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Acute renal injury induced by endotoxic shock in rats is alleviated via PI3K/Nrf2 pathway

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Abstract. – OBJECTIVE: To explore the effect of PI3K/Nrf2 pathway on acute kidney injury (AKI) induced by endotoxic shock in rats by construction of the endotoxic shock rat model.

MATERIALS AND METHODS: A total of 30 Sprague Dawley (SD) rats were randomly assigned into three group, namely the control group (group C), endotoxic shock model group (group L) and wortmannin + endotoxic shock model group (group WL), with 10 rats in each group. Pathological lesions in renal tissues were evaluated by histological score of kidney (HSK). Biochemical indicators including blood urine nitrogen (BUN), creatinine (Cr) and urinary α1-microglobulin (α1-MG) in renal tissues were accessed. Activities of superoxide dismutase (SOD) and malondialdehyde (MDA) were detected by relative commercial kits. Expression levels of Nrf2, Heme oxygenase 1 (HO-1) and Akt in renal tissues were determined by Western blot and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), respectively.

RESULTS: HSK, levels of BUN, Cr and α1-MG and activities of SOD and MDA were significantly increased in group L comparing to those in group C ($p<0.05$). The above-mentioned indicators were also remarkably higher in group WL than those of group L ($p<0.05$). There were significant differences in expression levels of Nrf2, HO-1 and Akt between group L and group WL ($p<0.05$). In particular, lower mRNA levels of Nrf2 and HO-1, as well as protein levels of p-Akt, Nrf2 and HO-1 were observed in group WL compared with those in group L ($p<0.05$).

CONCLUSIONS: The present study showed that AKI induced by endotoxic shock in rats was regulated through PI3K/Nrf2 pathway. HO-1 acts as the effector protein, might serve as an essential factor in protecting AKI induced by endotoxic shock.

Key Words
PI3K/Nrf2 pathway; Acute kidney injury; Endotoxic shock

Introduction

Endotoxin is an exogenous pyrogen released after bacterial disintegration, which is also known as lipopolysaccharides (LPS). LPS activates the macrophage system and plasma protein system, thereby causing a series of pathological reactions[1,2]. Activated macrophage further releases a large amount of proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), which in turn lead to fever, increased vascular permeability and circulatory dysfunction. Overdose of LPS stimulates a variety of immune cells and thus leads to endotoxin shock, which is characterized as bleeding, disseminated intravascular coagulation (DIC), hypotension, hypoxia, metabolism acidosis, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS)[3]. Acute kidney injury (AKI) is a common complication induced by endotoxic shock. The mechanism of AKI, however, is unknown. It has been reported that the mortality rate of AKI caused by endotoxic shock is as high as 74.5%. Therefore, AKI is considered to be an independent risk factor for predicting the development and prognosis of endotoxemia[4,5]. Scholars have suggested that oxidative stress, decreased renal blood flow, renal tubular circulation disorder, cell apoptosis, mitochondrial dysfunction and inflammatory response may explain the pathogenesis of AKI[6,7]. Although great advances have been made on AKI treatment, effective treatments are still lacking[8]. It is of great importance in clarifying the mechanism of AKI induced by endotoxic shock, so as to provide more effective treatments.

PI3K, a lipid kinase that is widely expressed in various types of cells, activates downstream genes by phosphorylating phosphatidylinositol

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family members on the cell membrane[9,10]. Akt, also known as protein kinase B (PKB), is a threonine/serine (Thr/Ser) protein kinase, which is also an essential downstream factor in PI3K pathway. PI3K is activated when cells are stimulated by the internal and external environment. Subsequently, 3-phosphoinositide dependent protein kinase1 (PDK1) and Akt are simultaneously located on the cell membrane to promote cell proliferation and inhibit apoptosis[12-14]. Nrf2, as a key factor in cellular oxidative stress, is overexpressed in metabolic and detoxifying organs. It interacts with ARE to regulate the transcription and expression of antioxidant proteins, detoxification enzymes and proteasome chaperones. Under normal physiological conditions, cytoplasmic Nrf2 binds to Keap1 (Kelch-like ECH-associated protein 1) in an inactive state. However, Nrf2 dissociates from the Nrf2-Keap1 complex under oxidative stress and translocates into the nucleus, which in turn forms hybrid dimers with small nuclear proteins, such as JunD, Maf, c-Jun and ATF4. Heme oxygenase 1 (HO-1), catalase (CAT) and superoxide dismutase (SOD) together to resist the internal and external stimuli[15,16]. HO-1 is confirmed as heat shock protein 32, which is overexpressed when cells are stimulated by oxidative stress, cytokines, growth factors and endotoxin[17, 18]. Currently, MAPK, PI3K/Akt and PKC pathway are widely involved in activation and nuclear translocation of Nrf2[19]. Recent investigations have shown that the activation of PI3K/Nrf2 pathway may enhance the antioxidant and anti-inflammatory ability of cells, thus protecting the physiological functions of lungs, heart, kidney and liver[20-22].

