EVALUATION OF THE ANTIOXIDANT AND RENOPROTECTIVE EFFECTS OF ELLAGIC ACID ON ISCHEMIA / REPERFUSION INDUCED NEPHROPATHY IN RATS

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Abstract

Aims: Renal ischemia-reperfusion (I/R) injury is one of the important cause of acute kidney injury (AKI). Reactive oxygen species and inflammatory cytokines play major role in the pathogenesis of IR injury. Ellagic acid (EA), a phenolic compound, have shown to exert antioxidants, anti-inflammatory, anticarcinogenic, antihyperlipidemic effects. We aim to evaluate, the effect of EA on renal I/R induced nephropathy in rats.

Materials and methods: Twenty-eight male Sprague-Dawley rats were divided into four groups; control, control + EA, I/R, and EA+I/R. EA (85 mg/kg, perorally) was administered 30 min prior to the ischemia. Rats were unilaterally nephrectomized and subjected to 45 min of renal pedicle occlusion followed by 60 min of reperfusion. Both groups were subsequently studied by renal function tests, oxidant and antioxidant parameters, and kidney histology.

Results: Serum/kidney TAC, NO and paraoxonase levels were significantly higher, while serum urea and creatinine, serum/kidney MDA and TOS were significantly lower in EA+I/R group compared to I/R group (p<0.05). Histopathologic examination revealed that the severity of damage was significantly lower in the EA+I/R group compared to the I/R alone group.

Conclusion: Administration of EA appears to have beneficial effects on I/R induced renal injury by reducing oxidative stress, thus preventing histological injuries and bringing about an improvement in renal function.

Key words: Ischemia-reperfusion injury, kidney, ellagic acid, rats.

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Introduction

Despite advances in critical care medicine, acute renal failure (ARF) which is characterized by a rapid decline in kidney function over a period of hours to days to weeks remains a serious clinical condition. Acute renal ischemia reperfusion (I/R) injury may occur in several clinical situations, some examples are kidney transplantation, renal artery revascularization, hemorrhage, severe injury, shock, and elective urological operations. ARF is associated with a high mortality and morbidity. I/R injury of donor organs during renal transplantation may lead to delayed graft function in the postoperative period, and may even result in loss of the graft. It is also known that interventions for the prevention I/R injury have positive effects on graft function and survival. Therefore, it is essential to explore preventive and therapeutic treatments in this regard.

Although reperfusion is crucial for an ischemic organ to prevent irreversible cellular injury, sometimes it may result in excess tissue damage, even more than that of ischemia alone.

I/R injury is a complex pathophysiologic process and reactive oxygen species (ROS) and inflammatory cytokines play a major role in the pathogenesis of I/R injury.
Renal ischemia induces oxidative stress, which results in an aggravated and prolonged systemic inflammatory response after reperfusion\(^{(10)}\).

Ellagic acid (EA), a phenolic compound, has been shown to provide antioxidant, anti-inflammatory, anticarcinogenic, and antihyperlipidemic effects. EA is known to increase antioxidant activity in the face of cellular lipid peroxidation, thus protecting cells from oxidative damage\(^{(12)}\).

In the present study, we aimed to evaluate the effect of EA on kidney function disturbances, oxidative stress and histological damages in renal ischemia reperfusion (I/R) induced nephropathy in rats.

**Materials and methods**

**Animals**

A total of 28 male Sprague-Dawley rats, weighing 250–280 g, were used in this experimental study. Animals were fed standard rat food and water ad libitum. The experimental protocol for this study was approved by the local committee on animal research ethics of the Dicle University School of Medicine.

**Surgery and Experimental protocol**

Twenty-eight rats were divided into four groups as follows (n=7 per group):

- **Control group**, in which rats did not receive any vehicle or drugs and underwent only nephrectomy without occlusion.
- **Control + EA group**, EA was administered as an 85 mg/kg dose, by mouth 30 minutes prior to ischemic insult. Rats underwent nephrectomy without occlusion.
- **I/R group**, in which the animals were exposed to unilateral ischemia and reperfusion.
- **EA + I/R group**, EA was administered as an 85 mg/kg dose, 30 minutes prior to unilateral ischemia and reperfusion.

Rats were anaesthetized with an intramuscular (i.m.) injection of xylazine (10 mg/kg) and ketamine (70 mg/kg). The abdominal area was shaved and cleaned with povidone-iodine solution, a 3-cm midline incision was made. A right nephrectomy was performed and then, the left renal pedicle was occluded by placing a microvascular clamp for 45 minutes to induce ischemia. After 45 minutes, the clamp was removed and kidney color change was observed for a period of two minutes and then subjected to reperfusion for one hour with saline (2 ml/kg).

At the end of ischemia-reperfusion period, all rats were sacrificed by intracardiac puncture and left kidneys were harvested. Blood samples were immediately centrifuged and plasma samples were stored at -70°C until assayed. Kidneys were divided into 2 pieces from the pelvis for histopathological examination and laboratory analysis (stored at -70°C until biochemical analysis).

**Assessment of renal function**

Serum urea and creatinine were measured by the AEROSET c8000 analyzer.

