**Cordyceps Sobolifera** Extract Ameliorates Lipopolysaccharide-Induced Renal Dysfunction in the Rat

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Abstract: **Cordyceps Sobolifera** (CS), an economic traditional Chinese herb, may ameliorate nephrotoxicity-induced renal dysfunction in the rat via antioxidant, anti-apoptosis, and anti-autophagy mechanisms. We investigated the water extract of fermented whole broth of CS on lipopolysaccharide (LPS)-induced renal cell injury *in vitro* and *in vivo*. CS effect on LPS-induced epithelial Lilly pork kidney (PK1) and Madin-Darby canine kidney epithelial (MDCK) cell death was detected with MTT assay. Two-month treatment of CS effects on renal blood flow (RBF), glomerular filtration rate (GFR), plasma blood urea nitrogen, creatinine level and leukocytes (WBC) count were determined in the LPS-treated rats. We further examined the effects of CS supplement on renal tubular oxidative stress, endoplasmic reticulum stress, apoptosis and autophagy by Western blot analysis. LPS dose-dependent PK1 and MDCK cell death, which can be ameliorated by CS treatment. LPS significantly decreased RBF and GFR and increased blood leukocyte counts, plasma blood urea nitrogen and creatinine level in the rat after 24 hours of injury. LPS enhanced renal tubular ER stress, autophagy and apoptosis via by increase protein expressions of GRP78, caspase 12, Beclin-1 and Bax/Bcl-2 ratio. These findings are associated with the significant
staining in renal proximal and distal tubular ED-1, GRP78, Beclin-1 autophagy, and TUNEL apoptosis in the LPS-treated kidneys. Two months of CS supplement significantly improved RBF, GFR and WBC values and reduced ED-1, GRP78, Beclin-1 autophagy and TUNEL apoptosis in the LPS-treated kidneys. Long-term CS treatment reduced LPS-induced stress responses and tissue damage possibly via blocking LPS-triggered signaling pathways.

**Keywords**: Cordyceps Sobolifera; Reactive Oxygen Species; Acute Renal Failure; Lipopolysaccharide; Rat.

**Introduction**

There are about 260 species of *Cordyceps* according to biological classification (Jiang, 1996). Among these, *Cordyceps sobolifera* (CS) is one of the economic traditional Chinese herbs and is as famous as *Cordyceps sinesis*. Based on the traditional Chinese medicine, the therapeutic effects of *Cordyceps* are described as including nutritious materials, enhancing physical strength, reducing of fatigue syndrome like calm and sleep, acting as an analgesic and antipyretic agent, regulating the immune system, anti-oncogenesis and improving renal function (Russell et al., 2008). A previous report indicated that CS treatment could improve the clearance of creatinine and blood urea nitrogen (BUN) as well as the decrease in urinary protein (Jin et al., 2005). CS (30 g) also can prevent the progression of chronic renal failure in early or intermediate stage in humans (Jin et al., 2005). In addition, CS may play an improving role in reduction of tubulointerstitial lesions, which may be contributed to the damage of nephron tubular Na⁺/K⁺ pump, cell-mediated oxidation to endothelium of vessel and tubular cell, and decreased renal blood flow (Wang et al., 2000). A possible mechanism for renal protection might be that pre-treatment of CS attenuates glomerulosclerosis by activating urokinase type plasminogen activator which in turn by decreasing the accumulation of extracellular matrix (Jin et al., 2005). Wang et al. (2006) indicated that CS significantly decreased fibronectin and collagen IV in primary culture of human embryonic mesangial cells. However, all these studies are preliminary and limited by language (published in Chinese). In the international literature, there is no report of CS, particularly its chronic protective effects on nephrotoxicity-induced acute renal failure.

Toxicity like lipopolysaccharide (LPS) or ischemic/hypoxic status increased oxidative stress production contributing to abnormal signal transduction, cellular dysfunction and cascade of autophagy, apoptosis and necrosis (Chien et al., 2001; 2007; Ho et al., 2009; Yeh et al., 2010). The alteration in caspase-dependent apoptosis pathway, an endoplasmic reticulum (ER) stress response, and Beclin-1/LC-3-dependent autophagy has been suggested to play a role in the tissue injury (Chien et al., 2007; Yeh et al., 2010). In this study, we investigated whether CS can protect the kidney after endotoxin insult. The cell lines, the epithelial Lilly pork kidney (PK1) cell and Madin-Darby canine kidney epithelial (MDCK) cell, from proximal and distal tubular culture of kidney, respectively, were used for examining the location of protection of CS after lipopolysaccharide (LPS) treatment in vitro. Renal blood flow (RBF), glomerular filtration rate (GFR), and white blood cell (WBC) counts were analyzed for renal and immune functions in the rat in vivo. Autophagy,
apoptosis and ER stress were further evaluated in the damaged kidney by Western blot and immunostains.

