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Comparison between iloprost and alprostadil for protection against ischemia/reperfusion injury in a rat model

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1. Introduction
Ischemia/reperfusion (IR) injury refers to a series of pathophysiologic processes that occur along with the return of blood to tissues after an ischemic period. The reperfusion of ischemic tissue can lead to catastrophic damage to cellular structures, membrane lipids, and proteins (1). Multiple interactions between free oxygen radicals, lymphocytes, cytokines, the complement system, and endothelial cells play a major role in the reperfusion process (2,3). Alprostadil (PGE1) is a vasodilator agent that has an inhibitory effect on platelet aggregation. It is also used as a regulatory element in IR injury for inhibition of the immune system and reduction of free oxygen radicals (4). Iloprost has shown favorable results in critical limb ischemia (5). It also decreases neutrophil adhesion and platelet aggregation, helping to prevent organ damage in IR injury (6).

Background/aim: Alprostadil and iloprost are successful agents used for both pulmonary hypertension and extremity ischemia treatment. Different systemic effects of these agents may change the preferences of clinical usage. Superiority of preventing ischemia/reperfusion (IR) injury is a criterion for clinical preference of these agents. The present study was designed to compare the protective effects of alprostadil and iloprost in a rat model of IR injury.

Materials and methods: Twenty-three male Sprague Dawley rats were used (aged 8-12 weeks, mean weight 230 ± 30 g). They were randomized into 4 groups: Group 1 (iloprost + IR), Group 2 (alprostadil + IR), Group 3 (saline + IR), and Group 4 (control). Under general anesthesia, in all groups except Group 4, the abdominal region was explored and the abdominal aorta was temporarily clamped for 60 min. After the clamp was removed, 120 min of reperfusion was applied. In Group 4, the rats were placed under general anesthesia and abdominal exploration was performed without the IR procedure. For all rats, body temperature was kept at 36 °C with a heater pad through the whole procedure. The rats were euthanized under general anesthesia to remove the kidneys and lungs for study. Histopathological and biochemical analyses were conducted with kidney and lung tissues. Histopathological scoring was done by analyzing cellular damage at tissue level. Malondialdehyde (MDA), superoxide dismutase, and glutathione levels were studied for biochemical analysis.

Results: Histopathologic analysis showed that, as compared with alprostadil, iloprost provided a significantly higher level of renal protection against IR injury (P < 0.01). Renal tissue levels of MDA were significantly lower in the alprostadil group as compared to Group 3 (P < 0.05).

Conclusion: Alprostadil and iloprost seem to provide protection against IR injury, with iloprost being more protective in renal tissue. Alprostadil is more effective than iloprost in protecting lung tissue against IR injury.

Key words: Reperfusion injury, alprostadil, iloprost, lung, kidney
information about the extent of damage (7,8). The present study was designed to compare the use of alprostadil and iloprost in a rat model of IR injury.

2. Materials and methods

2.1. Study design

This randomized, controlled, single-blind, interventional animal study was conducted in the Experimental Research Center of the Yeditepe University Faculty of Medicine (Istanbul, Turkey) after obtaining approval from the local ethics committee at the same center. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (9).

Twenty-three male Sprague Dawley rats were used. These rats were aged 8-12 weeks and weighed 230 ± 30 g. The rats were kept free in cages on a 12-h light-dark cycle under constant environmental conditions (temperature 22 ± 2 °C, humidity 50 ± 5%) and given ad libitum feed and water. The rats were randomized into 4 groups: Group 1 (iloprost + IR) and Group 2 (alprostadil + IR) each included 7 rats receiving iloprost or alprostadil, respectively. The 5 rats in Group 3 (saline + IR) received 0.9% isotonic saline solution, and there were 4 rats in Group 4 (control).

2.2. Surgical procedure

All rats underwent inhalational anesthesia with isoflurane (Isoflurane USP, Adeka, Turkey). The induction and maintenance of anesthesia was performed with a mixture of 2% isoflurane and 100% O2 delivered at a rate of 2 L/min through a breathing mask. The body temperature of all rats was kept at 36 °C with a heater pad through whole procedure.

Temporary abdominal aorta clamping and removal of the clamp to reperfuse the lower extremities for 120 min has been defined as the IR rat model (10,11).

