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Original Article

HBSP attenuates lipopolysaccharide-induced inflammatory response in human renal proximal tubular epithelial cells by induction of Nrf2 via PI3K/Akt pathway

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Abstract: The present study aimed to investigate the effects of HBSP on lipopolysaccharide (LPS)-induced oxidative stress and cell apoptosis in renal tubular epithelial cells, and explore its signaling mechanisms. Cultured human proximal tubule epithelial cell line HK-2 cells were treated with various concentrations of LPS and HBSP, and the conditioned mediums were collected. Then, cell viability and apoptosis were detected by MTT and flow cytometry; Reactive oxygen species (ROS) and lipid peroxidation were tested to study the oxidative stress response in renal injury; Western bloting was used to detect the expression of several related proteins. SPSS 14.0 was employed to perform statistical analyses. The results showed that HBSP attenuated LPS-induced injury in HK-2 cells in a dose-dependent manner. Oxidative stress was involved in the tubular dysfunction and injury, and HBSP could reversed and attenuated LPS-induced intracellular oxidative stress. Meanwhile, HBSP treatment resulted in the enhanced level of nuclear translocation and transcriptional activity of Nrf2, and further induced the enhanced expression of HO-1 and NQO1. Activation of HO-1 and NQO1 were regulated by Nrf2 via PI3K/Akt pathway. HBSP could be served as a good candidate for renal protection. It can attenuate LPS-induced oxidant damage by activating antioxidative protein via PI3K/Akt signaling pathway.

Keywords: Helix B surface peptide, lipopolysaccharide, acute kidney injury, sepsis, oxidative stress, PI3K/Akt pathway

Introduction

Sepsis is a leading predisposing factor of acute kidney injury (AKI), contributing to approximately 50% of AKI cases in critically ill patients [1, 2]. Septic AKI, a dire clinical situation, is associated with a very high hospital mortality rate, partially due to the poor understanding for the pathogenesis of AKI [3, 4]. Ample evidence has put forward apoptosis and inflammation as more critical players in the pathophysiology of sepsis-induced AKI, rather than pure necrosis [5-7]. During sepsis, inflammatory mediators derived from bacterial products as well as released by immune cells, such as lipopolysaccharide (LPS) and cytokines, could guide the immune system to fight infection and also can potentially injure the renal function [8]. LPS is the dominant cell wall molecule of Gram-negative bacteria, contributing to inflammatory responses [9, 10], and could be constantly released by leukocytes at a low level throughout infection [11]. Increasing proof demonstrates that LPS is related to the pathogenesis of septic AKI [12, 13]. Recently, LPS has been widely used as a model of experimental sepsis-induced AKI.

Oxidative stress is a hallmark of septic AKI, and this sepsis-induced oxidative stress may contribute to the development of renal dysfunction and injury [14, 15]. Biancone et al. [16] indicated that bacteria components or plasma from patients with septic AKI could prompt cultured tubular cells to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). These oxidants could lead to the degradation of cellular components (such as DNA, proteins, lip-

ids, etc.), thereby accelerating the loss of tubular function [17]. Moreover, increasing number of studies have demonstrated that renal tubular epithelium is the major target of oxidative stress, resulting in renal dysfunction as sepsis progresses [18, 19].

Erythropoietin (EPO), a critical regulator of hematopoiesis, has been proven to play a renoprotective effect in renal dysfunction and injury [20], and can slow the progression of renal injury [21]. However, the hypertension and thrombosis adverse effects of EPO *in vivo* hinder its application in clinic [22]. Therefore, helix B surface peptide (HBSP), one EPO derivative derived from the aqueous face of helix B of EPO, is developed to resolve such problem, and has been proven to exert tissue-protective activities [23].

In the present study, we hypothesized that HBSP exerted renoprotective effects in sepsis-induced AKI mediated through amelioration of oxidative stress-induced cell injury. Thus, we employed human proximal tubule epithelial cell line HK-2 cells as cell model and LPS-mediated oxidant injury as an in vitro model of septic AKI to test the antioxidant activity of HBSP in HK-2 cells and explore the underlying intracellular signaling mechanisms. This study will facilitate our better understanding of the molecule mechanisms underlying proximal tubular injury during sepsis and might contribute to the development of more efficient preventive and therapeutic strategies.

Materials and methods

Materials

HBSP (QEQLERALNSS) was purchased from Scipeptide Biotechnology, Ltd, Shanghai, China. LPS purchased from Sigma-Aldrich (St. Louis, MO, USA). MTT is from Boster (Wuhan, China). An-nexinV-FITC is from KeyGEN BioTECH (Nanjing, China). All antibodies in this study were from Bioss (Beijing, China).

Cell culture

Primary human proximal renal tubular epithelial cells (HK-2) were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle medium and Nutrient Mixture F12 (DMEM/F12) (Gibco, Eggenstein, Germany) supplemented with 10% fetal bovine serum (Gibco) at 37°C under 5% CO_2 humidified atmosphere. Cultures were re-fed with fresh media every 2 to 3 days. For experiments, cells were grown in 6-well plates. To ascertain the effects of LPS concentration, cells were firstly incubated with various concentrations of LPS for 24 hours. Then, HBSP was added under various concentrations in the presence or absence of LPS. The conditional media and cells were obtained for further analysis.

Cell viability assay

The condition-specified HK-2 cells were processed for cell viability assay by MTT test, which is a sensitive measurement of the metabolic status of cells. Briefly, HK-2 cells were plated in 96 well plates for 24 h at a density of 2×10^3 cells/well. Afterwards, cells were washed twice with phosphate-buffered saline (PBS) and then exposed to 0.5 mg/ml MTT stock solution at 37°C for 4 h. Then, MTT solution was removed and replaced with 10% dimethyl sulfoxide (DMSO) to solubilize the formazan crystals. The absorbance of each sample was measured at a wavelength of 570 nm with a microplate reader (Molecular devices, Sunnyvale, CA, USA).