**Methods and Materials**

**CS Extract Preparation**

CS water extract was purchased from Biotechnology Center of Grape King Inc. (Chung-Li City, Taiwan). Briefly, *Cordyceps sobolifera* GK 0156 (BCRC 37801) was grown on PDA medium in a petri dish, and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture. The seed culture was grown in 2.0 L flask containing 1.0 L of base medium at 22°C on a rotary shaker incubator at 80 rpm for three days. The 1.0 L flask inoculated into a 200 L fermentor (Bio Top) agitated at 60 rpm with an aeration rate of 0.5 vvm at 25°C for three days. After then, the 200 L fermentor was put into a 5 Ton fermentor for three days. The 5 Ton fermentation product was heated at 100°C for one hour and then centrifuged at 5000 g for ten minutes. The cultured broth was then concentrated under vacuum and freeze-dried to powder form.

**Cell Culture and Cytotoxicity Assay**

Porcine kidney epithelial cells (PK1) and Madin darby canine kidney (MDCK) cells were grown in 100 mm diameter tissue culture dishes for three days in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 IU/ml), streptomycin (100 μg/ml) at 37°C under 5% CO₂ air. When cells grew into 80% confluence, cytotoxicity assays were performed.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow tetrazole purchased from Sigma, is reduced to purple formazan in living cells. Therefore, we used MTT assay to evaluate the viability (cell counting) spectrophotometrically by using a microtiter plate reader. We explored the effects of CS extract (1.5 mg/200 μl) treatment on PK1 and MDCK cell viability after 24 hours of low (25 μg/ml) and high doses (250 μg/ml) of LPS injury. After CS/LPS treatment, PK1 and MDCK cells were incubated with 0.5 mg MTT/ml medium at 37°C for one hour, the supernatants were then discarded and the metabolized MTT was solubilized with 400 μl DMSO, and color development was measured at 570 nm.

**Animal Preparation**

Female Wistar rats weighing 293 ± 10 g were housed at the Experimental Animal Center, National Taiwan University, at a constant temperature and with a consistent light cycle (light from 0700 to 1800 hours). Animal care and the experimental protocol were in accordance with the guidelines of the National Science Council of the Republic of China (NSC, 1997). All efforts were made to minimize both animal suffering and the number of animals used throughout the experiment. We used intraperitoneal lipopolysaccharide
(LPS; Sigma, St Louis, MO, USA) from Escherichia coli O127:B8 for induction of renal injury in the rat. Thirty rats were separated into three groups, including control, LPS and LPS + CS groups (n = 10 each). In addition to normal diet supplied by the Animal Center to the control and LPS groups, animals of the LPS + CS group were fed with CS over two months, while those in the control and LPS groups CS was replaced by water. The rats were fed with 2 ml CS powder suspension (75 mg/ml H2O) or water by way of oral feeding in the morning of each day. The rat was administered LPS (20 mg/kg body weight) via peritoneal injection 24 hours before the onset of each study. After LPS injury, rectal temperature of the rat was measured, followed by anesthetized with urethane (1.2 g/kg body weight), and then trachea was intubated using PE-240 tubing. The femoral artery and vein were cannulated (PE-50) for blood collection and administration of 6 ml inulin solution, respectively. The inulin solution contains NaCl 0.3 g/dl H2O and inulin 25 mg/ml. The left ureter and renal artery were exposed via a lateral incision of the back for cannulation with one silastic catheter (SILASTIC® medical grade tubing, 0.305 mm ID, 0.635 mm OD) and mounting of an ultrasonic probe (1RB series, Transonic System Inc). The probe was connected to a transonic flowmeter (T206, Transonic System Inc) for measurement of renal blood flow (RBF). The inulin infusion (50 μl/min) was started after femoral vein cannulation. At the end of 30 minutes baseline period after surgery, two blood (0.5 ml/each time) and urine samples were collected at each 30 minutes interval.

