Original Paper

Administration of Curcumin Protects Kidney Tubules Against Renal Ischemia-Reperfusion Injury (RIRI) by Modulating Nitric Oxide (NO) Signaling Pathway

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Key Words

Riri • Renal tubule • Curcumin • NO • NOS • cGMP • PKG • Signal transduction

Abstract

Background/Aims: To explore the protective effect of curcumin on renal ischemia-reperfusion injury (RIRI) in rats, and its influence on nephridial tissue's NO and cGMP levels as well as downstream signaling pathway, to elucidate the possible mechanism of curcumin on RIRI. **Methods:** 36 *Sprague Dawley* rats (SD rats) were randomly divided into Sham group, Model group, curcumin (CUR +) Model group, 12 rats per group. They were all given RIRI model preparation by unilateral artery occlusion method. All groups' β_2 -MG in urine in 24h, serum Cr and BUN were compared, and UAER were calculated. Nitric oxide synthase (NOS), cGMP-dependent protein kinase (PKG), Caspase-3 expression were all determined by western blot. Nitric oxide (NO), NOS and cGMP levels were also examined by using ELISA. All groups' nephridial histomorphology and kidney tubules score were evaluated and compared. **Results:** β_2 -MG and UAER in urine, serum Cr and BUN, in renal tissue were all elevated in Model of RIRI, indicating the success of animal model of RIRI establishment, and above index in CUR + Model group were all lower than those in Model group. Furthermore, iNOS, NO, cGMP, PKG and Caspase-3 in renal tissue were all increased in Model of RIRI, indicating the NO signaling pathway was activated, which is one of the pathogenesis of RIRI, and above index in CUR + Model group were all lower than those in Model group, suggesting that inactivation of iNOS/NO/cGMP/PKG signaling pathway is one of the reasons that explain the protective effect of curcumin in RIRI. **Conclusion:** The activation of iNOS/NO/cGMP/PKG signaling pathway and the consequent promoted apoptosis of renal tubules are significantly involved in the pathogenesis of development of RIRI, and curcumin treatment could protect renal tubules against RIRI, at least partially, by suppressing the activated iNOS/NO/cGMP/PKG signaling pathway.

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Introduction

RIRI is known as the underlying pathophysiology of a variety of severe clinical disorders, such as the sudden decrease of renal function (AKI) observed in patients with renal transplantation, sepsis, and other ischemic damages [1]. The incidence of death is high for these patients, particularly for patients included in the intensive care unit, where the incidence of in-hospital death rate is more than 50%. Patients who survive the acute event are still at high risk of development of chronic renal disorder that may evolve into end-stage renal disorder [2]. The release of cytokines, a buildup of toxic products in renal cells, and reduced oxygen and nutrient delivery have key effects [3].

Curcumin [diferuloylmethane, 1, 7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is a polyphenol compound obtained from the plant *Curcuma longa* (turmeric). Curcumin has immunomodulatory, anti-proliferative, antimicrobial, anti-inflammatory and antioxidant effects and hence has potential therapeutic value in IRI. Curcumin is insoluble in water with a high level of octanol: water partition coefficient (logPow= 3.29), which limits its application as an orally delivered agent [4-7]. Earlier studies determining the effectiveness of curcumin on IR injury in animal models have used oral delivery with slight functional salvage [8-10].

As a product of reaction catalyzed by NOS, a gaseous molecule known as NO is released from L-arginine, and it is important in the immune, cardiovascular and nervous systems [11]. A range of agonists, including bacterial lipopolysaccharides and pro-inflammatory cytokines, elevate iNOS expression, while the activities of eNOS are triggered by activators, including *estrogen* and *estradiol*, suggesting that eNOS and iNOS may have an effect on physiology and bone inflammation [12]. The function of NO in RIRI has been well studied [13]. Nevertheless, what has not been investigated is the implication of the NO downstream signaling pathway, cGMP and PKG, in RIRI. PKG is a serine/threonine kinase consisted of a catalytic and a regulatory domain in one polypeptide chain [14, 15]. Two genes that encode PKG have been found in mammalian cells including type I and type II [16]. The alternative splicing of Type I occurs at the first exon, encoding two isoforms known as I α and I β . These enzymes have identical catalytic domains [17]. PKG-I is produced in macrophages, renal tubular cells, mesangial cells, endothelial cells, cardiomyocytes, vascular smooth muscle cells and other cell types [18]. Recently, studies indicated the protective role of the cGMP/PKG signaling pathway on IRI in cardiomyocytes by modulating several signaling pathways including increased phosphorylation of GSK-3 β , ERK and Akt, or modulation of mitochondria K_{ATP} channels [19-21].

