Leukocyte Involvement in Renal Reperfusion-Induced Liver Damage

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Abstract

Backgrounds/Aims: Renal ischemia–reperfusion (IR) induces organ damage in remote organs. The aim of this study was to assess the role of leukocytes in the induction of liver damage after renal IR injury. Methods: Inbred mice were subjected to either sham operation or bilateral renal IR injury (60 min ischemia followed by 3 h reperfusion). Mice were then anesthetized for collection of leukocytes by heart puncture. Isolated leukocytes were transferred to two other groups: intact recipient mice that received leukocytes from IR mice and intact recipient mice that received leukocytes from sham-operated control mice. After 24 h, recipient mice were anesthetized and samples were collected. Results: Alanine aminotransferase, aspartate aminotransferase, and hepatic malondialdehyde increased significantly, and hepatic glutathione decreased significantly in intact recipient mice that received leukocytes from IR mice in comparison with intact recipient mice that received leukocytes from sham-operated control mice. Loss of normal liver architecture, cytoplasmic vacuolization, and focal infiltration of leukocytes were seen. Conclusion: These results suggest that leukocytes are one of the possible factors that contribute to liver damage after renal IR injury and this damage is partly due to the induction of oxidative stress.

Keywords: renal ischemia, remote organs, liver damage, leukocyte, oxidative stress

INTRODUCTION

Renal ischemia–reperfusion (IR) is one of the most important causative mechanisms of acute kidney injury (AKI). Renal IR is associated with various clinical settings including shock, sepsis, kidney transplantation, vascular surgery, and elective urological operations.¹–³

The renal IR injury has multifactorial and interdependent causes such as inflammatory responses and leukocyte infiltration.⁴,⁵ Activation and migration of leukocytes into the kidney and liver have been demonstrated in renal IR injury. Activated leukocytes produce reactive oxygen metabolites as well as proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α).²,⁵,⁶

Studies have identified a close relationship between renal injury and other organ system failures. High mortality rate during AKI is partly due to remote organ manifestation. Lung functional and structural changes, brain inflammation and functional changes, liver apoptosis, and tissue damage and inflammation were seen after AKI.¹,²,⁷ The mortality rate of AKI is increased by multiple organ failure. Humoral or cellular factors are thought to be the causes of remote organ failure but their exact pathophysiological mechanisms are not completely understood.⁸–¹⁰

It is demonstrated that AKI results in an increase in leukocyte infiltration not only in the kidney but also in the liver and spleen. It has been reported that renal IR increased hepatic tumor necrosis factor levels, myeloperoxidase activities and thiobarbituric acid reactive substance concentrations and decreased superoxide dismutase, catalase and hepatic glutathione (GSH) levels.¹¹–¹³ Golab et al. showed that during renal IR, liver functional indices such as blood aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated and level of spermine–spermidine acetyl transferase, an enzyme upregulated in early phases of hepatic injury, was increased.²,¹⁴ To date, the mechanism of liver dysfunction in renal IR setting is not completely understood.
According to the similarities between the IR-induced kidney damage and the IR-induced liver damage as a remote organ, we decided to investigate leukocyte involvement in liver injury after induction of renal IR.

MATERIALS AND METHODS

Animal Procedures
Male BALB/c mice were purchased from Pasteur Institute (Jehran, Iran). They (weight = 25–35 g) were maintained at room temperature (22 ± 2°C) in a 12:12-hour light–dark cycle and with free access to standard diet and water. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Tehran University of Medical Sciences.

Mouse Renal IR Model
An established model of renal IR injury in mice was used. Briefly, mice (n = 9) were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg; Sigma-Aldrich, Steinheim, Germany). Systolic blood pressure was measured by the tail-cuff method connected to a pneumatic transducer using a PowerLab/4sp data acquisition system (software Chart, version 5, AD Instruments, Castle Hill, Australia).

Then a midline incision was made and the renal pedicles were bluntly dissected and occluded with nontraumatic vascular clips (Biemer-Clip, Aesculap, Tuttlingen, Germany) for 60 min. After the allotted ischemia time, clamps were gently removed. The kidneys were observed for a further 5 min to ensure reflow. Occlusion was verified visually by a change in the color of the kidneys to a paler shade and reperfusion by a blush. Sixty minutes of ischemia was chosen because it produces more severe damage to the kidney compared with a shorter ischemia time.