**White Cell Count**

To avoid red blood cell interference, 20 μl of blood was added to 0.38 ml dilution fluid for lysing the erythrocyte. The dilution fluid contained 2 ml glacial acetic acid and 1–2 drops of 1% methylene blue in 98 ml distilled water. White cell counts in 10 μl samples were counted manually by using a hemocytometer.

**Measurement of GFR**

The concentration of inulin in the plasma and urine was determined by Anthrone reaction. Briefly, to prepare one liter of anthrone reagent, 500 ml of concentrated sulfuric acid was diluted with 250 ml of water, and the mixture was cooled to room temperature. Anthrone (4 g; Sigma, I2255) was dissolved in 250 ml of concentrated sulfuric acid and added to 750 ml of diluted sulfuric acid. Twenty μl plasma or urine (diluted for ten times) was mixed with 260 μl H2O, 20 μl ZnSO4-7H2O (10%), and 20 μl NaOH (0.5N) shaking for 30 min for protein removal. After shaking, 80 μl NaOH (4N) was added to the mixture and boiled for 15 min and centrifuged for 10 min at 15,000 rpm. Afterward, the anthrone solution (0.2 ml) was added to supernatant fluid (0.8 ml) and mixed well. The mixture was heated in a 75°C water bath for 5 min. The mixture was cooled at room temperature and read at 630 nm in a spectrophotometer. The inulin concentration was measured by using a standard curve (from 16 to 128 μg/ml). GFR was calculated according to the following equation: 

\[
GFR = \frac{(Ux \times V)}{Px}
\]

\(Ux\) is the urine concentration of inulin, \(V\) is urine volume per minute, and \(Px\) is the concentration of inulin in plasma. Arterial blood was collected for
renal functional determination. Blood urea nitrogen (BUN) and plasma creatinine were analyzed using a commercial kit from Sigma. After sacrifice with KCl, the kidney was removed and divided into two parts. One part was stored in 10% neutral buffered formalin for immunocytochemical assay, and another was quickly frozen in liquid nitrogen and stored at −70°C for protein isolation.

Renal Tissue Protein Extraction and Western Blotting

The Western blotting method was described previously (Yeh et al., 2010). The renal tissues were homogenized and lysed in RIPA lysis buffer (20 mM MOPS pH 7.0, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% nonidet P-40, 1% deoxycholic acid and 0.1% sodium dodecyl sulfate (SDS) at 4°C. The protein concentration was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). The lysates (30 μg/ lane) were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) in 12% gels and electroblotted onto a nitrocellulose membrane (Hybond™-C Extra; Amersham Biosciences Corp., Hong Kong, China). The expression levels of autophagy related protein, Beclin-1 and LC3, apoptosis-related protein Bax, Bcl-2, caspase 3 and poly-(ADP-ribose)-polymerase (PARP) fragments, and ER stress related markers GRP78 and caspase 12 were analyzed by Western blot in kidney samples. Primary antibodies raised against mouse anti-human Bcl-2 (Transduction, Bluegrass-Lexington, KY), Bax (Chemicon, Temecula, CA), the activation fragments of caspase 3 (CPP32/Yama/Apopain, Upstate Biotechnology, Lake Placid, NY), PARP (N-terminal peptide from the p85 fragment, Promega, Madison, WI, USA), GRP78 (Santa Cruz Biotechnology, CA, USA), caspase 12 (Santa Cruz), Beclin-1 (AnaSpec, Inc., San Jose, CA, USA), LC3 (MLB), and β-actin (Clone AC-74, Sigma) were used. All of these antibodies cross-react with the respective rat antigens. The samples were then treated with horseradish peroxidase-conjugated secondary antibodies at room temperature for two hours. After washing, the membrane was developed using SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemical Co., Rockford, IL, USA). The density of the band with the appropriate molecular mass was determined semi-quantitatively by densitometry using an image analyzing system (Alpha Innotech, San Leandro, CA).

In situ Demonstration of ED-1, Apoptosis and Autophagy Formation

LPS might enhance renal apoptotic and autophagic cellular death and monocyte/macrophage (ED-1) infiltration in rats. We performed apoptosis (Chien et al., 2001), autophagy (Chien et al., 2007) and ED-1 (Yu et al., 2005) staining in the paraffin-embedded sections of renal tissue. For this oxidative stress measurement, the rats (n = 3 in each group) were sacrificed at the end of LPS injury. Renal sections were deparaffinized, rehydrated, and stained immunohistochemically for presence of an autophagy marker of Beclin-1 diluted at 1:50. The percentage of Beclin-1/autophagy was calculated as Beclin-1-stained area/total area ×100% and analyzed by Adobe Photoshop 7.0.1 image software analysis.