In this study, we systematically investigated the roles of NO, NOS and its downstream signaling pathway such as cGMP, or PKG in the pathogenesis of the development of RIRI both *in vivo* and *ex vivo*.

Materials and Methods

Animals

36 healthy male SD rats in a SPF grade, with body mass of 230 to 260 g and age of 8 to 12 weeks, were provided by Zhejiang Academy of Medical Sciences (Animal License No.: syck (Zhejiang) 2014-0008). 36 SD rats were divided into sham-operated group (Sham group), renal ischemia-reperfusion model control group (Model group), *curcumin* + model (CUR + Model group) by random number table, 12 rats per group.

RIRI model preparation

The RIRI model in rats was established by referring to the method described in literature [3]. In detail, following SD rats were anesthetized by intraperitoneally injecting 3.5% *chloral hydrate* (10 mL $\times$ kg⁻¹), the skin and muscular layers of the right abdomen were cut layer by layer to expose the right kidney and renal pedicle vessels, and a blunt dissection was performed for the renal pedicle. Later, an atraumatic bulldog clamp was used to block the renal pedicle vessels for 45 min and then was removed to restore the renal

blood flow. A ruddy renal surface indicated a successful reperfusion. Nevertheless, in Sham group, only the right renal pedicle vessels were exposed, without blocking the renal pedicle vessels using an atraumatic bulldog clamp, and the wound was sutured 45 min later.

Treatment methods

In Sham group, only resection of right kidney and dissociation of left renal pedicle was performed, and no occlusion of left renal artery was given; at 2 h before the surgery, 1 ml of 0.1% *DMSO* solution was injected into the caudal veins of rats. In Model group, only IRI model was established; at 2 h before the surgery, 1 ml of 0.1% *DMSO* solution was injected into the caudal veins of rats. In CUR group, at 2 h before preparation of IRI model, the *curcumin-DMSO* solution (dissolve a proper amount of *curcumin* in 1 ml of 0.1% *DMSO* solution, 100 mg/kg body weight) was injected into the caudal veins. While in CUR + model group, the *curcumin-DMSO* solution (dissolve a proper amount of *curcumin* in 1 ml of 0.1% *DMSO* solution, 100 mg/kg body weight) was injected into the caudal veins at 2 h prior to preparation of RIRI model.

Observation indexes

a) Urine test indexes: 5 ml of urine was taken from that within 24 h after the surgery collected using metabolic cages and β 2-MG level was determined. The 24-hour UAER was calculated. b) Biochemical indexes of renal function: at 24 h after the surgery, 2-5 ml of inferior vena cava blood was collected and the serum Cr and BUN levels were measured on a Hitachi 7170A automatic biochemical analyzer. c) Renal pathomorphology as well as score of kidney tubules: after blood collection, the rats were sacrificed. The left kidney was excised and divided into two segments: one was used to make paraffin-embedded sections for observation of the renal tissue morphology under a light microscope and scoring of renal tubules. d) NO level, NOS activity and cGMP level: the other segment was used to prepare renal tissue homogenate, the activities were determined using ELISA kits.

Cell culture

RPMI1640 contained 10% FBS was utilized to maintain HK-2 cells (human proximal tubule cell line) in 5% CO₂ incubator. Cells from passage 3 to 8 were utilized in our research, and Lipofectamine 2000 (Invitrogen, CA, US) was utilized to transfect cells.

