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ORIGINAL ARTICLE

Propofol Protects Rats and Human Alveolar Epithelial Cells Against Lipopolysaccharide-Induced Acute Lung Injury via Inhibiting HMGB1 Expression

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Abstract—High-mobility group box 1 (HMGB1) plays a key role in the development of acute lung injury (ALI). Propofol, a general anesthetic with anti-inflammatory properties, has been suggested to be able to modulate lipopolysaccharide (LPS)-induced ALI. In this study, we investigated the effects of propofol on the expression of HMGB1 in a rat model of LPS-induced ALI. Rats underwent intraperitoneal injection of LPS to mimic sepsis-induced ALI. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS administration, followed by infusion at 5 mg/(kg · h) through the left femoral vein cannula. LPS increased wet to dry weight ratio and myeloperoxidase activity in lung tissues and caused the elevation of total protein and cells, neutrophils, macrophages, and neutrophils in bronchoalveolar lavage fluid (BALF). Moreover, HMGB1 and other cytokine levels were increased in BALF and lung tissues and pathological changes of lung tissues were excessively aggravated in rats after LPS administration. Propofol inhibited all the above effects. It also inhibited LPS-induced toll-like receptor (TLR)2/4 protein upexpression and NF-κB activation in lung tissues and human alveolar epithelial cells. Propofol protects rats and human alveolar epithelial cells against HMGB1 expression in a rat model of LPS-induced ALI. These effects may partially result from reductions in TLR2/4 and NF-κB activation.

KEY WORDS: propofol; HMGB1; LPS; NF-κB; TLR4; acute lung injury.

INTRODUCTION

Acute lung injury (ALI) is a major consequence of septic shock and contributes to the high morbidity and mortality of sepsis [1]. Previous studies have demonstrated that, after severe injury or infectious challenge, some patients respond by activating pro-inflammatory signaling pathways and overexpressing inflammatory mediators that result in a systemic inflammatory response that culminates in severe shock, multi-organ failure, and death [2]. Despite extensive investigation, the cellular and molecular mechanisms that mediate ALI during septic shock have remained largely unknown. Furthermore, developing effective methods for preventing and/or treating sepsis-induced ALI has proven to be difficult. A growing body of evidence suggests that there is a link between the innate immune response and ALI in several important disease states, including ischemia-reperfusion injury [3], traumatic brain injury [4], and septic shock [5].

High mobility group box 1 (HMGB1) is an evolutionarily conserved protein present in virtually all types of cells, where it functions to stabilize nucleosomal structure and regulate gene expression [6]. Recent evidence suggests that extracellular HMGB1 functions as a danger-associated molecular pattern [7] and is actively secreted by

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immunologically activated immune cells or passively released from pathologically damaged cells [8]. *In vivo* neutralization of HMGB1 by specific antibodies protects mice against lethal sepsis [9], as well as lipopolysaccharide (LPS)-induced ALI [10]. In a more clinically relevant animal model of sepsis (induced by cecal ligation puncture, CLP), delayed administration of HMGB1-specific neutralizing antibodies, beginning 24 h after CLP, dose dependently rescue rodents from lethal sepsis [11]. Moreover, targeted inhibition of HMGB1 expression in innate immune cells (e.g., macrophages and dendritic cells) reduces systemic HMGB1 accumulation and similarly rescues mice from sepsis [12]. Taken together, these experimental data establish extracellular HMGB1 as a critical late mediator of experimental sepsis. *In vitro* studies have demonstrated that the HMGB1-stimulated inflammatory response could be mediated through several pattern-recognition receptors including the receptors for advanced glycation end products [13], toll-like receptor 2 (TLR2) [14], TLR4 [15], and TLR9 [16].

Propofol is an anesthetic agent with sedative properties that modulates innate immunity and pro-inflammatory signaling in sepsis [17, 18]. Propofol administration downregulates sepsis-induced increases in TLR2/4 expression [19, 20], attenuates sepsis-induced NF-κB activity [21], and activates heme oxygenase-1 expression [22]. Previous studies demonstrated that propofol inhibits sepsis-induced ALI in rats [17, 20]. Therefore, we hypothesized that propofol would down-regulate HMGB1 expression and that the inhibition of HMGB1 expression would be associated with the inhibition of TLR2/4 and NF-κB activation by propofol during sepsis. The aim of the present study was to determine whether propofol attenuates ALI by the inhibition of HMGB1 expression in a rat LPS model.