Flow cytometric detection of cell apoptosis

Currently, flow cytometry is widely used to quantify apoptotic cells by determining DNA content of cells by propidium iodide (PI) staining. Briefly, cells were treated at confluence on 6-well culture dishes, harvested by trypsinization, and subsequently washed twice with PBS. Then, cells were incubated in 50 μg/ml PI in the presence of 200 μg/ml RNase for 30 min at room temperature, protected from light. The analysis was performed on Becton Dickinson FACS Calibur flow cytometer (Becton Dickinson, Heidelberg, Germany) with laser excitation at 488 nm using a band-pass filter to collect red PI fluorescence. The percentages of cells in different phases of the cell cycle were analyzed using CELLQuest software version 3.0 (Becton Dickinson).

ROS accumulation and lipid peroxidation

HK-2 cells were initially plated in 6 well plates for 24 h at a density of 5×10^6 cells/ml. To moni-

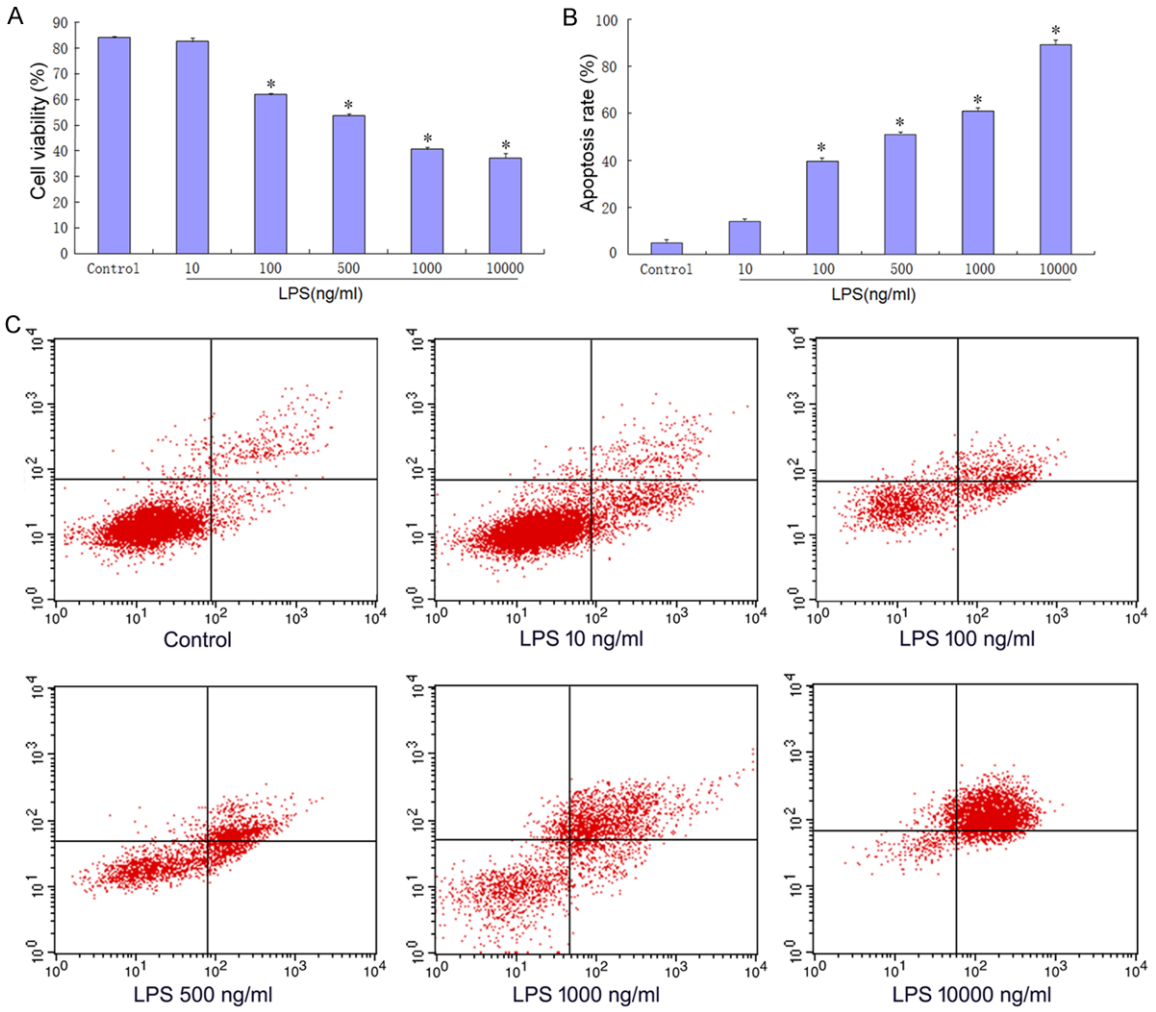

Figure 1. Dose response of HK-2 cells to various concentrations of LPS exposure. HK-2 cells were cultured in medium containing escalating doses of LPS for 24 hours. LPS exposure reduces cell viability and induces cell apoptosis in a dose-dependent manner. The results show that 500 ng/ml of LPS exposure results in moderate cell injury, and is selected for the subsequent study. A: Effect of various concentrations of LPS on cell viability by MTT assay. B: Effect of various concentrations of LPS on cell apoptosis rate by flow cytometry. C: Flow cytometric analysis of HK-2 cells stained with various concentrations of LPS. *$P < 0.05$ compared to control group.

tor intracellular accumulation of ROS, the cultured HK-2 cells were incubated with 10 μM DCFH-DA (GENios, USA) at 37°C for 30 min. Cells were subsequently washed thrice with medium to eliminate residual DCFH-DA. Fluorescence intensity of 100 μL cell suspension was quantified with a fluorometer (GENios) using 488 nm excitation and 525 nm emission filters. The results were given as percents relative to the oxidative stress of the control cells set to 100%.