Histological analysis

Harvested kidney tissue samples were fixed using 10% *formalin*, embedded in paraffin, followed by sliced into 2.5 μ m sections, which were next stained with H&E. The severity of RIRI was scored by evaluating the necrosis status of the tubules, as described by the following 4 grades: absence of necrosis (grade 0); presence of necrosis of individual cells (grade 1); presence of necrosis of PCT (grade 2); presence of necrosis confined to distal 1/3 of PCT (grade 3); presence of necrosis of all through PCT (grade 4).

Luciferase assay

The promoter region of *iNOS* and *eNOS* were subcloned into pGL3.0 vector, upstream of luciferase gene, respectively. The construct was then transfected into renal tubule cells, with empty vector transfected in parallel as a control. The cells transfected with the constructs were pre-treated with normal saline (NS) or *curcumin*, and the relative luciferase activity by using a Dual-Luciferase Reporter Assay System (Promega, Massachusetts, USA) 48 hours post-transfection. Three independent experiments were repeated.

RNA isolation and real-time PCR

RNAeasy Total RNA isolation kit (Qiagen, Valencia, CA) was used to purify RNA from kidney tissues based on the instruction by the supplier. 10 μ L of *DEPC* (*diethylpyrocarbonate*) was utilized to elute the total RNA purified, and 1 μ g RNA was used to synthesize cDNA in 20 μ L reverse transcriptase reaction with 1X First Strand Synthesis buffer (Invitrogen, CA, USA). SYBR® Green PCR system (Applied Biosystems, Bedford, MA, USA) was utilized to perform the real-time PCR, and the reaction was carried out at 95°C for 10 min, then 95°C for 15 for 40 cycles and 60°C for 1 min. Small nuclear RNA U6 and β -actin were served as internal control. The cycle threshold (Ct) value was utilized to express and analyze the relative *iNOS*, *eNOS* or *PGK-I*. Primer sequences were as follows: *iNOS*, forward: 5'-GTG GTG ACA AGC ACA TTT GG-3'; *iNOS*, reverse: 5'-GGC TGG ACT TTT CAC TCT GC-3'; *eNOS* forward: 5'-ATCAGAATTCATGGGCAACCTGAA-3';

eNOS, reverse: 5'-ATCAGAATTCTCATCAGGGGCTGT-3'; PGK1 forward : 5'-AACCAGAGGATTAAGGCTGC-3'; PGK1 reverse: 5'-GCCTACACAGTCCTTCAAGA-3'; β -catenin forward: 5'-GTCTTACCTGGACTCTGGAATCC-3'; β -catenin reverse: 5'-GGTATCCACATCCTCTTCTCAG-3'. 2- $\Delta\Delta$ CT was utilized to present the expression of target genes. All reactions were performed in triplicate.

Western blot analysis

Ice-cold PBS was used to wash the kidney tissues harvested, and M-PER mammalian protein extraction reagents (Upstate Biotechnology, Lake Placid, NY, USA) was used to extract the total protein following instruction provided by the supplier. 10 % SDS-PAGE gel was utilized to separate the lysates, and electro-transferred to a PVDF (polyvinylidenedifluoride) membrane (Millipore, Billerica, MA, USA). Tris-buffered saline (150 mM NaCl, 10 mM Tris) supplemented with 0.1 % Tween 20 and 5 % non-fat milk was utilized to block the blots avoid unspecific binding site. Subsequently, the primary antibodies against *PKG-I* (1:4000, Santa Cruz, Santacruz, CA), *iNOS* (1:1000, Santa Cruz, Santacruz, CA), *eNOS* (1:2000, Santa Cruz, Santacruz, CA), Capase-3 (1:3000, Santa Cruz, Santacruz, CA) and anti- β -actin (1:8000, Santa Cruz, Santacruz, CA) were utilized to incubate the membrane for 12 hours at 4°C, and then horseradish peroxidase (HRP)-conjugated secondary antibody (1:13000 dilution, Boster, China, BA1066) was maintained for 60 minutes at room temperature. The bands and immunoblotting were detected and quantified with the use of ECL plus detection reagents and Quantity One software (Pierce, Rockford, IL, USA). All experiments were run in triplicate.