In the present study, Sprague Dawley (SD) rats were randomly assigned into three group, namely the control group (group C), shock model group (group L) and wortmannin + endotoxic shock model group (group WL). Pathological lesions, biochemical indicators, oxidative stress and expressions of key factors in PI3K/Nrf2 pathway in renal tissues were detected, so as to clarify the underlying mechanism of PI3K/Nrf2 pathway in AKI induced by endotoxic shock in rats.

Materials and Methods

Experimental Animals

Male SD rats aged 8 week (180-220 g) were selected in this study. Each rat was individually housed in the environment with the temperature of 18-22°C and relative humidity of 40-70%. Food and water were supplied for one week before the experiment. Rats were denied access to food but had access to water 24 h prior to the rat model construction. This study was approved by the Animal Ethics Committee of E-Da hospital, I-Shou University Animal Center.

Totally 30 SD rats were randomly assigned into three group, namely the control group (group C), shock model group (group L) and wortmannin + endotoxic shock model group (group WL). Briefly, rats were anesthetized and placed on the sterile table at a supine position. The right carotid artery was cannulated after incision of the neck skin alongside the midline, which was then connected to a monitor to detect the vital signs. After recovering to a stable arterial blood pressure (MAP) for 30 min, rats in group WL and group C were injected with 0.6 mg/kg wortmannin and isodose normal saline, respectively. About 30 min later, 5 mg/kg LPS (diluted in 2 ml of normal saline) was injected in rats of group L and group WL. MAP was monitored during the procedure. The endotoxic shock rat model was considered to be successfully constructed when rat MAP dropped to 75% of baseline or below after LPS administration for 2 h. Rats died within 6 h after LPS administration and those without significant reduction of MAP were excluded.

Sample Collection and Preservation

After LPS (or normal saline) administration for 6 h, 5 mL of blood sample collected from the right internal carotid artery was placed in the procoagulant tube and centrifuged at 3000 rpm for 15 min at 4°C. The upper serum was collected in an EP tube and preserved in -80°C. Totally 3 mL of bladder urine was collected for the determination of BUN, Cr and α1-MG concentration using an automatic biochemical analyzer. Rats were then sacrificed for kidneys harvesting. Blood stains on the surface of renal tissues were washed with pre-cooled phosphate-buffered saline. The right kidney was fixed with 10% formalin and stained with Hematoxylin and Eosin (HE) (Beyotime, Shanghai, China) for immunofluorescence histochemistry. The left kidney was then quickly placed in cryogenic vials for subsequent determination.

Renal Histopathology

Right kidney tissue was fixed in 10% formalin for over 24 h before dehydrated by gradient ethanol, paraffin-embedded and sectioned in a 5-μm slice. Renal slice was stained with HE and observed under a CX41 light microscope. HSK was

evaluated according to the previously described method[23]. In brief, 10 high-power fields were randomly selected in each slice for injury evaluation, and the final HSK was calculated based on the average score of 10 evaluations.

Determination of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in Renal Tissues

Left kidney was prepared into homogenate, followed by centrifugation at 3500 rpm/min for 15 min. Activities of SOD and MDA were detected using the relative commercial kits (R&D Systems, Minneapolis, MN, USA).

RNA Extraction and Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR)

The mRNAs from renal tissues were extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then reversely transcribed to complementary Deoxyribose Nucleic Acid (cDNA). The reaction conditions for subsequent amplification were as follows: denaturation at 95°C for 15 s, followed by annealing at 58°C for 20 s and extension at 72°C for 15 s, for a total of 40 cycles. Each sample was repeatedly detected for 3 times. Primers sequences used in this study were as follows: HO-1, forward: 5'-GTCTACGCCCCGCTCTACTTCCCG-3', reverse: 5'-TAGCCTCTTCCACCACCCTCTGCC-3'; β-actin; forward: 5'-AAACGAGACGAGATTGGCATGGCTTTA-3'; reverse: 5'-GGGATGCTCGCTCCAACGACTGCT-3'; Nrf2, forward: 5'-TTAGTGCTTTTGAGGATTCTTTCGG-3'; reverse: 5'-AATTCTGTGCTTTCAGGGTGGTTCT -3'.

Western Blot

The total protein of the renal tissues was extracted. The concentration of each protein sample was determined by a bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, USA). A total of 50 μg total protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were then blocked with 5% skimmed milk, followed by the incubation of specific primary antibodies (p-Akt, Nrf2, HO-1) overnight at 4 °C. Membranes were then incubated with the secondary antibody at room temperature for 1 h. Immunoreactive bands were exposed by enhanced chemiluminescence (ECL) method.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 statistical software (Chicago, IL, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparison of measurement data between different groups was conducted using one-way ANOVA test, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Pathological Observation of Renal Tissues

HE staining of renal tissues showed clear nephron structure, normal glomerular morphology and complete tubular structure in renal tissues of group C. Moreover, no significant infiltration of inflammatory cells was observed in the renal interstitium of group C (Figure 1A). However, there were significant pathological lesions in the renal tissues of group L and group WL, including destructed nephron structure, contracted glomerulus, various infiltration of inflammatory cells, microthrombus formation in glomerular capillary, serious degeneration and necrosis of renal tubular epithelial cells, renal interstitial edema and haemorrhage (Figure 1B-C). HSK in group C was remarkably lower than that in group L and group WL ($p<0.05$), and HSK in group WL was the highest compare to the other two groups ($p<0.05$, Figure 1D).

