Effects of Renal Ischemia Reperfusion on Brain, Liver & Kidney Tissues in Adult Male Rats.

Nahed Salah El-din Mohamed* and Hanan A. Mubarak

Department of Physiology, Kasr Al-Aini Faculty of Medicine, Cairo University, Cairo, Egypt
nahedsm4@hotmail.com

Abstract: Several studies suggest that renal ischemia reperfusion (RIR) can induce acute kidney injury (AKI). However, remote effects of RIR injury need further investigations. Renal injury associated with liver disease or neurological manifestations is a common clinical problem. The aim of this study was to examine the effects of RIR on brain and liver tissues in rats by inducing bilateral renal ischemia for 30, 45 and 60 minutes followed by one hour reperfusion and measurement of renal functions, liver functions & tumor necrosis factor alpha (TNF-α) in addition to histological examinations of kidney, liver and brain tissues. 40 rats were subjected to either sham operated (control group-1) or 30 min RIR (group-2), 45 minRIR (group-3), 60 min RIR (group-4).

The results demonstrated that compared to sham rats, serum creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), increased significantly 45 min & 60 min of RIR (P<0.05). There was a significant increase (P<0.05) in TNF-α in kidney, liver and brain tissues after 30 min, 45 min, and 60 min RIR compared to shame rats (p<0.01) and the rise of TNF-α after 60 min RIR is significantly higher (p<0.05) than that after 30 min RIR. Histological examination of brain tissues showed mild pyknosis after 45 min RIR and patches of vacuolization after 60 min RIR. In liver tissues there were congestion & hydropic degeneration after 45 min RIR and there was leucocyte infiltration in addition to congestion after 60 min RIR. Stained kidney tissues showed mild glomerular collapse after 30 min RIR, mild necrosis of tubular cells after 45 min RIR and periarteriolar neutrophilic infiltration in addition to mild tubular necrosis after 60 min RIR. It is concluded that RIR causes inflammation in liver & brain tissues which was much more after 60 min. The effects of RIR on remote organs need to be investigated for a long period after RIR

Key words: Renal ischemia reperfusion, brain, liver, tumor necrosis factor-alpha (TNF-α), rats

1. Introduction:
Ischemia reperfusion is a frequently encountered phenomenon in organisms. Prolonged ischemia followed then by reperfusion results in severe oxidative injury in tissues and organs. Renal ischemia reperfusion (RIR) is a common cause of acute kidney injury (AKI). RIR injury occurs in many clinical situations, such as transplantation, partial nephrectomy, sepsis, hydronephrosis, or elective urological operations. Mortality during AKI is largely due to extrarenal manifestations. Several studies have begun to investigate mechanisms that underlie distant organ effects of RIR injury and found a significant inflammatory effect of RIR on the lung and the heart.

Liver and kidneys are both involved in the regulation of body homeostatic responses, metabolism and excretion of drugs and toxic products. Many studies have suggested cross-talk between the liver and kidneys. Central nervous system changes, the signs of which range from decreased mental status to obtundation and seizures, are one of the classic indications to begin dialysis during AKI. An increasing body of evidence suggests that the deleterious effects of AKI on remote organ function could, at least in part, be due to loss of the normal balance of immune, inflammatory, and soluble mediator metabolism that attends injury of the tubular epithelium. Such dysregulation, acting at least in part on endothelium, leads to compromise of remote organ.

Kielar et al have evaluated the extrarenal regulation of AKI. This regulation may be as a result of increased production of cytokines such as tumor necrosis factor-alpha (TNF-α) and growth factors such as hepatocyte growth factor produced by extrarenal organs.

The aim of the present study was to assess the changes in brain, liver and kidney tissues after different periods of renal ischemia followed by reperfusion.

2. Material and Methods:
Experimental Animals

Forty male Albino rats belonging to local strain weighing between 180-220 gm were obtained from the Animal House of Faculty of Medicine, Cairo University and included in this study. The animals were housed in wire mesh cages at room temperature with 12:12h light-dark cycles and maintained on standard rat chow and tap water. Veterinary care was provided by Animal House Unit of Cairo University. The animals were divided into 4 groups of 10 animals each.

