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Original Article

Anti-inflammatory and anti-apoptotic effects of oxysophoridine on lipopolysaccharide-induced acute lung injury in mice

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Abstract: Oxysophoridine (OSR) is an alkaloid with multiple pharmacological activities. This study aimed to investigate the protective effects and underlying mechanisms of OSR on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. Here, we found that OSR treatment markedly mitigated LPS-induced body weight loss and significant lung injury characterized by the deterioration of histopathology, histologic scores, wet-to-dry ratio, exduate volume, and protein leakage. OSR dramatically attenuated LPS-induced lung inflammation, as evidenced by the reduced levels of total cells, neutrophils, lymphocytes, and macrophages and pro-inflammatory cytokines (i.e., tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein-1) in bronchoalveolar lavage fluid and of their mRNA expression in lung tissues. OSR also inhibited LPS-induced expression and activation of nuclear factor-κB p65 in pulmonary tissue. Additionally, OSR administration markedly prevented LPS-induced pulmonary cell apoptosis in mice, as reflected by the decrease in expression of procaspase-8, procaspase-3, cleaved caspase-8, and cleaved caspase-3, and Bcl-2-associated X/B-cell lymphoma 2 ratio. These results indicate that OSR is a potential therapeutic drug for treating LPS-induced ALI.

Keywords: Acute lung injury, lipopolysaccharide, oxysophoridine, inflammation, apoptosis

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), characterized by interstitial edema, neutrophil recruitment, disruption of epithelial integrity, parenchymal injury, and inflammatory stress-induced cell apoptosis, are life-threatening problems with significant morbidity and mortality [1]. Despite considerable research, the mechanism underlying inflammation-induced ALI and appropriate treatment approaches are still unknown.

ALI is generally defined as uncontrolled and excessive production of inflammatory mediators, such as cytokines, chemokines, adhesion molecules, and bioactive lipid products [2, 3]. Lipopolysaccharide (LPS), a pathogenic component of endotoxin in the outer membrane of Gram-negative bacteria, is widely used to induce animal models of ALI by intratracheal instillation. These animal models have characteristics similar to human ALI, including lung edema, endothelial and epithelial injury, neutrophil and macrophage accumulation, and pro-inflammatory mediator release [4]. LPS can induce an inflammatory response and disturb the immune system when it enters into the host by activating toll-like receptors (TLRs) [5, 6]. The binding of LPS to TLR4 leads to IκB-α phosphorylation and degradation, promotes nuclear translocation and activation of nuclear factor-kappaB (NF-κB), and subsequently causes the excessive release of pro-inflammatory cytokines, chemokines, and adhesion molecules [(e.g., tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1)] [7, 8]. It has been reported that increased epithelial/endothelial cell apoptosis significantly promotes the damage of the lung alveolar-capillary interface in ALI [9]. LPS-induced ALI involves mitochondrial dys-

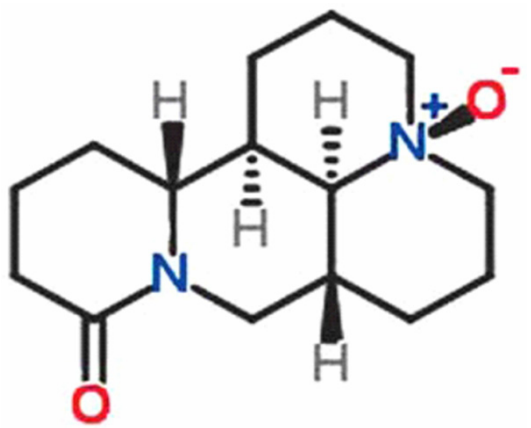

Figure 1. Chemical structure of OSR. The molecular formula of OSR is $C_{15}H_{24}N_2O_2$ and the molecular weight is 264.3633.

function and cell apoptosis, and inhibition of apoptosis increases the survival rate of animals [10, 11]. Together, LPS-induced inflammatory and apoptotic responses are critical contributors to the development of ALI.

Oxysophoridine (OSR), a derivative of sophoridine, with two piperidine rings (**Figure 1**), is a natural alkaloid extracted from *Siphocampylus verticillatus*. OSR has been shown to exhibit various pharmacological activities, including anti-depression [12], anti-nociception [13], anti-inflammation [14], and anti-apoptosis [15]. Despite the several known protective effects of OSR, few studies focused on the prevention of ALI by this compound. Thus, the present study was designed to evaluate the protective effects of OSR administration on LPS-induced ALI in mice by its anti-inflammatory and anti-apoptotic activities.

Materials and methods

Reagents

OSR was supplied by Zi Jing Hua Pharmaceutical Co. (Yanchi, Ningxia, China). LPS (*Escherichia coli* 055:B5) was purchased from Sigma-Aldrich (St. Louis, MO, USA). OSR and LPS were dissolved in sterile PBS (pH 7.4) before use. Pentobarbital sodium was purchased from the National Institutes for Food and Drug Control (Beijing, China). Mouse TNF-α and IL-1β, IL-6, and MCP-1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D (Minneapolis, MN, USA). Rabbit anti-mouse NF-κβ p65, Iκβ-α, procaspase-3, cleaved caspase-3, procaspase-8, and cleaved caspase-8 polyclonal antibodies (pAbs) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Rabbit anti-mouse Bax and Bcl-2 pAbs were obtained from Cell Signaling Technology Inc. (Danvers, MA, USA). Rabbit anti-mouse b-actin and histone-3 monoclonal antibodies were from Sigma. Horseradish peroxidase-conjugated anti-rabbit IgG was obtained from Chemicon (Temecula, CA, USA). Other reagents were purchased from Sigma unless otherwise stated.