MATERIALS AND METHODS

Materials

Propofol and LPS (*Escherichia coli* 055:B5) were obtained from Sigma (St. Louis, MO, USA). HMGB1, TNF-α, IL-6, and myeloperoxidase (MPO) enzyme-linked immunosorbent assay (ELISA) kit were obtained from Invitrogen (Carlsbad, CA, USA). Anti-TLR2, anti-TLR4, anti-p-NF-κB p65, and anti-NF-κB p65 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-IκB-α and anti-p-IκB-α antibodies were obtained from Cell Signaling Technology Inc (Beverly, MA, USA).

Animals

Adult male Sprague–Dawley rats (weighing 250 to 300 g) were provided by the Experimental Animal Center of Shandong University, kept in a 12-h dark/12-h light cycle in a temperature- and humidity-controlled room and fed on standard laboratory diet and water. All procedures were performed in accordance with the Declaration of Helsinki of the World Medical Association. The study was approved by the ethics committee of Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China.

Animal Experimental Design

Animals were randomly divided into six groups and each group contained 20 rats: (1) control group (normal saline, NS), (2) propofol (10 mg/kg) group, (3) LPS group (5 mg/kg), (4) LPS + propofol (1 mg/kg) group, (5) LPS + propofol (5 mg/kg) group, and (6) LPS + propofol (10 mg/kg) group. In another experiments, animals were randomly divided into six groups and each group contained 20 rats: (1) control group (NS), (2) propofol (10 mg/kg) group, (3) HMGB1 group (5 mg/kg), (4) HMGB1 + propofol (1 mg/kg) group, (5) HMGB1 + propofol (5 mg/kg) group, and (6) HMGB1 + propofol (10 mg/kg) group. LPS (5 mg/kg), HMGB1 (5 mg/kg), or vehicle (NS) was intraperitoneally administered to induce ALI [23]. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg·h) through the left femoral vein cannula. The doses of these drugs were on the basis of previous studies and our preliminary experiments [17, 18, 20, 24, 25]. At 24 h after LPS/HMGB1 administration, the rats were sacrificed and samples were collected.

Cell Culture and Treatment

\The human alveolar epithelial cells A549 were obtained from the Typical Species Preservation Center of Wuhan University (Wuhan, Hubei, China). A549 cells were seeded into 6-well plates and were cultured in DMEM supplemented with 10 % FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5 % CO_2. Cells were grown until 70 % confluence before drug treatments. Cells were divided into four groups: (1) control group (NS), (2) propofol (100 μM) group, (3) LPS group (1 μg/mL), and (4) LPS + propofol (100 μM) group. A549 cells were treated with propofol (100 μM) 24 h after LPS (1 μg/mL) stimulation. The doses of these drugs were on the basis of previous

studies and our preliminary experiments [26]. The cell samples were harvested at 24 h after the addition of LPS to analyze the expressions of TLR2, TLR4, p-NF-κB p65, and p-IκB-α.

Lung Wet/Dry Weight Ratio *In Vivo*

The water content of lungs was determined by calculating the wet/dry weight ratio of lung tissues. The inferior lobe of right lung was excised, rinsed briefly in phosphate-buffered saline (PBS), blotted, and then weighed to obtain the "wet" weight. The lung was then dried at 80 °C for 72 h to obtain the "dry" weight. The wet/dry ratio was calculated by dividing the wet weight by the dry weight.

Determination of Bronchoalveolar Lavage Proteins and Cell Counts

Bronchoalveolar lavage (BAL) was performed by intratracheal injection of 5-mL ice-cold PBS followed by gentle aspiration. The recovery ratio of the fluid was about 90 %. Then, the recovered fluid was pooled and centrifuged at 1200×*g* for 10 min at 4 °C. Supernatants were preserved for the measurement of total protein concentration by the Bradford method with bovine serum albumin (BSA) as a standard. The cell pellet was re-suspended in 50 μL PBS, and total cells recovered in bronchoalveolar lavage fluid (BALF) were counted. The cell differentiation was determined for 200 cells by examination of the hematoxylin and eosin (H&E)-stained smears.

