Original Research Article

Curcumin prevents renal oxidative stress and tissue damage induced by renal ischemia/reperfusion in rats

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ABSTRACT

Background: There is increasing evidence to suggest that curcumin has antioxidant efficacy in renal ischemia reperfusion injury (IRI). However, it has not been investigated whether this effect is dose-dependent or not. The aim of this study is to investigate the dose-dependent effect of curcumin on renal IRI in an experimental rat model.

Methods: The rats (n=32) were separated into four groups: sham, I/R, I/R+CUR-50, I/R+CUR-100. Rats were subjected to renal ischemia by clamping bilateral renal pedicles for 60 min, and then reperfused for 3 h. Animals in treatment groups received 50 mg/kg/day and 100 mg/kg/day curcumin orally for 5 days before IRI, respectively. MDA, GSH, SOD, and CAT activities were determined in renal tissue. Renal tissue also evaluated histopathologically for mean histopathological damage score.

Results: The mean MDA levels in the I/R+CUR-50 and I/R+CUR-100 groups were significantly decreased when compared with the I/R group (p=0.038 and p=0.016, respectively). SOD, CAT and GSH levels of all treatment groups were significantly increased in comparison to that of I/R group (p<0.05, for all). No statistically significant difference between treatment groups were detected (p>0.05). In histological examination, the rats treated with curcumin had nearly normal morphology of the kidney.

Conclusions: Curcumin significantly ameliorates the damage of renal IRI by its antioxidant activity. We detected the highest intraperitoneal dose of curcumin reduced the IRI induced oxidative stress as 50 mg/kg per day.

Keywords: Antioxidant, Curcumin, Ischemia reperfusion injury, Kidney, Oxidative stress

INTRODUCTION

Ischemia reperfusion induced acute kidney injury is one of the leading cause of renal failure with high morbidity and mortality.1 Recent studies have shown that apoptosis plays an important role in renal ischemia reperfusion injury (IRI) and is attenuated by an imbalance in scavenging and generation of reactive oxygen species.2,3 During ischemia a small amount of free radicals occur, however, the amount of free radical formation is much more higher in the reperfusion period, which leads to lipid peroxidation and increases the damage.4 Currently, effective preventive/treatment strategies for renal ischemia reperfusion induced acute kidney injury are neither ideal nor optimistic.

Curcumin (CUR) is a yellowish and highly lipophilic pigment obtained from the seed of the "Curcuma longa"
plant. Recent in vivo and in vitro studies have demonstrated the antioxidant properties of CUR as well as its anti-inflammatory, immunomodulatory, antitumoral and antipsoriatic efficacy. Although CUR has been shown to have antioxidant and anti-inflammatory efficacy in renal IRI, it has not been investigated whether this effect is dose-dependent or not. Herein, the aim of this study was to investigate the dose-dependent effect of CUR on renal IRI in an experimental rat model.

**METHODS**

**Ethics statement**

This study was approved by the Adnan Menderes University (ADU) Institutional Animal Care and Use Committee (IACUC) and performed according to the criteria provided by the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals (64583101/2015/027-27.03.2015).

**Experimental design**

32 female Wistar albino rats (Adnan Menderes University Animal Research Center, Aydin, Turkey), weighing between 250-300 g, were housed in individual cages for 5 days in a well-ventilated room with a 12:12-hour light/dark cycle at 21°C. Animals were fed with standard rat chow and tap water ad libitum.

The animals were randomly divided into 4 groups as follows (n=8 in each group):

Group 1 (Sham): A blunt dissection was performed to expose the renal pedicles, but IRI was not performed.

Group 2 (I/R): Both kidney pedicles were clamped for 60 minutes. The clamp was removed subsequently to restore perfusion for 3 hours.

Group 3 (I/R+CUR-50): After the administration of CUR (Sigma Aldrich-Alfa B21573-10G) orally for 5 days at a dose of 50 mg/kg/day, subjects underwent ischemia for 60 minutes and then reperfusion for 3 hours.

