Iloprost, a prostacyclin (PGI\textsubscript{2}) analogue, reduces liver injury in hepatic ischemia–
reperfusion in rats\textsuperscript{1}

Iloprost, um análogo da prostacicлина (PGI\textsubscript{2}), reduz danos da isquemia/reperfusão hepática
em ratos

Ercan Gedik\textsuperscript{I}, Sadullah Girgin\textsuperscript{I}, Basra Deniz Obay\textsuperscript{II}, Hayrettin Ozturk\textsuperscript{III}, Hulya Ozturk\textsuperscript{IV}, Huseyin Buyukbayram\textsuperscript{V}

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

ABSTRACT

\textbf{Purpose:} To evaluate the effects of iloprost a prostacyclin analogue on the hepatic IR injury in rats. \textbf{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\textsuperscript{-1}, i.v). Liver tissues were taken to determine SOD, CAT, GSH, and MDA levels and for biochemical and histological evaluation. \textbf{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. \textbf{Conclusion:} We demonstrated hepatoprotective effects of iloprost against severe ischemia and reperfusion injury in rat liver.

Key words: Liver. Ischemia. Reperfusion. Iloprost. Rats.

RESUMO

\textbf{Objetivo:} Avaliar os efeitos do iloprost, um análogo da prostacicлина nos danos causados ao fígado de ratos pela lesão de IR. \textbf{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 (10 µg kg\textsuperscript{-1}, i.v). Tecidos hepáticos foram retirados para determinar os níveis de SOD, CAT, GSH, e MDA e para avaliação bioquímica e histológica. \textbf{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 edemacizados e vacuolizados. No grupo 4, havia estruturas sinusoidais regulares, apresentando morfologia normal, sem sinais de congestão. \textbf{Conclusão:} Demonstaramos os efeitos hepato-protetores do Iloprost contra a isquemia grave e o dano de reperfusão no fígado de ratos.


\textsuperscript{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\textsuperscript{1-5}.

Data obtained by several researchers indicate that the generation of oxygen-derived free radicals is probably the most important factor involved\textsuperscript{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\textsuperscript{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-\textgreek{a}, interleukin-1,-8, platelet-activating factor and arachidonic acid metabolites\textsuperscript{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$_2$. 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 ± 2°C) 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.

**Biochemical analyses**

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 100°C. 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–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. 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. The assay mixture contained 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM NaN3, 1 mM reduced glutathione, 0.2 mM NADPH, 1 U glutathione reductase, and tissue (0.05–0.2 mg). After a 5-min pre-incubation (20–25°C), the reaction was initiated by adding 0.25 mM H2O2. 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.

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).
Iloprost, a prostacyclin (PGI₂) analogue, reduces liver injury in hepatic ischemia–reperfusion in rats

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).

(a) MDA

(b) SOD

(c) CAT

(d) GSH

* $p$<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

FIGURE 2 - Effects of ischemia/reperfusion and iloprost on the MDA (a), SOD (b), CAT (c), and GSH (d) levels in liver tissue.
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 in the 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 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 liver22-24. During ischemia, cells can not keep their membrane integrity; and this causes release of calcium and phospholipid A₄, 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 activated25-26. Despite their important initiative function ROS are not responsible for the whole pathophysiological process by their own namely, other inflammatory mediators originating from post-ischemic tissues, such as eicosanoids, can also, contribute significantly to the pathophysiology of I/R injury27. Especially PGI₂ and PGE₁ induce vasodilatation, inhibit platelet and leukocyte aggre-
Iloprost, a prostacyclin (PGI₂) analogue, reduces liver injury in hepatic ischemia–reperfusion in rats

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 PGI₂ analogue iloprost in treating hepatic ischemia.

References


Acta Cirúrgica Brasileira - Vol. 24 (3) 2009 - 231

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@yahoo.com.br

Conflict of interest: none
Financial source: none

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

How to cite this article

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