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Protective effect of sophocarpine on lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Sophocarpine (SOP) is a tetracyclic quinolizidine alkaloid isolated from *Sophora alopecuroides* L. A number of studies have shown that SOP has anti-inflammatory actions and protects against a variety of tissue and organ injuries. The purpose of this study was to investigate the protective effects of SOP on LPS-induced acute lung injury (ALI) in mice. Lung histological alterations, edema, and MPO activity were measured in this study. Furthermore, the production of inflammatory cytokines and the expression of NF-κB and MAPKs signaling pathways were measured. The results showed that the LPS-induced lung histological alterations, edema, protein concentration, inflammatory cell level in BALF, MDA content, and MPO activity were significantly attenuated by SOP. The LPS-induced inflammatory cytokine TNF-α, IL-1β, and IL-6 were also inhibited by SOP. SOP also inhibited the LPS-induced IL-6 and IL-8 production in A549 cells. Western blot analysis demonstrated that SOP remarkably inhibited the phosphorylation of IκBα and NF-κB. LPS-induced MAPKs activation and TLR4 expression were also suppressed by treatment with SOP. In conclusion, the results indicate that SOP protects against LPS-induced ALI by inhibiting TLR4 signaling pathway.

1. Introduction

Acute lung injury (ALI) is one of the most common acute critical diseases [1]. Its pathogenesis is complex and its mortality is high. ALI is characterized by the extensive damage of pulmonary endothelial cells and alveolar epithelial cells caused by excessive inflammation [2,3]. The imbalance of the inflammatory response induced by bacterial infection is an important factor in causing ALI [4]. LPS, as the major component of the outer membrane of *gram-negative* bacteria, induces the excessive activation and release of various inflammatory factors, which is a key factor in the development of ALI [5]. LPS has the ability to activate TLR4 signaling pathway, which subsequently leads to the production of inflammatory cytokines [6]. A large body of studies demonstrated that the inhibition of the inflammatory response had protective effects against lung injury [7].

Sophocarpine (SOP), a tetracyclic quinolizidine alkaloid isolated from *Sophora alopecuroides* L., has been reported to have anti-inflammatory effects [8]. A previous study demonstrated that SOP inhibited LPS-induced inflammatory cytokine production in RAW264.7 cells [9]. SOP also had the ability to attenuate liver fibrosis in rats [10]. SOP has been reported to protect mice from ConA-induced hepatitis [11]. Furthermore, SOP was found to inhibit the inflammatory response in synoviocytes and protected mice against collagen-induced arthritis [12]. However, the molecular mechanisms underlying the lung-protective properties of SOP are currently unclear. In the present study, the mechanism and the protective effects of SOP on lung injury were investigated.

2. Materials and methods

2.1. Reagents

SOP, purity 98%, was purchased from Shanghai Aladdin Biochemical Technology Co. (Shanghai, China). LPS (*E. coli* O55:B5) and DMSO were purchased from Sigma (St. Louis, MO, USA). MPO test kit was purchased from Jiancheng Institute of Biotechnology (Nanjing, China). TNF-α, IL-1β, and IL-6 ELISA kits were purchased from Biolegend (San Diego, USA). The antibodies used in this study were purchased from Cell Signaling Technology Inc. (Beverly, MA).

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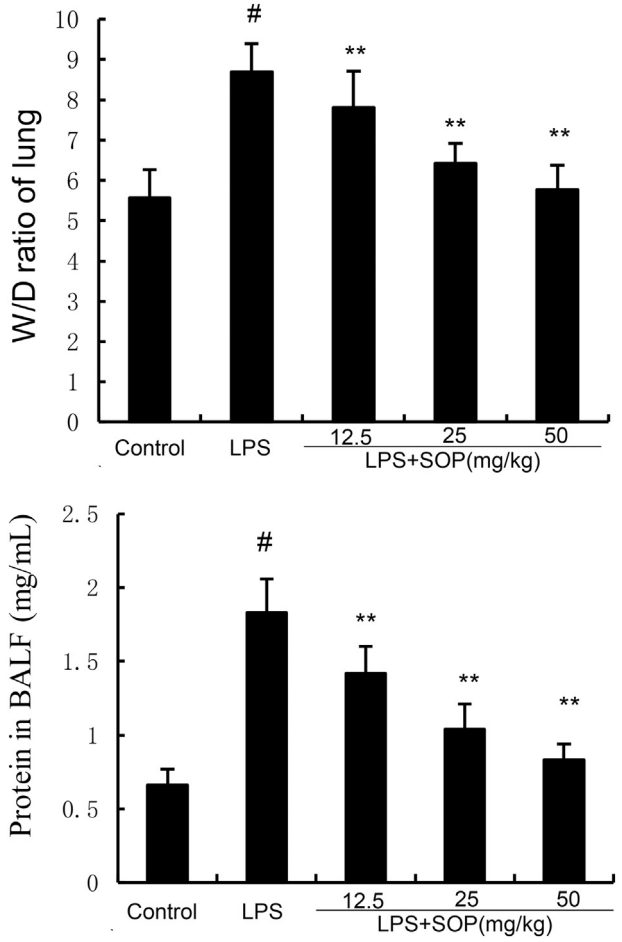