At the beginning of reperfusion, 1 mL of sterile saline at 37°C was injected intraperitoneally, the incision was closed in two layers with 4-0 silk suture, and the animals were allowed to recover. Sham-operated mice underwent surgical procedure identical to those of IR mice except that clamps were not applied. During anesthesia, animals were kept well hydrated with warm sterile saline and were maintained at a constant body temperature (∼37°C) on a heating pad.

Sample Collection and Preparation
After 3 h of reperfusion or sham operation, both IR and sham-operative donor mice were anesthetized and blood and liver tissue were collected. Blood samples were obtained from heart with heparinized syringes and centrifuged at 4000 g for 10 min at 4°C. Plasma samples were collected for biochemical analysis.

Adoptive Transfer of Leukocyte
To test the hypothesis that leukocytes have a role in liver injury after renal IRI, leukocytes from donor mice were transferred to recipient intact mice. In this model, for transferring of the leukocytes, donor and recipient mice must be congenic to avoid any transplantation problem.

Leukocytes from donor mice with ischemic injury and sham-operated mice were injected (5 × 10⁶ cells) through the tail vein to two intact recipient groups (intact recipient mice receiving leukocyte from IR mice and intact recipients mice receiving leukocyte from sham-operated controls mice, n = 9).

Sample Collection from Recipient Mice
After 24 h, recipient mice were anesthetized and blood and liver tissue were collected. Blood samples were obtained from heart with heparinized syringes and centrifuged at 4000 g for 10 min at 4°C. Plasma samples were collected for biochemical analysis.

Biochemical Assay
Plasma concentrations of Blood urea nitrogen (BUN), plasma creatinine and liver function indices – ALT and AST – were evaluated by colorimetric methods using commercially available kits.

Measurement of Liver Oxidative Stress Indices
Liver tissue malondialdehyde (MDA) level was evaluated by its reaction with thiobarbituric acid according to the Esterbauer and Cheeseman method. Liver GSH was assayed according to the Tietze method.

Histological Procedures
Liver was fixed in 10% formalin buffer and then embedded in paraffin. Hepatic sections (4 μm) were stained by hematoxylin and eosin. Hepatic sections were evaluated for the presence of congestion, cellular degenerative changes, cytoplasmic vacuolization, and leukocyte infiltration.

Statistical Analysis
Means and standard errors were calculated. Unpaired Student t-test was used for comparison, and statistical significance was determined as p < 0.05.

RESULTS
Sixty minutes of ischemia followed by 3 h of reperfusion significantly increased plasma BUN and creatinine levels in IR donor group compared with sham donor mice (Figure 1).
Liver function indices, AST and ALT, increased significantly after renal IR compared with sham donor mice (Figure 2). In IR donor mice, liver MDA increased significantly and GSH concentration decreased significantly in contrast with sham donor mice (Table 1).

Intact mice that received $5 \times 10^6$ leukocytes by i.v. injection from renal IR group showed significant elevated levels of AST and ALT compared with mice that received leukocytes from sham donor group (Figure 3). IR recipient mice showed elevated level of liver MDA and reduction in liver GSH concentration compared with sham recipient mice (Table 1).

Liver tissues in sham donor and sham recipient groups displayed normal histology. There was no sign of tissue or vascular congestion and necrosis was not detected. In IR donor mice livers, obvious sinusoidal and vascular congestion was seen. Frequent scattered leukocyte infiltration was detected. Cellular disintegration including nuclear and cytoplasmic degenerative changes was present along with interstitial edema. In IR recipient livers, loss of normal liver architecture was detected in some areas. Cytoplasmic vacuolization and focal infiltration of leukocytes was frequently seen. One of the most interesting features was the significant number of duplicated cells (Figure 4).

**DISCUSSION**

Liver and kidneys are important regulators of body homeostasis and are involved in the excretion of the toxic products of metabolism and exogenous drugs. Reperfusion of the ischemic kidney, resulting in AKI, is a serious problem that affects the outcome of various surgical operations such as organ transplantation and surgical revascularization. Kidney failure still continues to be associated with a high mortality rate, particularly when associated with multiple organ failure. However, the exact mechanisms leading to ischemic AKI have not been completely understood.\(^1\,^8\)

The observations described in this report identify leukocytes as recognized participants in the dysfunction

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**Table 1. GSH and MDA concentrations after renal IR.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham donor</td>
<td>24.95 ± 1.65</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>IR donor</td>
<td>18.43 ± 0.75*</td>
<td>1.05 ± 0.10*</td>
</tr>
<tr>
<td>Sham recipient</td>
<td>29.04 ± 1.60</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>IR recipient</td>
<td>23.26 ± 1.02**</td>
<td>0.88 ± 0.06**</td>
</tr>
</tbody>
</table>

Notes: Liver MDA contents increased and liver GSH decreased after 60 min ischemia followed by 3 h reperfusion in IR donor group compared with sham donor group. Similar changes were observed in IR recipient group compared with sham recipient group. Results are represented as mean ± SEM. MDA, malondialdehyde (μmol/100 mg tissue); GSH, glutathione (μmol/100 mg tissue).