The method for the terminal deoxynucleotidyl transferase-mediated nick-end labeling method (TUNEL) was performed as described previously (Chien et al., 2001). Sections of
the kidney were stained by methyl green and the TUNEL-avidin-biotin-complex method. Twenty high-power \((\times 400)\) fields were randomly selected, and the value of apoptotic cells/apoptotic cells and methyl green stained cells was counted. The number of apoptotic cells was expressed per 100 of the tubular cells in each section.

For renal monocyte/macrophage (ED-1) staining, the tissue sections were incubated overnight at 4°C with a mouse anti-rat antibody to ED-1 (CD68, 1:200, Serotec, Sydney, NSW, Australia). A biotinylated secondary antibody (Dako, Botany, NSW, Australia) was then applied followed by streptavidin conjugated to HRP (Dako). The chromogen used was Dako Liquid diaminobenzene (DAB). Twenty high-power \((\times 200)\) fields were randomly selected for each kidney section, and the value of ED-1 positive cells was counted.

**Semi-Quantitative Analysis of Tissue Slide**

The kidneys were cut using coronal or transverse section and then stained with hematoxylin and Eosin (H&E) in Taipei Institute of Pathology. We observed the damage of proximal and distal tubules of renal cortex in ten fields randomly. The degree of renal tubule necrosis was scored by a grading scale of 0–3 according to the method modified from Jablonski et al. (1983). Briefly, we characterized cytopathologic change as follows: tubular cell lost, tubular or cell swelling, exfoliated tubular cells in tubular lumen, pyknotic nuclei, and apical blebbing from the surface of tubular cells. We evaluated ten fields at random and characterized a score among three ranges: minimal was 0–10%, moderate was 11–20%, and severe was 21–30% of renal tissue change.

**Statistical Analysis**

All values are expressed as mean ± SE. For comparisons of group data, one-way analysis of variance and then the Student’s unpaired \(t\)-test were conducted. \(p < 0.05\) was considered to indicate statistical significance.

**Results**

In the *in vitro* cellular study, PK1 and MDCK cells were treated with low and high dosages of LPS in both groups for 24 hours. LPS decreased the viability of PK1 cells in a dose-dependent manner (Fig. 1 left panel). CS pre-treatment significantly improved the decreased viability of the PK1 cells. In MDCK cells, LPS also dose-dependently decreased the viability of MDCK cells (Fig. 1 right panel). CS pre-treatment also ameliorated the decreased viability of MDCK cells. The cell viability study implies that CS can protect PK1 and MDCK cells against LPS-induced injury.

The *in vivo* study showed that LPS treatment significantly decreased renal blood flow and GFR, and increased leukocyte counts in the blood when compared to the control group (Fig. 2). Two months of CS pre-treatment significantly restored LPS depressed RBF and GFR and reduced the leukocyte counts in the blood. LPS treatment significantly impaired the renal tubular structure (18% of total section, Fig. 3B) when compared to the control.
Figure 1. The effects of CS extract treatment on PK1 and MDCK cell viability after 24 hours of LPS injury. LL: low dose of lipopolysaccharide (25 μg/ml); HL: high dose of lipopolysaccharide (250 μg/ml); LL + CS: low dose of lipopolysaccharide with CS (1.5 mg/200 μl) treatment; HL + CS: high dose of lipopolysaccharide with CS (1.5 mg/200 μl) treatment; n = 5; mean ± SE. *p < 0.05 compared to the control group. (A) p < 0.05 compared to the LL group. (B) p < 0.05 LL + CS vs. LL group. (C) p < 0.05 L + CS vs. HL group.

Figure 2. The effects of CS on renal function and WBC change in rats treated with LPS. C: control group, LPS: lipopolysaccharide, CS: Cordyceps Sobolifer, n = 6, mean ± SE. *p < 0.05 compared to the control group. #p < 0.05 between LPS + CS and LPS group.
tissue (0.5%, Fig. 3A). For most cases, the major damage is the occurrence of blebbing cells and exfoliated cells in the tubular lumen. CS pre-treatment significantly attenuated LPS-induced injury to a degree of 7% of total section (Fig. 3C). The mean change of renal damage in three groups of kidneys is displayed in Fig. 3D. LPS-induced renal dysfunction was also indicated by the increase in BUN (Fig. 3E) and creatinine (Fig. 3F). CS pre-treatment significantly decreased LPS-enhanced BUN and creatinine level.