Animals

Male BALB/c mice (8 weeks age, weighing 22 g to 25 g) were obtained from Laboratory Animal Center of Henan Province (Zhengzhou, Henan, China). All animals were housed under specific pathogen-free conditions with a 12 h light/dark cycle at 22°C to 24°C. Standard laboratory chow and tap water were obtained *ad libitum*. All experimental protocols were approved by the Laboratory Animal Committee of Xinxiang Medical University. The mice were sacrificed under anesthesia with pentobarbital sodium, and all efforts were exerted to minimize suffering.

LPS-induced ALI and OSR treatment

Mice were randomly divided into five groups (n = 12/group): control group, vehicle group, and three OSR-treated groups (60, 120, and 180 mg/kg). OSR was administered intragastrically once per day for seven consecutive days. During this period, the control and vehicle groups were given equal volume of PBS. At 1 h after the last administration, ALI was induced by intratracheal instillation of LPS, as previously described [16]. In brief, animals were anesthetized with pentobarbital sodium (50 mg/kg) by intraperitoneal injection. The mice from the vehicle and OSR-treated groups were orally intubated with a sterile plastic catheter and intratracheally given a single dose of 5 mg/kg body weight LPS (2.5 mg/mL). Control mice were given an equal volume of PBS. At 24 h after LPS or PBS instillation, all animals were euthanized with pentobarbital sodium in accordance with the American Veterinary Medical Association Guidelines on Euthanasia, June 2007. Mice were weighted once a day for 4 days to evaluate the body weight change after LPS or PBS administration.

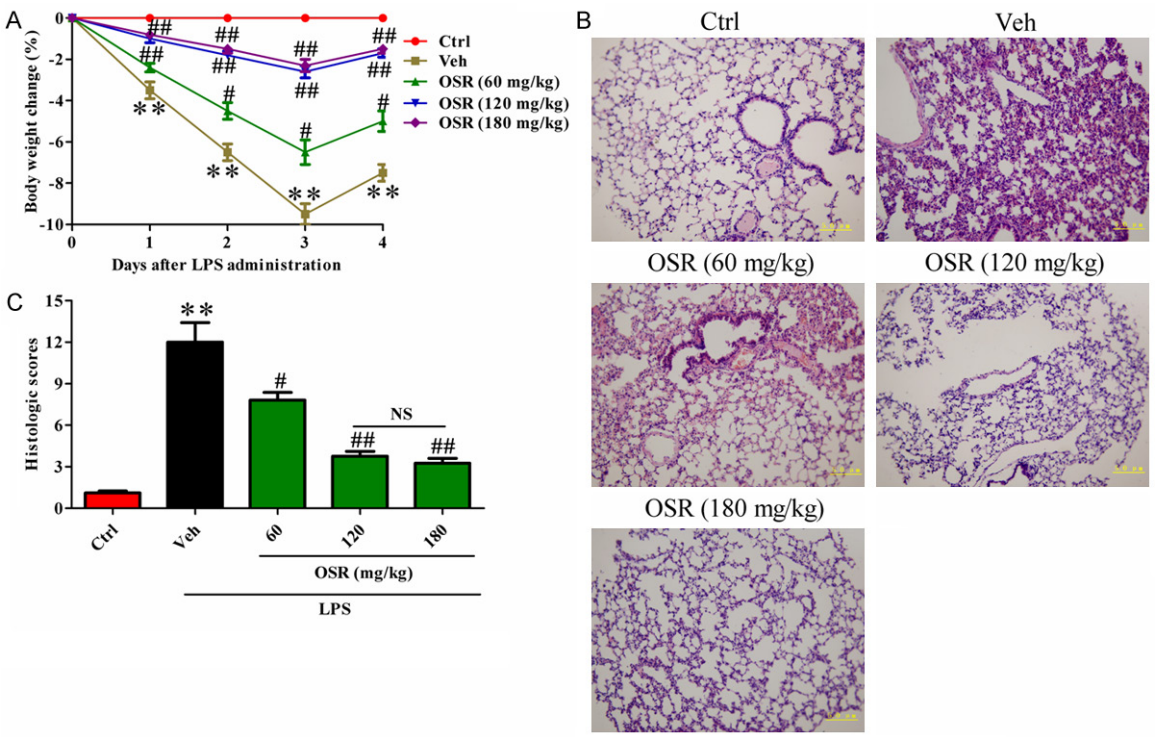

Figure 2. OSR treatment ameliorates body weight loss and lung histopathological change in LPS-challenged mice. Mice were treated with OSR (60, 120, and 180 mg/kg) once per day for 7 consecutive days prior to LPS or PBS administration. A. Body weight change was measured once a day for 4 days. B. Lung tissues were stained with H&E for histopathological evaluation at 24 h after LPS challenge. Scale bar: 5.0 mm. C. The slides were histopathologically assessed using a semi-quantitative scoring system based on the severity of lung damage. Data are expressed as means ± SD (n = 12 per group). **P < 0.01 vs. control (Ctrl) group; #P < 0.05 vs. vehicle (Veh) group; ##P < 0.01 vs. Veh group.