Group 1: Control group (sham-operated rats)
The other animals were subjected to renal ischemia followed by 1 hour reperfusion. According to periods of ischemia they were divided into

Group 2: 30 min ischemia followed by 1 hour reperfusion

Group 3: 45 min ischemia followed by 1 hour reperfusion

Group 4: 60 min ischemia followed by 1 hour reperfusion

Surgical procedure

Rats were anaesthetized with pentobarbital sodium 60 mg /kg. A midline laparotomy was performed and the renal arteries were carefully separated from around the tissues. In the RIR groups, renal arteries were occluded by a non traumatic microvascular clips for 30, 45, 60 min followed by 1 hr reperfusion. Occlusion was approved visually by color change of the kidneys to a paler shade and reperfusion by blushing. Sham-operated animals underwent identical surgical treatment, including isolation of both renal arteries. However, artery occlusion was not performed. At the end of the experimental procedure, blood samples were collected retro-orbitally for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine. All rats were scarificed and brain, liver and kidney tissues were removed and prepared for histological examination and measurement of TNF-α.

Assessment of renal and liver function

Determination of serum AST and ALT was carried out by colorimetric method. Kidney function tests (BUN & creatinine) were assessed by conventional available kits.

Histological procedures

Paraffin –embedded brain, liver & kidney sections after formalin fixation (10% phosphate-buffered) & dehydration were stained by Hematoxylin & Eosin. Histological examination of all tissues was evaluated per section in at least 10 randomly selected non-overlapping fields at x100, x 200 and x 400 magnification.

Measurement of TNF- α

A portion of the brain, liver and kidney were homogenized after the tissue samples had been diluted in 5 vol of homogenate buffer [10 mM HEPES (pH 7.9), 10 mM KCL, 0.1 mM EGTA, 1 mM DTT, and 0.5 mM phenylmethylene sulfonyl fluoride] using a vertishear tissue homogenizer. The homogenates were centrifuged at 3,000 g for 15 min at 4°C. The supernatants were subsequently stored at -80°C until the ELISA technique could be performed. TNF- α was determined in tissue homogenate using an ELISA. The ELISA was performed by adding 100 µl of each sample to wells in a 96-well plate of a commercially available rat ELISA kit (R&D system quantakine USA). The samples were tested in duplicate. The ELISA was performed according to the manufacturer’s instructions and final results were expressed as picograms per mg tissues.

Statistical analysis

The data was encoded and entered using the statistical package SPSS Version 15. The results are given as mean ± standard error (M±SE). Statistical analysis was performed by ANOVA (analysis of variance) and multiple comparison Post-Hoc Tests to determine significant differences between groups. Correlations were done to test for linear relations between variables using Pearson correlation test. Statistical significance of a difference was defined when p ≤ 0.05

3. Results:

The effect of RIR on the brain, liver and kidneys was investigated 30, 45 and 60 minutes of renal ischemia followed by 1 hour reperfusion.

The effect of RIR on biochemical parameters

Effect of clamping of both renal arteries was confirmed by a significant increase in serum creatinine and BUN after 45 min and 60 min RIR compared to 30 min RIR and control group (Table 1). RIR resulted in a significant increase in BUN after 45 min (70.04 ± 2.34 mg/dl) & 60 min RIR (79.06 ± 4.53 mg/dl) compared to control (37.70 ± 2.39 mg/dl p<0.05) & 30 min RIR groups (47.39 ± 3.81 mg/dl p<0.05) and there was a significant rise in serum creatinine after 45 min (p<0.05) & 60 min RIR (p<0.05) compared to sham control rats (sham versus 30 min RIR versus 45 min RIR versus 60 min RIR: 0.11 ± 0.01 vs. 0.27 ± 0.06 vs. 0.44 ± 0.08 vs 0.89 ± 0.05 mg/dl ). Serum creatinine
was significantly higher after 60 min RIR than that after 30 min 
& 45 min RIR (p<0.05)

The effect of RIR on liver function was demonstrated by a significant increase in liver enzymes ALT and AST (Table-1)

ALT was increased significantly (p<0.05) after 30 min, 45 min and 60 min RIR compared to control group (43.79±2.10, 68.04±4.67, 77.51±3.39 vs 23.37±1.71 U/L) respectively. The levels of ALT after 60 min RIR & 45 min RIR was significantly higher than that after 30 min RIR (p<0.05).