Cytokine Measurements

The levels of HMGB1, TNF-α, and IL-6 in the supernatants of BALF and lung tissues were measured with a commercially available ELISA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

MPO Activity Assay

Lung tissues were homogenized in hydroxyethyl piperazine ethanesulfonic acid (pH 8.0) containing 0.5 % cetyltrimethyl ammonium bromide and subjected to three freeze-thaw cycles. The homogenate was centrifuged (4 °C, 12,000×*g*, 30 min). The MPO activity was assayed using a commercially available ELISA kit (Invitrogen, Carlsbad, CA, USA). Samples were diluted in phosphate citrate buffer (pH 5.0), and the absorbance of the sample was measured at 460 nm using a microplate reader. The specific activity of MPO in the lung is expressed as unit per gram of the tissue.

Histological Examination

The right lobes were excised and fixed with 10 % neutral phosphate-buffered formalin, imbedded in paraffin and sliced. After H&E staining, pathological changes of lung tissues were observed under a light microscope. The standard lung injury score was performed by a blinded pathologist to objectively quantify the lung injury.

Western Blot Analysis

Protein concentrations were determined by BCA Protein Assay Kit, and 20 μg proteins was loaded per well on a 10 % sodium dodecylsulfate-polyacrylamide gel and transferred onto polyvinylidene difluoride
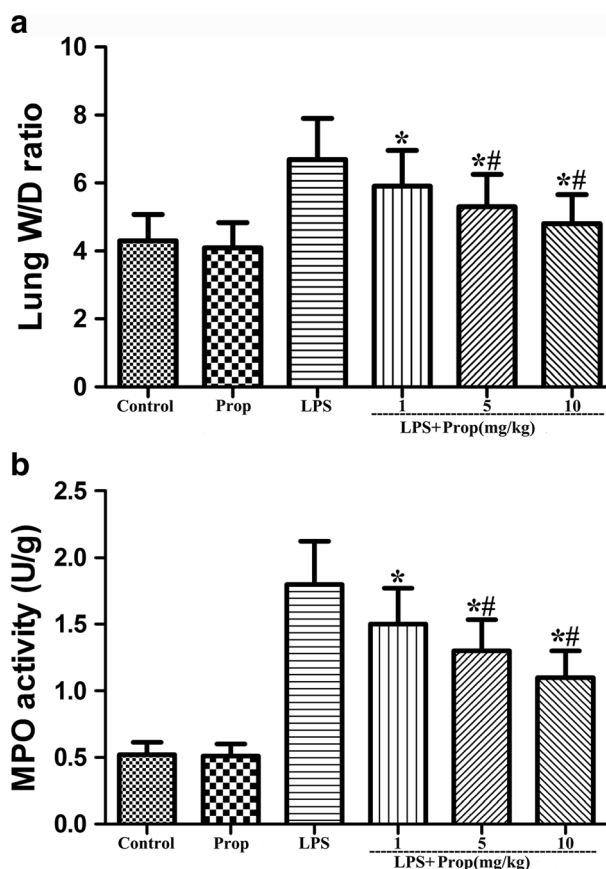
Fig. 1. Propofol reduced the lung wet/dry weight ratio and the MPO activity in LPS-induced ALI rats. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg·h) through the left femoral vein cannula. The lung wet/dry weight ratio (**a**) and the MPO activity (**b**) were determined at 24 h after LPS administration. The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with LPS group; #$P < 0.01$ compared with LPS group. *Prop* propofol.