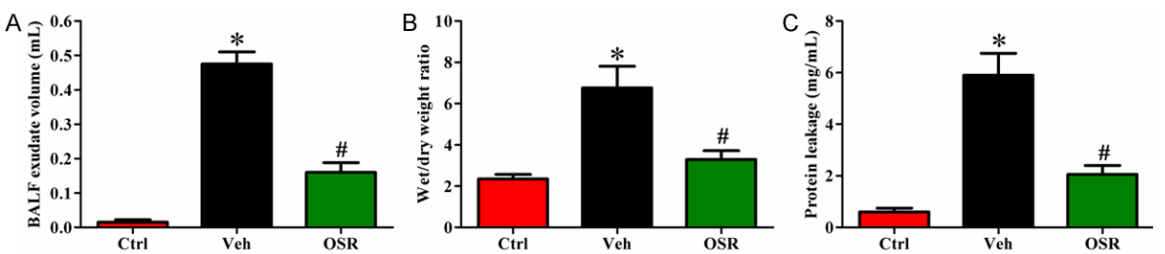

Figure 3. OSR treatment improves LPS-induced lung injury in mice. Mice were treated with 120 mg/kg OSR once per day for 7 consecutive days prior to LPS or PBS administration. BALF and lung samples were harvested to evaluate the hallmarks of lung injury at 24 h after LPS or PBS challenge. A. BALF exudate volume. B. Lung W/D weight ratio. C. Protein leakage in the BALF. Data are means ± SD (n = 12 per group). *P < 0.05 vs. Ctrl group; #P < 0.05 vs. Veh group.

Histopathological evaluation of the lung tissues

At 24 h after LPS or PBS administration, the lungs were harvested to observe the morphologic changes. The tissue samples were immersed in 10% formalin and fixed for 24 h at room temperature, embedded in paraffin, and sectioned into 5 mm-thick slices. The sections were deparaffinized, rehydrated gradually, and stained with hematoxylin and eosin (HE). Histological changes were evaluated blindly by two experienced pathologists. The degree of lung injury was graded using a scoring system based on histologic features, including edema, congestion and hyperemia, tissue infiltration and

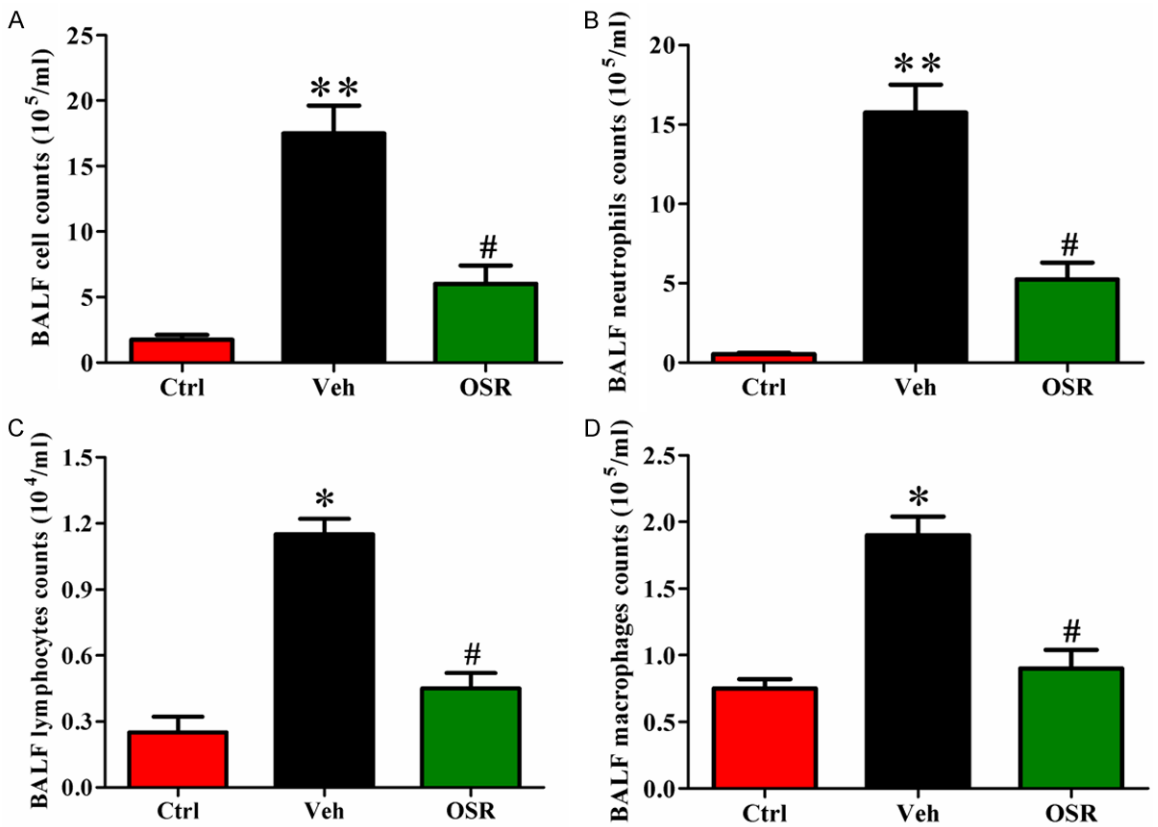

Figure 4. OSR treatment reduces inflammatory cell in BALF of LPS-challenged mice. Mice were treated with 120 mg/kg OSR once per day for 7 consecutive days prior to LPS or PBS administration. BALF was collected to measure the inflammatory cell counts at 24 h after LPS or PBS exposure. The number of (A) total cells, (B) neutrophils, (C) lymphocytes, and (D) macrophages in BALF were measured by a hemocytometer. Data are expressed as means ± SD (n = 12 per group). *P < 0.05 vs. Ctrl group; **P < 0.01 vs. Ctrl group; #P < 0.05 vs. Veh group.

