

# Protective effect of curcumin against cyclosporine A-induced rat nephrotoxicity

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**Abstract.** This study explored the potential value of curcumin, a natural product, in the protection of CsA-induced nephrotoxicity. The aim of the present study was to investigate the effects of curcumin on Cyclosporine A (CsA)-induced renal oxidative stress and determine the potential underlying molecular mechanisms of the renal protective effects of Cur. HK-2 human renal cells were co-treated with CsA and various doses of Cur. Cell survival rate was determined by an MTT assay, total cellular protein was collected and oxidative stress in cell homogenates was evaluated by determining the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), the levels of malondialdehyde (MDA) and reactive oxygen species (ROS), and total antioxidant capacity. Furthermore, Bcl-2 and Bcl-2-associated X (Bax) protein expression was measured by western blot analysis. In addition, a CsA-induced nephrotoxicity (CAN) rat model was also established. Renal function was analyzed by measuring creatinine (Crea) and blood urea nitrogen (BUN) in the serum of rats, and histopathological examination was performed on renal tissues using hematoxylin and eosin staining, periodic acid-Schiff staining and nuclear factor- $\kappa$ B (NF- $\kappa$ B) immunostaining. The results demonstrated that treatment of HK-2 cells with CsA significantly increased ROS and MDA levels, and decreased the activities of SOD, GSH-Px and CAT, compared with the control group. However, these effects of CsA were dose-dependently improved by treatment with Cur. In addition, Cur treatment increased Bcl-2 and decreased Bax protein in HK-2 cells, compared with cells treated with CsA alone. In the CAN rat model CsA (30 mg/kg) treatment significantly elevated serum Crea levels and BUN, but lowered endogenous Crea clearance rate, compared with the control group.

Co-administration of Cur with CsA significantly reversed the effects of CsA on serum Crea levels, BUN and Crea clearance rate (Ccr). Additionally, Cur alleviated CsA-induced renal cell injury, as less vacuolar degeneration of glomerular cells was observed compared with the CsA alone group. In conclusion, Cur may increase renal antioxidant capacity and reduce the Bax/Bcl-2 ratio, subsequently improving CsA-induced renal failure and renal tubular deformation and cell vacuolization.

## Introduction

Conventional use of cyclosporine A (CsA) following kidney transplantation is considered to be associated with the development of chronic allograft nephropathy, which leads to a gradual and irreversible loss of graft function and is a major cause of redialysis following renal transplantation (1). At present, the mechanism of CsA-induced nephrotoxicity (CAN) is not fully understood, however, results indicate that CsA may lead to an increase in reactive oxidative metabolites and reduced renal antioxidant capacity (2). Oxidative stress is a major trigger of CAN. CsA directly induces endothelial cell membrane lipid peroxidation and oxidative stress in cells, which enhances the production of oxygen free radicals (3,4). In addition, CsA also blocks the formation of nitric oxide, thereby increasing the damage caused by oxidative stress. Blocking the expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) reduces the production of reactive oxidative metabolites and improves kidney antioxidant capacity (5,6). Curcumin (Cur) has been reported to increase the proliferation, reduce the rate of apoptosis and reduce the Bcl-2-associated X (Bax)/Bcl-2 ratio in human umbilical vein endothelial cells (7).

Oxidative stress is also associated with the occurrence of CAN (8). Histological manifestations of CAN include progressive glomerulosclerosis, interstitial fibrosis associated with mononuclear cell infiltration and renal tubular atrophy, while the primary clinical manifestations are progressive deterioration of renal function, hypertension and proteinuria (9,10). These manifestations are consistent with renal injury in which oxidative stress is the major cause. There is also evidence that oxidative stress is involved in the glomerular atrophy observed in interstitial fibrosis of epithelial cells to fibroblasts during the process of metaplasia (11). Oxidative stress in chronic graft kidney glomerular atrophy interstitial degeneration in an animal experimental model has also been confirmed (12), increased

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cell membrane unsaturated fatty acids and cholesterol lipid peroxidation was observed, while the cell membrane fluidity was decreased and permeability was increased, affecting the membrane-associated enzyme involved in the biochemical process and ion pump function. In addition, oxidative stress may also induce the oxidation of biological macromolecules, and protein structure and conformational were altered through the direct effect on the sensitive amino acids (tryptophan, tyrosine, histidine and cysteine), or through lipid peroxidation products causing oxidative damage, leading to cell death or apoptosis, tissue and organ damage (13). Oxidative stress also leads to the dysfunction of important intracellular organelles, such as the mitochondrial inner membrane that functions in the oxidative phosphorylation process, which leads to mitochondrial dysfunction (14,15). Studies have demonstrated that oxidative stress is also an important intracellular messenger, activating various intracellular signaling pathways (16,17) and mediating cell stress responses and injury responses. Oxidative stress is an important pathogenic factor influencing the recovery of short- and long-term function following renal transplantation (18-20). The progression of CAN may be alleviated according to the characteristics of its different stages and the application of suitable antioxidants.