Fig. 1. Effects of SOP on the lung W/D ratio and vascular permeability. The values were presented as mean ± SEM of three independent experiments. #$p < 0.01$ vs. control group, *$p < 0.05$ and **$p < 0.01$ vs. LPS group.

2.2. Animals and treatment

BALB/c mice, weighing 18–20 g, were purchased from the Experimental Animal Center of Jilin University. Before the experiment, the mice were acclimated for 1 week and given free access to food and water. The animal experiment was performed in according to the US National Institutes of Health and approved by the Institutional Animal Ethics Committee of Jilin University (approval no. 20170016). The mice were randomly divided into five groups, each containing twelve mice: control group, model group (LPS group), and LPS + SOP (12.5, 25, and 50 mg/kg) groups. One hour before the intratracheal instillation of 10 mg LPS diluted in 50 ml PBS, SOP (12.5, 25, and 50 mg/kg) was administered to mice i.p. and 12 h after LPS administration, all animals were sacrificed. The SOP doses used in this study were based on a previous study [12].

2.3. Measurement of lung wet/dry ratio and protein content assay

The right lungs were obtained and weighted to calculate the "wet" weight. After placing the lung tissues in an oven at 60 °C for 48 h, we weighted them to record the "dry" weight. The lung W/D ratio was measured by dividing the wet weight by the dry weight. The protein concentration in BALF was measured by the BCA method according to the instructions recommended by the manufacturers.

2.4. Measurement of MPO and MDA

The lung tissues were collected and 100 mg lung tissues were homogenized and fluidized with extraction buffer to obtain 5% homogenate. The MPO activity and MDA content of the lung tissues were determined by commercial kits according to the instructions recommended by the manufacturers.

2.5. Cytokine assay

The contents of TNF-α, IL-1β, and IL-6 in the BALF were determined with the relevant ELISA kits. The optical density of each well was assayed at 450 nm with a microplate spectrophotometer. Finally, the contents were calculated according to the standard curves.