The expression of apoptosis-related proteins (Bax, Bcl-2, caspase 3 and PARP), ER stress proteins (GRP78 and caspase 12) and autophagy-related proteins (Beclin-1 and LC3) was explored in the kidneys of control, LPS and LPS plus CS pretreated groups. After 24 hours, LPS significantly increased proapoptotic signaling pathway (increased Bax/Bcl-2 ratio, caspase 3 and PARP expression) (Fig. 4A), enhanced ER stress GRP78 and caspase 12 expression (Fig. 4B) and increased Beclin-1 and LC3 protein expression (Fig. 4C) in the kidneys. CS pre-treatment significantly reduced the expression of apoptosis-related, ER stress-related and autophagy-related proteins after LPS stimulation. We used immunocytochemical stain with ED-1 to evaluate monocyte/macrophage infiltration, GRP78 to examine ER stress, TUNEL to evaluate apoptosis formation, and Beclin-1 to evaluate autophagy production in the kidneys. LPS significantly and consistently enhanced ED-1, GRP78, TUNEL and Beclin-1 expression in the kidneys (Fig. 5). Two months of CS treatment decreased all these parameters in the damaged kidneys.
Discussion

CS contains many components, including Cordycepin, adenosine, polysaccharides, cordycepic acid and lots of proteins. It is not known which components play roles in the improvement of RBF or GFR in this study. Our data showed that CS pre-treatment for two months ameliorated LPS-induced RBF and GFR reduction. To our knowledge, most of compositions in CS have not been reported to alter the RBF and GFR except adenosine. Yao et al. (2000) indicated that adenosine decreases systemic blood pressure and GFR by inducing contraction in afferent glomerular artery. Therefore, adenosine may not play a role

Figure 4. Comparison of (A) apoptosis-related proteins, (B) endoplasmic reticulum stress-related proteins, and (C) autophagy-related proteins in the control (Co), LPS (L) and LPS + CS (L + C) treated kidneys. LPS significantly increased renal Bax/Bcl-2 ratio, caspase 3 and PARP in (A) the apoptotic pathway, (B) GRP78 and caspase 12 in the endoplasmic reticulum stress, and (C) Beclin-1 and LC3 protein expression in the autophagy pathway. CS treatment significantly reduced LPS-enhanced proteins expression of the kidneys. *p < 0.05 when compared to Co group. #p < 0.05 L + Cs vs. L group.

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The active component in CS extract for renal protection requires to be explored in future.

In both cell lines and animal model, the doses of LPS used were rather high. The discrepancy for this dosage may be due to the low toxicity of LPS in its origin. We used

![Figure 5](image.png)

**Figure 5.** Effect of CS on LPS-induced leukocyte infiltration (ED-1 indicated with brown color), endoplasmic reticulum stress (GRP78 indicated with brown color), apoptosis (TUNEL indicated with brown color) and autophagy (Beclin-1 indicated with brown color) in the rat kidney. LPS treatment increased ED-1 (B), GRP78 (E), TUNEL (H) and Beclin-1 (K) expression when compared to the control stain of ED-1 (A), GRP78 (D), TUNEL (G) and Beclin-1 (J) in the control kidney. CS pre-treatment significantly attenuated ED-1 (C), GRP78 (F), TUNEL (I) and Beclin-1 (L) stain in the LPS-treated kidney.

in the mechanism of CS-increased RBF and GFR. The active component in CS extract for renal protection requires to be explored in future.

In both cell lines and animal model, the doses of LPS used were rather high. The discrepancy for this dosage may be due to the low toxicity of LPS in its origin. We used
1.5 mg/200 μl of CS on LPS-induced PK1 and MDCK cell injury because we found that this dosage of CS is safe and did not affect PK1 and MDCK cell viability in baseline culture condition. According to our data, CS > 1.5 mg/200 μl impaired PK1 cells, whereas CS > 3.0 mg/200 μl damaged MDCK cells. The dosage of 150 mg/per rat (250–300 g in body weight) was selected because previous clinical reports (Wang et al., 2000; Jin et al., 2005; Wang et al., 2006) had used 15–30 g of CS for therapeutic application in humans.