Cur is a yellow, acidic phenol that is extracted from *Curcuma longa* L., also termed Turmeric, is one member of ginger family (21). Cur is the major active ingredient that has a pharmacological role. Cur is a type of plant polyphenol that exhibits a wide range of biological activities, including anti-oxidative, anti-inflammatory, anticancer, anti-atherosclerotic, antimutagenic and immunoregulatory effects (22). Cur has antioxidant capacity in neutral and acidic environments, and interferes with cell signaling at various levels and affects biological enzyme activity, angiogenesis and cell adhesion (23,24). It was also reported in a preclinical study that Cur influences gene transcription and induces apoptosis (25). In rat kidneys, Cur alleviates damage caused by nephrotoxic substances, including doxorubicin, cyclosporine, gentamicin, chloroquine and ischemia-reperfusion injury due to its antioxidant properties (26-28). The potential of Cur in the prevention and treatment of diabetic nephropathy is primarily based on its antioxidative (29,30) and antifibrotic (31) properties; to the best of our knowledge, its role in inflammatory lesions has not previously been reported. Cur is reported to inhibit the activity of lipoxygenase and cyclooxygenase (32), reduce free radicals generated from the arachidonic acid pathway, suppress xanthine oxidase activity (33), decrease adenosine metabolism of free radicals and interfere with nitric oxide synthase activity (34) to reduce the arginine metabolism of nitric oxide caused by the generation of free radicals, thereby limiting oxygen free radical damage and exhibiting a protective role. Cur may function in the antioxidant process through various mechanisms (35,36): Cur and its derivatives inhibit metal ion ( $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ )-induced lipid peroxidation, inhibits oxidative modification of low density lipoprotein and protects DNA from oxidative lipid damage; Cur inhibits the production of reactive oxygen species and scavenging free radicals (including  $\cdot\text{OH}$ ,  $\text{DPPH}\cdot$  and  $\text{O}_2^-$ ) and peroxide. Cur reduces serum and tissue lipid peroxide levels and enhances superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity; Cur inhibits NADPH oxidase expression and the

activation of xanthine oxidase 8; and Cur inhibits the synthesis of nitric oxide or accelerates its clearance so that levels in the kidney are low to prevent nitric oxide toxicity. The effect of oxidative stress on chronic CsA renal injury in rats and the effect of Cur on renal injury have not been widely reported. Therefore, the present study aimed to provide an experimental basis for the further development and application of the natural active substance, Cur.

## Materials and methods

**Drugs.** Cur, with a purity of 99.8%, was purchased from Shijiazhuang spring letter Biotechnology Co., Ltd. (Shijiazhuang, China; <http://www.sjzcxswkj.com>) and CsA was obtained from Zhejiang Ruibang Pharmaceutical Co., Ltd. (Wenzhou, China).

**Primary reagents.** Rabbit anti-human Bax primary antibody and horseradish peroxidase-conjugated goat anti-rabbit secondary antibody were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Rabbit anti-human Bcl-2 primary antibody and  $\beta$ -actin primary antibody were purchased from Abcam (Cambridge, UK). SOD (cat no. A001-3), malondialdehyde (MDA; cat no. A003-1), GSH-Px (cat no. A005), reactive oxygen species (ROS; cat no. E004) and catalase (CAT; cat no. A007-1-1) detection kits were all purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents were of domestic and analytical grade.

**HK-2 human renal cells culture.** HK-2 cells were purchased from Beijing Zhongyuan Jinqiao Biotechnology Co., Ltd. (Beijing, China). The base medium for this cell line was provided by Invitrogen; Thermo Fisher Scientific, Inc. (Waltham, MA, USA) as part of a kit: Keratinocyte serum free medium (K-SFM; kit cat no. 17005-042). This kit was supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF)). The following components were added the base medium: 0.05 mg/ml BPE-provided with the K-SFM kit; 5 ng/ml EGF-provided with the K-SFM kit. Atmosphere: air, 95%; carbon dioxide ( $\text{CO}_2$ ), 5%, 37.0°C and humidity 70-80%.