*Denotes $p < 0.05$ by t-test compared with sham donor and **denotes $p < 0.05$ by t-test compared with sham recipient, $n = 9$. 

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of liver after renal IR. For prevention of leukocyte-transferring reaction side effect in recipient animal, we used inbred mice that have the same genotype. The significant increase in AST and ALT suggests liver functional injury in IR recipient mice and histological observation confirmed liver tissue damage in this group. Increase in liver MDA contents and decrease in liver GSH concentration in IR donor mice compared with sham donor animals are suggestive of oxidative stress in this remote organ. In addition, induction of oxidative stress in IR recipient mice suggests that leukocytes are involved in the induction of hepatic oxidative injury.

In previous studies, it has been clearly demonstrated that leukocytes are involved in organ injury such as kidney, liver, and heart damage as a result of IR. Neutrophils have a crucial role in the induction of renal IR. Activated neutrophils adhere to vascular endothelial cells in the vasa recta of the outer medulla. Activated neutrophils infiltrating into tissue release a variety of inflammatory cytokines and enzymes such as elastase and myeloperoxidase, leading to tissue injury.

In 2000, Rabb et al. reported that mice deficient in CD4+ and CD8+ T cells showed less renal IR injury. Serum creatinine was reduced at 24 h after renal IR in CD4+ T-cell-deficient mice compared with control mice with renal IR. They concluded that T cells are important mediators of ischemic AKI. Wild-type control mice had a markedly increased neutrophil.

Figure 3. Liver function after leukocyte transfer in recipient mice (mean ± SEM): (A) plasma AST, (B) plasma ALT. Note: *Denotes p < 0.05 versus sham operative group.

Figure 4. Hematoxylin- and eosin-stained sections of mice liver: (A) sham donor group; (B) sham recipient group; (C) IR donor group, sinusoidal and vascular congestion (small black arrows), cellular disintegration (small white arrow), interstitial edema, and infiltration of leukocytes were seen; (D) IR recipient group, loss of normal liver architecture, cytoplasmic vacuolization (big white arrow), and focal infiltration of leukocytes and duplicated cells (big black arrow) were frequent (H&E, ×400).
infiltration into renal tissue after IR, whereas neutrophil infiltration in the CD4+/CD8-deficient mice was decreased.5 Burne et al. showed that B-cell-deficient mice confer protection from renal IR injury. Postischemic serum creatinine was significantly reduced and survival rate was significantly higher in B-cell-deficient mice compared with wild-type mice. They also demonstrated that T-cell-deficient mice had a reduced renal IR injury, but combined B- or T-cell deficiency was not protective.21,22 Moreover, natural killer (NK) cells infiltrate into the kidney following IR injury and induce apoptosis in tubular epithelial cells. Adoptive transfer of NK cells worsened renal IR injury in NK-cell-deficient mice.3 After renal IR, activation of NKT cell results in the production of IFN-γ-mediated neutrophil infiltration and renal tissue injury.23

Renal IR induces dysfunction in remote organ. It has been suggested that kidneys and liver have crosstalk during AKI. It has been shown that 30 min ischemia and 1 h reperfusion is a minimum time to elicit remote effects of renal IR injury in rat.12 In this study, 60 min bilateral ischemia and then 3 h reperfusion was used, because the induction of higher degrees of renal damage was desired to stimulate more leukocyte activation. Studies in recent years showed that renal IR injury induces liver oxidative stress. Elevation in liver MDA and reduction in liver GSH concentration and superoxide dismutase activity were shown after renal IR injury. As the liver tissue represents one of the major vascular beds in the body, after renal IR injury, activated leukocytes infiltrate not only into the kidneys but also into the liver.2,12

These results suggest that leukocytes are one of the possible factors that contribute to liver damage after renal IR injury and this damage is partly due to the induction of oxidative stress.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES


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