neutrophil margination, intraalveolar hemorrhage and debris, and cellular hyperplasia. Each feature was graded as either absent = 0, mild = 1, moderate = 2, or severe = 3. The total score was obtained as the sum of a single evaluation of each animal [17].

Lung wet/dry (W/D) weight ratio

At 24 h after LPS or PBS challenge, lung W/D weight ratio was measured to evaluate the severity of pulmonary edema. After being harvested and cleaned from blood, the wet lung was weighed, placed in an oven for 24 h at 80°C, weighed again when it dried. The lung W/D ratio was then calculated [17].

Bronchoalveolar lavage fluid (BALF) collection and cell counts

At 24 h after LPS or PBS administration, BALF was obtained by intratracheal intubation as previously described [18]. After the mice were anesthetized, the tracheas were cannulated, and lungs were gently washed with 1 mL of PBS. Lavage samples were centrifuged at 1000 g for 10 min at 4°C, and the supernatant was stored at -20°C to measure protein leakage and cytokine and chemokine levels. Cell pellets were resuspended in PBS and then stained with Dif-Quick for cytospin preparations [19]. The total cells, neutrophils, lymphocytes, and macrophages were counted by a certified laboratory technologist using a hemocytometer.

Protein leakage

The total protein in the BALF was determined using a standard commercial kit (Bio-Rad Laboratories, Hercules, CA, USA) and expressed as milligram per milliliter of BALF.

Enzyme-linked immunosorbent assay (ELISA)

At 24 h after LPS or PBS administration, BALF was harvested to determine the levels of TNF-

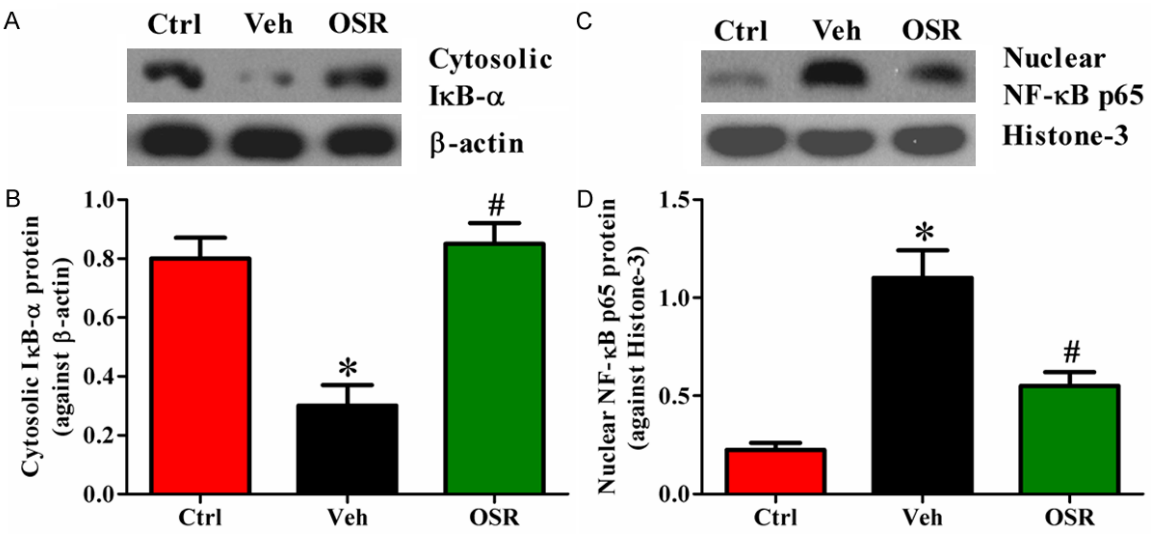

Figure 5. OSR treatment inhibits IκB-α degradation and NF-κB p65 nuclear translocation in the lung of LPS-stimulated mice. Mice were treated with 120 mg/kg OSR once per day for 7 consecutive days prior to LPS or PBS challenge. Western blot was used to analyze the expression of IκB-α and NF-κB p65 in mouse lung at 24 h after LPS or PBS treatment. A. Representative results of IκB-α expression in cytoplasm. B. Relative protein band densities of IκB-α normalized against b-actin. C. Representative results of nuclear NF-κB p65 expression. D. Relative protein band densities of NF-κB p65 normalized against histone-3. The respective densitometric analysis of protein bands was obtained from 3 independent experiments. Data are expressed as means ± SD (*n* = 12 per group). *P < 0.05 vs. Ctrl group; #P < 0.05 vs. Veh group.