membrane. After being blocked for 3 h in Tris-buffered saline with 0.1 % Tween 20 (TBST) and 3 % BSA, membranes were incubated overnight at 4 °C with primary antibodies in TBST containing 3 % BSA. Membranes were then washed and incubated with horse radish peroxidase-conjugated secondary antibodies in TBST for 2 h and developed using an ECL detection system. The density of the bands on the membrane was scanned and analyzed with an image analyzer (Lab Works Software, UVP Upland, CA, USA).
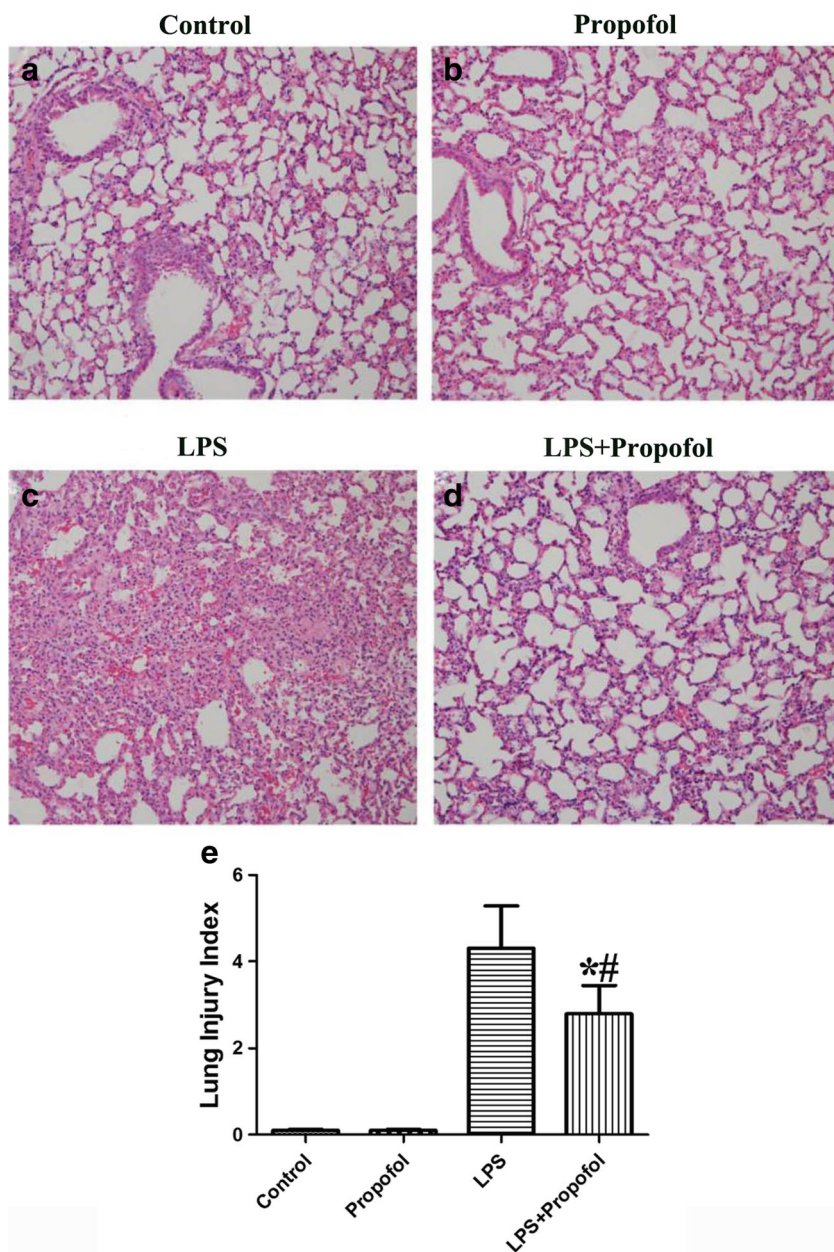

Fig. 2. Histologic assessment of the effect of propofol on LPS-induced ALI. Propofol bolus (10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg · h) through the left femoral vein cannula. Lungs from each experimental group were processed for histological evaluation at 24 h after LPS administration. **a** Control group, **b** propofol (10 mg/kg) group, **c** LPS group, **d** LPS + propofol (10 mg/kg) group (H&E staining, magnification ×200), and **e** lung injury index. The data are presented as means ± SD ($n = 20$).*$P < 0.05$ compared with LPS group; [#]$P < 0.01$ compared with LPS group.

Statistical Evaluation

Data are the mean ± standard deviation (SD) of results obtained from 20 rats in each group *in vivo* and three replicates *in vitro*. Statistical analysis of the results was carried out by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in SPSS 11.0 (Chicago, IL, USA), and P values < 0.05 were considered to be statistically significant.

RESULTS

Effects of Propofol on the Lung Wet/Dry Weight Ratio in Lung Tissues

To evaluate the LPS/HMGB1-induced changes in pulmonary vascular permeability, the lung wet/dry weight ratio in lung tissues was analyzed. As shown in Figs. 1 and 5, the lung wet/dry ratio in lung tissues was significantly increased after LPS/HMGB1 challenge compared with control group. However, propofol administration obviously decreased the wet/dry ratio in lung tissues in a dose-dependent manner ($P < 0.05$; Figs. 1a and 5a). There were no significant differences of the wet/dry ratio in lung tissues between control and propofol groups.