ELISA

iNOS, *eNOS*, NO and *cGMP* activities in the renal tissue samples from all three groups were assessed by using a commercially obtained ELISA kits according to the manufacturer's instructions (Jiancheng Bioengineering Institute).

Statistical analysis

The difference among groups was evaluated using one-way analysis of variance (ANOVA) followed by Tukey–Kramer test for multiple comparisons. The statistical analysis was performed using SPSS 15.0 (IBM, Chicago, IL), and a P value less than 0.05 is considered to be statistical significant.

Results

Influence of curcumin on urine β 2-MG, UAER and renal function index in RIRI rats

In addition to Sham group only undergoing resection of right kidney and dissociation of left renal pedicle, all the other groups successfully completed the preparation of RIRI model. As shown in Fig. 1A, urine β 2-MG levels of Model, and Model + CUR group were higher than that of Sham group (* $P < 0.05$), and this index in Model + CUR group was lower than that in Model group (# $P < 0.05$). Meanwhile, urine UAER levels of Model, and Model + CUR group were also higher than that of Sham (* $P < 0.05$), and this index in Model + CUR group was lower than that in Model group (# $P < 0.05$) (Fig. 1B). As shown in Fig. 2A and 2B, serum Cr and BUN levels of Model, and Model + CUR group were all higher than that of Sham group (* $P < 0.05$), and the index in Model + CUR group was lower than that in Model group (# $P < 0.05$).

Influence of curcumin on nephridial histomorphology as well as score of renal tubules damages in RIRI rats

After H&E staining, the following conditions were found under a light microscope: 1) in Sham group, glomerulus and renal tubules showed normal renal histological structure, and there was no hyperemia and edema as well as inflammatory cell infiltration in the interstitium, as shown in Fig. 3A. Model group showed macroscopic serious congestion and edema in the renal cortex and medulla and pale medulla, different levels of hyperemia in the glomeruli and renal interstitium, significant hydropic degeneration of renal tubular epithelial cells, and even vacuolar degeneration in some cells, as well as obvious mess, necrosis and falling off of brush border, narrowed renal tubules, and a variety of protein casts with necrotic and

Fig. 1. Comparison of urine $\beta 2$ -MG and UAER level between groups (n, mean $\pm$ s). A. Model, and Model + CUR groups exhibited higher urine $\beta 2$ -MG levels than that of Sham group (* $P < 0.05$), and Model + CUR group showed a lower urine $\beta 2$ -MG level than Model group (# $P < 0.05$) (n, mean $\pm$ s). B. Model, and Model + CUR groups exhibited higher urine UAER levels than that of Sham group (* $P < 0.05$), and Model + CUR group showed a lower urine UAER level than Model group (# $P < 0.05$) (n, mean $\pm$ s).

Fig. 2. Comparison of renal function indexes between groups (n, mean $\pm$ s). Model, and Model + CUR groups exhibited higher levels of serum Cr and BUN than Sham group (* $P < 0.05$), and Model + CUR group showed lower levels of serum Cr and BUN than Model group (# $P < 0.05$).

Fig. 3. Histological presentation and comparison between groups. A. Histological presentation of sham group, and renal tubules and glomerulus displayed normal renal histological structure B. Histological presentation of model group, medulla and renal cortex and pale medulla were serious edema and congestion; glomeruli and renal interstitium were hyperemia; renal tubular epithelial cells were evidently hydropic degeneration C. Histological presentation of CUR+Model group, showed more significantly decreased renal tissue damage in comparison with Model group

deciduous cells in the renal tubule cavity; also, pink serous fluid, cellular casts or albuminoid fluid accumulation were seen in some renal tubule cavities, and there was hyperemia, edema and a large number of inflammatory cell infiltration in the renal interstitium, and the vessels around the renal tubules were dilated obviously and showed congestion (Fig. 3B). CUR + Model group exhibited more obviously decreased renal tissue damage compared with Model group (# $P < 0.05$) (Fig. 3C). Furthermore, the damage of renal tubules was quantified, and we found that the score of renal tubule in Model, and Model + CUR group was higher than that of Sham group (* $P < 0.05$) (Fig. 4). The above index of Model + CUR group was lower than that of Model group (# $P < 0.05$) (Fig. 4).