**Detection of oxidative stress in HK-2 human renal cells.** HK-2 cells ( $2 \times 10^4$  cells/well) were assigned to the following groups for 48 h at 37°C in a 5%  $\text{CO}_2$  incubator: Control (equivalent volume of saline), 50  $\mu\text{M}$  Cur, 2  $\mu\text{M}$  CsA, 10  $\mu\text{M}$  Cur + 2  $\mu\text{M}$  CsA, 50  $\mu\text{M}$  Cur + 2  $\mu\text{M}$  CsA, 100  $\mu\text{M}$  Cur + 2  $\mu\text{M}$  CsA and 5  $\mu\text{M}$  N-acetyl-L-cysteine (NAC; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Cells were washed with PBS, collected in a test tube with 1 ml normal saline and lysed with an ultrasonic crusher. After centrifugation at 12,000 x g for 10 min at 4°C, SOD activity and MDA levels in the supernatant were measured by SOD and MDA kits, a GSH-Px detection kit was used to measure GSH-Px activity, an ROS detection kit was used to measure ROS levels and a CAT detection kit was used to determine CAT activity.

**MTT assay.** HK-2 cells in logarithmic growth phase were inoculated in 96-well plates ( $5 \times 10^3$  cells per well), added with

different concentrations of Cur (0.1, 1, 10, 100 and 1,000  $\mu\text{M}$ ) and then co-incubated with or without (2  $\mu\text{M}$ ) for 24 h at 37°C. After incubating at 37°C for 4 h with 20  $\mu\text{l}$  MTT (5 mg/ml), 200  $\mu\text{l}$  DMSO was added. After 10 min vibration, the absorbance value of the mixture was measured at 560 nm using a microplate reader.

**Western blot analysis.** HK-2 cells in the control and different treatment groups were washed three times with cold PBS and lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China; cat. no. P0013B) on ice. Lysates were collected and centrifuged at 12,000 x g for 10 min at 4°C, the supernatants were collected and protein concentration was determined by a BCA assay. A 10% SDS-PAGE gel was prepared and samples were loaded (80  $\mu\text{g}$  protein per lane), and run on the gel. After 2 h of SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes. Subsequently, blocking with 5% non-fat milk was performed for 1 h at room temperature and the membranes were incubated with diluted primary antibodies, including rabbit anti-human Bax (cat. no. 5023; 1:1,500 dilution), anti- $\beta$  actin antibody (cat no. ab227387; 1:2,000 dilution) and Bcl-2 (cat. no. ab194583; 1:2,000 dilution) antibodies overnight at 4°C. The membrane was washed three times with 1X TBS 0.1% Tween-20 and then the horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (cat. no. 7074; 1:4,000) was added and incubated with the membrane at room temperature for 2 h. Grey scale and area of the protein band were analyzed by gel imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with enhanced chemiluminescent kit (SuperSignal; Pierce, Thermo Fisher Scientific, Inc.; cat no. 34094). The protein expression level was indicated by the integral grey value (D, density) using Image J software (National Institutes of Health, USA).

**Apoptosis assay.** HK-2 cells treated with medium or different drugs for 48 h were collected and treated according to the Annexin V-FITC Apoptosis Detection kit (Vazyme Biotech; cat no. a211-01). After washing twice with PBS, cells were suspended with 1X binding buffer, added with 5  $\mu\text{l}$  of fluorescein isothiocyanate labeled Annexin V and 5  $\mu\text{l}$  of PI, and then mixed gently. After incubation at room temperature for 15 min, the cells were analyzed by flow cytometry. A total of 20,000 cells were analyzed each time.

**CAN rats model.** Male Sprague-Dawley rats (n=60; 7 weeks of age), clean grade and weighing 200-250 g, were purchased from the Experimental Animal Center of Changzhou Cavens Laboratory Animal Co., Ltd. (Changzhou, China) and kept under controlled conditions (temperature 23 $\pm$ 1.5°C, relative humidity 40-60%, 0.03-0.04% CO<sub>2</sub>, 12/12 light/dark cycle)- at the Animal Center of Ningbo University (Ningbo, China). Rats were allowed free access to drinking water and food. The present study was approved by the animal ethical committee of the Medical School of Ningbo university. The rats were randomly divided into four groups according to their body weight, with n=6 per group: Control group, Cur group (30 mg/kg/day) (37)-, CsA group (20 mg/kg/day) and Cur (30 mg/kg/day) + CsA (20 mg/kg/day) group, all of which were administered for 21 days. The control group received 0.9%

Table I. Effect of Cur on CsA-induced renal dysfunction in rats.