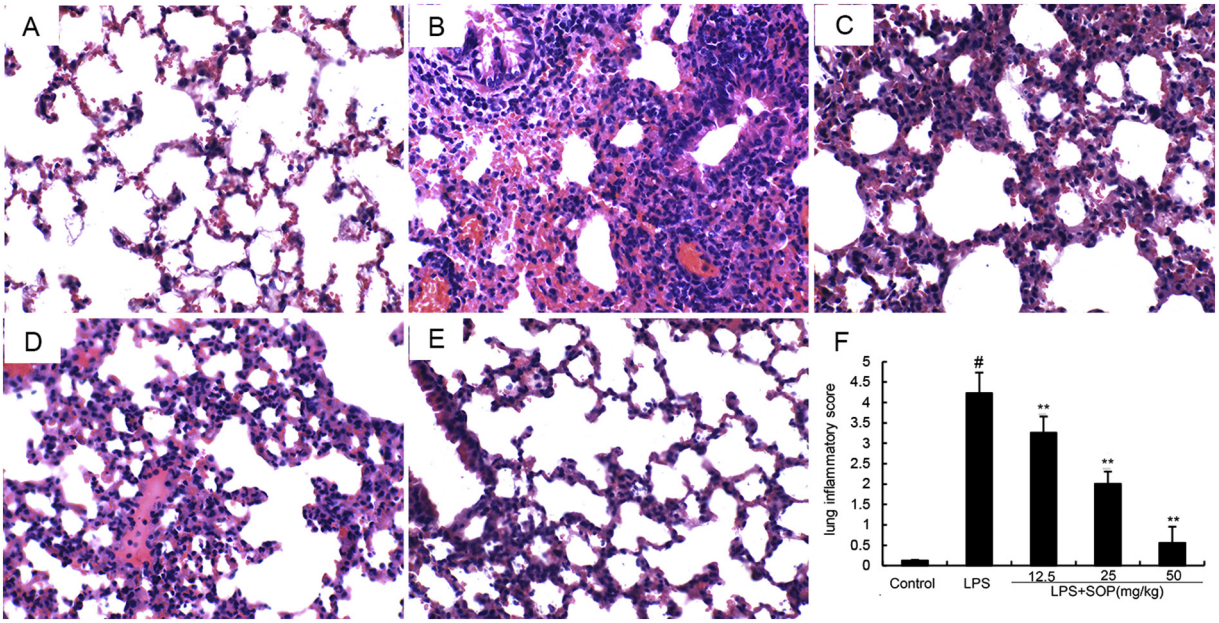

Fig. 2. Effects of SOP on lung histopathological changes. Representative histopathological changes of lung tissues were obtained from mice of different groups. A: Control group, B: LPS group, C: LPS + SOP (12.5 mg/kg) group, D: LPS + SOP (25 mg/kg) group, E: LPS + SOP (50 mg/kg) group, F: Lung score (Hematoxylin and eosin staining, magnification 200×).

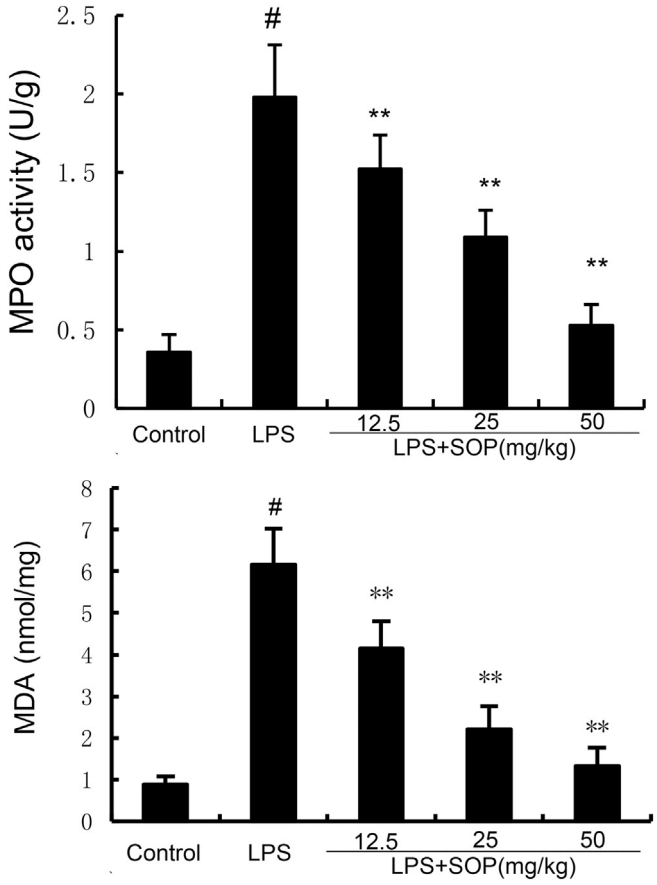

Fig. 3. Effects of SOP on MPO activity and MDA content. The values were presented as mean ± SEM of three independent experiments. $p\#$ < 0.01 vs. control group, p^* < 0.05, p^{**} < 0.01 vs. LPS group.

2.6. Lung histopathology

The effects of SOP on lung histopathology were measured by H&E staining. Formalin-fixed lung tissues were embedded in paraffin and cut into sections. Finally, the sections were stained with H&E (Sigma, USA) according to the instructions recommended by the manufacturer. The histological changes in the lungs were scored as previously described [13]. Each histological characteristic was scored 0 to 5.

2.7. Inflammatory cell counts

The BALF was collected and centrifuged at 3000 × *g* for 15 min at 4 °C. The number of total cells, neutrophils, and macrophages were counted with a hemocytometer.

2.8. Western blot assay

Proteins from lung tissues were extracted using RIPA buffer and quantified by BCA method (Beyotime, Nanjing, China). The proteins were subjected to 12% SDS-PAGE and electrotransferred onto PVDF membranes. After blocking in nonfat milk for 2 h, the membranes were incubated with antibodies against p38, JNK, ERK, IκBα, NF-κB p65, and TLR4 for 12 h at 4 °C. The membranes were washed three times with TBST and incubated with secondary antibodies. Finally, the bands were tested using the enhanced chemiluminescence reagent (KeyGEN Biotechnology, Nanjing, China) and quantified using ImageJ software.