In this study, we found that LPS increased the WBC counts, however, long-term CS pre-treatment attenuated LPS-enhanced WBC counts. Increased total WBC value in the blood by LPS stimulation may increase the number of polymorphonuclear leukocytes (PMN) which possibly contribute to inflammation, septic responses and renal dysfunction (Ge et al., 2009). Cohen et al. (1990) demonstrated that there is a decrease in GFR and renal function in the isolated kidney perfused with the serum-free perfusate containing LPS but not with serum-free perfusate alone. Evidence has also shown that there is a significant decrease of GFR in the perfusion with serum-free perfusate plus PMN, implying a participation of PMN in reduction of GFR (Linas et al., 1992). This extra-corporeal study might partially explain the relationship between GFR and leukocyte counts in our study.

ISP-1 (myriocin) might be the cause of CS to reduce the leukocyte counts. The speculation is supported by the evidence that it is a component extracted from fungal metabolites (Fujita et al., 1994) and from CS (Yu et al., 2008). FTY 720, which is a derivative from ISP-1, is effective in blocking egress from both thymus and lymph nodes in lymphocyte traffic. FTY 720 significantly decreased PMN percentage in bronchoalveolar lavage and ameliorated necrotizing pancreatitis-associated lung injury (Liu et al., 2008a). Also, it was found that CS could attenuate cytotoxic T cell line proliferation (Miyake et al., 1995). In our study, we found an increase of ED-1 positive cells in the LPS treated kidney, however, CS pre-treatment reduced the ED-1 cells, indicating an anti-inflammatory capability by CS extract.

In this study, LPS significantly impaired the renal tubular structure. The impairment of renal tissue may be induced by the enhancement of ER stress, apoptosis, or autophagy. In contrast, CS treatment caused a significant decrease in the ratio of Bax/Bcl-2 when compared to the LPS group. Basically, the rise in Bcl-2 and drop in Bax would prevent cytochrome C to move outside from the mitochondria, reduce the increased amount of reactive oxygen species (ROS), and thus attenuate programmed cell death in renal tubules (Chien et al., 2007). There is limited literature to evaluate the LPS effect on ER stress. According to our data, LPS increased the GRP78 and caspase 12 protein expressions and GRP78 immunostain in the kidney, indicating an increased ER stress by LPS. Chronic CS treatment reduced GRP78 and caspase 12 protein expressions and GRP78 stain in the kidney. Autophagy and apoptosis concomitantly occurred in the damaged distal tubule and proximal tubular cells of post-ischemia/reperfusion kidney (Chien et al., 2007). The enhanced response of apoptosis and autophagy to LPS injury primarily found in the renal cortex and outer medulla was diminished by long-term CS pre-treatment. In this investigation, 2 months of CS supplement could attenuate LPS-induced apoptosis, autophagy, ER
stress and ED-1 infiltration possibly by the upregulation of anti-apoptotic protein and downregulation of proapoptotic and autophagic proteins expression.

ER stress and oxidative stress are known to contribute to apoptotic cell death in response to several stresses (Hwang et al., 2008). Small interfering RNA-mediated inhibition of ER stress and inhibition of ER stress marker induction resulted in a significant decrease in apoptosis (Hwang et al., 2008). ER stress induced apoptosis in the diabetic kidney because three hallmarks of ER-associated apoptosis, C/EBP homologous protein, c-JUN NH2-terminal kinase and caspase 12, were upregulated in the diabetic kidney (Liu et al., 2008b). In renal tubules, Bcl-2 is localized in mitochondria and ER and upregulation of Bcl-2 significantly inhibited cisplatin- or ATP depletion-induced tubular apoptosis (Bhatt et al., 2008). In our LPS model, ER molecular stress chaperone GRP78 was increased, caspase 12 protein expression was enhanced and also increased level in autophagy-Beclin-1/LC3 and apoptosis-PARP protein expressions. These data suggest that LPS-induced ER stress, autophagy and apoptosis signaling in the damaged rat kidneys. We found that chronic CS treatment significantly attenuated ER stress, autophagy and apoptosis in the renal tubules subjected to LPS insults, leading to the improvement of renal dysfunction.

In conclusion, our study found that CS inhibited or reduced LPS-induced stress responses and tissue damage. The multiple beneficial effects of CS might be due to its blocking of LPS-triggered signaling pathways, thus reducing the subsequent variety of stress responses and renal tissue injury.

Acknowledgments

This work was supported partly by the National Taiwan University Hospital (NTUH-99-S-1391) and by the National Science Council of the Republic of China (NSC 98-2320-B-002-043-MY3). We thank Ms. Hung Yu-Kuen and Tsai Ching-I for technical support. We also thank Ms. Liu Fei-Yun for the consultation of white cell count.

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