Group	BUN, mmol/l	Serum	
		Crea, $\mu\text{mol/l}$	Ccr, ml/min
Cur	4.77 $\pm$ 0.52	53.12 $\pm$ 5.32	0.22 $\pm$ 0.08
CsA	10.47 $\pm$ 1.45 <sup>c</sup>	68.33 $\pm$ 10.12 <sup>b</sup>	0.17 $\pm$ 0.11 <sup>a</sup>
Cur+CsA	6.68 $\pm$ 0.91 <sup>b,e</sup>	57.98 $\pm$ 8.96 <sup>a,d</sup>	0.18 $\pm$ 0.09 <sup>d</sup>
Control	4.88 $\pm$ 0.71	49.62 $\pm$ 6.23	0.22 $\pm$ 0.06

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01 and <sup>c</sup>P<0.001 vs. control group; <sup>d</sup>P<0.05 and <sup>e</sup>P<0.001 vs. CsA alone group. n=6 per group. Cur, curcumin; CsA, cyclosporine A; BUN, blood urea nitrogen; Crea, creatinine; Ccr, Crea clearance rate.

sodium chloride injection (4 ml/kg/day, intragastric) + vegetable oil (2 ml/kg/day, subcutaneous), the Cur group received Cur (30 mg/kg/day, intragastric) + vegetable oil (2 ml/kg/day, subcutaneous), the CsA group received 0.9% sodium chloride injection (4 ml/kg/day, intragastric) + CsA (20 mg/kg/day dissolved in vegetable oil, subcutaneous) and the CsA + Cur group received Cur (30 mg/kg/day, intragastric) + CsA (20 mg/kg/day dissolved in vegetable oil, subcutaneous).

**Renal function test.** On the 22nd day, 24 h urine of rats was collected by a metabolic cage. The rats were anesthetized with ether and 3.0-4.0 ml blood was collected to obtain serum by centrifugation at 1,400 x g for 30 min at 4°C. Serum/urine creatinine (Crea) and blood urea nitrogen (BUN) levels were measured with a Hitachi Model 7060 automatic biochemical analyzer, and creatinine clearance (Ccr) was calculated as follows: Ccr=[urine Crea concentration x1 h urine volume (ml)]/serum Crea concentration.

**Histopathological examination.** On the 22nd day, rat kidneys were fixed with 5% formaldehyde at 4°C for 48 h to prepare paraffin blocks and tissue sections (5  $\mu\text{m}$ ) following dehydration. For histological investigation, the deparaffinized and rehydrated rat kidney tissue sections were stained with hematoxylin for 15 min and 1% eosin for 3 min at room temperature by the Hematoxylin and Eosin Staining kit (Beyotime Institute of Biotechnology; cat no. C0105). For periodic acid-Schiff (PAS) staining, the slides were immersed in periodic acid solution for 10 min, and then immersed in Schiff's solution for 30 min at 20°C after being rinsed with distilled water 4 times using a Periodic Acid Schiff (PAS) Staining kit (cat no. ab150680). After rinsing slides under hot running tap water, the slides were stained with hematoxylin for 3 min, rinsed in running tap water for 3 min and applied the bluing reagent for 30 sec. Finally, the distilled water rinsed slides were dehydrated through graded alcohols. Expression of NF- $\kappa$ B in kidney tissue was detected by immunohistochemistry. In brief, tissues in paraffin sections were treated with the following procedures, including dewaxing, incubation with anti-NF- $\kappa$ B p65 antibody (cat no. ab16502; 1:1,000 dilution) for overnight at 4°C and horse radish peroxidase-conjugated goat anti-rabbit secondary antibody (CST; cat no. 7074) for

