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Original Research

Protective role of curcumin on renal ischemia reperfusion injury via attenuating the inflammatory mediators and Caspase-3

F-H. Liu[1*], W-J. Ni[2], G-K. Wang[1], J-J. Zhang[1]

[1] College of Pharmacy, Zhejiang pharmaceutical college, Ningbo, Zhejiang, China
[2] Heading Ningbo Medicine Co., Ltd. Ningbo, Zhejiang, China

Abstract: Renal ischemia/reperfusion (I/R) damage may arise due to nephron sparing surgery in patient with a solitary kidney of restricted renal parenchymas. Apoptosis, inflammation and oxidative stress play a significant role in the expansion of renal dysfunction following renal I/R. The aim of the current investigation was to particularize the potential effect of curcumin against hypoxia induced renal injury. The albino Wistar rats divided into groups and each group contains six rats. They groups are normal control; disease control; curcumin (5 mg/kg per day) and another group orally treated with curcumin (10 mg/kg per day) for two weeks before induction of renal I/R. The renal and serum samples were collected and used for the biochemical estimation. The renal tissue was further used for the histopathological estimation. The result of the current investigation demonstrated that the curcumin significantly ($P<0.01$) attenuated I/R induced renal injury in a dose-dependent way. It also causes significant ($P<0.01$) reduction in the serum creatinine, blood urea nitrogen level and also suppressed the kidney injury molecules-1. Additionally, it also causes significant inhibition of the malonaldehyde, caspase-3, myeloperoxide, lactose dehydrogenase and interferon-gamma together with enhanced interlukin-10 content.

Key words: Curcumin, caspase-3, renal reperfusion, lactose dehydragenase.

Introduction

The renal ischemia/reperfusion (I/R) occurs during the transplantation or surgical intervention of the kidney. The situation has been further aggravated with the generation of the massive amount of free radicals generation and associated inflammatory response. Thus, to provide therapeutic relief, it is imperative to understand the exact mechanism behind the process. Recent studies have shown effectiveness of various anti-inflammatory and antioxidant products against renal ischemia reperfusion damage which provide mechanistic as well as therapeutic effects.

According the previous studies, the renal failures are resultant of complete degradation of cellular function which often leads to the disconnection of tubular cells from extracellular matrix. Moreover, during stage of restoration, the kidney cells showed dysregulation and disruption of actin cytoskeleton and loss of interaction between the extracellular and intracellular matrix and adenosine triphosphate reduction (1,13). The resident tissue and leukocytes cells confirmed the interaction in the host defense, while some of the inflammatory mediators showed the interaction between the cell-matrix and cell-cell (2,14).

Therefore, in the current investigation, we intended to explore the potential effect of curcumin against renal I/R injury and explore the probable mechanism of action of this protection.

Materials and Methods

Chemicals

Curcumin was purchased from the Sigma Aldrich, USA. All others chemical and reagents were used in the experimental study were analytical grade.

Experimental animal

Swiss Adult albino Wistar rats (150-200 g) were used for the current experimental study. The rats were received from the departmental animal house and stored in single cage. All rats were kept in an excellent conditions 12h light/dark and (23± 2°C). All rats were fed with the standard diet and *ad libitum*. The current experimental study was approved form the Institutional Animal Ethical Committee.

Experimental study

The Wistar were divided into following groups and each group contain six rats. Group I: sham control treated with the corn oil; Group II: I/R control group; Group III: I/R control received curcumin (5 mg/kg) and Group IV: I/R control received curcumin (10 mg/kg) for 2 weeks via oral gavage. The all group rats received the pre-determined treatment, after the treatment of the last dose, the rats were subjected to renal ischemia (45 min) followed via reperfusion for 6h except sham control.

Induction of Renal reperfusion/ischemia

Briefly, the overnight fasted rats were anesthetized using the anesthesia (Phenobarbital sodium). The both kidney of the rats were exposed via kidney pedicles with artery, nerve and vein. The renal bilateral pedicle was clamping for induction the renal ischemia using the arterial clips for 45 min, followed by removal the clips and allows for the reperfusion for 6 h. The change of the

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*** Corresponding author:** Dr. Fu-He Liu, College of Pharmacy, Zhejiang pharmaceutical college, China. Email: liufhe@hotmail.com

color pale to red confirmed the renal reperfusion of the tissue. The sham control group rats received the same type of the treatment except clamping the tissue. All the experimental period the temperature of the rat was maintained using the incandescent bulb or warm plate to maintain the temperature 37°C.

