Pioglitazone Attenuates Ischemia/Reperfusion–Induced Liver Injury in Rats


ABSTRACT

Introduction. Hepatic ischemia/reperfusion (I/R) injury leads to free radical generation and acute inflammatory responses that cause liver damage, an important problem for liver transplantation. Pioglitazone is known to protect I/R injury in various tissues; however, the mechanism of cytoprotection is not well understood. This study investigated the effects of pioglitazone administration in a warm hepatic I/R model on tumor necrosis factor (TNF)-α level, tissue injury, and antioxidant enzyme activity.

Materials and Methods. Eighty wistar strain rats were divided into 4 groups (n = 20): Group 1 sham hosts; Group 2 hepatic I/R; Group 3 hepatic I/R + pioglitazone (10 mg/kg); and Group 4 hepatic I/R + vehicle. Rat livers were subjected to 30 minutes of ischemia followed by 6 hours of reperfusion. After reperfusion rats were humanely killed to obtain liver tissue to study glutathione peroxidase (GPx), superoxide dysmutase (SOD), malondialdehyde (MDA) levels and for histopathologic assessment. TNF-α, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured in serum.

Results. Pioglitazone pretreatment significantly reduced liver enzyme content (ALT, 176.80 ± 13.75 vs 235.28 ± 31.92 and AST, 748.20 ± 79.29 vs 944.85 ± 101.87) and TNF-α level (9.86 ± 8.67 vs 138.28 ± 9.99) after I/R compared with the control group. MDA level (3.02 ± 0.37 vs 4.36 ± 0.38) and hepatocytic degeneration were reduced in the pioglitazone-treated group. GPx (2.40 ± 0.25 vs 1.36 ± 0.31) and SOD activity (2.22 ± 0.30 vs 1.40 ± 0.35) were significantly higher in the pioglitazone-treated group compared with the control group.

Conclusion. The present study showed that pioglitazone administration improved hepatic I/R injury that was associated with enhanced antioxidant enzyme activities and suppression of TNF-α, ALT, and AST levels. Because peroxisome proliferator-activated receptor-γ agonists are widely used to treat diabetic patients, it may be relatively easy to expand their clinical indication. However, further investigations will be required to delineate protective mechanisms by which pioglitazone attenuates hepatic tissue injury after I/R.
liver. In addition to Kupffer cell–induced oxidant stress, which increases with the length of the ischemic episode, intracellular generation of reactive oxygen by xanthine oxidase and by affected mitochondria may also result in liver dysfunction and injury during reperfusion. Also, the presence of a phagocyte-type NADPH oxidase has recently been recognized to be a major source of superoxide formation in endothelial cells and hepatocytes. Pharmacological preconditioning is known to trigger hepatic protection. Thiazolidinediones are a new class of antidiabetic drugs that improve insulin sensitivity and lipid metabolism, acting as agonists of peroxisome proliferator-activated receptor-γ (PPARγ). They change transcription of target genes that are involved in lipid metabolism, glucose homeostasis, cell proliferation, and differentiation, as well as inflammatory responses. There are 3 PPARγ class compounds: troglitazone, rosiglitazone, and pioglitazone. Although troglitazone administration produced severe liver toxicity in case reports, the new thiazolidinediones seem to be safe. However, in the last few years, it has become evident that the therapeutic effects of PPARγ ligands reach beyond their use as insulin sensitizers. Pioglitazone, (±)-5-[4-[(5-ethyl-2-pyridyl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride (C₁₉H₂₀N₂O₃S·HCl; Fig 1) is a synthetic ligand of PPARγ that has been used to treat patients with type 2 diabetes mellitus. In addition, pioglitazone may improve endothelial dysfunction in healthy humans with insulin resistance, decrease anti-inflammatory cytokines and improve cellular anti-oxidant systems. Furthermore, PPARγ agonists have been reported to reduce organ injury caused by I/R and attenuate drug-induced toxicity. Thus, we hypothesized that the use of pioglitazone could be associated with functional and histological benefits in the setting of hepatic I/R.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 230–280 g were used in all experiments, which were approved by our Committee for Animal Research. The animals were divided randomly into 4 experimental groups, each containing 20 rats, (total number, 80): (1) sham, laparotomy without I/R; (2) hepatic I/R; (3) hepatic I/R + pioglitazone (10 mg/kg); and (4) hepatic I/R + vehicle (0.5% dimethylsulfoxide [DMSO] in phosphate-buffered saline [PBS]).