Comparison of Biochemical Indicator Levels

Levels of serum BUN and Cr, and urine α1-MG were remarkably elevated in group L and group WL in comparison with those in group C ($p<0.05$). Moreover, higher levels of the above mentioned biochemical indicators were found in group WL than those of group L ($p<0.05$, Figure 2A-C).

Comparison of MDA and SOD Activities

To verify the oxidative stress in renal tissues caused by LPS, we next detected MDA and SOD activities. Our results showed that higher MDA activity was observed in group WL than that of group L and group C ($p<0.05$, Figure 2D). In addition, SOD activities in group L and group WL were remarkably lower than that in group C ($p<0.05$). Lower SOD activity was found in group WL than that of group L ($p<0.05$, Figure 2E), indicating that wortmannin was capable of increasing the LPS-induced oxidative stress.

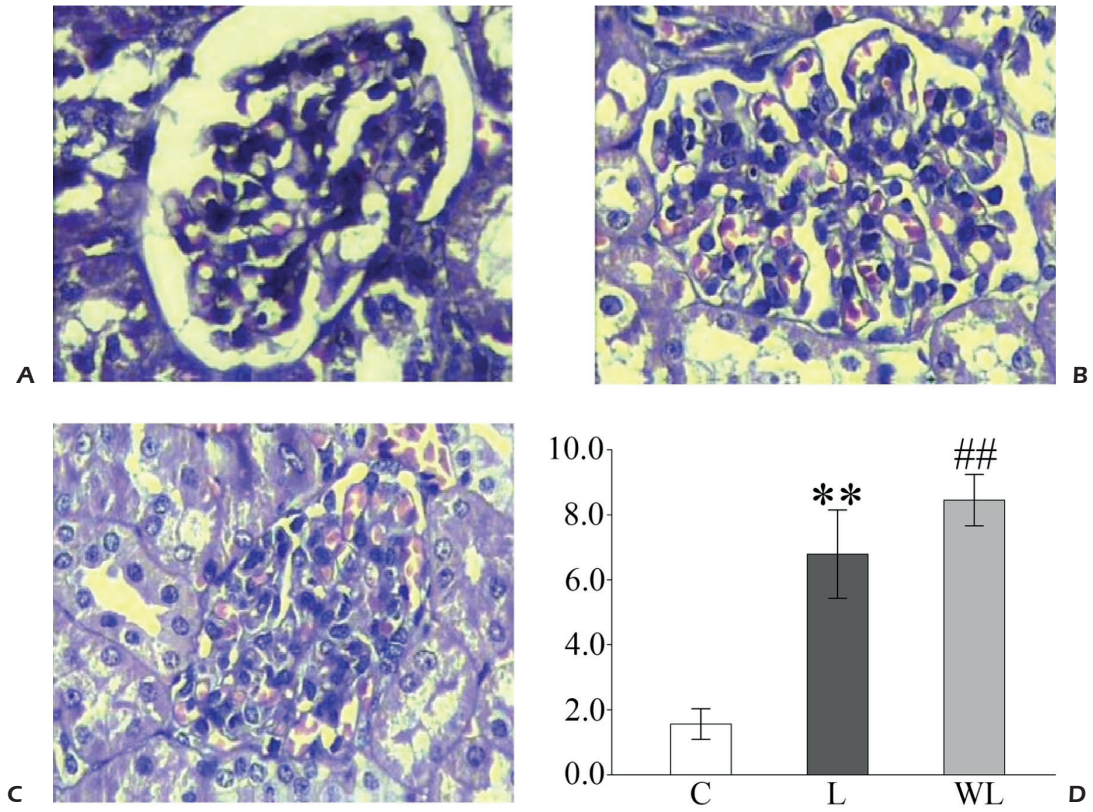

Figure 1. Pathological observation in rat renal tissues. **A**, Pathological observation in rat renal tissues of group C. **B**, Pathological observation in rat renal tissues of group L. **C**, Pathological observation in rat renal tissues of group WL. **D**, Comparison of HSK in each group (**p<0.05 *vs.* group C, $^{\#\#}p$<0.05 *vs.* group L).