Malonyl dialdehyde (MDA) is a terminal product of lipid peroxidation, which can be used to measure the content of intracellular lipid peroxidation. MDA concentration in cell homogenates was determined with commercial kits purchased from Jiancheng bioengineering Institute (Nanjing, China), using the thiobarbituric acid method. The assay was based on the conjugation ability of MDA with thiobarbituric acid, to form a red product which has maximum absorbance at 532 nm.

Western blotting

In the present study, western bloting analysis was used for the quantification of various protein expression. HK-2 cells were lysed with lysis buffer [62.5 mM Tris-HCl (pH 6.8), 10% glycer-

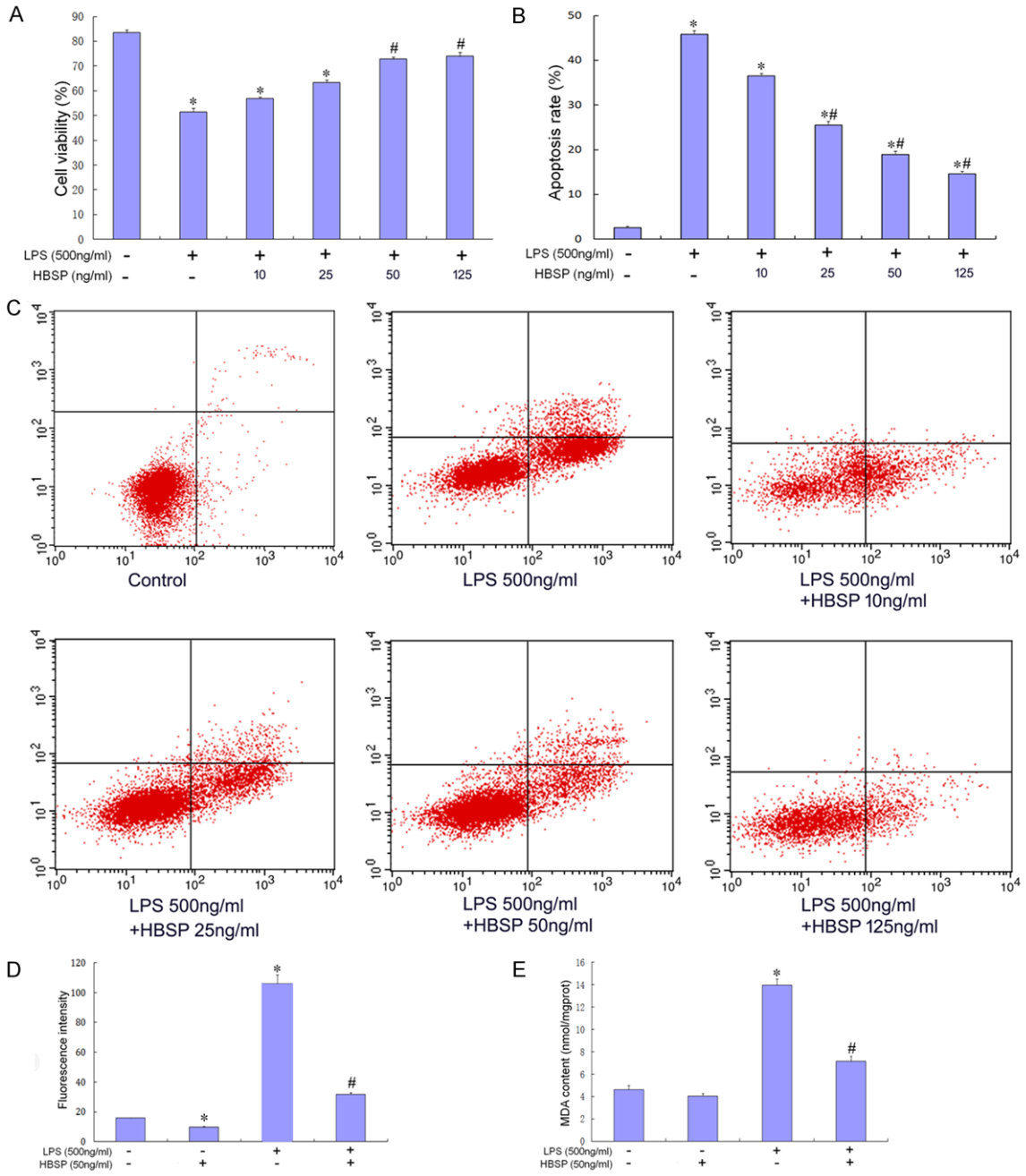

Figure 2. Effect of HBSP on cell injury caused by LPS exposure. HK-2 cells were pre-treated with HBSP for 2 h followed by 24 h of 500 ng/ml LPS. Treatment of HK-2 cells with HBSP attenuates LPS-induced cell injury. A: Dose response of cell viability to various concentrations of HBSP treatment against 500 ng/ml of LPS-induced cell viability reduction. B: Effect of various concentrations of HBSP on 500 ng/ml of LPS-induced cell apoptosis. C: Flow cytometric analysis of HK-2 cells stained with various concentrations of HBSP and 500 ng/ml of LPS exposure. The results show that pre-treatment with 50 ng/ml HBSP attenuates significantly LPS-induced cytotoxicity, and 50 ng/ml of HBSP was chosen in further experiments. D: Effect of HBSP on intracellular ROS level in HK-2 cells following LPS challenge. E: Effect of HBSP on the accumulation of lipid peroxidation induced by LPS exposure in HK-2 cells. The results show that HBSP inhibits LPS-induced ROS and MDA accumulation. *P < 0.05 compared to the untreated cells, #P < 0.05 compared to LPS group.