Collection of serum samples

At end of the experimental study, the rats were decapitated and the blood samples were collected and the collected samples were centrifuged at 5000 rpm for 10 min at 4°C. The all group rats were sacrificed at end of the study and the renal tissue samples were successfully removed, rinsed with the chilled saline and the tissue and serum samples were stored at -80°C for further used.

Estimation of biochemical parameters

The biochemical parameters viz., serum creatinine, blood urea nitrogen and lactate dehydrogenase were estimated according to the reported method of Anwar et al., 2015 (14).

Estimation the caspase-3 activity

The caspase-3 content in the renal was estimated via using the ELISA kit. Whereas, the caspase-3 content was estimated using the procedure provided by the manufacture's instruction (15).

Estimation of cytokines

The cytokines viz., IL-10 and IFN-γ was determined by the using the ELISA kit. The estimation of the proinflammatory cytokines was performed according to the manufacture's instruction.

Estimation of endothelin-1and myeloperoxidase (MPO)

ELISA kit was used for the estimation of the endothelin-1 and myeloperoxidase content. All the procedure was adopted according to the instruction provided by the manufactures.

Estimation of collagen-IV

The renal homogenate was used for the estimation of the collagen-IV. The collagen-IV content was estimated using the ELISA kit and we adopted the procedure provided by the manufactures.

Statistical analysis

The data of the current study was presented as mean± SEM. GraphPad prism was used for the analysis of the data by ANOOVA followed by Dunett's test. P<0.05 was considered as significant.

Results

Effect of curcumin on renal function test

Figure 1-3 demonstrated the effect of the curcumin on I/R rats. The renal function parameters viz., creatinine, LDH and BUN significantly found to be (P<0.05) modulated in I/R control group rats as compared to normal control group rats. I/R rats treated with the curcumin significantly (P<0.001) modulated the renal parameter almost near to the normal control level as compared to I/R control group at dose dependent manner.

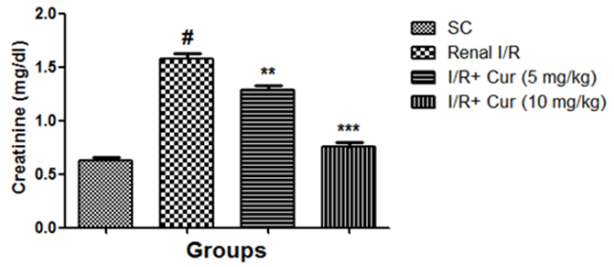

Figure 1. Effect of curcumin on the level creatinine in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin and SC=Sham control. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

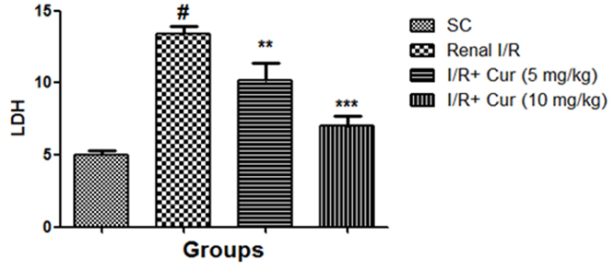

Figure 2. Effect of curcumin on the level of LDH in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control and LDH= Lactate dehydrogenase. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

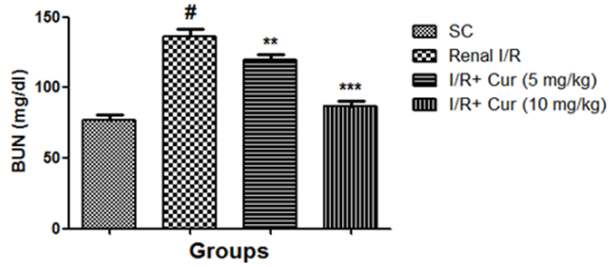

Figure 3. Effect of curcumin on the blood urea nitrogen level (BUN) in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin and SC=Sham control. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

Effect of curcumin on inflammatory marker

As presented in the figure 4, the level of MPO was significantly (P<0.05) increased in I/R control group rats as compared to normal group, which was then found to be significantly (P<0.001) inhibited by curcumin at dose dependently. The curcumin (10 mg/kg) reached the level of MPO almost near to the normal control level. I/R control rats revealed the enhanced content of IFN-γ

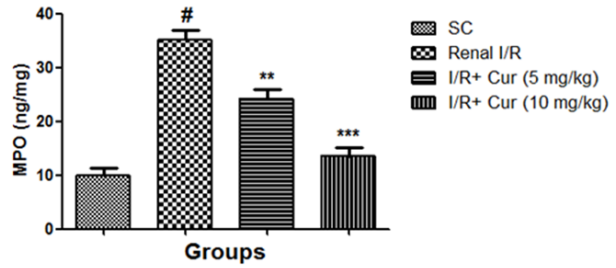

Figure 4. Effect of curcumin on the MPO content in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

as compared to normal level. The IFN-γ level found to be almost double as compared to the normal control. The enhanced level of IFN-γ significantly (P<0.001) declined by the curcumin in a dose dependent manner (figure 5).