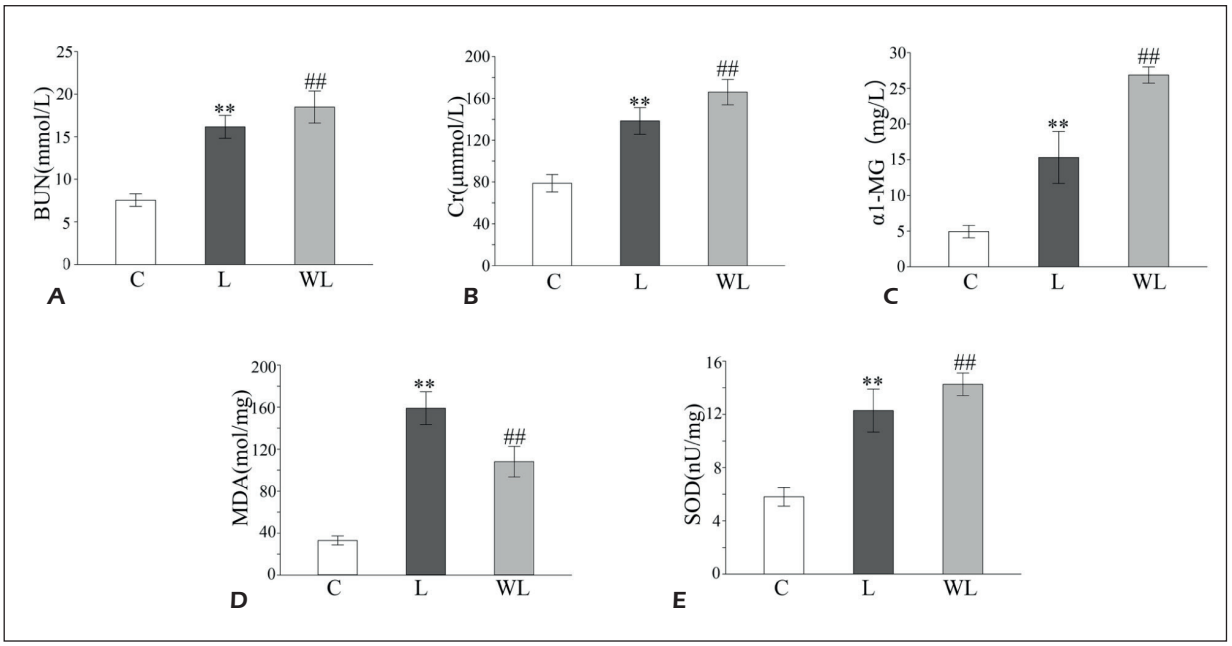

Figure 2. Comparison of biochemical indicator levels. **A**, Expression level of BUN in rats of three groups. **B**, Expression level of Cr in rats of three groups. **C**, Expression level of α1-MG in rats of three groups. **D**, MDA activity in rats of three groups. **E**, SOD activity in rats of three groups (**p<0.05 *vs.* group C, $^{\#\#}p$<0.05 *vs.* group L).

Nrf2 and HO-1 Were Involved in the Endotoxic-Induced AKI

Nrf2 and HO-1 are important antioxidant factors. Here, we detected mRNA levels of Nrf2 and HO-1 in rat renal tissues. QRT-PCR results showed that mRNA levels of Nrf2 and HO-1 were remarkably increased in group L and group WL compared with those of group C ($p<0.05$). However, lower mRNA levels of Nrf2 and HO-1 were found in group WL than those of group L, suggesting that Nrf2 and HO-1 were involved in the regulation of endotoxic-induced AKI ($p<0.05$, Figure 3A-D).

PI3K/Nrf2 Pathway Participated in the Regulation of Endotoxic-Induced AKI

To further explore the underlying mechanism of AKI, we detected key factors in PI3K/Nrf2 pathway. Western blot data showed that protein levels of p-Akt, Nrf2 and HO-1 in group L and group WL were remarkably increased compared with those of group C ($p<0.05$, Figure 4A-4C). In particular, lower protein levels of p-Akt, Nrf2 and HO-1 were found in group WL than those of group L ($p<0.05$, Figure 4D-F). These results demonstrated that wortmannin could exaggerate endotoxic-induced AKI *via* inhibiting PI3K/Nrf2 pathway.

Discussion

As one of the outer components of Gram-negative bacteria cell wall , LPS is released in the blood circulation when bacteria are dissolved or destroyed. LPS can lead to fever, septic shock, DIC, MODS, and even death. The mortality rate is as high as 70% in endotoxemia patients with AKI. Therefore, AKI is considered as an independent risk factor for predicting the progression and prognosis of endotoxemia[24-26]. LPS-induced endotoxic shock model is frequently used in *in vivo* experiments[27, 28]. In this experiment, 5 mg/kg LPS (dissolved in 2 ml of normal saline) was injected into rats. The results showed that rat MAP in both group L and group WL decreased to 75% of the baseline or