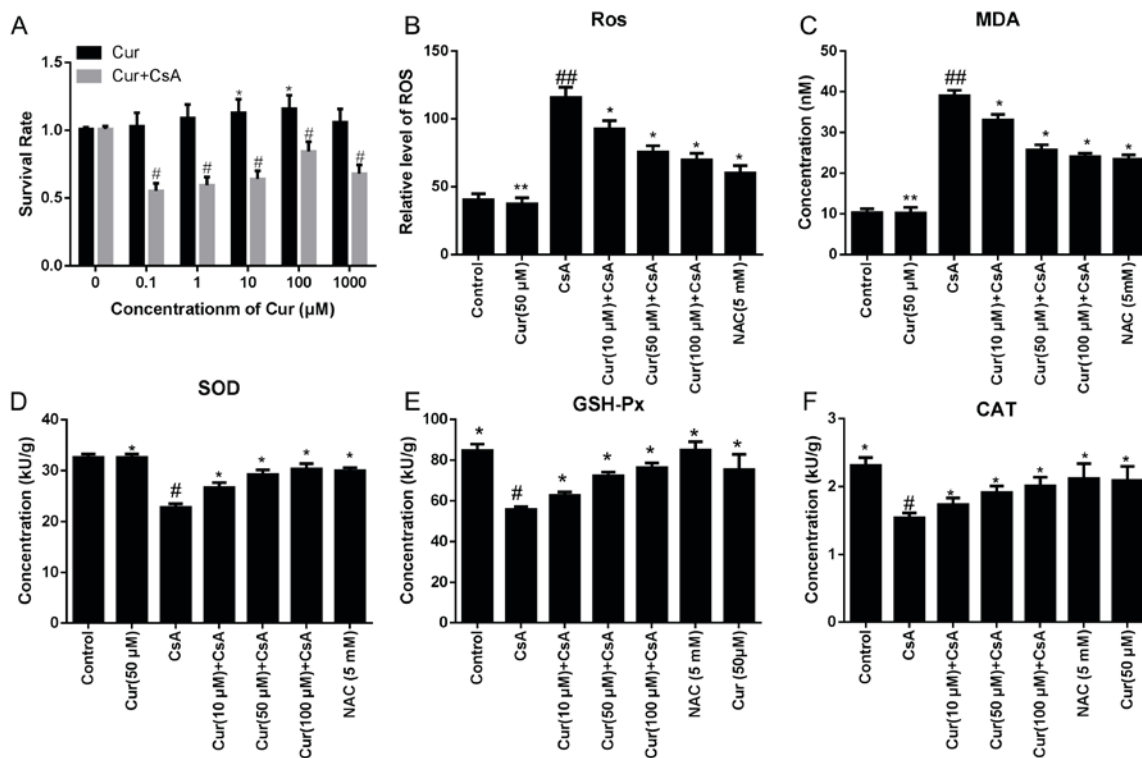


Figure 1. Effect of different concentrations of Cur on HK-2 cell viability, oxidative stress-associated factors and antioxidant enzyme activity. (A) An MTT assay was performed to determine the effect of Cur on the survival rate of HK-2 cells. (B) Effect of Cur on the production of ROS in HK-2 cells. (C) Effect of Cur on the production of MDA in HK-2 cells. (D) Effect of Cur on SOD activity in HK-2 cells. (E) Effect of Cur on the activity of GSH-Px in HK-2 cells. (F) Effect of Cur on the activity of CAT in HK-2 cells. # $P < 0.05$  and ## $P < 0.01$  vs. control cells; \* $P < 0.05$  and \*\* $P < 0.01$  vs. CsA only-treated cells. Cur, curcumin; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; CsA, cyclosporine A; NAC, N-acetylcysteine.

2 h at room temperature, stained with DAB and mounted with anti-fade oil. All sections were observed at  $\times 200$  magnification on a light microscope (37).

**Statistical analysis.** All statistical analyses were performed with PASW Statistics 18.0 software (SPSS, Inc., Somers, USA). Data are presented as the mean  $\pm$  standard deviation. One-way analysis of variance and the Tukey post-hoc test were used to analyze the significance between the groups.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

### *Cur alleviates oxidative stress in CsA-treated HK-2 cells.*

*In vitro*, a concentration of  $100 \mu\text{M}$  Cur improved cell viability when treated with CsA, compared with lower concentrations of Cur (Fig. 1A). The levels of ROS and MDA were significantly downregulated in a dose-dependent manner following treatment with Cur, compared with the CsA alone group, as demonstrated in Fig. 1B and C. The ROS level in the CsA alone group was  $115.67 \pm 7.66$  KU/g, while levels in the  $100 \mu\text{M}$  Cur + CsA group were  $71.67 \pm 5.35$  KU/g ( $P < 0.01$ ; Fig. 1B). MDA levels in the CsA alone group were  $39.01 \pm 1.36$ , while levels in the  $100 \mu\text{M}$  Cur + CsA group were  $23.85 \pm 1.22$  nM ( $P < 0.01$ ; Fig. 1C). In addition, the activity of SOD was increased in the  $100 \mu\text{M}$  Cur + CsA group ( $22.76 \pm 0.66$  KU/g in the CsA alone group vs.  $32.6 \pm 0.66$  KU/g in the  $100 \mu\text{M}$  Cur + CsA group;  $P < 0.05$ ; Fig. 1D) and the GSH-Px activity