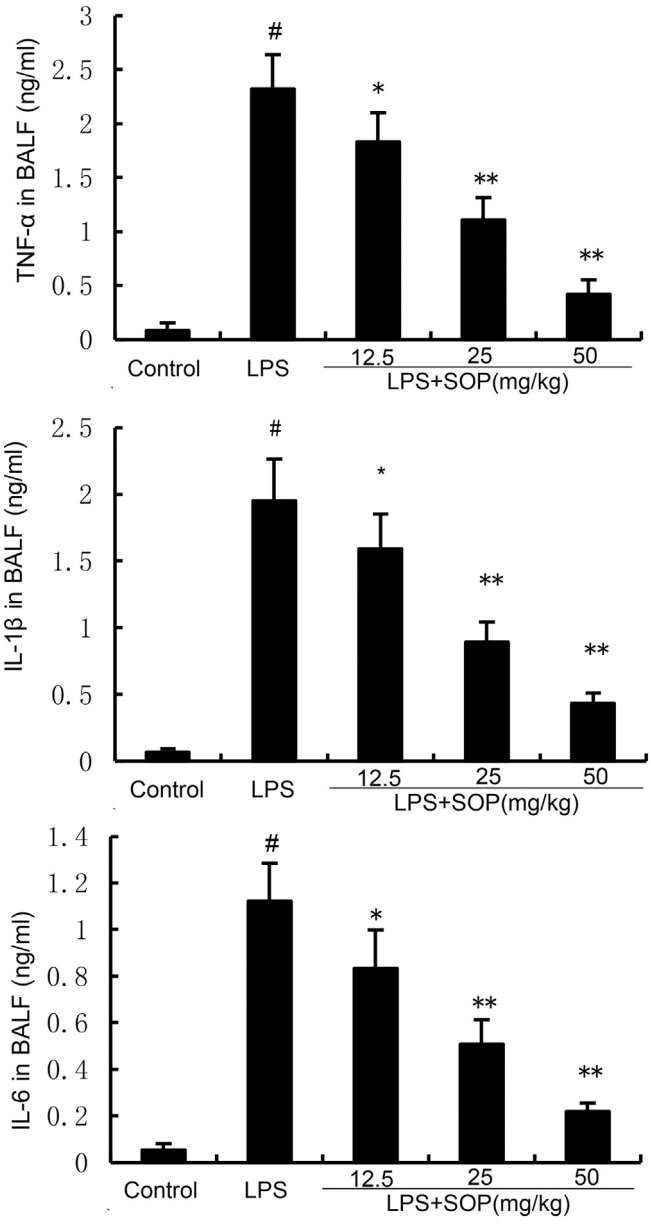

Fig. 4. Effects of SOP on TNF-α, IL-1β, and IL-6 production in the BALF. The values were presented as mean ± SEM of three independent experiments. $p\#$ < 0.01 vs. control group, p^* < 0.05, p^{**} < 0.01 vs. LPS group.

2.9. In vitro study

Human lung epithelial A549 cells were used in this study for in vitro study. The cells were cultured in DMEM supplemented with 5% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 °C in a humidified incubator under 5% CO_2. The cells were treated with SOP 1 h before LPS treatment. 24 h after LPS treatment, the culture supernatants were collected and the levels of IL-8 and IL-6 were measured by ELISA. NF-κB activation was measured by western blot analysis.

2.10. Statistical analysis

All the data were expressed as mean ± SEM. One-way ANOVA with Tukey multiple comparison test were used to perform the statistical analysis. Statistical significance was accepted at p < 0.05.