Technique of Operation

Rats were anesthetized with intraperitoneal ketamine (40 mg/kg weight) + xylazine (10 mg/kg weight). A midline laparotomy was performed after the skin had been shaved and sterilized with 10% povidone-iodine solution. For the hepatic I/R group, the liver was isolated, and hepatic ischemia induced by occluding the portal vein and hepatic artery with an atraumatic microvascular clamp. The abdominal cavity was closed temporarily with towel forceps and covered with gauze. After 30 minutes, reperfusion was initiated by removing the clamp. For the I/R + pioglitazone group, the drug was dissolved in 0.5% DMSO in PBS and administered intraperitoneally (10 mg/kg weight) 2 hours before induction of ischemia, followed by hepatic I/R. For the sham group, the hosts underwent the same technique of exposure, but without hepatic I/R. After 6 hours reperfusion, rats in all groups were humanely killed to obtain blood samples and to remove the livers for further analysis.

Histopathologic Analysis

Liver tissues embedded in paraffin were cut into 4-μm sections. After deparaffinization, the tissues were stained with hematoxylin and eosin (H&E) for histological examination. The liver samples were then graded histologically according to the severity of the injury using a predetermined scoring system by a pathologist blinded to the groups. The assessment was expressed as the sum of the individual scores of 0 (no findings), 1 (mild), 2 (moderate), or 3 (severe) for each of the following 6 parameters: cytoplasmic color fading, vacuolization, nuclear condensation, nuclear fragmentation, nuclear fading, and erythrocyte stasis.

Glutathione Peroxidase and Superoxide Dismutase Assessment

To measure cytosolic enzyme activity, the liver samples were homogenized in 1.15% KCl solution. Glutathione peroxidase (GPx) activity was measured according to Paglia and Valentine using Randox (United Kingdom). Tissue superoxide dismutase (SOD) was assayed by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation according to Paoletti et al.

Malondialdehyde Assessment

Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substances (TBARS) method.

Biochemical Analyses

Plasma was used to measure serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as indicative of hepatic function. The plasma AST and ALT activities were estimated using commercially available kits using an autoanalyzer. Plasma was also used to measure TNFα as an important inflammatory mediator using an ELISA device.

Statistical Analysis

All results are presented as mean values ± SD. Significance testing between experimental and control groups was performed using one-way analysis of variance (ANOVA) with SPSS 13.0 software. P < .05 was considered statistically significant.

RESULTS

Histological Changes

Upon histopathologic evaluation, there were no pathological changes in liver tissue among the sham group. Liver
specimens from rats after I/R showed vacuolization, nuclear fragmentation, and erythrocyte stasis. Pioglitazone treatment significantly decreased these pathological changes. Histological tissue damage was milder in the I/R + pioglitazone group than in the I/R group ($P < .05$; Table 1).

### SOD and GPx Level

GPx and SOD activities in liver tissue were significantly higher in the I/R + pioglitazone group than in the I/R group ($P < .05$). However, GPx and SOD activities in liver tissue were significantly lower in the I/R + pioglitazone than in the sham group ($P < .05$; Table 1).

### MDA Level

Hepatic tissue MDA levels were significantly lower in the I/R + pioglitazone group than in the I/R group ($P < .05$). Also, MDA levels in hepatic tissue were significantly lower in the I/R + pioglitazone than in the I/R + vehicle group ($P < .05$). However, MDA levels in hepatic tissue were significantly higher in the I/R + pioglitazone than in the sham group ($P < .05$ for all; Table 1).

### Hepatic Transaminases and TNFα Level

Plasma ALT, AST, and TNFα levels in the pioglitazone-treated group were significantly lower than those in the I/R or the I/R + vehicle groups ($P < .05$). They were significantly higher in the I/R group than in the sham group ($P < .05$ for all; Table 2).

There were no significant differences between the I/R group and the I/R + vehicle group with regard to antioxidant parameters, serum ALT, AST, and TNFα levels, as well as in tissue injury scores ($P > .05$).

### DISCUSSION

Several studies have previously reported the effects of pioglitazone on hepatic I/R injury. In the present study, we showed that administration of pioglitazone significantly reduced hepatic tissue concentrations of MDA, an end product of free radical formation and lipid peroxidation as well as an index of ROS-mediated injury, which results from an imbalance between radical generating and radical-scavenging systems leading to cell membrane impairment or DNA damage. ROS alters proteins, carbohydrates, and lipids, as well as inactivates enzymes and transporters, damages DNA and the transcriptional machinery, and initiates the chain of reactions that peroxidize polyunsaturated fatty acids in membrane phospholipids. MDA reflects lipid peroxidation, whereas SOD and GPx are important antioxidant enzymes involved in the clearance of superoxide and H$_2$O$_2$ to maintain the structure and function of biological membranes. SOD dysmutases superoxide H$_2$O$_2$ is a compound that is catabolized by catalase and GPx. In higher organisms, GPx appears to have largely supplanted the need for catalase in membranes. The lower activity of SOD in elderly rats may be a consequence of inhibitory effects due to excessive ROS generation.