was also increased following treatment with  $100 \mu\text{M}$  Cur ( $55.65 \pm 1.45$  in the CsA alone group vs.  $76.04 \pm 2.07$  KU/g in the  $100 \mu\text{M}$  Cur + CsA group;  $P < 0.05$ ; Fig. 1E). Furthermore, CAT activity was increased in the  $100 \mu\text{M}$  Cur-treated group compared with the CsA alone group ( $1.54 \pm 0.07$  in the CsA alone group vs.  $2.31 \pm 0.12$  KU/g in the  $100 \mu\text{M}$  Cur + CsA group;  $P < 0.05$ ; Fig. 1F). CsA-induced increases in ROS and MDA, and decreases in SOD, GSH-Px and CAT activity, were dose-dependently reversed by treatment with Cur (Fig. 1B-F).

**Effects of Cur on Bax and Bcl-2 protein expression.** The results in Fig. 2A demonstrate that the rate of apoptosis was gradually decreased with increasing concentrations of Cur in HK-2 cells, compared with the CsA alone group, which indicated that Cur may inhibit CsA-induced cell apoptosis. The ratio of Bax/Bcl-2 is an important indicator in the balance between cell apoptosis and survival. The ratio of Bax/Bcl-2 was decreased when CsA was combined with Cur treatment, compared with the CsA alone group, as demonstrated in Fig. 2B and C, which indicates that levels of the anti-apoptotic protein Bcl-2 were higher compared with the proapoptotic protein Bax, thus indicating that Cur may improve cell survival in CsA-treated HK-2 cells.

**Effect of Cur on CsA-induced CAN model in rats.** To further evaluate the protective effect of Cur in a CsA-induced rat nephrotoxicity model, renal histopathological analysis was performed. Histopathological results are presented in Fig. 3. Hematoxylin and eosin staining demonstrated that the tissue