AST was found to be significantly increased (p<0.05) after 45 min and 60 min RIR compared to control group (82.16±2.23 & 89.73±2.90 vs 69.39±3.61 U/L) respectively. AST was significantly higher (p<0.05) after 60 min RIR than that after 30 min RIR.

Effect of RIR on TNF-α in brain, liver and kidney tissues.

TNF-α was measured as one of proinflammatory mediators to determine whether RIR would lead to inflammatory changes in brain, liver or kidney tissues.

In brain tissues, RIR produced a significant increase (p<0.05) in TNF-α after 30 min, 45 min, and 60 min of RIR compared to control group (23.52±1.28, 30.94±1.79 & 33.27±3.39 pg/mg tissue vs 12.69±0.89 pg/mg tissue, respectively (Table-2, Fig-1).

In liver tissues, there was a significant increase (p<0.05) in TNF-α after 30 min (50.10±4.10), 45 min (58.44±2.49) and 60 min (70.47±3.51 pg/mg tissue) of RIR compared to control group (17.15±1.29 pg/mg tissue).

In brain and liver tissues, the increase of TNF-α after 60 min was significantly higher (p<0.05) than that after 30 min of RIR (Table-2, Fig-1).

In kidney tissues the levels of TNF-α was increased significantly (p<0.05) after 30 min of RIR (39.86±4.26), 45 min of RIR (65.16±1.97) and 60 min of RIR (71.42±3.34) compared to control group (17.13±1.04 pg/mg tissue). The increase after 45 min & 60 min was significantly higher than that after 30 min of RIR (p<0.05). Although the rise of serum creatinine and BUN was not significant after 30 min RIR, the TNF-α increased significantly after 30 min denoting presence of inflammatory changes after 30 min RIR (Table-2, Fig-1).

Statistical analysis showed positive correlation (p<0.05) between TNF-α in brain & kidney tissues (Fig 2-A) , between TNF-α in liver & serum creatinine (Fig 2-B) and between TNF-α in kidney tissues and serum creatinine (Fig 2-C).

The effect of RIR on brain, liver and kidney histopathological structures.

To determine whether RIR resulted in adverse effects on structure of the brain, liver and kidney tissues, Hematoxylin & Eosin–stained sections from RIR groups & control rats were examined. Changes were very mild after 30 min RIR in the three types of tissues.

Histological examination of brain tissues (Fig-3) showed normal brain tissues of sham operated rats (A), mild pyknosis 45 min RIR (B) and patches of vacuolization 60 min RIR (C). In liver tissues ( Fig-4), there was congestion & hydropic degeneration 45 min RIR (E) & there was leucocyte infiltration in addition to congestion 60 min RIR (F) when compared to normal liver tissues (D). Stained kidney tissues (Fig-5) showed normal kidney tissues (G), mild glomerular collapse 30 min RIR (H), mild necrosis of tubular cells 45 min RIR (I) & periarteriolar neutrophilic infiltration in addition to mild tubular necrosis 60 min RIR (J).

Table-1: Effects of different periods of renal ischemia (30, 45 and 60 min) followed by one hour reperfusion (RIR) on renal and liver function tests

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>ALT( U/L)</th>
<th>AST(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.70±2.39</td>
<td>.11±.01</td>
<td>23.37±1.71</td>
<td>69.39±3.61</td>
</tr>
<tr>
<td>30 min RIR</td>
<td>47.39±3.81</td>
<td>0.27±.06</td>
<td>43.79±2.10*</td>
<td>74.32±3.40</td>
</tr>
<tr>
<td>45 min RIR</td>
<td>70.04±2.34*</td>
<td>▲ 0.44±.08*</td>
<td>68.04±4.67*</td>
<td>82.16±2.23*</td>
</tr>
<tr>
<td>60 min RIR</td>
<td>79.06±4.53*</td>
<td>▲ 0.89±.05*</td>
<td>▲ 77.51±3.39*</td>
<td>▲ 89.73±2.90*</td>
</tr>
</tbody>
</table>

Results are mean ±SE  
- n: number of male rats in each group  
- * Significant compared to control value (p<0.05)  
- ▲ Significant compared to 30 min RIR (p<0.05)  
- □ Significant compared to 45 min RIR (p<0.05)
Table-2 : Effects of different periods of renal ischemia (30, 45 and 60 min) followed by one hour reperfusion on tumor necrosis factor - alpha (TNF-α pg/mg tissues) in kidney, liver and brain tissues in male rats

