects liver from severe ischemia/reperfusion injury. World J Surg. 2002;26:872-7.
3. Suzuki S, Toledo-Pereyra LH. Interleukin 1 and tumor necrosis factor production as the initial stimulants of liver ischemia and reperfusion injury. J Surg Res. 1994;57:253-8.
4. Shibuya H, Ohkohchi N, Tsukamoto S, Satomi S. Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfused rat liver. Hepatology. 1997;26:113-20.
5. Palma-Vargas JM, Toledo-Pereyra L, Dean RE, Harkema JM, Dixon RA, Kogan TP. Small-molecule selectin inhibitor protects against liver inflammatory response after ischemia and reperfusion. J Am Coll Surg. 1997;185:365-72.
6. Adkison D, Höllwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN. Role of free radicals in ischemia-reperfusion injury to the liver. Acta Physiol Scand Suppl. 1986;548:101-7.
7. Gonzalez-Flecha B, Cutrin JC, Boveris A. Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to *in vivo* ischemia–reperfusion. J Clin Invest. 1993;91.456–64.
8. Jaescke H. Mechanism of reperfusion injury after warm ischemia of the liver. J. Hepatobil Pancreat Surg. 1998;5:402–8.
9. Koksel O, Ozdulger A, Aytacoglu B, Tamer L, Polat A, Sucu N, Yildirim C, Degirmenci U, Kanik A. The influence of iloprost on acute lung injury induced by hind limb ischemia-reperfusion in rats. Pulm Pharmacol Ther. 2005;18:235-41.
10. Mazzone A, Mazzucchelli I, Fossati G, Gritti D, Girola S, Canale C, Cusa C, Ricevuti G. Iloprost effects on phagocytes in patients suffering from ischaemic diseases: in vivo evidence for down-regulation of aMb2 integrin. Eur J Clin Invest. 1996;26:860–6.
11. Thomson IA, Egginton S, Slimms MH, Hudlicka O. Effect of muscle ischemia and iloprost during femorodistal reconstruction on capillary endothelial swelling. Int J Microcirc Clin Exp. 1996;16:284-90.
12. McNamara DB, Champion HC, Kadowitz PJ. Nonoperative management of lower extremity arterial disease. Part I. Pharmocologic management of peripheral vascular disease. Surg Clin North Am. 1998;78:447-56.
13. Blebea J, Cambria RA, DeFouw D, Feinberg RN, Hobson RW, Duran WN. Iloprost attenuates the increased permeability in skeletal muscle after ischemia and reperfusion. J Vasc Surg. 1990;12:657-65.
14. Sepodes B, Maio R, Pinto R, Marques C, Mendes-do-Vale J, McDonald MC, Thiemermann C, Mota-Filipe H. Tempol, an intracelullar free radical scavenger, reduces liver injury in hepatic ischemia-reperfusion in the rat. Transplant Proc. 2004;36:849-53.
15. Ozturk H, Gezici A, Ozturk H. The effect of celecoxib, a selective COX-2 inhibitor, on liver ischemia/reperfusion-induced oxidative stress in rats. Hepatol Res. 2006;34:76-83.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
17. Wasowicz W, Neve J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. Clin Chem. 1993;39:2522–6.
18. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244:6049–55.
19. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195:133–40.
20. Lawrence A, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 1976;71:952–8.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193:265–75.
22. Ishizaki N, Zhu Y, Zhang S, Nemoto A, Kobayashi Y, Subbotin V, Starzl TE, Todo S. Comparison of various lazaroid compounds for protection against ischemic liver injury. Transplantation. 1997;63:202–8.
23. Yokota R, Fukai M, Shimamura T, Suzuki T, Watanabe Y, Nagashima K, Kishida A, Furukawa H, Hayashi T, Todo S. A novel hydroxyl radical scavenger, nicaraven, protects the liver from warm ischemia and reperfusion injury. Surgery. 2000;127:661–9.
24. Zar HA, Tanigawa K, Kim YM, Lancaster JR Jr. Rat liver postischemic lipid peroxidation and vasoconstriction depend on ischemia time. Free Radic Biol Med. 1998;25:255–64.
25. Akar H, Sarac¸ Konuralp C, Yildiz L, Kolbakir F. Comparison of histopathologic effects of carnitine and ascorbic acid on reperfusion injury. Eur J Cardiothorac Surg. 2001;19:500-6.
26. Baltalarli A, Ozcan V, Bir F, Aybek H, Sacar M, Onem G, Goksin I, Demir S, Teke Z. Ascorbic acid (vitamin C) and iloprost attenuate the lung injury caused by ischemia/reperfusion of the lower extremities of rats. Ann Vasc Surg. 2006;20:49-55.

27. Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. J Pathol. 2000;190:255–66.
28. Rowlands TE, Gough MJ, Homer-Vanniasinkam S. Do prostaglandins have a salutary role in skeletal muscle ischaemia-reperfusion injury? Eur J Vasc Endovasc Surg. 1999;18:439–44.
29. Vane J, Corin RE. Prostacyclin a vascular mediator. Eur J Vasc Endovasc Surg. 2003;26:571–8.
30. Grant SM, Goa KL. Iloprost. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in peripheral vascular disease, myocardial ischemia and extracorporeal circulation procedures. Drugs. 1992;43:889–924.
31. Kawashima M, Nakamura T, Schneider S, Vollmar B, Lausberg HF, Bauer M, Menger MD, Schäfers HJ. Iloprost ameliorates post-ischemic lung reperfusion injury and maintains an appropriate pulmonary ET-1 balance. J Heart Lung Transplant. 2003;22:794-801.
32. Chang EJ, Lee SH, Mun KC, Suh SI, Bae JH, Kim SP, Choi HJ, Cho KB, Hwang JS. Effect of artificial cells on hepatic function after ischemia-reperfusion injury in liver. Transplant Proc. 2004;36:1959-61.
33. Zimmerman BJ, Granger DN. Reperfusion injury. Surg Clin North Am. 1992;72:65-83.
34. Reilly PM, Schiller HJ, Bulkley GB. Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. Am J Surg. 1991;161:488-503.

Conflict of interest: none
Financial source: none

Correspondence:
Erkan Gedik, MD
Assistant Professor in General Surgery
Department of General Surgery, Medical School
Dicle University, Diyarbakir - Turkey
Phone: +90-412-2488001-4679
Fax: +90-412-2488440
ercan.gedik@yohoo.com.br

Received: December 11, 2008
Review: February 12, 2009
Accepted: March 18, 2009

How to cite this article
Gedik E, Girgin S, , Obay BD, Ozturk H, Ozturk H, Buyukbayram, H. Iloprost, a prostacyclin (PGI2) analogue, reduces liver injury in hepatic ischemia–reperfusion in rats. Acta Cir Bras. [serial on the Internet] 2009 May-Jun;24(3). Available from URL: http://www.scielo.br/acb

***Color figures available from www.scielo.br/acb**