α, IL-1β, IL-6, and MCP-1 using commercial ELISA kits from R&D Systems Inc. (Minneapolis, MN, USA) according to the manufacturers' instructions. The absorbance was read at 490 nm on an ELISA plate scanner (Molecular Devices, Sunnyvale, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

At 24 h after LPS or PBS treatment, total RNA was extracted from the lung tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. cDNA was synthesized from total RNA using a SuperScript Reverse Transcriptase kit (Invitrogen), and then used as a template to amplify TNF-α, IL-1β, IL-6, MCP-1, and b-actin (as an internal standard) genes by PCR. The following primers were used: TNF-α, 5'-TTGACCTCAGCGCTGAGTTG-3' (forward) and 5'-CC TGTAGCCCACGTCGTAGC-3' (reverse); for IL-1β, 5'-CAGGATGAGGACATGAGC ACC-3' (forward) and 5'-CTCTGCAGACTCAAACTCCAC-3' (reverse); for IL-6, 5'-GTACTCCAGAAGACCAGAGG-3' (forward) and 5'-TGCTGGTGACAACCACGG CC-3' (reverse); for MCP-1, 5'-CCAGCACCAGCACCAGCCAA-3' (forward) and 5'-TGGATGCTCCAGCCGGCAAC-3' (reverse); and for b-actin, 5'-TGAGAGGGAAATC GTGCGTG-3' (forward) and 5'-TTGCTGATCCACATCTGCTGG-3' (reverse). The relative mRNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method [20].

Western blot analysis

At 24 h after LPS or PBS challenge, fresh lung tissues were harvested and cytoplasmic and nuclear protein extracts were prepared with a cytoplasmic and nuclear extraction kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instruction. The expression of NF-κB p65 was quantified in nuclear fractions, whereas the other protein levels were quantified in cytoplasmic fractions. Protein concentrations were determined using the BCA protein assay kit (Beyotime, Haimen, China). Equal amounts of protein were added per well for 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Roche, Mannheim, Germany) through an electrophoresis system (Bio-Rad Co., Ltd., Hercules, USA). The membranes were rinsed in Tris-buffered saline with Tween-20 (TBS-T, 0.05%) and blocked in 5% skim milk at room temperature for 1 h on a

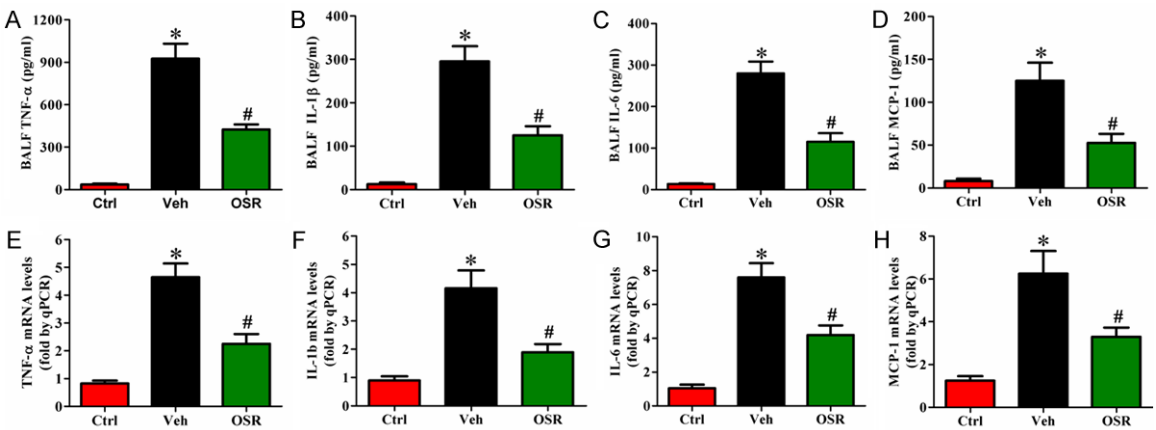

Figure 6. OSR treatment reduces the levels of inflammatory mediators in BALF and lung tissues of LPS-challenged mice. Mice were treated with 120 mg/kg OSR once per day for 7 consecutive days prior to LPS or PBS challenge. At 24 h after LPS or PBS treatment, the BALF and lung tissues were harvested to detect the levels of pro-inflammatory cytokines and chemokines. ELISA was performed to measure the production of (A) TNF-α, (B) IL-1β, (C) IL-6, and (D) MCP-1 in BALF. qRT-PCR was conducted to analyze the mRNA expression of (E) TNF-α, (F) IL-1β, (G) IL-6, and (H) MCP-1 in lung tissues. Data are expressed as means ± SD (n = 12 per group). *P < 0.05 vs. Ctrl group; #P < 0.05 vs. Veh group.