After the rats were anesthetized in the supine position, the skin over the abdominal area was shaved and cleaned with povidone iodine, and an abdominal incision of about 5 cm was made in the midline. The intestines were moved aside, and the abdominal aorta and inferior vena cava were exposed. Then a 22-G needle was inserted into the inferior vena cava for intravenous infusion. The abdominal aorta was clamped temporarily at the infrarenal aorta, sparing the visceral arteries just above the iliac bifurcation. The clamping lasted for 60 min, during which the ischemia of the lower limbs was monitored by color change in the limbs and pulselessness. The rats were monitored for pulse rate, saturation, and heart rate via a Covidien Nellcor. Both the upper and lower extremities were monitored during the ischemia and reperfusion period. The intestines were covered with isotonic solution-soaked wet gauzes to protect the abdominal cavity from intestinal ischemia and damage (Figure 1). Thereafter, the clamp was removed to allow reperfusion for 2 h. In the 3 study groups, the infusions lasted for 180 min, from the time of clamping to the end of the reperfusion period.

The infusion doses were as follows: Group 1, iloprost at 2 µg/kg hourly (iloprost + IR); Group 2, alprostadil at 0.1 mg/kg per minute (alprostadil + IR); Group 3, 0.9% isotonic saline solution at 2 mL/kg hourly (saline + IR). All infusions were delivered using an infusion pump (Aitecs 2016, Aitecs, Lithuania).

2.3. The control group

All the rats in Group 4 underwent general anesthesia via inhalation for 3 h (180 min) and an abdominal incision, without an IR process or inferior vena cava cannulation.

2.4. Sample collection and sacrifice procedure

After 3 h, each rat was euthanized under general anesthesia in compliance with the ethical standards for the care and use of laboratory animals. First the kidneys were removed,
followed by the removal of the lungs. The right kidney and the right lung were immediately frozen and stored at -80 °C for biochemical and marker analyses. The left kidney and left lung were fixed in 10% formaldehyde solution for histopathologic examination. The rats were euthanized through organ harvesting under general anesthesia with isoflurane.

2.5. Histopathologic evaluation
IR injury was performed according to previously published data (12).

After fixation in 10% formaldehyde solution and routine histological preparation (13), the kidney and lung tissues were embedded in paraffin. These paraffin-embedded tissue blocks were cut to a thickness of 5 µm and the slices were stained with hematoxylin and eosin. Slides were examined and photographed under a Leica photomicroscope (Germany). Histopathologic scoring was performed as follows according to previously published data (13), with total maximum histopathologic scores of 9 for the lung and the kidney separately: each criterion was rated with a score from 0 to 3, indicating no, slight, moderate, or severe damage.

The criteria for the microscopical histopathologic scoring for the lung included (i) interstitial edema and vascular congestion, (ii) alveolar structural disturbance, and (iii) inflammatory cell infiltration. The corresponding criteria for the kidney included (i) interstitial edema and vascular congestion, (ii) renal tubular degeneration, and (iii) renal corpuscle degeneration (Figures 2 and 3).

2.6. Biochemical analysis
The biomarkers analyzed in the tissue samples included MDA, SOD, and GSH. Biomarker quantification was performed according to the methods previously described by Akkaya et al. (14). Tissue MDA levels were determined spectrophotometrically via a modification to the method described by Placer et al. (15). During the peroxidation of fatty acids containing 3 or more double bonds, MDA is formed, which is measurable via the thiobarbituric

Figure 2. Histopathologic kidney samples. A) Normal glomeruli and tubular structures are seen in the control group. B) Severe degeneration of glomeruli (*) and tubules (→) with vascular congestion (h) and inflammatory cell infiltration (▲) in the IR group. C) Severe degeneration of glomeruli (*) and tubules (→) with vascular congestion (h), inflammatory cell infiltration (▲), and edema (e) in the alprostadil + IR group. D) Moderate degeneration of glomeruli (*) and tubules (→) with vascular congestion (h) and inflammatory cell infiltration (▲) in the iloprost + IR group.
Acid (TBA) technique. As an end product of fatty acid peroxidation, MDA reacts with TBA, forming a pink complex. This resulting pink color was read spectrophotometrically at 532 nm.

SOD activity was determined according to a modification of Sun et al’s method (16). It was based on the inhibition of nitroblue tetrazolium reduction by superoxide, which is formed by the xanthine-xanthine oxidase system. The determination of GSH was performed according to the method described by Ellman et al. (17). It was measured via an enzymatic cycling procedure in the presence of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), nicotinamide adenine dinucleotide phosphate (NADPH), and glutathione reductase.

2.7. Statistical analysis
The data were statistically evaluated using one-way analysis of variance (ANOVA), followed by a Tukey post hoc test (SPSS 18.0, SPSS Inc., USA). The results are presented as means ± standard deviations. P < 0.05 was considered statistically significant.