Influence of curcumin on NO, NOS and cGMP in renal tissues of RIRI rats

As shown in Fig. 5, the levels of NO, *iNOS*, *eNOS* and *cGMP* were measured using ELISA kits in the tissue samples collected from each treatment group, and we found that the NO, *iNOS* and *cGMP* levels of renal tissue homogenate in Model + CUR were higher than that of Sham group (* $P < 0.05$) but lower than that in Model group (# $P < 0.05$), except *eNOS*, which showed no significant difference among the three groups (Fig. 5). To confirm the above results regarding *iNOS* and *eNOS*, we performed real-time PCR and western blot to examine the expression of *iNOS* and *eNOS* in the tissue homogenates, and we found that the *iNOS*

Fig. 4. Comparison of the score of renal tubules between groups (n, mean $\pm$ s), score of renal tubule in Model + CUR group was higher than that of Sham group (* $P < 0.05$), and the above index of Model group was even higher than that of Model + CUR group (# $P < 0.05$)

Fig. 5. Comparison of the iNOS, eNOS, NO and cGMP by ELISA between groups (n, mean $\pm$ s), iNOS, NO and cGMP levels in Model + CUR group was much higher than sham group (* $P < 0.05$), and the above factors levels in Model group was even higher than that of Model + CUR group (# $P < 0.05$); whereas eNOS levels among sham, Model, Model + CUR were comparable with each other.

Fig. 6. Effect of curcumin on the expression of iNOS and eNOS by real-time PCR and western blot. A. Model, and Model + CUR groups exhibited higher levels of iNOS than Sham group (* $P < 0.05$), and Model + CUR group showed a lower level of iNOS than Model group (# $P < 0.05$). B. There is no obvious difference in eNOS mRNA and protein levels among sham, Model, Model + CUR.

expression in Model + CUR was higher than that of Sham group but lower than that in Model group (# $P < 0.05$) (Fig. 6A), but no difference was noted among the groups regarding the expression of eNOS (Fig. 6B).

Influence of curcumin on PKG and Caspase-3 in renal tissues of RIRI rats

To further explore the effect of curcumin on PKG and Caspase-3 activation, we performed western blot to determine the expression of those two genes in each group. As shown in Fig. 7, the PKG (Fig. 7A) and Caspase-3 (Fig. 7B) expression in Model + CUR were higher than that of Sham group (* $P < 0.05$) but lower than that in Model group (# $P < 0.05$).

Influence of curcumin on the transcription of the promoters of iNOS and eNOS

The promoter region of iNOS and eNOS were sub-cloned into pGL3.0 and transfected into cells pre-treated with NS or curcumin, and we found that even both promoter could

Fig. 7. Comparison of the expression of PKG and Caspase-3 between groups A. PKG level in Model + CUR group was higher than that of Sham group (* $P < 0.05$), and PKG level of Model group was even higher than that of Model + CUR group (# $P < 0.05$) B. Model, and Model + CUR groups exhibited higher levels of Caspase-3 than Sham group (* $P < 0.05$), and Model + CUR group showed lower levels of Caspase-3 than Model group (# $P < 0.05$).

Fig. 8. Effect of curcumin on the transcription of promoter of iNOS and eNOS using luciferase assay. A. iNOS promoter enhanced the transcription of luciferase compared with empty control (* $P < 0.05$), and luciferase activity of iNOS promoter and iNOS promoter plus CUR groups were comparable. B. eNOS promoter enhanced the transcription of luciferase compared with empty control (* $P < 0.05$), which was abolished by treating with CUR (* $P < 0.05$).