ol, and 2% sodium dodecyl sulfate (SDS)], and the supernatants were collected after centrifugation at 12,000 g at 4°C for 20 min. Protein concentration was determined by a BCA protein

assay kit (Sigma-Aldrich, Seelze, Germany). Equal amounts of protein (10 μg) were separated on pre-casted a 10% SDS-PAGE and electrotransferred onto the polyvinylidene fluoride membranes. The membrane was blocked by 5% skim milk in Tris-buffered saline containing 0.1% Triton X-100 (TBS-T) at room temperature for 2 h, and then incubated for 1 h with primary antibodies of AKT (1:1000), phospho-AktSer473 (1:1000), Nrf2 (1:1000), HO-1 (1:1000) and NQO1 (1:1000). The membrane was washed with Tris-buffered saline (TBS) and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:3000) at room temperature for 1 h. In this paper, enhanced chemiluminescence system (ECL, Amersham) was used for signal detection. Immunoreactive bands were scanned and densitometrically analyzed by Scion Image software (Scion, Frederick, Maryland, USA). The quantitative analyses were normalized to β-actin.

Statistical analysis

In the present study, all the data were presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using the SPSS 14.0 (SPSS Inc., Armonk, NY, USA). Statistical differences among groups were assessed using one way ANOVA analysis. $P < 0.05$ was considered as statistically significant.

Results

HBSP attenuates LPS-induced injury in HK-2 cells

In the present study, to determine the appropriate dose of exposure to LPS, we firstly incubated cells with various doses of LPS (0, 10, 100, 500, 1000, 10000 ng/ml) for 24 h. The results showed that LPS could reduce cell viability in a dose-dependent manner (**Figure 1A**), meanwhile, incubation of HK-2 cells with LPS induced dose-dependent cell apoptosis, as shown in **Figure 1B** and **1C**. Based on the cell viability and apoptosis test, treatment of HK-2 cells with 500 ng/ml of LPS resulted in moderate cell injury. Thus, 500 ng/ml of LPS was used in the subsequent study.

To evaluate the dose-dependent effects of HBSP against LPS-induced cellular injury, we pre-treated HK-2 cells with HBSP at concentration of 0, 10, 25, 50, 125 ng/ml for 2 h followed by 24 h of 500 ng/ml LPS, and tested the effects of HBSP by cell viability and apoptosis arrays. The results indicated that HBSP attenuated LPS-induced cellular injury in HK-2 cells in a dose-dependent manner. As illustrated, pretreatment with HBSP at concentration of 50 and 125 ng/ml significantly increased the viability of HK-2 cells (**Figure 2A**) and reduced the apoptosis of HK-2 cells against 500 ng/ml LPS-induced cytotoxicity (**Figure 2B** and **2C**). While relative to 50 ng/ml of HBSP, 125 ng/ml of HBSP could not present higher protective action, and higher concentration of HBSP might have somewhat side effects. Therefore, 50 ng/ml of HBSP was chosen to define the renoprotective effects on LPS-induced oxidative stress in HK-2 cells in further experiments.

HBSP inhibits LPS-induced ROS and MDA accumulation

In this work, in order to directly evaluate the effect of HBSP on LPS-induced oxidative stress, we tested the ROS production and lipid peroxidation of HK-2 cells in vitro. To achieve this, we pre-incubated HK-2 cells with 50 ng/ml of HBSP for 2 h followed by 500 ng/ml LPS for 24 h, and then incubated cells in fresh medium for 24 h. Relative to untreated cells, LPS-treated cells showed significantly high intracellular ROS level, indicating that LPS could increase ROS accumulation in HK-2 cells (**Figure 2D**). Pretreatment with HBSP significantly attenuated the increase of ROS level induced by LPS exposure in HK-2 cells (**Figure 2D**). Meanwhile, LPS exposure could significantly increase lipid peroxidation of HK-2 cells, and pre-treatment with HBSP significantly reduced LPS-induced MDA in HK-2, as shown in **Figure 2E**. Interestingly, in LPS-untreated cells, treatment with HBSP alone could slightly decrease the levels of intracellular ROS and MDA, suggesting that cytoprotective effect of HBSP, as illustrated in **Figure 2A-C**, involved in the antioxidant ability to decrease intracellular ROS and MDA.

HBSP exerts cytoprotective effect by activation of Nrf2 via PI3K/Akt pathway

PI3K/Akt signaling pathway is one of the most important pathways for cell survival. In this work, in order to investigate the necessity of PI3K/Akt pathway in HBSP-induced cytoprotec-