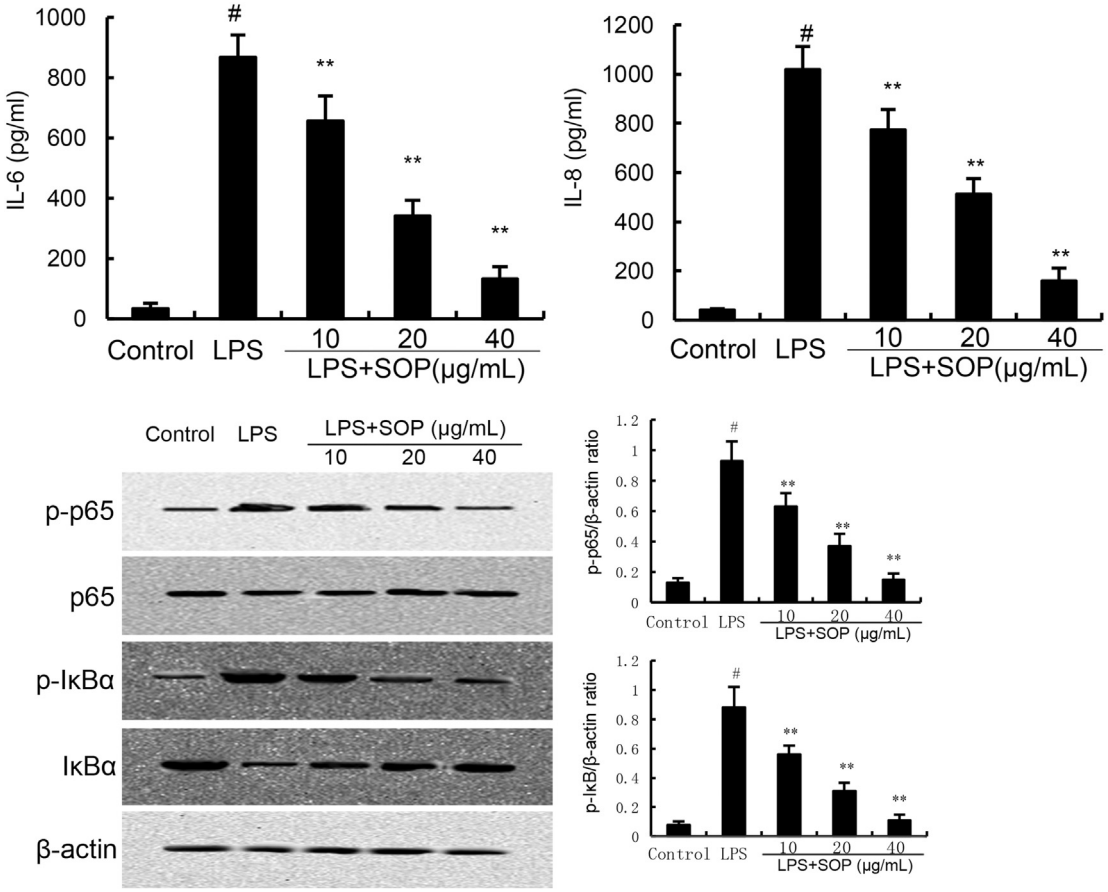

Fig. 5. Effects of SOP on LPS-induced IL-6 and IL-8 production, as well as NF-κB activation in A549 cells. The values were presented as mean ± SEM of three independent experiments. $p\# < 0.01$ vs. control group, $p^* < 0.05$, $p^{**} < 0.01$ vs. LPS group.

3. Results

3.1. Effects of SOP on lung edema and vascular permeability

The effects of SOP on LPS-induced lung W/D ratio were measured to assess lung edema in this study. The results demonstrated that LPS challenge significantly increased the lung W/D ratio. Compared to the LPS group, SOP remarkably inhibited LPS-induced lung W/D ratio (Fig. 1). The effect of SOP on vascular permeability was measured by detecting protein concentration in the BALF. The results demonstrated that LPS challenge significantly increased the protein concentration in the BALF. Compared to the LPS group, SOP remarkably inhibited LPS-induced protein concentration in the BALF (Fig. 1).

3.2. Effects of SOP on lung pathological changes

The lung pathological changes are shown in Fig. 2, no evident histological alteration was observed in lung specimens in the control group. The lung tissues of the LPS group exhibited severe pathological changes, such as alveolar hemorrhage, lung edema, and infiltration of inflammatory cells. Compared to the LPS group, SOP remarkably alleviated LPS-induced lung pathological changes.

3.3. SOP inhibits LPS-induced MPO activity and MDA content

The effects of SOP on LPS-induced MPO activity and MDA content were measured to assess the infiltration of neutrophils in lung tissues in this study. The results demonstrated that LPS challenge significantly increased the MPO activity and MDA content in lung tissues. Compared to the LPS group, SOP remarkably inhibited LPS-induced MPO activity and MDA content in lung tissues (Fig. 3).

3.4. SOP attenuates LPS-induced inflammatory cytokine production

The effects of SOP on LPS-induced TNF-α, IL-1β, and IL-6 production were measured in this study. The results demonstrated that LPS challenge significantly increased the production of TNF-α, IL-1β, and IL-6. Compared to the LPS group, SOP remarkably inhibited TNF-α, IL-1β, and IL-6 production in the BALF (Fig. 4). In vitro, SOP (10, 20, 40 μg/ml) significantly inhibited LPS-induced IL-6 and IL-8 production, as well as NF-κB activation (Fig. 5).

3.5. SOP attenuates LPS-induced inflammatory cells numbers in BALF

The number of inflammatory cells in BALF was counted in this study. As shown in Fig. 6, the numbers of total cells, neutrophils, and macrophages increased significantly after LPS treatment. Compared to the LPS group, SOP remarkably inhibited the numbers of total cells, neutrophils, and macrophages in the BALF (Fig. 6).