**Biochemical analysis**

After weighing the tissues, the samples were first homogenized by a homogenizer. Oxidant [total oxidant status (TOS), malondialdehyde (MDA)] and antioxidant parameters [total antioxidant capacity (TAC), nitric oxide (NO), paraoxonase] were measured in both serum and kidney tissue samples.

MDA levels were determined by the method based on the reaction of MDA with thiobarbituric acid\(^{(13)}\). TOS\(^{(14)}\), TAC\(^{(15)}\) were determined using a novel automated measurement method, developed by Erel. Paraoxonase was measured using a commercially available kit (Rel Assay Diagnostics, Gaziantep, Turkey). NO was measured using a commercial kit (Nitrate/Nitrite Colorimetric Assay kit, Cat No. 780001; Cayman Chemical, Ann Arbor, MI).

**Histopathological Examinations**

The kidneys were fixed in a 10% neutral buffered formalin solution and embedded in paraffin. Five µm thick sections were obtained for staining using periodic acid-Schiff and hematoxylin and eosin. Histopathological studies were performed under a light microscope. The following histological parameters were examined with respect to morphological kidney damage to the kidney after I/R: tubular necrosis and atrophy, regenerative atypia, hydropic degeneration, interstitial fibrosis and loss of brush border.

As shown in Table 1, these parameters were assessed for severity of changes using scores on a scale as follows: 0, absent; 1, mild (focal); 2, moderate (multifocal); 3, severe (diffuse).

**Statistical Analyses**

Data analyses were performed using Statistical Package for Social Sciences (SPSS), Version 16.0 for Windows. All the data are presented as mean ± standard error. Multiple groups were compared...
using one-way analysis of variance (ANOVA) with a post-hoc Bonferroni correction. P < 0.05 was considered statistically significant.

Results

The rats tolerated the study protocol well, and all survived until the end of the experiment.

**Renal function parameters**

Animals that underwent renal ischemia exhibited significant increases in serum urea and creatinine levels compared to control and EA group animals (p<0.05). Pre-treatment of rats with EA exhibited a significant reduction in the serum urea and creatinine levels compared with the reperfusion group (p<0.001). Table 2 shows the changes in renal function parameters induced by the experimental procedures in the groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>EA (n=7)</th>
<th>I/R (n=7)</th>
<th>EA+I/R (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>43.5±7.41</td>
<td>40.85±5.87</td>
<td>108.71±5.87</td>
<td>68.14±15.44</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.60±0.06</td>
<td>0.50±0.06</td>
<td>1.04±0.12</td>
<td>0.78±0.11</td>
</tr>
</tbody>
</table>

Table 2: The effect of EA on renal function tests in rats groups.

**Oxidant and Antioxidant parameters**

Serum and kidney tissue MDA and TOS levels were significantly higher in the I/R group compared to the control group. Kidney tissue TAC levels were significantly lower in the I/R group as compared to the control group.

Serum and kidney tissue TAC, NO and paraoxonase and serum TOS levels were found to be significantly higher, while kidney MDA levels were found to be significantly lower in EA+I/R group compared to the control group.

When compared with the I/R group, the rats that were administered EA produced a significant decrease in terms of serum and kidney tissue MDA and TOS levels and a significant increase in terms of serum and kidney tissue TAC, NO and paraoxonase levels. Table 3 shows the changes in Oxidant and Antioxidant parameters in the study groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>EA (n=7)</th>
<th>I/R (n=7)</th>
<th>EA+I/R (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (mmol/ml)</td>
<td>34.42±12.28</td>
<td>15.86±3.07</td>
<td>107.14±40.92</td>
<td>58.28±11.22</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>60.86±49.77</td>
<td>22.63±5.94</td>
<td>331.06±99.34</td>
<td>194.08±41.57</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>0.76±0.32</td>
<td>1.48±0.35</td>
<td>3.25±4.76</td>
<td>11.87±2.72</td>
</tr>
<tr>
<td>NO (µM)</td>
<td>4.57±2.07</td>
<td>60.42±10.73</td>
<td>29.85±18.09</td>
<td>140.14±138.36</td>
</tr>
<tr>
<td>Paraoxonase (U/L)</td>
<td>8.76±4.56</td>
<td>16.98±4.46</td>
<td>36.01±17.42</td>
<td>60.68±11.93</td>
</tr>
</tbody>
</table>

Table 3: Serum and kidney tissue oxidant and antioxidant parameters of the study groups.

**Histopathology**

Histological damage was not observed in neither the control nor the EA group (Figure 1A and B). The rats that underwent I/R showed significantly higher scores of tubular necrosis, tubular atrophy, regenerative atypia, hydropic degeneration and loss
of brush border. Pre-treatment of rats with EA exhibited a significant reduction in these histologic scores compared with the I/R group (Figure 1C and D). Histopathological findings of the groups are shown in table 4.

**Figure 1:** (A-D). Histopathological findings of the study groups (haematoxylin and eosin staining, original magnification, ×200). Normal histology in (a) control and (b) I/R caused severe deterioration in glomerular structure, atrophic structure, diffuse hydropic degeneration in the proximal and distal tubules (c). EA+I/R group shows relatively well-preserved architecture (d).