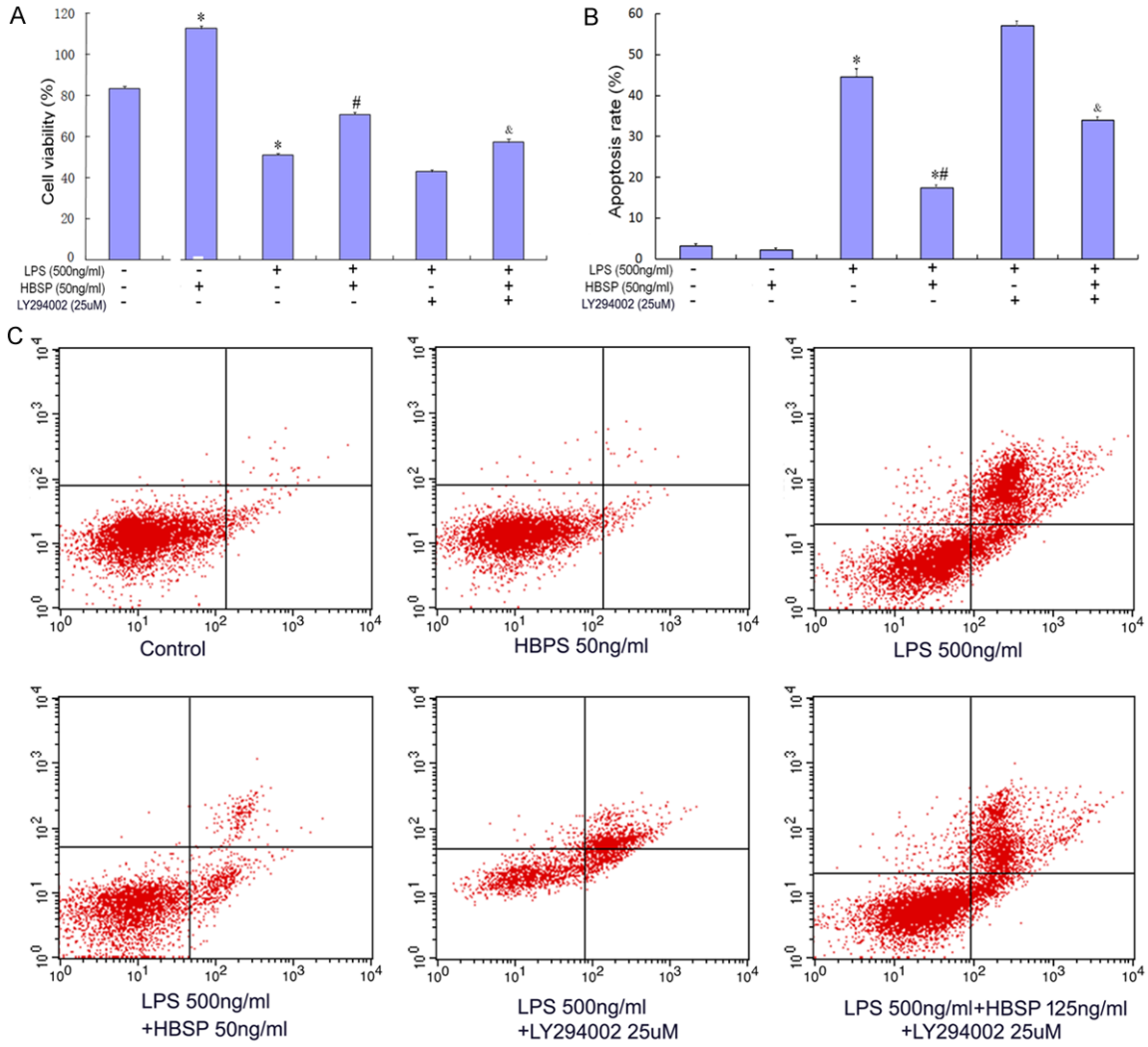

Figure 3. HBSP-induced cytoprotective effect in LPS treated cells is PI3K dependent. HK-2 cells were pre-treated 25 uM LY294002 for 1 h, followed by 50 ng/ml of HBSP for 2 h, then treated with 500 ng/ml LPS for 24 h. A: HBSP enhances the cell viability of LPS-treated HK-2 cells via PI3K/Akt pathway. B: HBSP reverses LPS-induced cell apoptosis via PI3K/Akt pathway. C: Flow cytometric analysis of HK-2 cells stained with LY294002 in the presence of HBSP and LPS. LY294002 reverses the cytoprotective effect of HBSP, suggesting that the cytoprotective effect of HBSP is mediated by the activation of PI3K/Akt pathway. *P < 0.05 compared to the untreated cells, #P < 0.05 compared to LPS group, &P < 0.05 compared to LPS+HBSP group.

tive effect, we pre-treated HK-2 cells with 25 uM LY294002 (a PI3K/Akt inhibitor) for 1 h, followed by 50 ng/ml of HBSP for 2 h, then treated with 500 ng/ml LPS for 24 h. The results showed that LY294002 could significantly reverse the cytoprotective effect of HBSP (**Figure 3**), suggesting that the cytoprotective effect of HBSP was mediated by the activation of PI3K/Akt signaling pathway.

Moreover, as shown in **Figure 4A**, Akt, the key player of PI3K/Akt signaling pathway, presented a stable expression level in each group. In the present study, both LPS exposure and HBSP treatment could enhance the expression of activated phosphorylated Akt (p-Akt), indicating that LPS only might stimulate bodies to response to oxidative stress via PI3K/Akt signaling pathway activation. While, this kind of activation was limited, and HBSP treatment resulted in the marked enhancement of the expression of p-Akt and the activation of PI3K/Akt signaling pathway. When HK-2 cells were pre-treated with LY294002, the PI3K inhibitor,