3.6. Effects of SOP on LPS-induced NF-κB activation

NF-κB signaling pathway is a well-known target for the regulation of inflammatory response. LPS challenge contributed to the upregulation of phosphorylated IκBα and NF-κB p65. Compared to the LPS group, SOP remarkably inhibited LPS-induced phosphorylation of IκBα and NF-κB p65 (Fig. 7). LPS-induced NF-κB nuclear translocation was also inhibited by SOP (Fig. 7). LPS also induced an increase in phosphorylated p38, JNK, and ERK. However, LPS-induced MAPKs activation was significantly inhibited by SOP (Fig. 8).

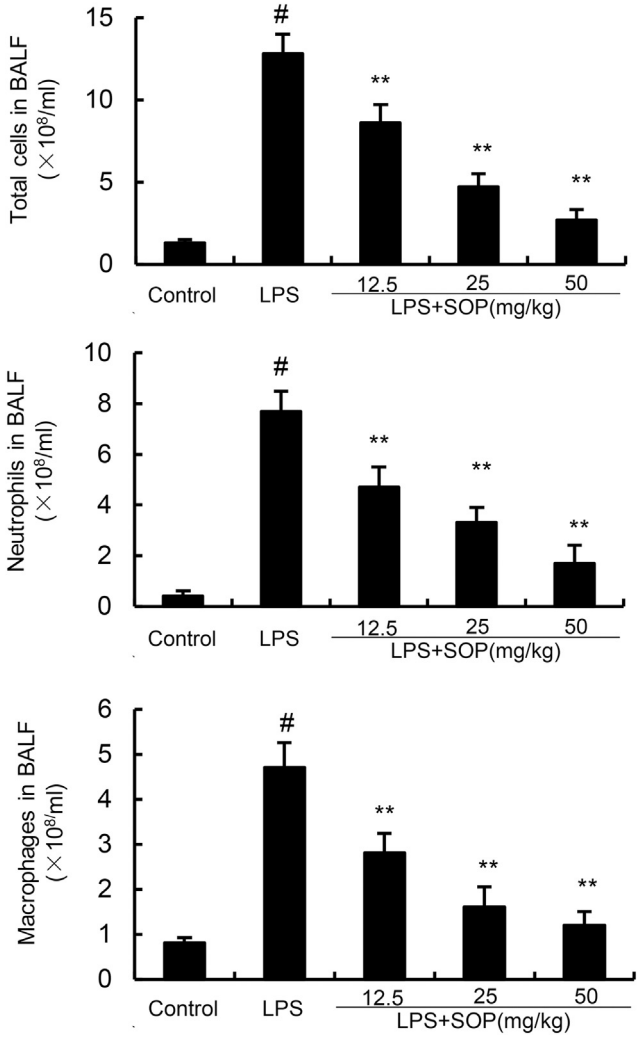

Fig. 6. Effects of SOP on inflammatory cells in the BALF. The values were presented as mean ± SEM of three independent experiments. $p\# < 0.01$ vs. control group, $p* < 0.05$, $p** < 0.01$ vs. LPS group.

3.7. Effects of SOP on LPS-induced TLR4 expression

TLR4 activation could lead to the activation of NF-κB signaling pathway. LPS challenge led to the upregulation of TLR4 expression. Compared to the LPS group, SOP remarkably inhibited LPS-induced TLR4 expression (Fig. 9).

4. Discussion

SOP has been reported to have anti-inflammatory effects. In the present study, we found that SOP alleviated LPS-induced ALI in mice, as confirmed by the decreases in lung pathological changes, MPO activity, lung edema, and inflammatory cytokine production. The mechanism may be attributed to the inhibition of TLR4 signaling pathway.

ALI is characterized by an excessive systemic inflammatory response. A significant elevation of the inflammatory cytokines TNF-α, IL-1β, and IL-6 was observed after LPS treatment [14]. These inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 could induce the injury of lung tissues and the necrosis of lung epithelial cells [15]. The increases in inflammatory cytokine production are dependent on the stimulation of lung macrophages or lung epithelial cells by LPS. In this study, our results showed that SOP could also inhibit LPS-induced IL-6 and IL-8 production in human lung epithelial cells (A549 cells). In previous studies, SOP has been reported to inhibit LPS-induced

