Curcumin alleviates ischemia reperfusion-induced acute kidney injury through NMDA receptor antagonism in rats

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ABSTRACT

Objective: The present study investigated the role of N-methyl-D-aspartate (NMDA) receptors in curcumin-mediated renoprotection against ischemia reperfusion (I/R)-induced acute kidney injury (AKI) in rats.

Methods: Rats were subjected to bilateral renal I/R (40 min I, 24 hours R) to induce AKI. Kidney injury was assessed by measuring creatinine clearance, blood urea nitrogen, plasma uric acid, potassium level, fractional excretion of sodium, and macroproteinuria. Oxidative stress in renal tissues was assessed by measuring myeloperoxidase activity, thiobarbituric acid reactive substances, superoxide anion generation, and reduced glutathione content. Hematoxylin & eosin staining was done to assess histological changes in renal tissues. Curcumin (30 and 60 mg/kg) was administered one hour before subjecting rats to AKI. In separate groups, NMDA receptor agonists, glutamic acid (200 mg/kg), and spermidine (20 mg/kg) were administered prior to curcumin treatment in rats followed by AKI.

Results: I/R-induced AKI was demonstrated by significant change in plasma and urine parameters along with marked increase in oxidative stress and histological changes in renal tissues that were aggravated with pretreatment of glutamic acid and spermidine in rats. Administration of curcumin resulted in significant protection against AKI. However, glutamic acid and spermidine pre-treatments prevented curcumin-mediated renoprotection.

Conclusion: It is concluded that NMDA receptor antagonism significantly contributes towards curcumin-mediated protection against I/R-induced AKI.

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Introduction

Acute kidney injury (AKI) is characterized by rapid decrease in renal excretory function and accumulation of creatinine, urea and other biochemical waste produced in the body. The renal ischemia reperfusion (I/R) is the most common cause of AKI. Clinically, the renal I/R-induced AKI is observed in conditions like renal transplantation, partial nephrectomy, sepsis, and other urological conditions. Presently, the treatment of AKI aims at reduction of volume overload, improvement of renal blood flow, and increase in GFR in patients. However, the existing therapy is considered insufficient to address the needs of all clinical cases effectively. Therefore, there exists a need for exploration of novel target sites and better therapeutic agents for effective treatment of AKI in clinics.

Curcumin is a biologically active anti-oxidant and anti-inflammatory ingredient of Curcuma longa and is widely used as coloring and flavoring agent in several foods in India. Renoprotective effects of curcumin has been evaluated in experimental animal models including I/R- and cisplatin-induced renal injury, which is attributed to its anti-oxidant potential.

N-methyl-D-aspartate (NMDA) is an excitatory neurotransmitter in the brain and is involved in learning and memory. NMDA receptors have also been detected in various parts of nephron including glomerulus, brush border membrane, outer medulla, macula densa and podocytes. It has been reported that hyperactivity of NMDA receptors in kidneys lead to renal damage in rodents. In rats, the antagonism of various modulatory sites of NMDA receptors affords protection against I/R-induced AKI.
In vitro studies showed that curcumin protects against neuronal cell death through NMDA antagonism.8 Further, curcumin inhibited glutamate release and protected against glutamate-induced excitotoxicity through enhanced expression of neurotrophic factors.9,10 However, curcumin-mediated modulation of NMDA receptors has not been explored in the in vivo model. Hence, the present study was designed to investigate the role of NMDA receptors in curcumin-mediated protection against I/R-induced AKI in rats.

Materials and methods
Curcumin was purchased from Central Drug House Pvt. Ltd., India. Spermidine was procured from Sigma Aldrich, India. Glutamic acid was obtained from SD Fine chemicals Ltd, India. All other reagents used in the study were of analytical grade.

Experimental protocol
Experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Adult female Wistar albino rats weighing 175–225 g were employed in the present study. Animals were kept under standard husbandry conditions with free access to food and water and were acclimatized in metabolic cages for 24 h prior to surgery. Rats were randomly assigned to seven groups, each comprising of 6 animals.

Group I (Control): Non surgery group.

Group II (Sham operated): Surgery was performed to expose both kidneys without ischemia. Group III (I/R): Both kidneys were occluded for 40 minutes followed by reperfusion for 24 h.

Group IV (I/R + Curcumin, 30 mg/kg): Curcumin was administered orally one hour before subjecting rats to AKI.

Group V (I/R + Curcumin, 60 mg/kg): Curcumin was given orally to rats one hour prior to surgery.

Group VI (I/R + Curcumin + glutamic acid): Glutamic acid (200 mg/kg) was injected intraperitoneally 30 minutes prior to administration of curcumin (60 mg/kg) and surgery was done one hour post treatment.