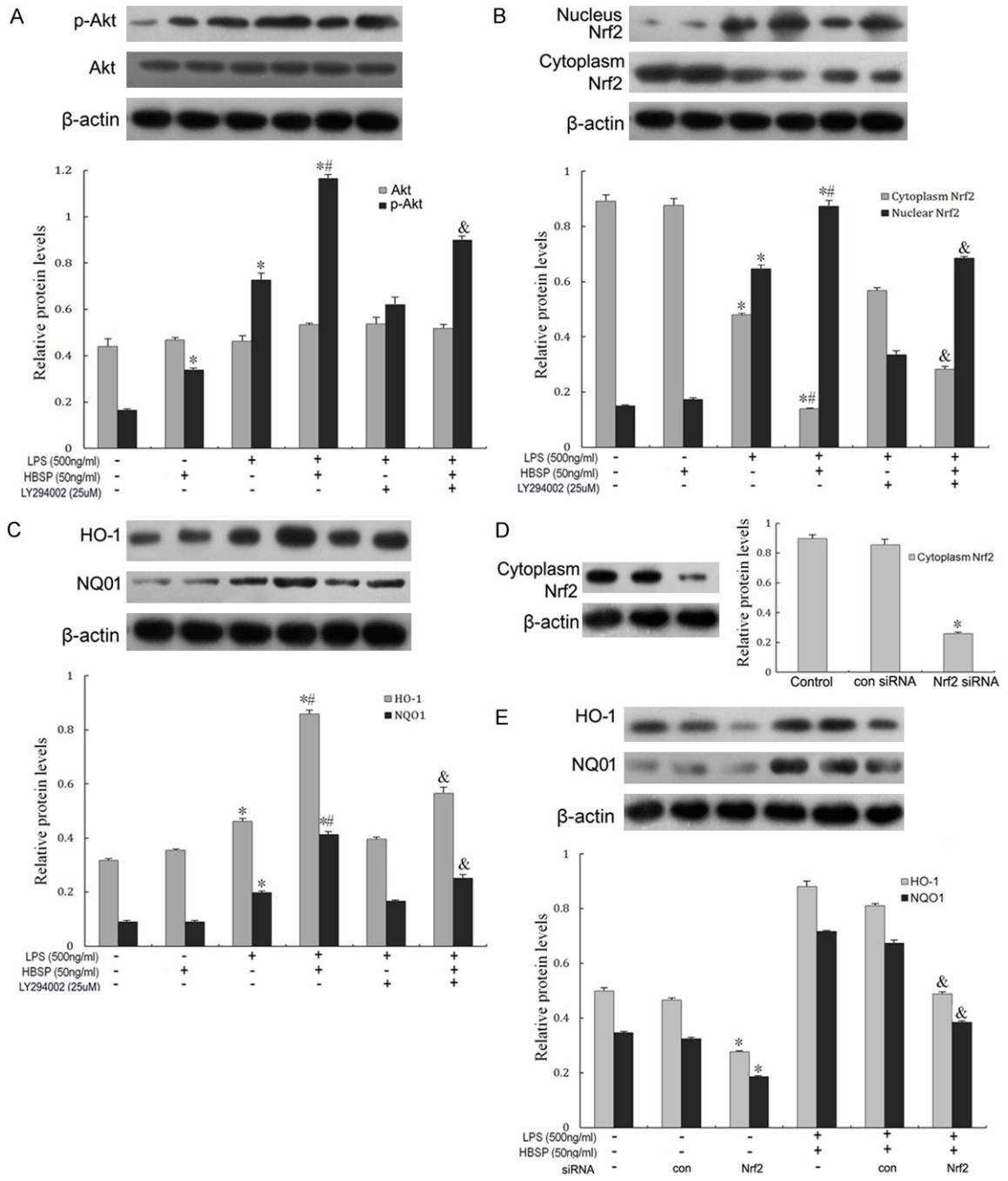

Figure 4. Western bloting analyzes the effects of HBSP treatment on the activation of PI3K/Akt pathway and the expression of antioxidant protein in LPS treated HK-2 cells. A: HBSP-induced Akt phosphorylation is PI3K dependent. The activation of Akt is prevented by co-incubation with LY294002. B: HBSP promotes the translocation of Nrf2 from cytoplasm to nuclear via PI3K/Akt signaling pathway. C: HBSP activates the expression of antioxidant proteins HO-1 and NQO1 in LPS-treated HK-2 cells via PI3K/Akt pathway. D: Specific Nrf2 siRNA inhibits the expression of Nrf2 in untreated HK-2 cells. E: HBSP enhances the expression of HO-1 and NQO1 regulated by Nrf2 in HK-2 cells under LPS exposure. The results shown are expressed as the ratio of specific proteins to β-actin. *P < 0.05 compared to the untreated cells, #P < 0.05 compared to LPS group, &P < 0.05 compared to LPS+HBSP group.

before HBSP and LPS treatment, the protective effects of HBSP were abolished obviously, and the expression of p-Akt induced by HBSP treatment was significantly reduced, confirming HBSP-induced Akt activation in a PI3K-dependent manner.

Meanwhile, the activation of PI3K/Akt signaling pathway promoted the translocation of Nrf2 from cytoplasm to nuclear. From **Figure 4B**, we can easily find that LPS exposure increased the nuclear translocation of Nrf2, and HBSP treatment further promoted the translocation. While, pre-treatment with LY294002 could significantly attenuated the nuclear translocation behavior of Nrf2 induced by HBSP in HK-2 cells (**Figure 4B**), suggesting that HBSP could activate the nuclear transition of Nrf2, which was regulated by PI3K/Akt signaling pathway. Similar to the content trend of nuclear Nrf2, the expression levels of antioxidant proteins HO-1 and NQO1 were increased in LPS-treated HK-2 cells, and were further enhanced by HBSP treatment (**Figure 4C**). Pre-treatment with LY294002 significantly attenuated the protein expression levels of HO-1 and NQO1 induced by HBSP (**Figure 4C**), indicating the important roles of PI3K/Akt signaling pathway in antioxidation effect.

Nrf2 regulates the expression of HO-1 and NQO1

Nrf2 is an important upstream modulator of peroxiredoxins HO-1 and NQO1. To verify the correlation between them, we performed the Nrf2 siRNA analysis. In untreated HK-2 cells, specific Nrf2 siRNA significantly inhibited the expression of Nrf2 (**Figure 4D**). Meanwhile, silencing of Nrf2 by specific siRNA significantly inhibited the expression of HO-1 and NQO1 in untreated HK-2 cells, and significantly reversed and attenuated LPS- and HBSP-induced high level expression of HO-1 and NQO1 (**Figure 4E**), indicating that HBSP enhanced the expression of HO-1 and NQO1 regulated by Nrf2 in HK-2 cells under LPS exposure in response to oxidative stress.

Discussion

Septic AKI is still an intractable clinical situation with poor prognosis. Recent research indicated that sepsis-induced oxidative damage contributed to the development of organ injury [24], which was also confirmed by our work. In the present study, LPS exposure of HK-2 cells obviously increased intracellular ROS and lipid peroxidation and further induced cell apoptosis and inflammation, suggesting that oxidative stress was involved in the tubular dysfunction and injury. While, HBSP could significantly reverse and attenuate LPS-induced intracellular oxidative stress, presenting the cytoprotective effect and antioxidant ability. Previous studies have showed the meaningful antioxidative effects of EPO [25, 26], the origin of HBSP. Although, few previous studies have proven the direct antioxidative effects of HBSP, our work indicated that HBSP reduced intracellular oxidative stress, and activated the expression of several peroxiredoxins (such as Nrf2, HO-1). These findings suggested that HBSP may exert renoprotective effect by reducing inflammatory mediators-induced oxidative stress.