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Control (n=7)</th>
<th>EA (n=7)</th>
<th>I/R (n=7)</th>
<th>EA + I/R (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>4.00±0.00</td>
<td>2.71±0.48</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>4.00±0.00</td>
<td>2.29±0.48</td>
</tr>
<tr>
<td>Regenerative atypia</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>3.71±0.48</td>
<td>2.57±0.53</td>
</tr>
<tr>
<td>Hydropic degeneration</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>4.00±0.00</td>
<td>2.71±0.48</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>(Ø)</td>
</tr>
<tr>
<td>Loss of brush border</td>
<td>(Ø)</td>
<td>(Ø)</td>
<td>3.86±0.37</td>
<td>2.71±0.48</td>
</tr>
</tbody>
</table>

**Table 4:** Histopathological findings of the groups.
a p<0.001 as compared to the control group
b p<0.001 as compared to the EA group
c p<0.001 as compared to the I/R group

**Discussion**

Reperfusion of ischemic renal tissue may have deleterious effects and initiates a series of cellular events that lead to necrotic and apoptotic cell death. Several mechanisms have been proposed in the pathogenesis of I/R injury, with free radicals and ROS being implicated to have a key role in the development of the damage. It is well known that deleterious effects such as a change in mitochondrial oxidative phosphorylation levels, decrease in adenosine triphosphate levels, increase in intracellular calcium, and activation of protein kinases, phosphatases, proteases, lipases and nucleases all cause disruption in cellular function and integrity. Re-oxygenation of ischemic tissue may contribute to the formation of ROS.

Although the effect of several agents such as antioxidants, antioxidant enzyme mimetics, nitric oxide and nitric oxide synthase inhibitors, erythropoietin, statins, beta-carotene and ascorbic acid compounds on IR injury have been examined, current therapeutic options are limited to supportive measures and renal replacement therapy. Thus, new therapeutic approaches are required.

EA, a phenolic compound is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage. In an experimental study, Pari et al. reported that EA might play an important role in protecting CsA-induced oxidative damage in the liver. In another study by Iino et al., they found that EA has a gastric protective effect against gastric lesions induced by NH4OH or reperfusion in the ischemic stomach, probably due to its anti-oxidative activity. Ateşșahin et al. showed that the EA might have a protective effect against cisplatin-induced nephrotoxicity and oxidative stress and also ameliorated cisplatin-induced pathological changes.

Renal I/R injury affects glomerular and tubular epithelium through ROS and leads to glomerular and tubular dysfunctions. In the present study, animals that underwent renal ischemia exhibited significant increases in serum urea and creatinine levels. On the other hand, administration of EA before ischemia exhibited a significant reduction in the serum urea and creatinine levels, suggesting an improvement in glomerular function.

Renal I/R injury is closely associated with increased oxidative stress and decreased antioxidant protective mechanisms in renal tissue.

Under normal conditions, naturally occurring antioxidant enzymes counteract the unfavorable cellular effects and protects against damage caused by oxygen free radicals. However, an imbalance between ROS generation and antioxidant capacity leads to oxidative injury. Several studies have shown the protective effects of antioxidants in renal I/R injury, suggesting an important role of ROS in the pathogenesis of I/R injury.
Bayrak et al. found significantly higher TOS and lower TAC levels in rats with I/R (9). Ahmadiasl et al. demonstrated that I/R injury was significantly increased in the plasma levels of TAC (10). Hosseini et al. revealed that I/R injury leads to increased oxidative stress and reduced TAC levels (11). In contrast to the findings by Ahmadiasl et al., but in agreement with the findings of Bayrak et al. and Hosseini et al., we found that kidney tissue TAC levels were significantly lower in the I/R group in the present study. Compatible with the results of Bayrak et al., we observed that serum and kidney tissue TOS levels were significantly higher in I/R group rats compared to control group rats. Furthermore, the rats that were administered EA produced a significant decrease in terms of serum and kidney tissue TOS levels and a significant increase in terms of serum and kidney tissue TAC, NO and paraoxonase levels.

It is known that ROS also cause renal-cell injury by lipid peroxidation, which results in increased membrane permeability in cells, mitochondria, and lysosomes (12, 13). MDA is one of the most frequently used indicators of lipid peroxidation (14). It has been demonstrated in several studies that I/R injury leads to increased levels of MDA (15, 16, 17). Compatible with the findings of previous studies, we observed that serum and kidney tissue MDA levels were found to be significantly higher in the I/R group compared to the control group, supporting increased lipid peroxidation. In addition, pretreatment of EA before ischemia reperfusion resulted in a reduction of MDA levels in comparison with the I/R group.

Histopathological examination also demonstrated the protective effect of EA. In the EA+I/R group, the severity of damage was significantly lower compared to the I/R alone group.

In conclusion, administration of EA appears to have beneficial effects on I/R induced renal injury by reducing oxidative stress, thus preventing histological injuries and bringing about an improvement in renal function. EA may be a promising agent for protecting tissues from oxidative damage and preventing renal I/R injury, but further clinical studies are warranted.

References