Group VII (I/R + Curcumin + spermidine): Spermidine (20 mg/kg) was given intraperitoneally to rats 30 minutes prior to administration of curcumin (60 mg/kg) and surgery was done one hour post treatment.

Curcumin was suspended in 0.5% carboxy methyl cellulose. Glutamic acid and spermidine was dissolved in distilled water. Bilateral renal ischemia was induced by occluding renal pedicles for 40 minutes followed by reperfusion for 24 h to induce I/R-induced AKI, as previously described.2,3 After surgery, the rats were returned to metabolic cages for urine collection. After 24 h, the rats were anesthetized with ether and blood samples were collected using retro-orbital puncture in heparinized tubes. The plasma isolated by centrifugation was used for the estimation of creatinine, blood urea nitrogen (BUN), uric acid, sodium, and potassium concentrations. The creatinine, sodium, and proteins in urine were quantified. The rats were humanly euthanized by cervical dislocation. The kidneys were removed and a portion was preserved in 10% formalin for histopathological examination. The remaining portion of kidneys was used for the quantification of oxidative stress parameters, such as myeloperoxidase (MPO) activity, superoxide anion generation (SAG), lipid peroxides, and reduced glutathione (GSH) levels.

Estimation of creatinine clearance (CrCl), BUN, and uric acid levels
Creatinine in plasma and urine samples was estimated using commercially available colorimetric kit from Crest Biosystems, Goa, India. CrCl was calculated using the following formula: \[ \text{CrCl} = \frac{\text{urine creatinine} \times \text{urine flow rate}}{\text{plasma creatinine}} \]. CrCl was expressed as milliliter per minute per kilogram of rat body weight. BUN and uric acid levels in plasma were estimated using kits (Transasia Biomedicals Pvt. Ltd., Surat, India) and values were expressed as milligram per deciliter of plasma.

Estimation of sodium/potassium levels and macroproteinuria
Potassium level in plasma was estimated by colorimetric kit (Crest Biosystems) and expressed as millimoles per liter of plasma. Sodium levels were estimated from plasma and urine samples. The FeNa was calculated using the following formula: \[ \text{FeNa} = \frac{\text{urine sodium} \times \text{plasma creatinine}}{\text{plasma sodium} \times \text{urine creatinine}} \]. Value of FeNa was expressed as percentage change. Urinary microproteins were assayed using a commercial kit (Crest Biosystems) and expressed as milligram per day.

Estimation of renal oxidative stress parameters
The myeloperoxidase (MPO) activity was measured using the standardized method.2,3 Lipid peroxides were quantified in kidney tissues using thiobarbituric acid reactive substances (TBARS) assay. The protein content in renal tissues was quantified by Lowry’s method and
results of TBARS were expressed as nanomoles per milli-
gram of protein. The SAG in renal tissue was assayed in
terms of measuring reduced nitroblue tetrazolium (NBT)
as reported earlier.2,3 The GSH content in renal tissue
was estimated and expressed as micromoles of reduced
glutathione per milligram of protein.

Histological studies

Kidney sections fixed in 10% formalin were embedded
in paraffin. Sections of 4 μm thickness were cut and
stained with hematoxylin & eosin (H & E) for gross histo-
pathological changes in renal tissues.

Statistical analysis

Data were analyzed using one-way analysis of variance
followed by the Tukey–Kramer post hoc test. Results
were expressed as mean ± standard error of mean
(SEM). Group differences were considered statistically
significant, when \( p < 0.05 \).

Results

No significant difference between control and sham
operated group was observed in various parameters
determined in this study. Therefore, the data obtained
in the control group were used for making further stat-
istical comparisons between different groups.

Effect of pharmacological interventions on renal
oxidative stress parameters

Results displayed in Figure 2 indicate significant oxida-
tive stress in the I/R group as opposed to the control
group. The oxidative stress was revealed by increases in
MPO, TBARS, and SAG accompanied with decrease in
the GSH level. Once again, curcumin treatment showed
abolition of I/R-induced increase in oxidative stress.
Glutamic acid and spermidine pretreatment reversed
the anti-oxidant activity of curcumin.

Histological examination of renal tissue

In control rats, H & E staining of renal tissue showed
normal integrity of glomerulus surrounded by Bowman
capsule and convoluted tubules. The I/R caused mor-
phological changes such as detachment of basement
membrane from glomeruli, increased tubulo-interstitial
spaces and dilatation of tubules as well as accumulation
of neutrophils in renal sections. Curcumin treatment
protected against I/R-induced kidney injury. Pretreatment with glutamic acid and spermidine abol-
ished curcumin-mediated protection against AKI
(Figure 3).