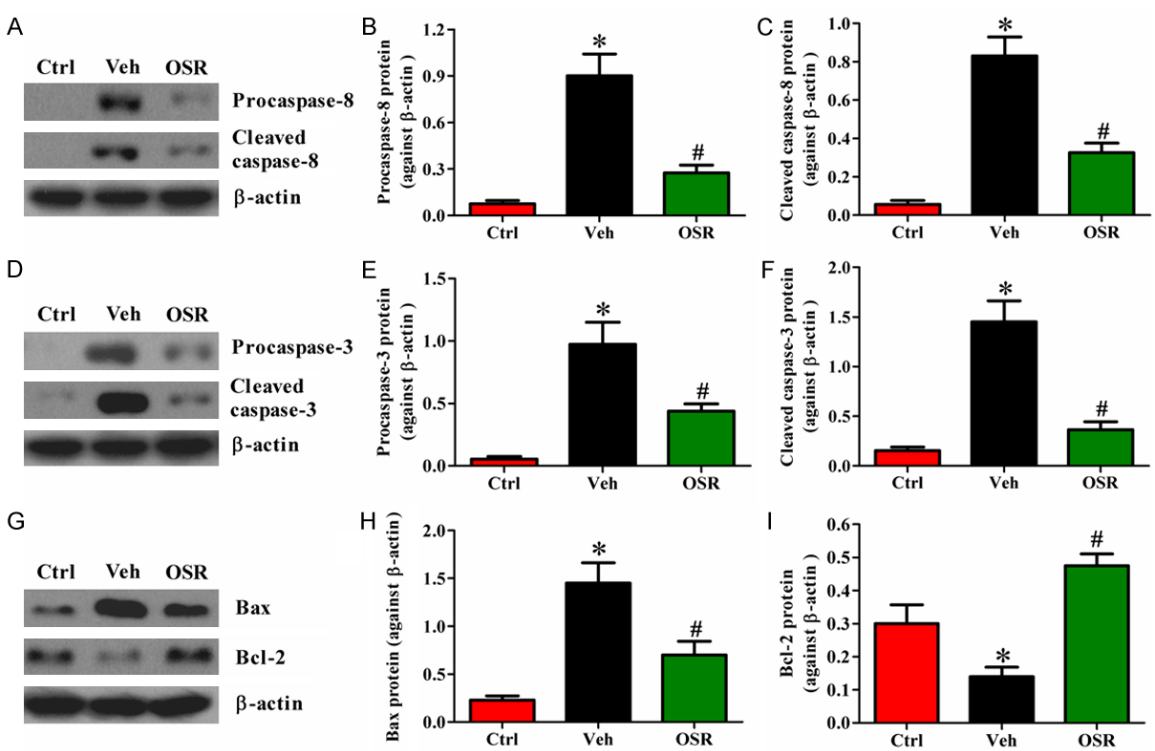

Figure 7. OSR treatment inhibits lung cell apoptosis in LPS-stimulated mice. Mice were treated with 120 mg/kg OSR once per day for 7 consecutive days prior to LPS or PBS challenge. Lung tissues were collected to assess the expression of apoptosis-associated molecules at 24 h after LPS or PBS exposure. Western blot was performed to analyze the expression of (A) procaspase-8 and cleaved caspase-8; (D) procaspase-3 and cleaved caspase-3; and (G) Bax and Bcl-2. Protein bands were densitometrically quantiied and relative amounts of procaspase-8 (B), cleaved caspase-8 (C), procaspase-3 (E), cleaved caspase-3 (F), Bax (H), and Bcl-2 (I) are shown. The respective densitometric analysis of protein bands is from three separate experiments. Data are expressed as means ± SD (n = 12 per group). *P < 0.05 vs. Ctrl group; #P < 0.05 vs. Veh group.

shaker. Subsequently, the membranes were incubated with primary antibodies (IκB-α, NF-κB p65, procaspase-3, cleaved caspase-3, procaspase-8, cleaved caspase-8, Bax, Bcl-2, b-actin, and histone-3) at 4°C overnight, followed by washing with TBS-T and incubating with the peroxidase-conjugated secondary antibody at room temperature for 1 h. b-actin and histone-3 were used as the internal loading controls. Detection was performed with an enhanced chemiluminescence assay kit (Pierce, Rockford, IL, USA). Protein bands were quantified using Quantity One software (BioRad, USA).

Statistical analysis

All data in the tests and figures were presented as means ± SD, except for histologic scores. The histologic scores were analyzed with the Kruskal-Wallis test, followed by the Mann-Whitney U test with a Bonferroni correction. The intergroup differences were tested by one-way ANOVA, followed by the Tukey multiple comparison test. Survival data were calculated using the Fisher exact probability test and are expressed as percentages. Statistical analyses were accomplished by GraphPad Software (San Diego, CA, USA). In all tests, $P < 0.05$ was considered to be statistically significant.

Results

OSR treatment reduces LPS-induced body weight loss and lung damage in mice

In this study, we found that LPS challenge led to significant body weight loss in mice ($P < 0.01$, **Figure 2A**). Treatment with different doses of OSR (60, 120, and 180 mg/kg) significantly mitigated the loss caused by LPS (**Figure 2A**). However, no significant change in body weight was observed in control-treated mice (**Figure 2A**). We also investigated the effects of OSR on lung histopathology in mice with either PBS or LPS challenge. HE staining showed that LPS-treated mice exhibited severe lung injury characterized by interstitial edema, consolidation, alveolar hemorrhage, alveolar wall thickening, and prominent inflammatory cell infiltration into the interstitial and alveolar spaces, which was markedly improved by OSR administration (**Figure 2B**). By contrast, no histologic change was observed in the lungs from the control group (**Figure 2B**). A scoring system was used to grade the degree of lung injury. LPS-challenged mice showed a remarkable increase in lung histologic scores but were reduced by OSR treatment (**Figure 2C**). Collectively, 120 and 180 mg/kg OSR treatment showed more protective effects on LPS-induced lung injury than 60 mg/kg OSR did; however, no significant difference was noted in the above two groups (**Figure 2A-C**). Thus, we selected 120 mg/kg OSR for further studies. Several well-known hallmarks of lung injury such as BALF exudate volume, lung W/D weight ratio, and protein leakage in BALF were significantly increased in LPS-stimulated mice compared with those in the control group, which were remarkably reduced by OSR treatment (**Figure 3A-C**). These results demonstrated that OSR treatment mitigated lung injury in LPS-challenged mice.