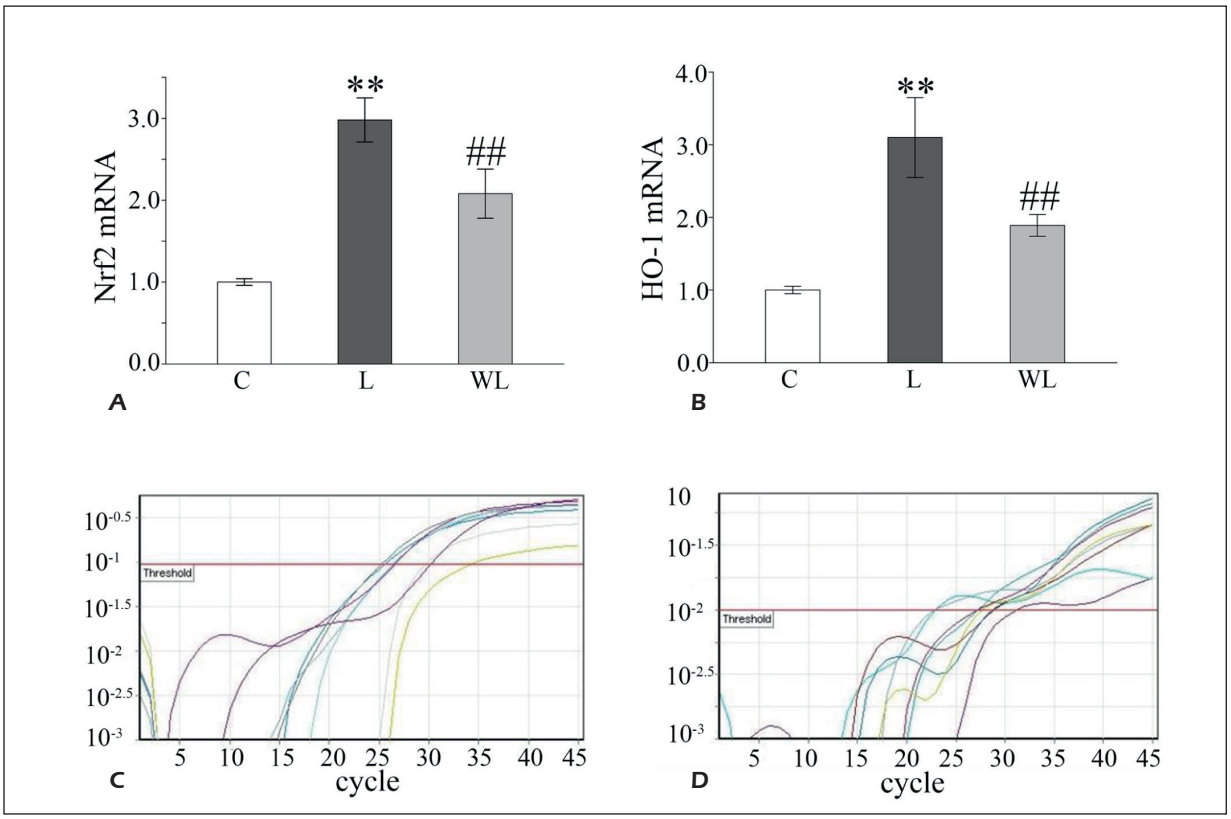

Figure 3. The mRNA levels of Nrf2 and HO-1 in renal tissues. **A**, The mRNA level of Nrf2 in renal tissues of three groups. **B**, The mRNA level of HO-1 in renal tissues of three groups. **C**, Quantitation of mRNA level of Nrf2. **D**, Quantitation of mRNA level of HO-1 (**$p<0.05$ *vs.* group C, $^{\#\#}p<0.05$ *vs.* group L).