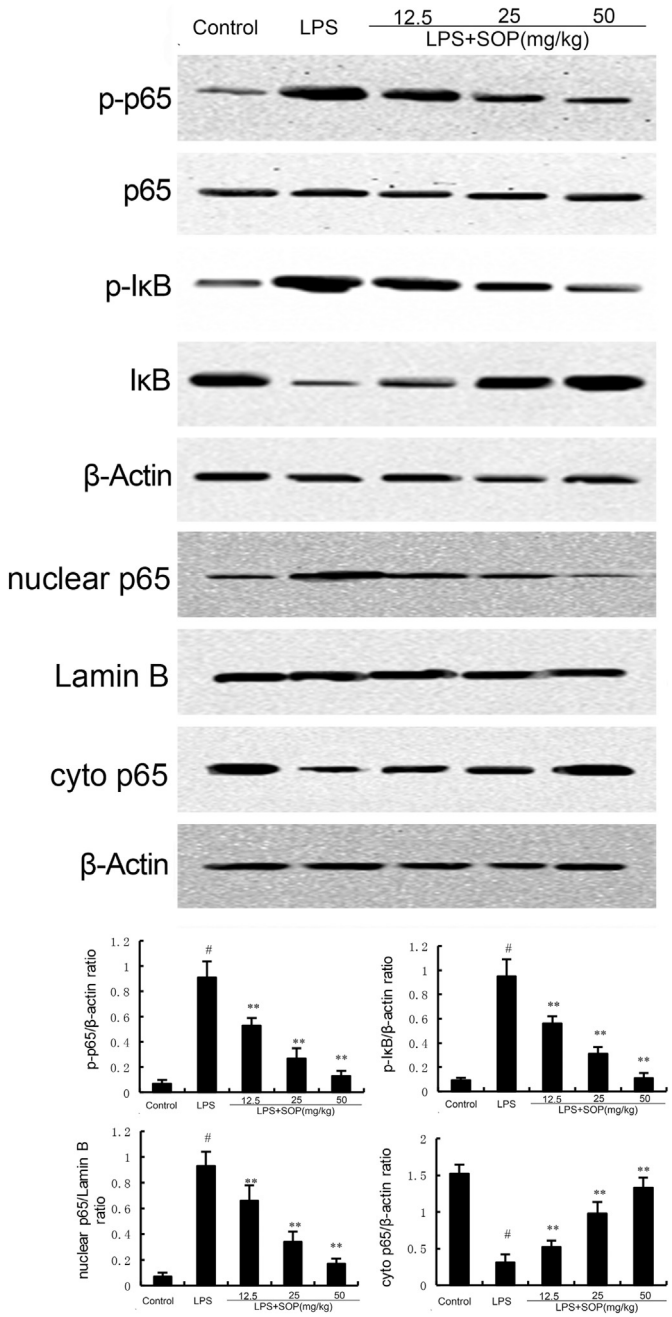

Fig. 7. SOP inhibits LPS-induced NF-κB activation. The values were presented as mean ± SEM of three independent experiments. $p\# < 0.01$ vs. control group, $p* < 0.05$, $p** < 0.01$ vs. LPS group.

inflammatory cytokine production both in vivo and in vitro. Our results were consistent with these studies. Our results suggested that SOP exhibited anti-inflammatory effects in lung injury. Moreover, the increased inflammatory cytokines could further promote NF-κB signaling pathway activation and lead to the further release of inflammatory cytokines and oxidative mediators [16,17]. Additionally, these inflammatory cytokines could induce the infiltration of neutrophils into lung tissues [18]. The infiltrated neutrophils are the major cells that release oxidative mediators. In this study, LPS-induced inflammatory cell infiltration was significantly attenuated by SOP. Furthermore, MDA, the biomarker of oxidative stress, was also significantly inhibited by SOP. These results suggested that SOP protected against LPS-induced ALI by inhibiting inflammatory and oxidative stress.