OSR treatment reduces inflammatory cells in BALF of LPS-challenged mice

To confirm the efficacy of OSR treatment on LPS-induced inflammatory response in lung, the total cells, neutrophils, lymphocytes, and macrophages in BALF were measured using a hemocytometer. We found that LPS-treated group showed significant increases in the total cells (**Figure 4A**), neutrophils (**Figure 4B**), lymphocytes (**Figure 4C**), and macrophages (**Figure 4D**) in BALF compared with those of the control group, which were hindered by OSR administration (**Figure 4A-D**). These results suggested that OSR treatment reduced inflammatory cells in BALF of LPS-treated mice.

OSR treatment inhibits LPS-induced lung NF-κB p65 activation

NF-κB is a critical transcription factor required for the expression of numerous pro-inflammatory mediators in LPS-induced ALI [21]. To explore the molecular mechanisms by which OSR treatment ameliorates LPS-induced lung injury, we evaluated the levels of IκB-α in the cytoplasm and NF-κB p65 accumulation in the nuclei. A basal level of IκB-α was observed in the cytoplasm of lung samples in the control group (**Figure 5A**, **5B**). The expression of IκB-α substantially decreased in the lungs of LPS-stimulated mice, which was significantly improved by OSR treatment (**Figure 5A**, **5B**). The lung NF-κB p65 nuclear accumulation was significantly elevated in LPS-challenged mice compared with that in the control group. However, OSR administration significantly reduced the

level of nuclear NF-κB p65 after LPS stimulation (**Figure 5C**, **5D**). These results indicated that OSR treatment inhibited LPS-induced IκB-α degradation and NF-κB p65 nuclear translocation in mouse lung.

OSR treatment reduces the levels of cytokines and chemokines in BALF and lung tissues of LPS-challenged mice

To analyze the effects of OSR treatment on the LPS-stimulated expression and release of pro-inflammatory cytokines and chemokines, we measured the levels of TNF-α, IL-1β, IL-6, and MCP-1 in BALF by ELISA and of their mRNAs in lung tissues by qRT-PCR. As shown in **Figure 6**, LPS stimulation resulted in significant increases in TNF-α, IL-1β, IL-6, and MCP-1 in BALF when compared with the control group, which was markedly reduced by OSR treatment (**Figure 6A-D**). Similarly, the mRNA expression of TNF-α, IL-1β, IL-6, and MCP-1 in the lung tissues of LPS-treated mice was enhanced, which was improved by OSR treatment (**Figure 6E-H**). These results suggested that OSR treatment significantly prevented the increases of pro-inflammatory cytokine and chemokine in BALF and of mRNA expression in lung tissues of LPS-challenged mice.

OSR treatment inhibits LPS-induced lung cell apoptosis in mice

Inflammatory stress often causes lung cell apoptosis [22]. To evaluate the effect of OSR treatment on lung cell apoptosis in LPS-challenged mice, the protein expressions of pro-apoptotic (procaspase-8, cleaved caspase-8, caspase-3, cleaved caspase-3, and Bax), and anti-apoptotic (Bcl-2) in lung tissues was analyzed by Western blot. As shown in **Figure 7A-C**, the expression of procaspase-8 and cleaved caspase-8 was dramatically higher in the lungs of LPS-stimulated mice than that in the control group, whereas the increase was significantly reduced by OSR treatment. Similar effects of OSR on procaspase-3 and cleaved caspase-3 expression were observed in the lungs of LPS-challenged mice (**Figure 7D-F**). In addition, LPS stimulation markedly increased Bax expression and decreased Bcl-2 expression in the lungs, whereas the expression changes of the 2 proteins were reversed by OSR treatment (**Figure 7G-I**). These results indicated that LPS challenge increased lung cell apoptosis, which was significantly alleviated by OSR treatment.

Discussion

ALI and ARDS are the two main causes of disability and death in clinical settings because of lacking effective therapy strategies [23]. Thus, identifying novel drugs with protective effect against ALI are an urgent need. In this study, we found that OSR treatment alleviated LPS-induced ALI in mice via its anti-inflammatory and anti-apoptotic effects. The key findings are as follows. First, OSR treatment significantly mitigated the body weight loss of LPS-challenged mice. Second, OSR administration improved LPS-induced lung injury, as evidenced by the changes in histopathology, lung W/D weight ratio, BALF exudate volume and protein leakage, and the numbers of inflammatory cells in BALF. Third, OSR administration inhibited LPS-induced lung IκB-α degradation and NF-κB p65 nuclear translocation. Fourth, the levels of pro-inflammatory cytokine and chemokine in BALF and of their mRNA expressions in lung tissues in LPS-challenged mice were reduced by OSR treatment. Finally, OSR treatment prevented lung cell apoptosis by downregulating pro-apoptotic proteins (procaspase-8, caspase-8, procaspase-3, caspase-3, and Bax) and upregulating anti-apoptotic protein Bcl-2 in the lungs of LPS-stimulated mice.