Fig. 9. Schematic description of the effect of curcumin on iNOS/NO/cGMP/PKG/Caspase-3 signaling pathway.

promoted the transcription of luciferase compared with the control (* $P < 0.05$) (transfected with empty pGL3.0 vector), but the inhibitory effect was only noted in *iNOS* promoter, as shown in Fig. 8A and 8B.

All in all, as one of major pathogenesis of RIRI, activation of *iNOS*, instead of *eNOS*, produces plenty of NO, which subsequently activates cGMP, and its downstream effector, PKG. PKG is a stimulator of caspase-3, an executor of apoptosis. In such way, RIRI causes damages to renal tubules. The preventive role of curcumin in RIRI could be explained by its ability to suppress the transcription of *iNOS*, and attenuate the downstream signaling pathway of iNOS/NO/cGMP/PKG, resulting in a suppressed apoptosis and less damages to renal tubules during RIRI (Fig. 9).

Discussion

Curcumin exhibits its protective effect against ischemia or reperfusion injury by lowering different cytokines such as $\text{INF-}\gamma$, $\text{IL-1}\beta$, and $\text{TNF-}\alpha$, and suppressing 5-lipoxygenase [22]. Kuhad et al. indicated that application of curcumin two days before and three days after the delivery of cisplatin substantially reduced systemic inflammation, oxidative stress and

renal injury in rats [23]. Tirkey et al. suggested that curcumin was capable to decrease oxidative stress, promote renal function and also improve tissue injuries caused by chronic application of cyclosporine [24]. In addition, *curcumin* has been demonstrated to have a suppressive effect against the apoptosis by suppression of caspase-3 and TGF- β [25]. In fact, a lot of studies have demonstrated that *curcumin* prevents against inflammation, oxidative stress, tissue damages and functional disturbances resulted from RIRI in the kidney and other organs [9, 26]. The application of *curcumin* in this study resulted in partially restored kidney function and less damages to renal tubules, as well an overall inactivation of iNOS/NO/cGMP/PKG signaling pathway [27].

RIRI is a multi-factorial/multi-pathway complex pathological and physiological process and may involve complicated occurrence and development mechanisms, but the exact mechanism is not fully elucidated [28]. In recent years, research hotspots focus on energy metabolism disorder, reactive oxygen, signaling pathways, etc. There into, energy metabolism disorder is considered to be the initiation link of RIRI, while the signaling pathway of NO/cGMP/PKG, all which [NO, cGMP and PKG (cGMP-dependent protein kinase)] play important biological functions in a variety of histocytes, is considered as the key link to induce IRI [29-31]. PKG is an extensively existed serine/threonine kinase in eukaryocytes, while cGMP plays the role as the second messenger through the interaction between intracellular receptor proteins, in which the most key receptor of cGMP in the vascular smooth muscle cells is serine/threonine kinase (i.e., PKG) [32]. NO can excite various signal transduction pathways and stimulate the soluble guanyl cyclase (rGCs) to catalyze the synthesis of intracellular cGMP; then, cGMP activates PKG to regulate local and systemic signals. The influence of NO/cGMP/PKG signal pathway activation on RIRI is rarely seen in literatures. However, studies about cardiac IRI suggested that NO/cGMP/PKG signal transduction mechanism might be associated with activation of PKG and opening of ATP-sensitive K^+ channel in the cell membrane [31]; PKG as the downstream signaling protein of cGMP could activate ATP-sensitive K^+ channel. In summary, we speculated that curcumin may activate the downstream PKG-regulated ATP-sensitive K^+ channel through NO/cGMP/PKG signaling pathway to ultimately reduce the degree of IRI and induce the renal protection. In this study, we found the iNOS/NO/cGMP/PKG signaling pathway was highly activated in the animal model of RIRI, and treatment with curcumin substantially suppressed this signal activation, and partially but significantly restored it, indicating that the preventive effect of curcumin against RIRI could be mediated by its ability to suppress the transcription of *iNOS*, and attenuate the downstream signaling pathway of iNOS/NO/cGMP/PKG, resulting in a suppressed apoptosis and less damages to renal tubules during RIR.