The level of IL-10 was found significantly reduced in I/R control rats. The oral administration of curcumin significantly (P<0.001) boosted the level of IL-10 in a dose dependent manner. The curcumin at the dose of 10 mg/kg increased the level of the IL-10 almost near to the normal control rats.

Effect of curcumin on antioxidant parameters

The figure 6 demonstrated the increased content of MDA (lipid peroxidation parameters) in I/R control group rats as compared with normal control. On the other hand, I/R rats treated with the curcumin showed the significantly decreased content of MDA as compared with I/R control.

Effect of curcumin on KIM-1 and Caspase-3

Figure 7 demonstrated the effect of the KIM-1 content in the sham control and I/R rats. I/R rats revealed the increased content of KIM-1, which was significantly (P<0.001) reduced by the curcumin in dose dependent manner. The caspase-3 content was found to be significantly increased in I/R rats as compared to the sham control. On the other hand, the enhanced content of caspase-3 significantly (P<0.001) declined by the curcumin in a dose dependent manner as compared to I/R rats (figure 8).

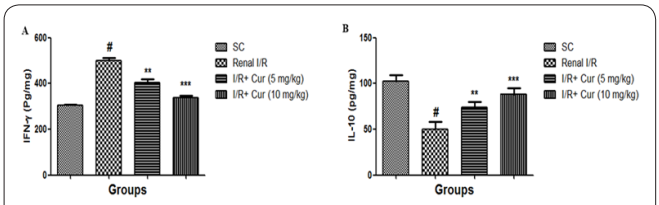

Figure 4. Effect of curcumin on the inflammatory mediators (IFN—γ and IL-10) content in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control, IFN—γ=Interferon-gamma and IL-10= Interleukin-10. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

Discussion

Several researches claimed that the uses of the phyto-therapy increase day by day due to their biological potential with remarkable safety. Several experimental studies are carried out to investigate the effectiveness of diverse herbs in the treatment of I/R. Kidney I/R results in declined filtration rate, massive tubular injury, acute renal damage and deposition of nitrogenous wastages in the blood. The I/R is related to the oxidative stress

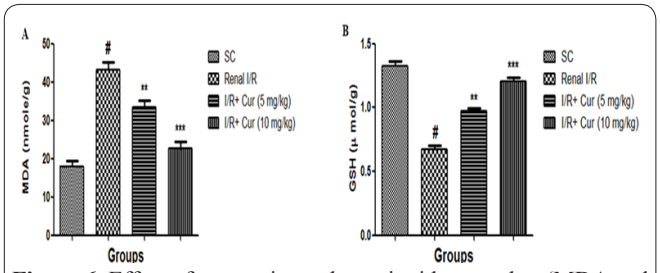

Figure 6. Effect of curcumin on the antioxidant marker (MDA and GSH) content in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control, MDA=Malonaldehyde and GSH= Glutathione. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

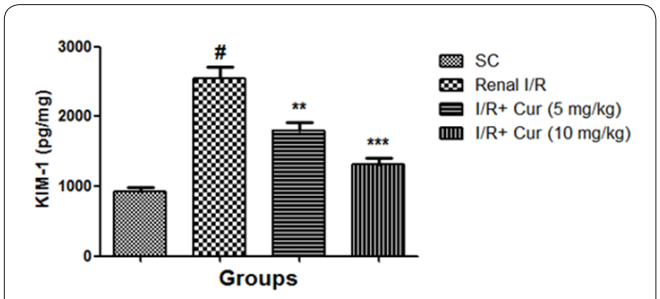

Figure 7. Effect of curcumin on the KIM-1 content in sham control and I/R rats I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