3. Results
3.1. Results of histopathologic analyses
For the kidney, in Group 4 (control), no histopathologic damage was observed. The highest damage score was found in Group 3 (saline + IR). The damage scores for Group 1 (iloprost + IR) and Group 2 (alprostadil + IR) were substantially higher as compared to Group 4 (P < 0.001). Comparing Groups 2 and 3, the damage score for Group 2 was significantly lower than that for Group 3 (P < 0.05). In addition, comparing Group 1 and Group 3, the damage score for Group 1 was found to be significantly lower than that for Group 3 (P < 0.001). Comparing Group 1 and Group 2, the damage score for Group 1 was significantly lower than that for Group 2 (P < 0.001). The comparative histopathologic results of the renal tissue samples of the 4 groups are shown in Table 1 and Figure 4.

For the lung, the damage score for Group 4 (control) was the lowest. The damage scores for Groups 1 (iloprost) and 3 (alprostadil) were close to one another, and the difference between them was not statistically significant (P > 0.05). The damage score for Group 2 (alprostadil)
was significantly lower than that for Group 3 (saline + IR) (P < 0.05). In addition, comparing Group 2 (alprostadil) and Group 1 (iloprost), the damage score for Group 2 (alprostadil) was found to be significantly lower than that of Group 1 (P < 0.01) (Table 2 and Figure 5).

3.2. Results of biochemical analyses
Regarding GSH, in Group 4 (control), the GSH levels in the kidney tissue were the highest, whereas in Group 3 (saline + IR), GSH levels were the lowest. The difference between Group 4 and Group 3 was statistically significant (P < 0.05). The GSH level for Group 1 (iloprost) was higher than that for Group 3 (P < 0.05). Comparing Group 2 (alprostadil) and the other groups, GSH levels were not statistically significantly different (P > 0.05). For the lung tissue, there were no statistically significant differences between the groups (P > 0.05).

Regarding SOD, both lung and kidney tissues showed no statistically significant differences between the groups.

Regarding MDA, in the kidney tissue, the MDA level of Group 3 (saline + IR) was higher than that of Group 4 (control), but this finding was not statistically significant. The MDA levels of Group 1 (iloprost) and Group 2 (alprostadil) were found to be lower than that of Group 3, but a statistically significant difference was found only between Group 2 and Group 3 (P < 0.05). In the lung tissue, no statistically significant differences were found between groups regarding MDA levels (Tables 3 and 4).

4. Discussion
IR injury is a pathological process affecting many organs in the body, particularly the kidneys, lungs, and heart (18). In clinical practice, the treatment of this damaging process remains palliative. Iloprost is beneficial in ischemic injury, as well as in other pathological processes, such as infections. It is also an effective agent against pulmonary hypertension (19,20). Alprostadil has similar effects, and its use has been examined in the literature (21,22). Protection against lower limb ischemia, considered in the context of a multiorgan preservation strategy and the broad-spectrum preventive effects of both agents, may represent a challenge in terms of the choice of treatment.

Table 1. Histopathological results for kidney tissue.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>0</td>
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<tr>
<td>2</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>0</td>
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<td>7</td>
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<td>7</td>
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</tbody>
</table>

Groups: Mean Std. deviation

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.28</td>
<td>7.00</td>
<td>8.80</td>
<td>0.00</td>
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<tr>
<td>Std. deviation</td>
<td>0.75</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Figure 4. Comparison of histopathological kidney scores between groups.

Group 1: Iloprost + IR, Group 2: Alprostadil + IR, Group 3: Saline + IR, Group 4: Control.
PGE1 has a vasodilative effect on the pulmonary arteries. For this reason, PGE1 is used for pulmonary hypertension treatment. In addition to vasodilation, PGE1 has a bronchodilative effect, and it is superior to PGI2 in terms of its vasodepressive effect in lung tissues, as reported in animal studies (23,24). A histopathologic study in lung tissue showed that the tissue damage score of Group 2 (alprostadil + IR) was lower than those of Group 1 (iloprost + IR) and Group 3 (saline + IR) (P < 0.005). However, no protective effect of iloprost against IR was found in lung tissue.

Alprostadil interacts with specific G protein-coupled receptors to increase plasma levels of adenosine, which in turn decreases leukocyte activity, provides endothelial protection, and inhibits platelet aggregation. These result in improvements in blood flow and increased oxygen delivery to tissues (25). Having similar effects, iloprost is an analog of endogenous PGI2. It provides endothelial protection via decreasing neutrophil adherence to the vascular endothelium. Neutrophil stabilization limits tissue damage by reducing the release of free radicals. Histopathological studies of tissue samples have shown that both iloprost and alprostadil protect the kidney from IR injury. The damage scores for Group 1 (iloprost) and Group 2 (alprostadil) were lower than that for Group 3 (saline + IR). Although alprostadil was found to be protective in kidney tissue, when comparing Group 1 (iloprost) and Group 2 (alprostadil), iloprost was found to protect kidney tissue better than alprostadil did (P < 0.001).