LPS is a well-established pathogen leading to ALI [24]. The characteristics of LPS-induced mouse ALI by intratracheal instillation closely resemble the observed symptoms in humans [25]. Thus, we investigated the protective effects of OSR on ALI using a mouse model induced by intratracheal LPS instillation. As expected, lung inflammatory responses and injuries were observed in LPS-treated mice, including lung edema, alveolar distortion, hyaline membrane formation, hemorrhage, and necrosis, which were weakened by OSR treatment. In detail, OSR administration significantly inhibited LPS-induced lung edema, as shown by the decrease in BALF protein leakage and exudate volume and lung W/D weight ratio. Moreover, LPS challenge resulted in the infiltration of inflammatory cells, thereby causing excessive production of inflammatory mediator and ultimately leading to lung damage [26]. We here demonstrated that LPS-challenged mice

presented massive infiltration of inflammatory cells, including neutrophils, lymphocytes, and macrophages in BALF. However, OSR treatment markedly reduced LPS-induced inflammatory cell infiltration into lung. These results suggested that OSR exerted protective effects on LPS-induced ALI in mice.

In LPS-induced ALI, neutrophils are the earliest immune cells recruited into the injury sites. After LPS instillation, neutrophils are activated and cause the release of inflammatory cytokines and chemokines. In neutrophils, LPS is recognized by TLR4 and subsequently triggers a signal cascade for NF-κB activation [7]. NF-κB is a critical transcription factor in the pathogenesis of ALI. Once activated, NF-κB p65 dissociates from IκBs and translocates from the cytoplasm to the nucleus where it may trigger the transcription of inflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and MCP-1 [27]. Inactivation of NF-κB p65 prevents inflammation-induced increase in edema, inflammatory cell infiltration, and pro-inflammatory cytokine expression in the lungs [28]. We showed that LPS activated NF-κB in lungs by promoting IκB-α degradation and NF-κB p65 nuclear translocation, and subsequently the release of inflammatory mediators in turn stimulated neutrophils to migrate into the lung tissues to initiate, enlarge, and perpetuate the local or systematic inflammation, which ultimately contributed to the overall outcome and severity of ALI [29]. TNF-α is the earliest and primary endogenous cytokine that elicits the inflammatory cascade. Elevation of TNF-α results in vascular endothelial cell damage, induces alveolar epithelial cells to produce other cellular factors, leads to enzyme leakage out of the organelle, and eventually damages the lung parenchyma [30]. IL-1β can lead to surfactant abnormalities and increase protein permeability across the alveolar capillary barrier [31]. IL-6 has been shown to be an excellent predictor of the severity of ALI [32]. MCP-1 is an important chemokine in the recruitment and adherence of monocytes and neutrophils to the endothelium [16]. In the present study, OSR administration remarkably inhibited LPS-induced increase in levels of TNF-α, IL-1β, IL-6, and MCP-1 in BALF and of their mRNAs expression in lung tissues, paralleling the decrease in NF-κB activation. Therefore, the inhibitory effects of OSR on LPS-enhanced levels of TNF-α, IL-1β, IL-6, and MCP-1 may be attributed to its suppression of NF-κB activation.

Cell apoptosis also plays an essential role in the pathogenesis of ALI [33]. LPS-triggered cell apoptosis is partly dependent on the mitochondria pathway [11]. Mitochondria-mediated apoptosis is steered by pro- and anti-apoptotic Bcl-2 family proteins, specifically Bax and Bcl-2. Bcl-2 prevents the release of cytochrome c into the cytosol from mitochondria and subsequent caspase-3 activation, whereas Bax has the opposite function [34]. Caspase-3 is a key player in the execution phase of cell apoptosis [35]. Caspase-8 as an important initiating caspase of the death receptor-dependent pathway can activate caspase-9, which is involved in the process of caspase-3 activation [36]. A previous study showed that LPS enhances the release of cytochrome c from mitochondria to the cytoplasm, which activates the caspase cascade and ultimately leads to cell apoptosis [37]. In this study, we found that OSR administration markedly inhibited the enhancement of the pro-apoptotic proteins (procaspase-8, procaspase-3, cleaved caspase-8, cleaved caspase-3, and Bax), and restored anti-apoptotic protein Bcl-2 expression. These findings suggested that OSR treatment prevent LPS-induced ALI via its anti-apoptotic activity.

The results presented here showed that OSR treatment ameliorated LPS-induced ALI in mice. OSR administration significantly mitigated the body weight loss, improved lung histopathology, decreased lung W/D weight ratio, and reduced exudate volume and protein leakage and inflammatory cells in BALF in LPS-stimulated mice. The levels of pro-inflammatory cytokines and chemokines in BALF and lung tissues of LPS-treated mice were significantly reduced by OSR treatment, paralleling the inhibition of NF-κB p65 activation in lung tissues. Moreover, OSR treatment markedly downregulated pro-apoptotic protein expression, whereas it upregulated Bcl-2 expression in the lung tissues of LPS-challenged mice. Together, OSR exerts protective effect on LPS-induced ALI possibly through its anti-inflammatory and anti-apoptotic activities, which may be a potential therapeutic agent for ALI.

Disclosure of conflict of interest

None.

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