Group 4 (I/R+CUR-100): After the administration of CUR (Sigma Aldrich-Alfa B21573-10G) orally for 5 days at a dose of 100 mg/kg/day, subjects underwent ischemia for 60 minutes and then reperfusion for 3 hours.

**Surgical procedure**

Animals were fasted for 12 hours before the experimental procedures. Animals were anesthetized with ketamine hydrochloride (50 mg/kg, intraperitoneally, Ketalar®, Pfizer-Istanbul, Turkey) and xylazine (10 mg/kg, intraperitoneally, Rompun®, Bayer-Istanbul, Turkey). After abdominal skin shavings and povidone iodine clearance were performed in all groups, laparotomy was performed in a sterile environment via a midline abdominal incision.

Ischemia was induced by bilateral renal pedicle clamping with smooth vascular clamps in I/R and I/R + CUR groups. Bilateral nephrectomies were carried out immediately after 4 h of observation in sham group, after IRI in I-R and I/R + CUR groups. The left kidney was separated and then stored at 80°C until the analysis time used for further biochemical evaluations, whereas the right kidney was stored in 10% formalin for histological examination.

**Histopathological evaluations**

Paraffin blocks were cut at 5 μm thick, mounted on slides, stained with hematoxylin-eosin (H-E) and Periodic acid schiff (PAS). Histopathological changes including tubular cell swelling, tubular cell atrophy, tubular dilatation, infiltration, loss of brush border were semiquantitatively graded as follows: (0) normal, (1) mild, (2) moderate, (3) severe at a maximum score of 15. The microscopic scoring of the kidney sections was carried out in a blinded fashion. All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system.

**Biochemical evaluations**

**Preparation of tissue homogenates**

Tissues were homogenized (PCV Kinematica Status Homogenizator) in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Bronson sonifier 450) by 3 cycles (20-s sonications and 40-s pause on ice) and then was centrifuged (15,000 xg, 10 min, 4°C). Cell-free supernatant was subjected to enzyme assay immediately.

**Malondialdehyde levels and glutathione assay**

Malondialdehyde (MDA), referred to as thiobarbituric acid reactive substances (TBARS), was measured with thiobarbituric acid at 535 and 520 nm in a spectrophotometer as previously described. Results were reported as nmol/g wet tissue. Glutathione (GSH) concentrations in the homogenates were measured according to the spectrophotometric colorimetric method of Ellman. Results were reported as nmol/g wet tissue.

**Superoxide dismutase assay**

Superoxide dismutase (SOD) enzyme activity was measured according to the method of Sun et al, which is based on the formation of colored formazone by O2 produced with xanthine oxidase system, by minimizing nitroblue tetrazolium (NBT). The results are reported as units per gram protein.
Determination of catalase activity

Catalase (CAT) activity was measured according to Aebi’s method which is based on recording the decrease of absorbance at 240-nm wavelength for 1 min that was obtained by spiking sample to 50-mM phosphate buffer at pH 7 whose absorbance was adjusted to 0.500 with H2O2.13 Activity was reported as k (constant rate) per gram (U/g) protein.

Statistical analysis

Statistical analysis was carried out using the SPSS for Windows version 14.0 (SPSS Inc., Chicago, Ill, USA) statistical program. All data are expressed as arithmetic mean ± standard error of mean (SE). Normality for continued variables in groups were determined by the Shapiro Wilk test. The variables didn’t show normal distribution (p<0.05). Kruskal-Wallis and Mann Whitney U tests were used for comparison of variables among the studied groups. p<0.05 was regarded as significant.

RESULTS

Histopathological findings

There were no noticeable kidney changes in sham group. Glomerules and tubules had normal histological appearance (Figure 1).

By contrast, I/R group revealed significant renal tissue damage including tubular cell swelling, focal tubular cell atrophy, tubular dilatation and mixed inflammatory cells infiltration of the interstitium.