The production of inflammatory cytokines was regulated by the NF-

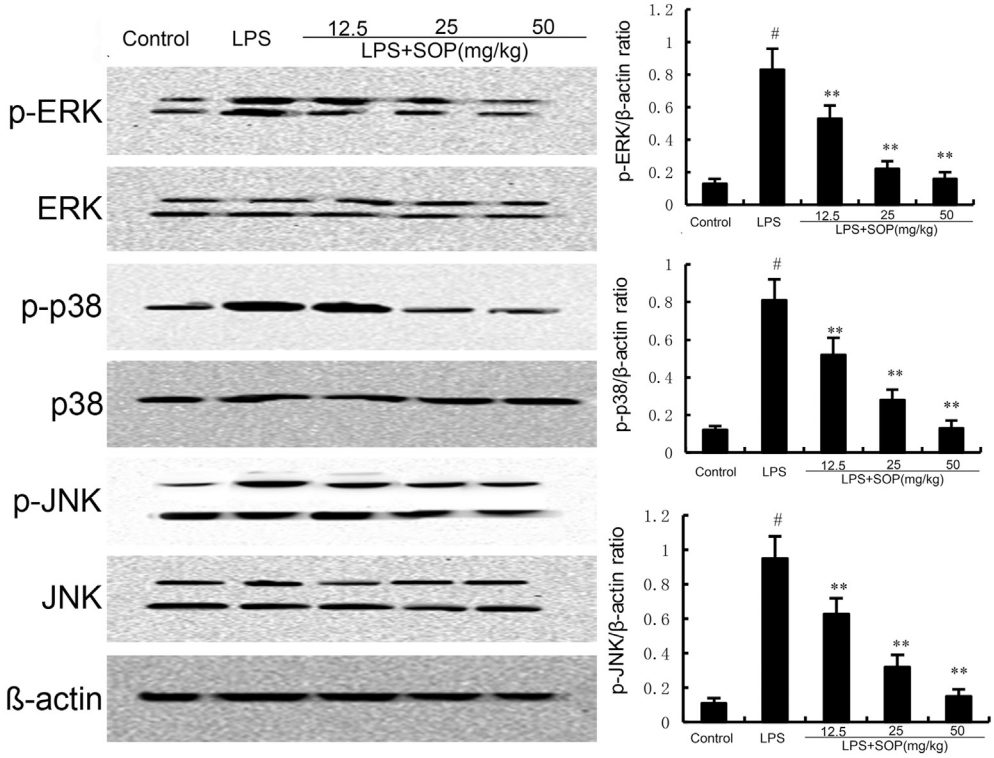

Fig. 8. SOP inhibits LPS-induced MAPKs activation. The values were presented as mean ± SEM of three independent experiments. $p\#$ < 0.01 vs. control group, p^* < 0.05, p^{**} < 0.01 vs. LPS group.

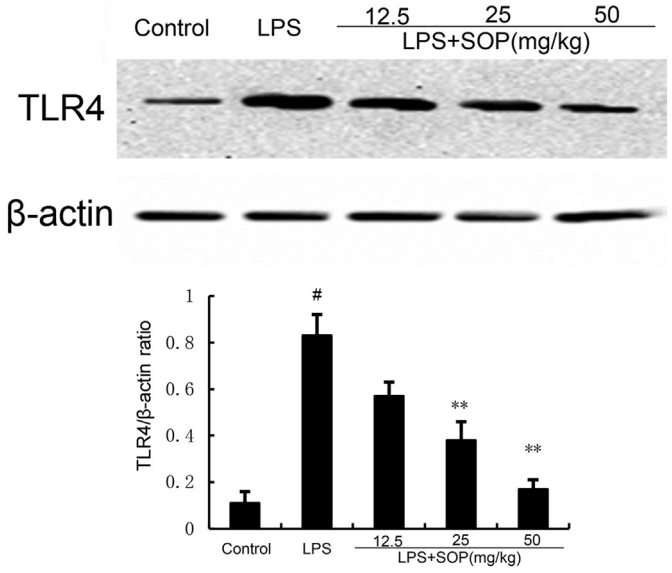

Fig. 9. Effects of SOP on TLR4 expression. The values were presented as mean ± SEM of three independent experiments. $p\#$ < 0.01 vs. control group, p^* < 0.05, p^{**} < 0.01 vs. LPS group.

κB and MAPKs signaling pathways [19]. LPS significantly increased the expression of TLR4, which results in the activation of NF-κB and MAPKs. NF-κB is an important molecule that plays a critical role in the regulation of inflammatory cytokine production [20,21]. Emerging evidence has demonstrated that the TLR4 and NF-κB signaling pathway were involved in the development of ALI [22]. Many agents protected mice against LPS-induced ALI through inhibiting TLR4 signaling pathway [23,24]. Furthermore, studies showed that TLR4 could be used as a target for the treatment of ALI. Therefore, we investigated whether SOP protected mice against LPS-induced ALI by regulating TLR4 signaling pathway. Our results demonstrated that SOP remarkedly inhibited LPS-induced TLR4 expression. Meanwhile, LPS-induced NF-κB and MAPKs activation was also suppressed by SOP. These results suggested SOP inhibited LPS-induced ALI through inhibiting TLR4 signaling pathway.

In conclusion, SOP exhibited its anti-inflammatory effect against LPS-induced ALI. The mechanism may be through the inhibition of TLR4 expression and NF-κB and MAPKs activation. SOP could be used as a potential anti-inflammatory agent for the treatment of lung injury.

Conflict of interest statement

All authors declare that they have no conflict of interest.

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