Discussion

Results of the present study indicated that curcumin
ameliorates I/R-induced AKI in a dose-dependent man-
ner as depicted by significant changes in plasma, urine,
renal oxidative stress parameters and histological ex-
amination of kidney tissue in rats. These observations cor-
roborate the findings of previous workers, indicating
the protective effect of curcumin in renal I/R injury.5
Administration of glutamic acid and spermidine abol-
ished curcumin-mediated renoprotective effect in the
rat model. To the best of our knowledge, this study
demonstrates the novel finding that NMDA receptor
antagonism plays a key role in curcumin-mediated
reno-protection in rats.

Curcumin is a biologically active ingredient of tur-
meric powder and has several medicinal properties,
such as anti-oxidant, anti-inflammatory and anti-arth-
ritic, and anti-cancer activity. Several investigators have
reported that curcumin has cardio-, hepato-, neuro- as
well as reno-protective properties.11 Curcumin did not
show any adverse effects in patients given dosage up
to 8 g/day for three months.12 Curcumin is purported to
have clinical therapeutic relevance and is undergoing
clinical trials for the treatment of various disorders,
such as Alzheimer disease, inflammatory bowel disease,
pancreatic and colon cancer, rheumatoid arthritis,
and psoriasis. The reno-protective effects of curcumin have been evaluated in different animal models of renal injury, such as I/R and cisplatin-induced nephrotoxicity. Protective effect of curcumin in all these models of renal injury has been attributed to its anti-inflammatory and anti-oxidant effects.

Under normal physiological conditions, the reactive oxygen species (ROS) are involved in intra- and intercellular communications. However, the excessive generation of ROS during I/R causes cellular/tissue damage and fibrosis through activation of transforming growth factor-β and mitogen-activated protein kinase. In the present study, I/R-produced oxidative stress was revealed by increase in SAG, MPO, TBARS and decreased level of GSH in renal tissues. Pretreatment with curcumin alleviated I/R-induced oxidative stress in a dose-dependent manner. The therapeutic actions of curcumin are attributed to its anti-oxidant and ROS scavenging properties. The phenolic hydroxyl groups in curcumin’s structure are responsible for its anti-oxidant activity, and the resultant phenoxy radicals formed with free radicals are stabilized by extended conjugation. The metabolic products of curcumin such as vanillic acid and ferulic acid are also documented to have an anti-oxidant effect. It has been reported that curcumin reacts with manganese and copper to form ion complexes that exhibit superoxide dismutase-like activity. In addition, curcumin alleviates ROS formation by inhibiting calcium entry into the cell and reduced activity of protein kinase C enzyme within the cells.

Glutamate is a potent agonist of NMDA receptor, whereas spermidine acts as agonist at polyamine binding site of the NMDA receptor. Our previous findings along with other studies indicated that activation of NMDA receptors is associated with significant oxidative stress and tissue damage in various experimental conditions.

Figure 1. Effect of pharmacological treatments on renal parameters in rats. Values represent mean ± SEM. a = p < 0.05 vs. control; b = p < 0.05 vs. I/R group; c = p < 0.05 vs. I/R + curcumin (30 mg/kg); d = p < 0.05 vs. I/R + curcumin (60 mg/kg).
models of renal injury. The activation of NMDA receptors in AKI leads to calcium influx and cellular overload causing generation of ROS and up-regulation of COX-2. In addition, the activation of NMDA receptors leads to renal cell damage through endothelin–nitric oxide pathway. Activation of NMDA receptors on renal podocytes causes oxidative stress and glomerular damage. In the rat model of hyperhomocysteinemia-induced glomerulosclerosis, the activation of NMDA receptors with homocysteine at the glutamate binding site leads to up-regulation of NADPH oxidase, consequently producing oxidative stress.
Interaction between curcumin and NMDA receptors has been reported in in vitro studies. Curcumin is known to protect hippocampal neurons against NMDA-induced cell death. Treatment with curcumin afforded retinal protection by reducing NMDA-induced increase in intracellular calcium load, and consequently inhibited ROS production and cell death. Curcumin decreases the activity of protein kinase C, which is involved in the upregulation of NMDA receptors. It was found that curcumin exposure decreases the phosphorylation of NR1 subunit of NMDA receptor, thereby limiting its biological activity. In rats, the inhibition of glutamate release in the nerve terminals of prefrontal cortex is considered to be the key mechanism for curcumin’s anti-depressant action. In our study, the abolition of curcumin-mediated renoprotection with glutamic acid and spermidine suggests that NMDA receptor antagonism plays an important role in its reno-protective action. However, the exact underlying mechanism of this interaction between curcumin and NMDA receptor still remains to be elucidated.

In summary, curcumin ameliorates I/R-induced renal oxidative stress and AKI in rats. The antagonism of NMDA receptors appears to be one of the mechanisms in curcumin-mediated renoprotection.

Disclosure statement
The authors do not have any conflict of interest.

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