Numerous studies have indicated that the PI3K/Akt signaling pathway plays important roles in regulating a variety of cellular processes [27-29]. A growing body of evidence suggests that the PI3K/Akt signaling pathway, as a negative regulator, plays a crucial role in regulating LPS-induced acute inflammatory responses [30, 31]. To further elucidate the intracellular signaling mechanisms of HBSP against LPS exposure, we focused on the PI3K/AKT pathway. In the present study, treatment of HBSP resulted in the marked enhancement of the expression of p-Akt and the activation of the PI3K/Akt signaling pathway; while, when HK-2 cells were pre-treated with the PI3K inhibitor before HBSP and LPS treatment, the protective effects of HBSP were abolished obviously, indicating that the renoprotective effects of HBSP were mediated by the activation of PI3K/Akt signaling pathway. Meanwhile, treatment of HBSP could significantly up-regulated the content of nuclear Nrf2, and pre-treatment with LY294002 could significantly attenuate the nuclear translocation behavior of Nrf2 induced by HBSP in HK-2 cells. These findings suggested that HBSP could activate the nuclear translication of Nrf2, which was regulated via PI3K/Akt pathway. In support of this conclusion, PI3K/Akt pathway was proven to be a downstream effector of NADPH oxidase, contributing to the activation of Nrf2 in response to hyperoxia [32].

Nrf2, also known as nuclear factor erythroid derived 2-related factor 2, is a basic leucine zipper protein. In cellular injury and inflammation, Nrf2 regulates the expression of antioxidant proteins, and protects against oxidative damage triggered by inflammatory mediators. Under

oxidative stress condition, Nrf2 is translocated from cytoplasm into the nucleus to initiate the expression of cytoprotective proteins. Liu et al. [33] reported that Nrf2 was protective during ischemic and nephrotoxic AKI in mice. Meanwhile, several clinical drug targets have focused on the activation of Nrf2 and Nrf2 related pathway [34, 35]. While the expression content of Nrf2 is limited under untreated condition. As shown in our study, HBSP treatment resulted in the enhanced level of nuclear translocation, antioxidant response element, and transcriptional activity of Nrf2 via PI3K/Akt pathway, and further induced the enhancement of downstream antioxidative protein expression.

Activation of Nrf2 contributes to the induction of many target antioxidative proteins, such as Heme oxygenase-1 (HO-1) and NADH quinone oxidoreductase 1 (NQO1). HO-1 and NQO1 are important antioxidant enzymes that exert cytoprotective effects against oxidative stress. Numerous studies have suggested the protective effects of HO-1 on a variety of pathologies, including sepsis and renal injury [36, 37]. NQO1 is also considered as a desirable therapeutic strategy to protect cells from oxidative damage [38]. In our study, Nrf2 siRNA suppressed the HBSP-induced activation of Nrf2, and resulted in the decreased expression of HO-1 and NQO1, indicating that the expression of HO-1 and NQO1 enhanced by HBSP was regulated by Nrf2. These findings were consistent with previous study [39], in which NQO1 and HO-1 were significantly lower in Nrf2-knockout mice, suggesting that Nrf2 regulated the induction of antioxidative proteins in response to hyperoxia.

In conclusion, the present results suggested that HBSP protected HK-2 cells against LPS-induced oxidative stress. This behavior attributed to the activation of Nrf2 via PI3K/Akt pathway and the induction of antioxidative proteins HO-1 and NQO1. Relative to EPO, HBSP could be served as a better candidate for renal protection. This work facilitates our better understanding of underlying mechanisms in septic AKI and the development of more efficient therapeutic targets.

Disclosure of conflict of interest

None.

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References

[1] Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A and Ronco C. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 2005; 294: 813-818.

[2] Silvester W, Bellomo R and Cole L. Epidemiology, management, and outcome of severe acute renal failure of critical illness in Australia. Crit Care Med 2001; 29: 1910-1915.

[3] Bagshaw SM, Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Oudemans-van Straaten HM, Ronco C and Kellum JA. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. Clin J Am Soc Nephrol 2007; 2: 431-439.

[4] Wan L, Bagshaw SM, Langenberg C, Saotome T, May C and Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? Crit Care Med 2008; 36: S198-203.

[5] Bonegio R and Lieberthal W. Role of apoptosis in the pathogenesis of acute renal failure. Curr Opin Nephrol Hypertens 2002; 11: 301-308.

[6] Abraham E and Singer M. Mechanisms of sepsis-induced organ dysfunction. Crit Care Med 2007; 35: 2408-2416.

[7] Rana A, Sathyanarayana P and Lieberthal W. Role of apoptosis of renal tubular cells in acute renal failure: therapeutic implications. Apoptosis 2001; 6: 83-102.

[8] Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J and Kellum JA. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock 2014; 41: 3-11.

[9] Akira S, Uematsu S and Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124: 783-801.

[10] Cohen J. The immunopathogenesis of sepsis. Nature 2002; 420: 885-891.

[11] Jacob AI, Goldberg PK, Bloom N, Degenshein GA and Kozinn PJ. Endotoxin and bacteria in portal blood. Gastroenterology 1977; 72: 1268-1270.