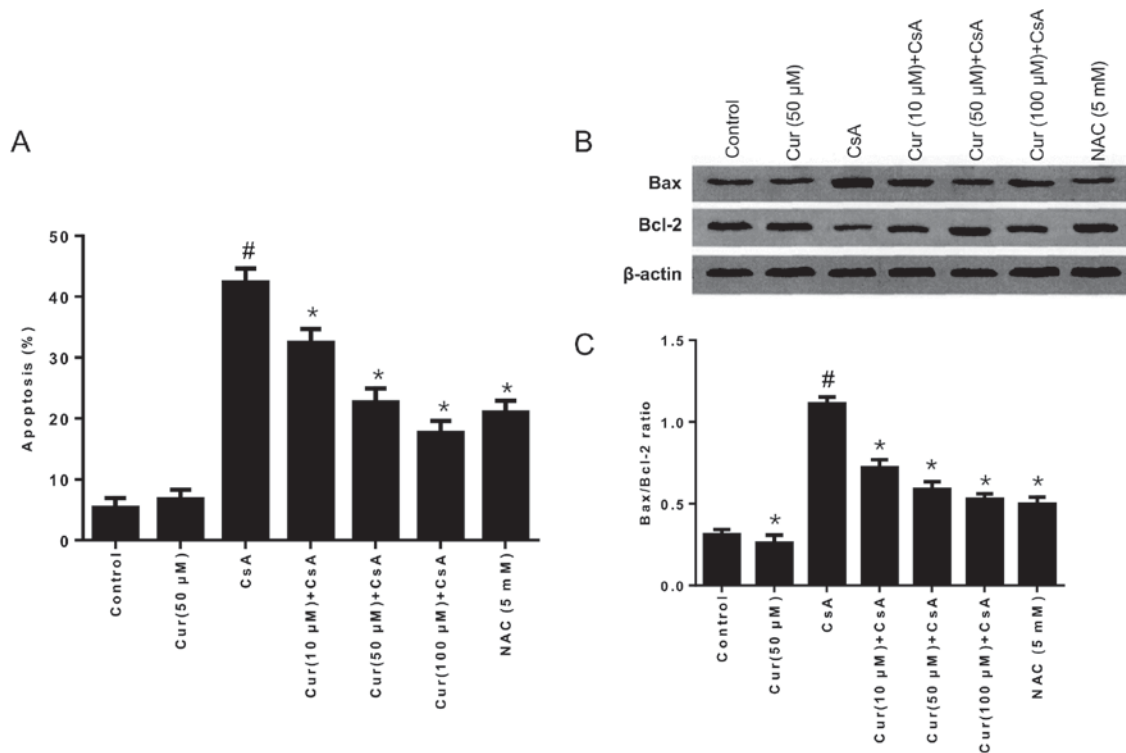


Figure 2. Effect of different concentrations of Cur on HK-2 cell apoptosis and the expression of apoptosis-associated proteins. (A) Effect of Cur on the apoptosis of HK-2 cells induced by CsA. (B) Effect of Cur on the protein expression of Bcl-2 and Bax in HK-2 cells. (C) Densitometric analysis was performed to determine the effect of Cur on the Bax/Bcl-2 ratio in HK-2 cells induced by CsA. <sup>#</sup>P<0.05 vs. control cells. <sup>\*</sup>P<0.05 vs. CsA only-treated cells. Cur, curcumin; CsA, cyclosporine A; Bax, Bcl-2-associated X; NAC, N-acetylcysteine.

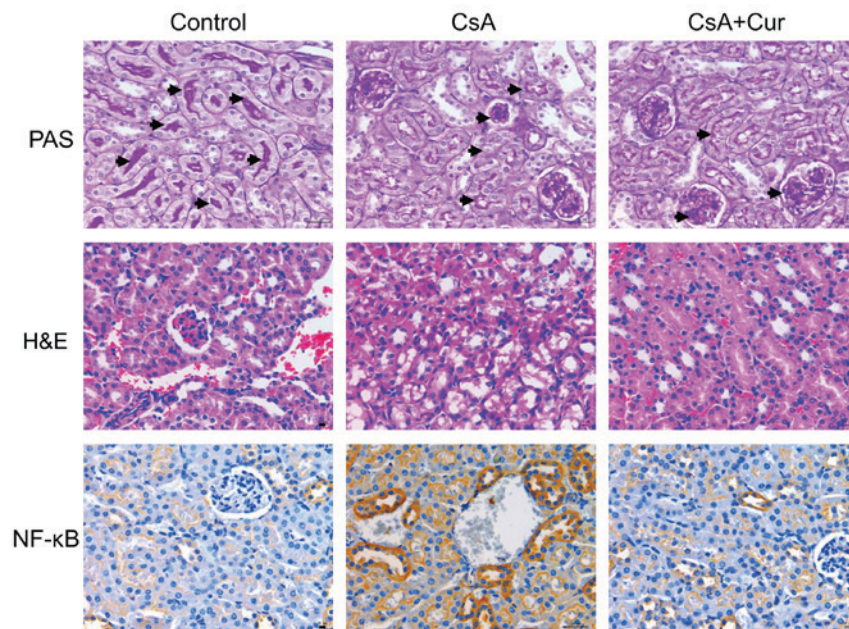


Figure 3. Effect of Cur on the CsA-induced CAN rat model. Renal PAS staining indicated that renal fibrosis was alleviated following administration of Cur in rats treated with CsA. Arrow, positive PAS staining. Renal H&E staining indicated that renal structure was improved following administration of Cur in CsA-treated rats. Immunostaining of NF-κB indicated that Cur reduced the inflammatory response in CsA-treated rats. Scale bar, 30 μm. Cur, curcumin; CsA, cyclosporine A; CAN, CsA-induced nephrotoxicity; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; NF-κB, nuclear factor-κB.

sections of the control group exhibited normal structure. By contrast, the kidneys of rats treated with CsA exhibited marked histological changes, cellular edema, vacuolar deformation

and dissolution. These changes were reduced when CsA was combined with Cur treatment. The nucleus presented blue and the glomerular basement membrane was purple. The

glomerular volume was markedly increased in the CsA group, with mesangial matrix hyperplasia (positive PAS staining), glomerular swelling, an irregular morphology of certain glomeruli, lobulated capillary loops and a markedly increased renal capsular space. NF- $\kappa$ B-positive cell cytoplasm or nuclei were stained brown. The results demonstrated that NF- $\kappa$ B-positive cells, and therefore inflammation, were markedly increased in the CsA group compared with the control group, which was reduced when CsA was combined with Cur treatment.