In addition, pyknotic nuclei and cast formation were observed in some areas. In PAS-stained sections, loss of brush border of proximal tubules was commonly detected (Figure 2). The mean histopathological damage score (MHDS) of I/R group was 9.75 ± 0.45. Statistically significant increase in MHDS was detected in I/R group in comparison with the Sham group (p=0.001).

Histopathological damage in I/R+CUR-50 and I/R+CUR-100 groups were markedly reduced (Figure 3).
MHDSs were 6.37±0.32 in I/R+CUR-50 and 5.62±0.26 in I/R+CUR-100 groups. MHDS of I/R group was significantly higher from those of I/R+CUR-50 and I/R+CUR-100 groups (p=0.001 and p=0.001, respectively).

Although, the lowest MHDS among groups were detected in I/R+CUR-100 group, no significant difference was detected between the treatment groups (p>0.05).

MHDSs of the groups were summarized in Table 1.

<table>
<thead>
<tr>
<th>Group 1 sham</th>
<th>Group 2 I/R</th>
<th>Group 3 I/R+CUR-50</th>
<th>Group 4 I/R+CUR-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHDS</td>
<td>1.50±1.18</td>
<td>9.75±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.37±0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed mean±SE of eight animals; <sup>a</sup>p = 0.001 vs group 1; <sup>b</sup>p = 0.001 vs group 2

Table 2: The tissue oxidant-antioxidant parameters of all groups.

<table>
<thead>
<tr>
<th>Group 1 sham</th>
<th>Group 2 I/R</th>
<th>Group 3 I/R+CUR-50</th>
<th>Group 4 I/R+CUR-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g wet tissue)</td>
<td>628.24±64.34</td>
<td>1446.8±242.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>717.06±93.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (nmol/g wet tissue)</td>
<td>2034.9±133.03</td>
<td>1380.14±90.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2200.83±137.04&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/gr protein)</td>
<td>80.96±5.50</td>
<td>46.23±3.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.14±6.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (K/g protein)</td>
<td>29.36±2.87</td>
<td>18.71±1.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>30.29±3.92&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed mean±SE of eight animals; <sup>a</sup>p = 0.005 vs group 1; <sup>b</sup>p = 0.038 vs group 2; <sup>c</sup>p = 0.016 vs group 2; <sup>d</sup>p = 0.001 vs group 1; <sup>e</sup>p = 0.001 vs group 2; <sup>g</sup>p = 0.002 vs group 1; <sup>f</sup>p = 0.002 vs group 2

**Biochemical findings**

The mean tissue MDA level of I/R group was significantly increased when compared with the sham group (p=0.005). However; mean MDA levels in the I/R+CUR-50 and I/R+CUR-100 groups were significantly decreased when compared with the I/R group (p=0.038 and p=0.016, respectively).

The lowest tissue MDA level was detected in I/R+CUR-50 group. The mean tissue SOD, CAT and GSH levels of I/R group were significantly decreased with the sham group (p=0.001, p=0.002 and p=0.002, respectively). SOD, CAT and GSH levels of all treatment groups were significantly increased in comparison to that of I/R group (p<0.05, for all). No statistically significant difference between treatment groups were detected (p>0.05).

The mean tissue MDA, SOD, CAT and GSH levels of all groups were shown in Table 2.

**DISCUSSION**

Renal IRI which commonly occurs due to septicemia, obligatory interruption of blood flow in the peri-operative kidney transplant period, nephron sparing surgery in patient with a solitary kidney, hemorrhagic shock, cardiovascular surgery or other ischemic damages, is a significant cause of acute kidney disease. Renal IRI remains an important factor in acute renal transplant rejection, delayed allograft function and long-term graft survival. Apoptosis, inflammation and oxidative stress play a significant role in the extension of renal dysfunction following renal IRI. The ischemic insult leads to depletion of adenosine triphosphate that results in tubular epithelial cell injury and apoptosis. Furthermore, reperfusion process causes a paradoxical increase in injury induced by reactive oxygen species generation, leukocyte infiltration and cytokine production.