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>TNF-α in brain (pg/mg tissue)</th>
<th>TNF-α in liver (pg/mg tissue)</th>
<th>TNF-α in kidney (pg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.69 ± 0.89</td>
<td>17.15 ± 1.29</td>
<td>17.13 ± 1.04</td>
</tr>
<tr>
<td>30 min RIR</td>
<td>23.52 ± 1.28 *</td>
<td>50.10 ± 4.10 *</td>
<td>39.86 ± 4.26 *</td>
</tr>
<tr>
<td>45 min RIR</td>
<td>30.94 ± 1.79 *</td>
<td>58.44 ± 2.49 *</td>
<td>65.16 ± 1.97 * ▲</td>
</tr>
<tr>
<td>60 min RIR</td>
<td>33.27 ± 3.39 *▲</td>
<td>70.47 ± 3.51*▲</td>
<td>71.42 ± 3.34 *▲</td>
</tr>
</tbody>
</table>

Results are mean ±SE
n: number of male rats in each group
* Significant compared to control value (p < 0.01)
▲ Significant compared to 30 min RIR (p< 0.05)

Fig-1 : Tumor necrosis factor -alpha (TNF-α) in brain , liver and kidney tissues after different periods of renal ischemia (30, 45 & 60 min) followed by one hour reperfusion (RIR)

Results are mean ± SE
* Significant compared to control group (p < 0.01)
▲ Significant compared to 30 min RIR (P< 0.05)
Figure 3. Hematoxylin & Eosin stained sections of rat brain. (A) Normal brain of sham operated rats (x200). Sections B & C from renal ischemia reperfusion groups (RIR) showed pyknosis & shrinkage of cytoplasm (B) 45 min RIR (x200) & patches of vacuolization (C) 60 min RIR (x100).
Figure 4. Hematoxylin & Eosin stained sections of rat liver. (D) Normal liver of sham operated rats (x200). Sections (E&F) from renal ischemia reperfusion groups (RIR) showed congestion with hydropic degeneration (E) 45 min RIR & congestion with leucocyte infiltration (F) 60 min RIR (x 400).

Figure 5. Hematoxylin & Eosin stained sections of rat kidney. (G) Normal kidney of sham operated rats (x400). Sections (H,I&J) from renal ischemia reperfusion groups (RIR) showed mild glomerular collapse (H) 30 min RIR (x200), mild necrosis of tubular cells (I) 45 min RIR (x400) & moderate periarteriolar neutrophilic infiltrate(J) 60 min RIR (x 400).
4. Discussions:

Acute renal ischemia is associated with a high mortality rate. Most of mortality during RIR injury despite dialysis is from extrarenal organ dysfunction. This initiated investigation into the underlying mechanisms. The present study was conducted to determine whether an experimental RIR would lead to any measurable short term changes in liver or brain tissues as remote organs. The changes in the brain, liver and the kidneys were examined after induction of various periods of renal ischemia (30, 45 & 60 min) followed by reperfusion for one hour in male rats.

The present study have demonstrated that 30min, 45min, & 60 min RIR resulted in a significant increase of TNF-α in brain tissues compared to sham operated group. The histological examination showed pyknosis & vacuolization of nerve cells. The changes were very mild at 30 min and increased after 45 & 60 min RIR

Liu et al examined brain histology in mice that underwent 60 min of bilateral renal ischemia followed by 24 h of reperfusion. Compared with sham-operated control mice, they found that mice with ischemic AKI developed marked brain changes evidenced by increased soluble inflammatory proteins, increased cellular inflammation and increased microglial cells and pyknotic neuronal cells in the hippocampus. However there was a trend toward an increase in TNF-α in kidney but in contrast to our results, there was no change in TNF-α in the brain. This might be due to measurement of TNF-α in mice 24 hr after ischemia (TNF-α was measured one hour after ischemia in the present study) or may be due to differences between mice & rats.

Because previous studies have demonstrated that ischemic AKI leads to inflammatory response in the blood and lung. Luis et al hypothesized that AKI would also lead to brain inflammatory changes.