**Renal function in Cur-treated CAN rats.** As demonstrated in Table I, renal failure was induced by continuous administration of CsA (20 mg/kg/day) for 21 days. The serum Crea and BUN were significantly elevated and the Ccr rate was significantly reduced ( $P < 0.05$ ) in the CsA alone group compared with the control group. Cur (30 mg/kg/day) alone did not significantly reduce renal function in rats compared with the control group, however, Cur significantly improved CsA-induced renal failure.

## Discussion

CsA is a cyclic polypeptide composed of 11 amino acids. As a potent immunosuppressive agent, CsA specifically acts on lymphocytes and inhibits the synthesis and release of lymphokines, such as interleukin-2 (38). Quiescent lymphocytes are in the G0 phase of the cell cycle and the early G1 phase. Animal experiments have demonstrated that CsA extended the survival time of allogeneic organ transplantation and inhibited the cell-mediated immune response (39). CsA-induced dose-dependent renal toxicity is the primary reason for its limited clinical application, which results in renal tubular atrophy, vacuolar degeneration and renal failure (40). In the present study, rats were given CsA for 21 consecutive days, and the results demonstrated marked nephrotoxicity, with increased levels of Crea and BUN, reduced Ccr and marked vacuolar degeneration and tubular atrophy in the kidney. A previous study demonstrated that CsA-induced increases in ROS, leading to cell membrane lipid peroxidation, is one potential mechanism of CsA-induced nephrotoxicity, while another study reported that CsA induced *in vivo* inducible nitric oxide synthase expression, resulting in high concentrations of nitric oxide and ROS generation, and increased free radical activity (2). Peroxynitrite, a powerful free radical, has been reported to regulate the tricarboxylic acid cycle, mitochondrial function, electron transfer and affect DNA synthesis, resulting in increased pathological damage (34,41). The results of the present study, from experiments on HK-2 cells, also confirmed that CsA treatment significantly increased the levels of MDA and Bax protein expression, and decreased SOD activity and Bcl-2 expression. Cur is an excellent hydrogen or neutron donor and, during redox reactions generated as a result of excessive free radicals, the body converts free radicals into phenolic oxygen free radicals, which protects the organism against free radical damage. In the body, Cur reacts with free radicals to generate more stable phenolic oxygen free radicals, thereby inactivating free radicals (27). In addition, Cur has been reported to exhibit a protective effect against CsA-induced nephrotoxicity in rats, as demonstrated by histological alterations and Glutathione S-transferase immune expression (42). Tirkey *et al* (42) reported that, through its

antioxidant activity, Cur effectively salvaged CsA-induced nephrotoxicity. Furthermore, Hu *et al* (43) demonstrated that the protective effect of Cur may be mediated by inhibiting the hypermethylation of the klotho promoter. The present study confirmed that Cur significantly increased renal antioxidant capacity and decreased the Bax/Bcl-2 ratio in CsA-treated HK-2 cells, which may be associated with the improvements in CsA-induced renal failure and renal tubular deformation and cell vacuolization following Cur treatment in rats. In addition, Tirkey *et al* (42) demonstrated that Cur markedly reduced elevated levels of thiobarbituric acid reactive substances, significantly attenuated renal dysfunction and increased the levels of antioxidant enzymes in CsA-treated rats and normalized the altered renal morphology. The development of renal diseases has been previously associated with NF- $\kappa$ B activation. NF- $\kappa$ B regulates the transcription of inflammatory factors in mesangial and tubular epithelial cells; therefore, it has a central role in the development and progression of renal disease (44). Wang *et al* (45) demonstrated that limb ischemic preconditioning-induced renoprotection in contrast-induced nephropathy may be dependent on increased renalase expression via activation of the tumor necrosis factor- $\alpha$ /NF- $\kappa$ B pathway. Renalase may contribute to the renal protective effect of delayed ischaemic preconditioning via the regulation of hypoxia-inducible factor-1 $\alpha$  (45,46). In conclusion, the present study demonstrated that Cur exhibited a protective effect on CsA-induced renal dysfunction, and the underlying mechanism may be associated with the antioxidant capacity of Cur in experimental animals. However, further investigation is required to determine whether Cur may be used as a clinical adjuvant to reduce renal toxicity induced by CsA.

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