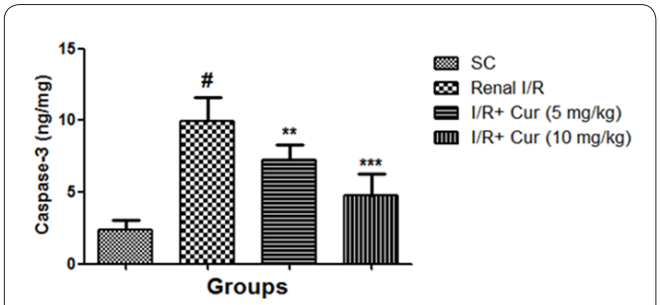

Figure 8. Effect of curcumin on the caspase-3 content in sham control and I/R rats. I/R=renal ischemia/reperfusion, Cur=Curcumin, SC=Sham control. Results were presented as mean ± S.E.M. ANOVA and Dunnett's tests were used for statistical analysis of data. # significantly different from sham control group at p 0.001 respectively. *,**,*** significantly different from I/R group at p< 0.05, p<0.01 and p < 0.001 respectively.

induced by the tissue injury and outflow of the cellular contents such as LDH. Ragab et al., (3) confirmed that the level of KIM-1 was found to be increased during the I/R renal injury. It has been well established that, KIM-1 (glycoprotein) promotes ischemic damage via improvement of tissue re-epithelization and apoptosis. The result of the current investigation illustrates that curcumin significantly reduces I/R damage in rat kidney with enhanced renal function, as proof by reduction in BUN, creatinine level and enhanced antioxidant status (GSH level) together with the reduced lipid peroxidation (MDA level), as well as improvement in the renal histopathological condition. Furthermore, increase proinflammatory mediators and LDH observed in I/R control group rats played a significant role in the expansion of inflammatory reaction and the curcumin treatment significantly attenuated I/R induced inflammatory processes. I/R induce renal damage, initiated via infiltration of the macrophages and neutrophils into the injured kidney, which induces the secretion of proinflammatory cytokines and IFN-γ that again promotes the secretion of more macrophages and neutrophils into the injured tissue and potentiate the inflammatory response. Furthermore, reperfusion subsequent to ischemia may induce a quick secretion of proinflammatory mediators. Formerly, IFN-γ injection encouraged the oxidative stress and also enhanced the cytokines receptor expression which increases their activity. Additionally, I/R induced renal injury via generation of cytotoxic proteases and free radicals, after the initiation of tissue injury and neutrophils mediates the cellular death. Neutrophil causes the secretion of the myeloperoxidase via hypochlorous, which was generated by free radicals. Consequently, myeloperoxidase is considered as the indicator of the neutrophils granulation infiltration into the damaged tissue and also considered as the indicator of renal injury associated with free radical generation. Vinuesa et al., (4) proved that the proinflammatory cytokines existed during the primary phase of I/R, while proinflammatory cytokines IL-10 releases during the last phase. Proinflammatory cytokines IL-10, which represses natural immune system and helps in the functional recovery after renal ischemia by inhibition of inflammation induced via monocyte and reduction of TNF- α. Few researchers have also claimed that the increased content of myeloperoxidase, IFN-γ reduces content of IL-10 and was observed in I/R rats (7-9). The result of the current study proved the renal protective effect curcumin via reduction of IFN-γ and myeloperoxidase and enhanced the IL-10 content.

The content of endothelin receptor (ETA), endothelin-1 and gene expression was increased during I/R renal damage. ETA blockade modulated the apoptosis and inflammation in renal ischemic. The possible mechanism of action for enhanced content of ET-1 due to free radical generation induced via renal I/R (5). ET-1 arbitrated diminution of renal blood flow and renal vasoconstriction contributed to the acute renal failure induced by ischemia and progressed into the chronic renal injury (6,7). The current study showed that, curcumin significantly ($P<0.001$) reduced the ER-1 content and also modulated the antioxidant parameters.

I/R induced rats showed the increased content of renal caspase-3. The increased apoptosis following renal I/R can be arbitrated via both intrinsic and extrinsic pathways. The reduced content of Bcl-2 and increased content of p53-Bax-caspase-3 was founded in the renal I/R. additionally, I/R induced ATP reduction and oxidative generation may cause the caspase-3 activation, endorse apoptosis and mitochondrial deterioration leading to the apoptosis in tubular cells and DNA fragmentation. I/R control rats treated with curcumin confirmed the diminution of caspase-3 content (10-12).

Conclusively, the current investigation explicated the potential effect of curcumin in the renal ischemia/reperfusion model. Several clinical studies strongly recommended to develop the preventive effect of plant based phyto-constituents against the renal ischemia/reperfusion damage during transplantation of kidney to avoid implant dysfunction and acute renal dysfunction.

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