[12] Xu C, Chang A, Hack BK, Eadon MT, Alper SL and Cunningham PN. TNF-mediated damage to glomerular endothelium is an important de-

terminant of acute kidney injury in sepsis. Kidney Int 2014; 85: 72-81.
[13] Kim DH, Jung YJ, Lee AS, Lee S, Kang KP, Lee TH, Lee SY, Jang KY, Moon WS, Choi KS, Yoon KH, Sung MJ, Park SK and Kim W. COMP-angiopoietin-1 decreases lipopolysaccharide-induced acute kidney injury. Kidney Int 2009; 76: 1180-1191.
[14] Gupta A, Rhodes GJ, Berg DT, Gerlitz B, Molitoris BA and Grinnell BW. Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. Am J Physiol Renal Physiol 2007; 293: F245-254.
[15] Wu L, Gokden N and Mayeux PR. Evidence for the role of reactive nitrogen species in polymicrobial sepsis-induced renal peritubular capillary dysfunction and tubular injury. J Am Soc Nephrol 2007; 18: 1807-1815.
[16] Biancone L, Conaldi PG, Toniolo A and Camussi G. Escherichia coli porin induces proinflammatory alterations in renal tubular cells. Exp Nephrol 1997; 5: 330-336.
[17] Galley HF. Bench-to-bedside review: Targeting antioxidants to mitochondria in sepsis. Crit Care 2010; 14: 230.
[18] Yasuda H, Yuen PS, Hu X, Zhou H and Star RA. Simvastatin improves sepsis-induced mortality and acute kidney injury via renal vascular effects. Kidney Int 2006; 69: 1535-1542.
[19] Wu L, Tiwari MM, Messer KJ, Holthoff JH, Gokden N, Brock RW and Mayeux PR. Peritubular capillary dysfunction and renal tubular epithelial cell stress following lipopolysaccharide administration in mice. Am J Physiol Renal Physiol 2007; 292: F261-268.
[20] Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, Kieswich J, Allen D, Harwood S, Raftery M, Thiemermann C and Yaqoob MM. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol 2004; 15: 2115-2124.
[21] Aydin Z, Duijs J, Bajema IM, van Zonneveld AJ and Rabelink TJ. Erythropoietin, progenitors, and repair. Kidney Int Suppl 2007; S16-20.
[22] Khorana AA, Francis CW, Culakova E and Lyman GH. Risk factors for chemotherapy-associated venous thromboembolism in a prospective observational study. Cancer 2005; 104: 2822-2829.
[23] Brines M, Patel NS, Villa P, Brines C, Mennini T, De Paola M, Erbayraktar Z, Erbayraktar S, Sepodes B, Thiemermann C, Ghezzi P, Yamin M, Hand CC, Xie QW, Coleman T and Cerami A. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proc Natl Acad Sci U S A 2008; 105: 10925-10930.
[24] Barichello T, Fortunato JJ, Vitali AM, Feier G, Reinke A, Moreira JC, Quevedo J and Dal-Pizzol F. Oxidative variables in the rat brain after sepsis induced by cecal ligation and perforation. Crit Care Med 2006; 34: 886-889.
[25] Katavetin P, Tungsanga K, Eiam-Ong S and Nangaku M. Antioxidative effects of erythropoietin. Kidney Int Suppl 2007; S10-15.
[26] Katavetin P, Inagi R, Miyata T, Shao J, Sassa R, Adler S, Eto N, Kato H, Fujita T and Nangaku M. Erythropoietin induces heme oxygenase-1 expression and attenuates oxidative stress. Biochem Biophys Res Commun 2007; 359: 928-934.
[27] Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002; 296: 1655-1657.
[28] Liu C, Su T, Li F, Li L, Qin X, Pan W, Feng F, Chen F, Liao D and Chen L. PI3K/Akt signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by apelin-13. Acta Biochim Biophys Sin (Shanghai) 2010; 42: 396-402.
[29] Hanada M, Feng J and Hemmings BA. Structure, regulation and function of PKB/AKT–a major therapeutic target. Biochim Biophys Acta 2004; 1697: 3-16.
[30] Williams DL, Li C, Ha T, Ozment-Skelton T, Kalbfleisch JH, Preiszner J, Brooks L, Breuel K and Schweitzer JB. Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. J Immunol 2004; 172: 449-456.
[31] Guha M and Mackman N. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 2002; 277: 32124-32132.
[32] Papaiahgari S, Zhang Q, Kleeberger SR, Cho HY and Reddy SP. Hyperoxia stimulates an Nrf2-ARE transcriptional response via ROS-EGFR-PI3K-Akt/ERK MAP kinase signaling in pulmonary epithelial cells. Antioxid Redox Signal 2006; 8: 43-52.
[33] Liu M, Grigoryev DN, Crow MT, Haas M, Yamamoto M, Reddy SP and Rabb H. Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. Kidney Int 2009; 76: 277-285.
[34] Prince M, Li Y, Childers A, Itoh K, Yamamoto M and Kleiner HE. Comparison of citrus coumarins on carcinogen-detoxifying enzymes in Nrf2 knockout mice. Toxicol Lett 2009; 185: 180-186.
[35] Zhang Y and Gordon GB. A strategy for cancer prevention: stimulation of the Nrf2-ARE signaling pathway. Mol Cancer Ther 2004; 3: 885-893.

[36] Chintagari NR, Nguyen J, Belcher JD, Vercellotti GM and Alayash AI. Haptoglobin attenuates hemoglobin-induced heme oxygenase-1 in renal proximal tubule cells and kidneys of a mouse model of sickle cell disease. Blood Cells Mol Dis 2015; 54: 302-306.

[37] Jarmi T and Agarwal A. Heme oxygenase and renal disease. Curr Hypertens Rep 2009; 11: 56-62.

[38] Lim JH, Kim KM, Kim SW, Hwang O and Choi HJ. Bromocriptine activates NQO1 via Nrf2-PI3K/Akt signaling: novel cytoprotective mechanism against oxidative damage. Pharmacol Res 2008; 57: 325-331.

[39] Cho HY, Jedlicka AE, Reddy SP, Kensler TW, Yamamoto M, Zhang LY and Kleeberger SR. Role of NRF2 in protection against hyperoxic lung injury in mice. Am J Respir Cell Mol Biol 2002; 26: 175-182.