Some investigators have found that NO from iNOS is harmful in I/R, since iNOS contributes to the generation of large amount of NO during I/R [33]. Chatterjee and colleagues have suggested that selective suppression of iNOS by using L-N6-(1-iminoethyl) lysine (L-NIL) can decrease renal injury and dysfunction related to I/R of the kidney by suppression of *iNOS* activity and consequent decrease of NO production [34]. Additionally, Mark and colleagues suggested that blocking of *iNOS* after I/R led to a helpful effect on renal function [34]. Additionally, NO itself together with the superoxide radical generates a strong cytotoxic metabolite known as peroxynitrite, resulting in tissue damage by decomposition into NO and hydroxyl radicals or by protein tyrosine nitration [35]. In this study, we evaluated the role of *iNOS* and *eNOS* in our animal model of RIRI. In line with the above statement, we found that iNOS rather than eNOS is the major NOS that is responsible for the dramatic increase in the production of NO and activation of the signaling pathway, and inhibition of *iNOS* by the presence of *curcumin* could be explained its ability to reduce the transcription ability of *iNOS* promoter, while *curcumin* did not show any effect on that of *eNOS* promoter.

According to Chinese medicine researches, *curcumin* possesses the functions, such as dispelling Qi-stagnation and blood-stasis, clearing away wind-cold, wind-heat and wind-damp to disperse blood stasis, promoting blood circulation to arrest pain, and so on, and is widely used for removing heat from the liver and cholagogue effect as well as antiseptics and anti-inflammation [36]; Modern pharmacological studies have shown that *curcumin*

has anti-oxidation, anti-tumor, anti-inflammation, anti-virus, immune regulation, promoting wound healing, anti-coagulant effects [37, 38]. Based on fundamental research results on the prevention of RIRI, *curcumin* had a potential effect in scavenging free radicals, inhibiting inflammation and reducing apoptosis to exert a protective effect on the myocardium, liver and kidney of murines, rabbits and canines. Pre-conditioning of *curcumin* was reported to decrease the RIRI via anti-apoptotic effects [25]; it also reduced renal ischemia reperfusion-induced lung injury and myocardial failure and injury [39, 40].

Notably, the present study is only to investigate the effects of *curcumin* on iNOS/NO/cGMP/PKG signaling pathway, and further study comprehensively converts all involved signaling pathways or networks is warranted. In addition, the results of this study are only derived from animal experiments. Because human body has more complex and sophisticated regulatory mechanism and system, the effect of *curcumin* on RIRI in human still needs to be further investigated.

Conclusion

In summary, the activation of iNOS/NO/cGMP/PKG signaling pathway and the consequent promoted apoptosis of renal tubules are significantly involved in the pathogenesis of development of RIRI, and *curcumin* treatment could protect renal tubules against RIRI, at least partially, by suppressing the activated iNOS/NO/cGMP/PKG signaling pathway.

Abbreviations

SD rats (Sprague Dawley rats); CUR (curcumin); UAER (urinary albumin excretion rates); RIRI (renal ischemia-reperfusion injury); IRI (ischemia-reperfusion injury); NO (nitric oxide); cGMP (cyclic guanosine monophosphate); NOS (nitric oxide synthase); eNOS (endothelial nitric oxide synthase); iNOS (inducible nitric oxide synthase); PKG (cGMP-dependent protein kinase); β 2-MG (β 2-microglobulin); ELISA (Enzyme-Linked ImmunoSorbent Assay); AKI (acute renal injury); GSK (glycogen synthase kinase); SPF (Specific-pathogen-free); DMSO (Dimethyl sulfoxide); DEPC (diethylpyrocarbonate); INF- γ (interferon- γ); IL-1 β (interleukin-1 β); TNF- α (tumor necrosis factor- α); TGF- β (Transforming (growth factor- β); L-NIL (L-N6-(1-iminoethyl) lysine); FBS (fetal bovine serum); H&E (hematoxylin-eosin); PCT (Proximal Convoluted Tubule).

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Disclosure Statement

Authors claim no conflict of interest.

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