It is difficult to explain why iloprost has more beneficial effects on renal injury than alprostadil according to the literature. Konstantinos et al. found iloprost to be successful among patients with contrast nephropathy. It was shown in animal studies that PGI2 may reverse renal toxic injury and attenuate the contrast substance’s effect on the kidneys via interactions at the cellular level (26). Similarly, it has been shown that PGI2 has therapeutic effects on anoxic renal injury in rabbits whose renal arteries were clamped (27). Vascular modulation in response to ischemic renal injury is implemented mainly by PGI2 (28). This may be why iloprost has an effect on renal tissue.

Table 2. Histopathological results for lung tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9</td>
<td>0.0</td>
</tr>
<tr>
<td>Group 2</td>
<td>7.57</td>
<td>0.55</td>
</tr>
<tr>
<td>Group 3</td>
<td>8.80</td>
<td>0.45</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.25</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Group 1: Iloprost + IR, Group 2: Alprostadil + IR, Group 3: Saline + IR, Group 4: Control.

Figure 5. Comparison of histopathological lung scores between groups.
Ischemic injury results in oxygen free radical production, leading to cellular damage and decreased GSH levels (29). MDA is an end product of lipid peroxidation whose levels correlate with the severity of the resulting damage (30). No significant difference was found when comparing the GSH levels of kidneys after administration of iloprost and alprostadil. However, the GSH level of Group 1 (iloprost + IR) was significantly higher than that of Group 3 (saline + IR). Likewise, comparing the kidney MDA levels of the groups, the MDA level of Group 2 (alprostadil) was lower than that of Group 3 (saline + IR) (P < 0.05). However, the lung levels of GSH and MDA remained similar for both iloprost and alprostadil (P > 0.05).

Table 3. Biochemical results for kidney tissue.

<table>
<thead>
<tr>
<th>Kidney</th>
<th>*SOD</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.673 ± 0.13</td>
<td>19.947 ± 2.15</td>
<td>1.609 ± 0.60</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.600 ± 0.17</td>
<td>15.735 ± 5.27</td>
<td>1.231 ± 0.33</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.576 ± 0.15</td>
<td>10.138 ± 4.63</td>
<td>1.986 ± 0.37</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.627 ± 0.19</td>
<td>20.524 ± 3.38</td>
<td>1.736 ± 0.29</td>
</tr>
</tbody>
</table>

Group 1: Iloprost + IR, Group 2: Alprostadil + IR, Group 3: Saline + IR, Group 4: Control.

*SOD level between groups is not statistically significant (P > 0.05).

Table 4. Biochemical results for lung tissue.

<table>
<thead>
<tr>
<th>*Lung</th>
<th>*SOD</th>
<th>*GSH</th>
<th>*MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.896 ± 0.51</td>
<td>20.03 ± 3.7</td>
<td>1.344 ± 0.49</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.896 ± 0.12</td>
<td>20.73 ± 4.3</td>
<td>1.367 ± 0.53</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.800 ± 0.07</td>
<td>21.21 ± 2.8</td>
<td>1.671 ± 0.94</td>
</tr>
<tr>
<td>Group 4</td>
<td>1.182 ± 0.24</td>
<td>21.50 ± 7.2</td>
<td>1.544 ± 0.41</td>
</tr>
</tbody>
</table>

Group 1: Iloprost + IR, Group 2: Alprostadil + IR, Group 3: Saline + IR, Group 4: Control.

*Difference between groups is not statistically significant (P > 0.05).

Inhalation anesthesia with isoflurane is used safely for general anesthesia procedures in rats. Both iloprost and alprostadil exert a therapeutic effect through vasodilation at the tissue level. Vascular effects on smooth muscle and vasodilation improve blood flow and microcirculation, protecting tissues from ischemic effects. It has previously been described that isoflurane has therapeutic effects on IR injury via vasodilation (31). In our study, all groups inhaled 2% isoflurane at 2 L/min mixed with 100% oxygen.

In conclusion, alprostadil and iloprost are commonly used for the treatment of pulmonary hypertension. These 2 drugs, which can successfully treat pulmonary hypertension treatment, are also successful in protecting renal tissue against IR damage. Iloprost has more beneficial effects in terms of protecting renal tissue against IR damage than alprostadil. In contrast, alprostadil has beneficial effects in terms of protecting lung tissue against IR damage, but no such effect is present for iloprost.
References