In the present study, the changes in liver functions (serum ALT and AST), histology and TNF-α in liver tissue were examined after induction of various periods of rat renal ischemic injury

Liver functions were reduced 30 min and 45 min after renal ischemia but showed a maximum reduction in the 60-min ischemia group. Liver histology showed congestion and hydropic degeneration 45 min RIR and there was leucocyte infiltration 60 min RIR. The TNF-α in the liver tissue was significantly increased 30 min, 45 min and 60 min RIR compared to control group and the increase after 60 min was significantly higher than that after 30 min of RIR.

The results of the present study are consistent with other investigators who reported that renal ischemia caused changes in liver histology, function, oxidative stress and inflammatory status, which led to a reduction in hepatic antioxidant capacity. With 30 min ischemia, the magnitude of these changes was less than those with 45 or 60 min ischemia. Their results showed a significant decrease in liver glutathione GSH, as well as a significant increase in TNF-α and IL-10 concentrations.

Serteser et al demonstrated some changes in hepatic TNF-α levels and oxidation products after RIR injury in mice. They have suggested that 30 min ischemia and 60 min reperfusion is sufficient to elicit remote effects of RIR injury.

Gurley et al found that RIR injury reduced hepatic oxidative drug metabolism, as determined by reduction of antipyrine clearance, 4 and 24 hours post IR injury & the peak level of TNF-α occurred one hour post ischemia reperfusion.

In a more recent study, Vaghiasiya et al demonstrated that serum concentrations of ALT & AST were significantly increased after renal ischemia for 30 min followed by reperfusion for 24 hours in normal & diabetic rats but the changes were much more in diabetic than in normal rats.

Yuce et al concluded that RIR may affect distant organs such as liver and oxidative stress may play role on this injury.

In the present study, as expected, RIR caused a reduction in renal functions, an increase in TNF-α and structural alteration in kidney tissues (necrosis of tubular cells & periarteriolar neutrophilic infiltration) in an ischemia-time-dependent manner.

These results are consistent with several studies that reported decreased renal functions in RIR in rats & mice. Rodent studies have demonstrated that the inflammatory response to hypoxia contributes to the resultant tissue injury. Vaghiasiya et al initiated changes in vascular endothelial cells, tubular epithelial cells and leukocytes that resulted in the loss of immune system homeostasis in the kidney. One of the hallmarks of RIR, in mouse models, was neutrophilic accumulation in the post-ischemic kidney and depletion of neutrophils prevented AKI.

In addition to the direct cytotoxic effects of hypoxia, RIR induces an inflammatory reaction within the renal parenchyma. RIR causes renal synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. ATP depletion causes tubular epithelial cells to undergo apoptosis or necrosis in vitro, and both apoptotic and necrotic tubular epithelial cells may be seen in ischemic acute renal failure. Necrosis of cells causes the release of a number of factors. High mobility group 1 protein, for example, is a nuclear factor that is released by necrotic cells and promotes inflammation.
released, it stimulates TNF-α production and leukocyte infiltration.

Macrophages infiltrate the injured kidney shortly after neutrophils (within 1 h of reperfusion). Intracellular cytokine staining of kidney infiltrating macrophages by flow cytometry demonstrated that these leukocytes are significant producers of the cytokines IL-1α, IL-6, IL- and TNF-α.20

Dong et al. 22 demonstrated that after RIR, renal dendritic cells produce the pro-inflammatory cytokines/chemokines TNF-α and IL-6 and that depletion of dendritic cells prior to RIR significantly reduced the kidney levels of TNF-α produced after RIR.

Critical early roles for neutrophils, macrophages & lymphocytes have been established in mouse models of AKI. Laboratory and clinical evidence suggests that the inflammatory milieu associated with RIR leads to dysfunction of renal cells and this may be the key factor leading to acute kidney injury. Cells in injured tissues release immunological signals which communicate with remote organs including the kidney.28

The results of the present work showed that renal ischemia followed by reperfusion caused detrimental changes in the brain, liver & kidney histology & function. After 60 min, the magnitude of these changes is much more than after 45 or 30 min. The effect of RIR on remote organs may need to be investigated for a long period after RIR. Further studies are needed to explore the mechanisms and pathophysiological pathways that mediate these changes in brain, liver and kidneys after RIR. Care should be taken to protect organs remote from sites of ischemia reperfusion especially during renal surgery.

Correspondence author
Nahed Salah El-din Mohamed
Department of Physiology, Kasr Al-Aini Faculty of Medicine, Cairo University, Cairo, Egypt nahedsm4@hotmail.com

References:

12/6/2010