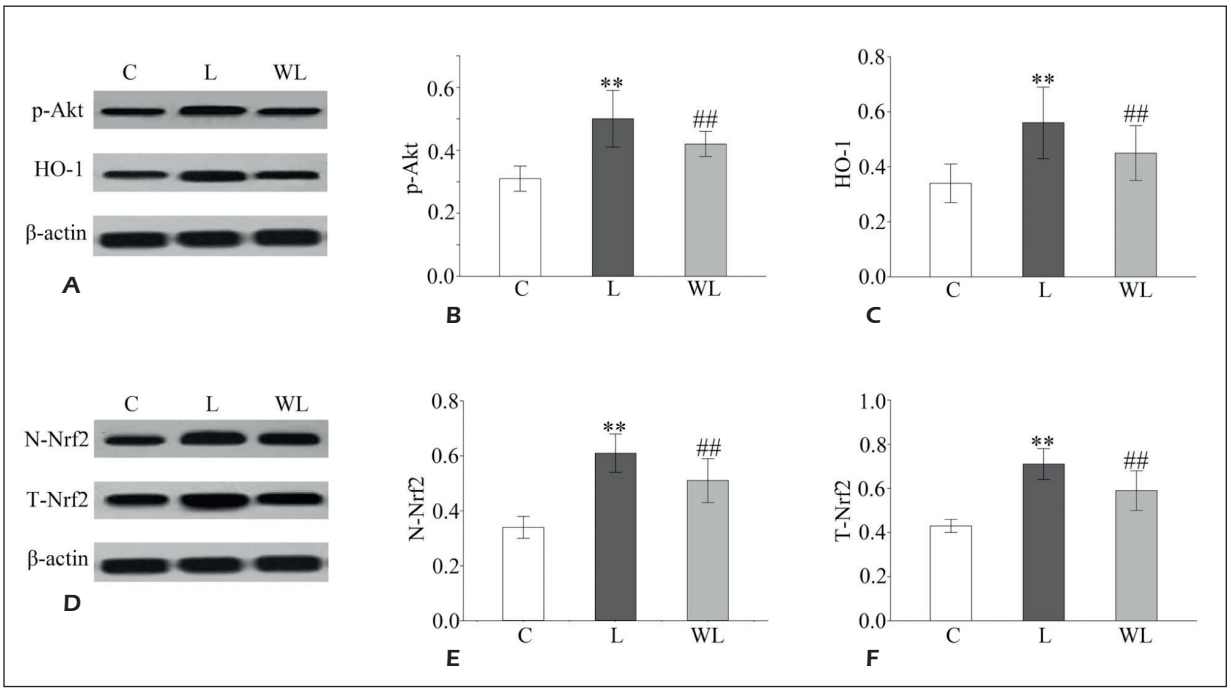

Figure 4. Protein levels of p-Akt, Nrf2 and HO-1 in renal tissues. **A**, Protein levels of p-Akt and HO-1 in renal tissues of three group. **B**, Comparison of protein level of p-Akt in three groups. **C**, Comparison of protein level of HO-1 in three groups. **D**, Protein level of Nrf2 in renal tissues of three group. **E**, Comparison of nuclear level of Nrf2 in three groups. **F**, Comparison of total level of Nrf2 in three groups (**$p<0.05$ *vs.* group C, $^{\#\#}p<0.05$ *vs.* group L).

below after LPS administration for 2 h. Additionally, lower HSK and biochemical indicator levels, as well as more severe oxidative stress was observed in rats administrated with LPS compared with those in negative controls, indicating the successful construction of rat shock model.

Wortmannin, a fungal metabolite extracted from the fungus Penicillium wortmanni, is a specific PI3K inhibitor. Functionally, wortmannin permeabilizes the cell membrane and irreversibly binds to Serine-833 in PI3K, thereby inhibiting the activation of PI3K/Akt signaling pathway[29,30]. In this study, 0.6 mg/kg wortmannin (diluted in 0.08 ml/kg solvent dimethylsulfoxide) was injected into rats in group WL.

Pathological lesions of endotoxin-induced AKI include decreased renal blood flow, cell apoptosis, circulatory disturbance of glomerular tubules, oxidative stress, mitochondrial dysfunction and inflammatory responses. Relative researches have illustrated that LPS initiates the inflammatory reaction and oxidative stress *via* NF-κB signaling pathway, thus resulting in renal damage[31]. Other studies also suggested that LPS induces the binding of endothelin (ET) to ET1 receptor in vascular smooth muscle cells and glomerular mesangial cells, which results in the reduction of renal blood flow[32].

PI3K/Akt pathway is an important survival pathway, and Nrf2-ARE is an endogenous defensive pathway[33, 34]. HO-1, as a crucial anti-oxidative effector protein in Nrf2/ARE pathway, can improve microcirculation and inhibit inflammation, oxidative stress and cell apoptosis in tissues[35,36]. Scholars have confirmed the protective effect of HO-1. For example, Li et al[37] found that pigment glycosides play a protective role in hepatotoxicity by upregulating HO-1 *via* Akt/Nrf2 pathway. Sahin et al[38] found that catechin could prevent cisplatin-induced nephrotoxic response *via* Nrf2/HO-1 pathway. Li et al[39] demonstrated that oxymatrine has a neuroprotective effect on the ischemia-reperfusion rat model by activating Nrf2/HO-1 pathway. In addition, Nrf2/ARE pathway may also regulate the inflammatory response and maintain the redox balance *via* inhibiting the NF-κB activation[40]. Hepatocyte growth factor is capable of reducing necrosis and apoptosis of epithelial cells, thereby protecting renal function through PI3K/Akt signaling pathway[41].

PI3K/Nrf2 pathway is involved in the regulation of oxidative stress *via* up-regulation of antioxidant proteins. Our results showed that HSK, biochemical indicators and oxidative stress-related indicators were increased in group L and

group WL, indicating that AKI was induced by LPS in rats. The above indicators were remarkably increased in group WL compared with those of group L, suggesting that inhibition of PI3K/Akt pathway exaggerated AKI in rats. Furthermore, we found that expressions of Nrf2 and HO-1 were increased in endotoxic-induced rat model, which were then reversed by wortmannin. Wortmannin administration further exaggerated AKI *via* inhibiting PI3K/Akt pathway. However, protein expressions of Nrf2 and HO-1 in renal tissues were not completely blocked after the inhibition of PI3K/AKT pathway, indicating that there might be other regulatory factors in renal tissues, which needs further exploration.