In contrast, our results showed a decrease in GPx and SOD activities in hepatic tissue of rats subjected to I/R compared with the sham-operated group. Besides, the activities of GPx and SOD were significantly higher in hepatic tissues of pioglitazone-treated rats. Even with excellent surgical techniques, ischemic periods created during surgery may result in increased morbidity and mortality. Therefore, various antioxidant agents have recently been tested to overcome the injury in various experimental and clinical models.

Pioglitazone is an anti-diabetic agent with well-known antioxidant and anti-inflammatory properties. In the current study, we observed significantly reduced MDA, GPx, and SOD levels in an experimental hepatic I/R model after pioglitazone administration. Significant elevations in serum transaminases occur only in the face of active cellular destruction as a harbinger of injury progression toward tissue necrosis. However, they may be construed as indicating recovery, although they merely represent a lack of ongoing hepatocyte death. TNFα was increased at 6 hours after reperfusion, but pioglitazone administration decreased this serum cytokine, which is an important inflammatory mediator that activates neutrophils and macrophages, causing more injury to tissues.

In summary, this work suggested that hepatic I/R results in a significant decrease in MDA, GPx, and SOD levels in hepatic tissue of Wistar rats. Administration of pioglitazone markedly increased the activities of SOD and GPx in hepatic tissue. Akahori et al. reported that pioglitazone treatment decreased expression of several cytokines (TNF-α, IL-1β, IFN-γ, and IL-10), chemokines (MCP-1, MIP-2, and IP-10), adhesion molecules (ICAM-1 and E-selectin), NO synthase (iNOS and eNOS), and caspase 3, thereby suppressing apoptosis and necrosis in hepatic tissue after I/R. They have also indicated that pioglitazone administration enhanced PPARγ messenger RNA (mRNA) expression in hepatic tissue.

### Table 1. Hepatic Tissue GPx, SOD, and MDA Content and Hepatic Tissue Injury Index

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control Mean ± SD</th>
<th>I/R Mean ± SD</th>
<th>I/R + Pioglitazone Mean ± SD</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (U/mg protein)</td>
<td>3.82 ± 0.35</td>
<td>1.36 ± 0.31</td>
<td>2.40 ± 0.25</td>
<td>1.36 ± 0.27</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>4.64 ± 0.35</td>
<td>1.40 ± 0.35</td>
<td>2.22 ± 0.30</td>
<td>1.52 ± 0.43</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>2.08 ± 0.37</td>
<td>4.36 ± 0.38</td>
<td>3.02 ± 0.37</td>
<td>3.98 ± 0.25</td>
</tr>
<tr>
<td>Liver tissue injury index</td>
<td>1.36 ± 0.50</td>
<td>16.21 ± 1.21</td>
<td>12.40 ± 1.51</td>
<td>16.16 ± 1.47</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean ± SD. GPx and SOD levels were statistically lower in I/R + pioglitazone group compared with I/R group ($P < .05$). MDA levels in the I/R group were higher than in the I/R + pioglitazone group significantly ($P < .05$). Tissue injury index in the I/R + pioglitazone group was higher than the I/R group ($P < .05$).
tissue. PPARγ agonists have shown beneficial effects to regulate physiological as well as pathological disease processes. As shown in our study, pioglitazone administration attenuated liver injury and TNF-α release as well as enhanced cellular anti-oxidant properties compared with the control group. This study further supports its therapeutic potential in acute hepatic injury such as liver transplantation and surgery. Because PPARγ agonists are widely used to treat diabetic patients, it may be relatively easy to expand their clinical indications.33,34

Thus, the present study suggested a physiological regulatory role of pioglitazone in the maintenance of oxidant/antioxidant balance in liver tissue. However, further investigations will be required to delineate the underlying mechanisms of pioglitazone action to improve SOD and GPx activities and MDA levels in hepatic tissues after I/R.

REFERENCES


19. Pioglitazone I/R


Table 2. Serum Levels of ALT, AST, and TNF-α

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>I/R</th>
<th>I/R + Pioglitazone</th>
<th>I/R + Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>38.95 ± 4.77</td>
<td>235.28 ± 31.92</td>
<td>176.80 ± 13.75</td>
<td>229.28 ± 26.23</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>137.18 ± 16.95</td>
<td>944.85 ± 101.87</td>
<td>748.20 ± 79.29</td>
<td>938.00 ± 55.55</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>25.09 ± 3.59</td>
<td>138.28 ± 9.99</td>
<td>98.60 ± 8.67</td>
<td>134.71 ± 10.88</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean ± SD. ALT, AST, and TNF-α levels were statistically different between the I/R (hepatic I/R) group and the I/R + pioglitazone group (P < .05). ALT and AST levels were not statistically different between the I/R group and the I/R + vehicle group.