Curcumin [diferuloylmethane,1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is a type of fat-soluble phenolic pigment extracted from the turmeric rhizome. Curcumin is used in traditional medicine, particularly as an antimicrobial, anti-inflammatory and antioxidant reagent.

Since the first scientific demonstration of the protective efficacy of CUR on renal ischemia-reperfusion injury and its inflammatory sequelae in 1998, several studies have directed their attentions on the effects of CUR against renal functional alterations, tissue damages, oxidative stress, and inflammation caused by hypoxia.

Najafi et al. demonstrated the attenuation of leukocytes infiltration in addition to renal oxidative stress as well as improvement in kidney function following the administration of CUR during 72-h reperfusion following 30 minutes of ischemia.

Curcumin exhibit a multifunctional antioxidant activity, including prevention of lipid peroxidation. Furthermore, it has been reported that CUR reacts directly with reactive oxygen species and reactive nitrogen species by means of the phenolic groups in its structure and induces...
the expression of various cytoprotective and antioxidant proteins such as SOD, CAT, glutathione reductase, glutathione peroxidase, hemeoxygenase1, glutathione-S-transferase, NAD(P)H: quinone oxidoreductase1 and γ-glutamylecysteine ligase. The protective effect of CUR against oxidative stress has also been reported in a study which examined in vitro renal effects of CUR in cultured HK-2 cells exposed to IRI. Indeed, recent studies have focused on the molecular mechanisms mediating antioxidant responses. Administration of CUR has been shown to protect kidney against IRI by suppressing the activated nitric oxide signaling pathway and upregulation of APPL1 that subsequently inhibits Akt signaling pathway activation and downregulation of the NFkB signaling pathway which induced apoptosis.

Awad and El-Sharif also demonstrated significant reduction in tissue level of TNF-α, IL-1β, IL-12, IL-18, INF-γ, TGF-β and caspase-3 and concluded that CUR protects the kidneys against IRI via immune-mediated and the new identified anti-apoptotic mechanisms.

On the other hand, Hammad et al. examined the effect of high dose (200 mg/kg/day) CUR against 30 or 45 min of renal IRI and concluded that CUR significantly improved the ischemia induced alterations in serum TNF-α and associated morphological features but did not have significant protective effect on the total renal artery blood flow immediately after reperfusion or on the glomerular and tubular functions.

It has been shown that CUR reduced the IRI induced cerebral, myocardial and retinal injuries in a dose-dependent manner. Furthermore, Liu et al. administered CUR with doses of 5 mg/kg per day and 10 mg/kg per day orally for two weeks before induction of renal IRI in rats. They reported the protective effect of CUR by attenuating the inflammatory mediators and caspase-3 in a dose-dependent way. These studies encouraged us to evaluate the protective effect of various doses of CUR against renal IRI.

Curcumin is insoluble in water with a high level of octanol: water partition coefficient (logPow= 3.29), however, antioxidant effects of CUR administered by oral gavage has been demonstrated in renal IRI. Therefore, we preferred oral administration of CUR.

Present study demonstrated no significant difference between CUR treatment groups in terms of oxidative stress biomarkers, antioxidant proteins and histopathological features. Administration time of CUR may be the reason for the difference in present study. Besides that, Liu et al. preferred the comparison of low doses. Curcumin with a dose of 50 mg/kg per day administered orally in present study may be the highest dose reduced the IRI induced oxidative stress.

On the other hand, we did not administer the CUR with dosages between 50 mg/kg per day and 100 mg/kg per day. This limitation can be overcome by further investigations.

CONCLUSION

In conclusion, the current study demonstrated that the CUR significantly ameliorates the damage of renal IRI and this nephroprotective effect is mediated through its antioxidant activity. However, there is no significant difference between groups treated with high dose CUR. We detected the highest intraperitoneal dose of CUR reduced the IRI induced oxidative stress as 50 mg/kg per day.

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Ethical approval: This study was approved by Adnan Menderes University (ADU) Institutional Animal Care and Use Committee (IACUC) (6458/3101/2015/027-27.03.2015)

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