Conclusions

We showed that acute kidney injury induced by endotoxic shock in rats was alleviated by PI3K/Nrf2 pathway. Effector protein HO-1 might serve as an essential factor in protecting endotoxin-induced acute kidney injury.

Conflict of Interest

The authors declared no conflict of interest.

References

1) Zhang X, Xiong H, Li H, Cheng Y. Protective effect of taraxasterol against LPS-induced endotoxic shock by modulating inflammatory responses in mice. Immunopharmacol Immunotoxicol 2014; 36: 11-16.
2) Kim SJ, Lee S, Kwak A, Kim E, Jo S, Bae S, Lee Y, Ryoo S, Choi J, Kim S. Interleukin-32gamma transgenic mice resist LPS-mediated septic shock. J Microbiol Biotechnol 2014; 24: 1133-1142.
3) Li W, Zhang H, Niu X, Wang X, Wang Y, He Z, Yao H. Effects and mechanisms of cavidine protecting mice against LPS-induced endotoxic shock. Toxicol Appl Pharmacol 2016; 305: 46-54.
4) Van Biesen W, Van Massenhove J, Lameire N, Vanholder R. Does urinary neutrophil gelatinase-associated lipocalin really solve the issue of discriminating prerenal from intrinsic acute kidney injury? Kidney Int 2012; 81: 321, 321-322.
5) Liu J, Su Z, Zhang ZC. [a multiplicity of morbidity and prognostic factors in patients with septic and acute kidney injury]. Zhonghua Yi Xue Za Zhi 2011; 91: 1112-1114.
6) Pathak E, Mayeux PR. In vitro model of sepsis-induced renal epithelial reactive nitrogen species generation. Toxicol Sci 2010; 115: 475-481.
7) Tran M, Tam D, Bardia A, Bhasin M, Rowe GC, Kher A, Zsengeller ZK, Akhavan-Sharif MR, Khankin EV, Saintgeniez M, David S, Burstein D, Karumanchi SA, Stillman IE, Arany Z, Parikh SM. PGC-1alpha promotes recovery after acute kidney injury during systemic inflammation in mice. J Clin Invest 2011; 121: 4003-4014.
8) Bansal S, Wang W, Falk S, Schrier R. Combination therapy with albumin and pentoxifylline protects against acute kidney injury during endotoxemic shock in mice. Ren Fail 2009; 31: 848-854.
9) Foster FM, Traer CJ, Abraham SM, Fry MJ. The phosphoinositide (PI) 3-kinase family. J Cell Sci 2003; 116: 3037-3040.
10) Karlsson R, Engstrom M, Jonsson M, Karlberg P, Pronk CJ, Richter J, Jonsson JI. Phosphatidylinositol 3-kinase is essential for kit ligand-mediated survival, whereas interleukin-3 and flt3 ligand induce expression of antiapoptotic Bcl-2 family genes. J Leukoc Biol 2003; 74: 923-931.
11) Eisenreich A, Rauch U. PI3K inhibitors in cardiovascular disease. Cardiovasc Ther 2011; 29: 29-36.
12) Xie F, Huang Q, Liu CH, Lin XS, Liu Z, Liu LL, Huang DW, Zhou HC. MiR-1271 negatively regulates AKT/MTOR signaling and promotes apoptosis via targeting PDK1 in pancreatic cancer. Eur Rev Med Pharmacol Sci 2018; 22: 678-686.
13) Slomovitz BM, Coleman RL. The PI3K/AKT/mTOR pathway as a therapeutic target in endometrial cancer. Clin Cancer Res 2012; 18: 5856-5864.
14) Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. Mol Biosyst 2015; 11: 1946-1954.
15) Kwak MK, Wakabayashi N, Kensler TW. Chemoprevention through the Keap1-Nrf2 signaling pathway by phase 2 enzyme inducers. Mutat Res 2004; 555: 133-148.
16) Min KJ, Kim JH, Jou I, Joe EH. Adenosine induces hemeoxygenase-1 expression in microglia through the activation of phosphatidylinositol 3-kinase and nuclear factor E2-related factor 2. Glia 2008; 56: 1028-1037.
17) Calay D, Mason JC. The multifunctional role and therapeutic potential of HO-1 in the vascular endothelium. Antioxid Redox Signal 2014; 20: 1789-1809.
18) Khitan Z, Harsh M, Sodhi K, Shapiro JI, Abraham NG. HO-1 upregulation attenuates adipocyte dysfunction, obesity, and isoprostane levels in mice fed high fructose diets. J Nutr Metab 2014; 2014: 980547.
19) Guo C, Wang S, Duan J, Jia N, Zhu Y, Ding Y, Guan Y, Wei G, Yin Y, Xi M, Wen A. Protocatechualdehyde protects against cerebral Ischemia-Reperfusion-Induced oxidative injury via protein kinase Cepsilon/Nrf2/HO-1 pathway. Mol Neurobiol 2017; 54: 833-845.
20) Yoon HY, Kang NI, Lee HK, Jang KY, Park JW, Park BH. Sulforaphane protects kidneys against ischemia-reperfusion injury through induction of the Nrf2-dependent phase 2 enzyme. Biochem Pharmacol 2008; 75: 2214-2223.
21) Dai C, Yang J, Liu Y. Single injection of naked plasmid encoding hepatocyte growth factor prevents cell death and ameliorates acute renal failure in mice. J Am Soc Nephrol 2002; 13: 411-422.

22) Dobrzynski E, Montanari D, Agata J, Zhu J, Chao J, Chao L. Adrenomedullin improves cardiac function and prevents renal damage in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 2002; 283: E1291-E1298.
23) Blydt-Hansen TD, Katori M, Lassman C, Ke B, Coito AJ, Iyer S, Buelow R, Ettenger R, Busuttil RW, Kupiec-Weglinski JW. Gene transfer-induced local heme oxygenase-1 overexpression protects rat kidney transplants from ischemia/reperfusion injury. J Am Soc Nephrol 2003; 14: 745-754.
24) Hoste EA, Kellum JA. Acute kidney injury: Epidemiology and diagnostic criteria. Curr Opin Crit Care 2006; 12: 531-537.
25) Kanagasundaram NS. Pathophysiology of ischaemic acute kidney injury. Ann Clin Biochem 2015; 52: 193-205.
26) Venkatachalam MA, Griffin KA, Lan R, Geng H, Saikumar P, Bidani AK. Acute kidney injury: A springboard for progression in chronic kidney disease. Am J Physiol Renal Physiol 2010; 298: F1078-F1094.
27) Kung CW, Lee YM, Cheng PY, Peng YJ, Yen MH. Ethyl pyruvate reduces acute lung injury via regulation of iNOS and HO-1 expression in endotoxemic rats. J Surg Res 2011; 167: e323-e331.
28) Itoh K, Tong KI, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med 2004; 36: 1208-1213.
29) Wymann MP, Zvelebil M, Laffargue M. Phosphoinositide 3-kinase signalling--which way to target? Trends Pharmacol Sci 2003; 24: 366-376.
30) Wipf P, Halter RJ. Chemistry and biology of wortmannin. Org Biomol Chem 2005; 3: 2053-2061.
31) Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: Evidence for TLR4 as the Lps gene product. J Immunol 1999; 162: 3749-3752.
32) Divino JN, Chawla KS, Da SC, Bjorge AM, Brittingham A. Endothelin-1 production by the canine macrophage cell line DH82: Enhanced production in response to microbial challenge. Vet Immunol Immunopathol 2010; 136: 127-132.
33) Huang K, Chen C, Hao J, Huang J, Wang S, Liu P, Huang H. Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-beta1 in rat glomerular messangial cells. Mol Cell Endocrinol 2015; 399: 178-189.
34) Wang W, Wu Y, Zhang G, Fang H, Wang H, Zang H, Xie T, Wang W. Activation of Nrf2-ARE signal pathway protects the brain from damage induced by epileptic seizure. Brain Res 2014; 1544: 54-61.
35) Yin XP, Wu D, Zhou J, Chen ZY, Bao B, Xie L. Heme oxygenase 1 plays role of neuron-protection by regulating Nrf2-ARE signaling post intracerebral hemorrhage. Int J Clin Exp Pathol 2015; 8: 10156-10163.
36) So H, Kim H, Kim Y, Kim E, Pae HO, Chung HT, Kim HJ, Kwon KB, Lee KM, Lee HY, Moon SK, Park R. Evidence that cisplatin-induced auditory damage is attenuated by downregulation of pro-inflammatory cytokines via Nrf2/HO-1. J Assoc Res Otolaryngol 2008; 9: 290-306.
37) Li H, Wu S, Shi N, Lian S, Lin W. Nrf2/HO-1 pathway activation by manganese is associated with reactive oxygen species and ubiquitin-proteasome pathway, not MAPKs signaling. J Appl Toxicol 2011; 31: 690-697.
38) Sahin K, Tuzcu M, Gencoglu H, Dogukan A, Timurkan M, Sahin N, Aslan A, Kucuk O. Epigallocatechin-3-gallate activates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats. Life Sci 2010; 87: 240-245.
39) Li M, Zhang X, Cui L, Yang R, Wang L, Liu L, Du W. The neuroprotection of oxymatrine in cerebral ischemia/reperfusion is related to nuclear factor erythroid 2-related factor 2 (nrf2)-mediated antioxidant response: Role of nrf2 and hemeoxygenase-1 expression. Biol Pharm Bull 2011; 34: 595-601.
40) Minelli A, Grottelli S, Mierla A, Pinnen F, Cacciatore I, Bellezza I. Cyclo(His-Pro) exerts anti-inflammatory effects by modulating NF-kappaB and Nrf2 signalling. Int J Biochem Cell Biol 2012; 44: 525-535.
41) Dai C, Yang J, Liu Y. Single injection of naked plasmid encoding hepatocyte growth factor prevents cell death and ameliorates acute renal failure in mice. J Am Soc Nephrol 2002; 13: 411-422.