Effects of Propofol on MPO Activity in Lung Tissues

Neutrophils are the major components of inflammatory and immunological reactions in injured lungs. MPO activity in lung tissues is known as a reliable marker of neutrophil infiltration. In this study, MPO activity in the homogenates of lung tissues was detected at 24 h after LPS/HMGB1 administration. As shown in Figs. 1 and 5, LPS/HMGB1 administration significantly increased MPO activity compared with control group. However, propofol treatment apparently decreased MPO activity in lung tissues of LPS/HMGB1-challenged rats in a dose-dependent manner ($P < 0.05$; Figs. 1b and 5b).
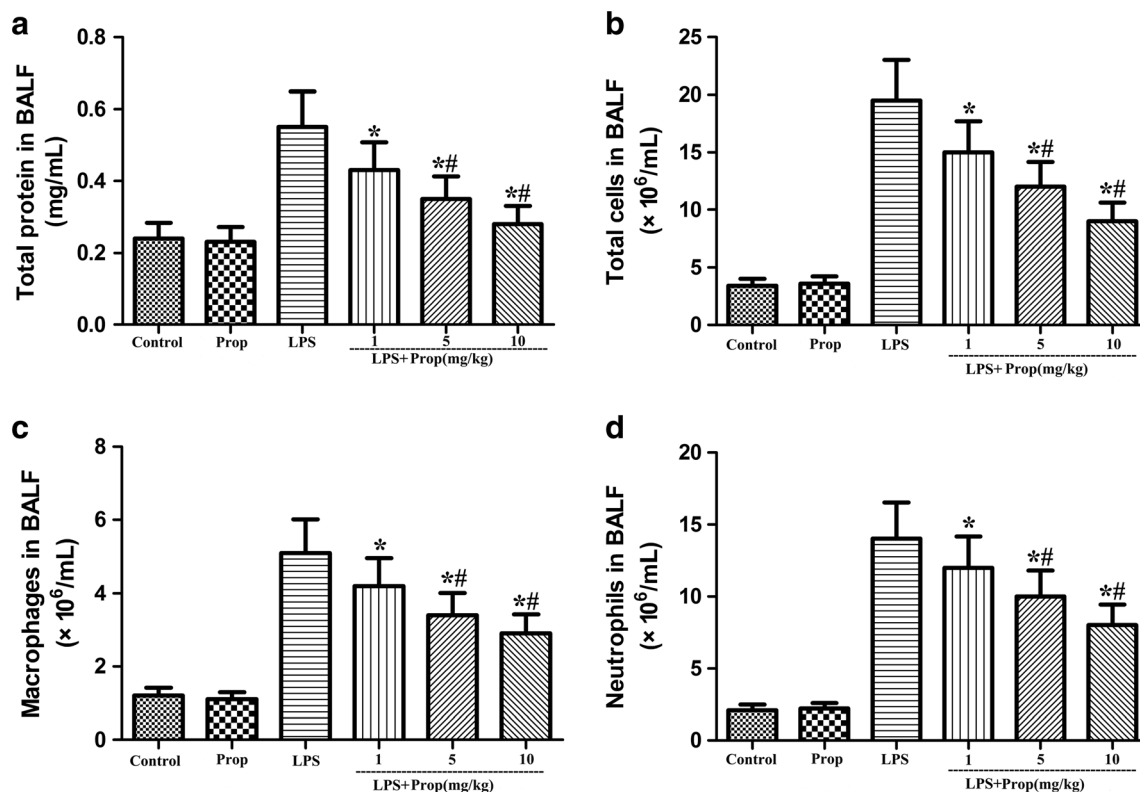

Fig. 3. Effects of propofol on the total protein concentration and the numbers of total cells, neutrophils, and macrophages in BALF. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg · h) through the left femoral vein cannula. BALF was collected at 24 h after LPS administration to measure the total protein concentration (**a**) and the numbers of total cells (**b**), neutrophils (**c**), and macrophages (**d**). The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with LPS group; #$P < 0.01$ compared with LPS group. *Prop* propofol.

Effects of Propofol on LPS-Mediated Lung Histopathologic Changes

To evaluate the histological changes after propofol treatment in LPS-treated rats, lung tissues were harvested at 24 h after administration of LPS. As shown in Fig. 2a, lung tissues from control group showed a normal structure and no histopathologic changes. In LPS group, histological examination revealed serious lung destruction, as indicated by the H&E assay, which manifest as serious pulmonary edema, hemorrhagia in stroma, alveolar collapse, and massive inflammatory cell infiltration (Fig. 2c). However, propofol treatment effectively alleviated the destruction of lung structure (Fig. 2d). Furthermore, propofol treatment group had a significantly lower lung injury score than control group ($P < 0.05$; Fig. 2e).
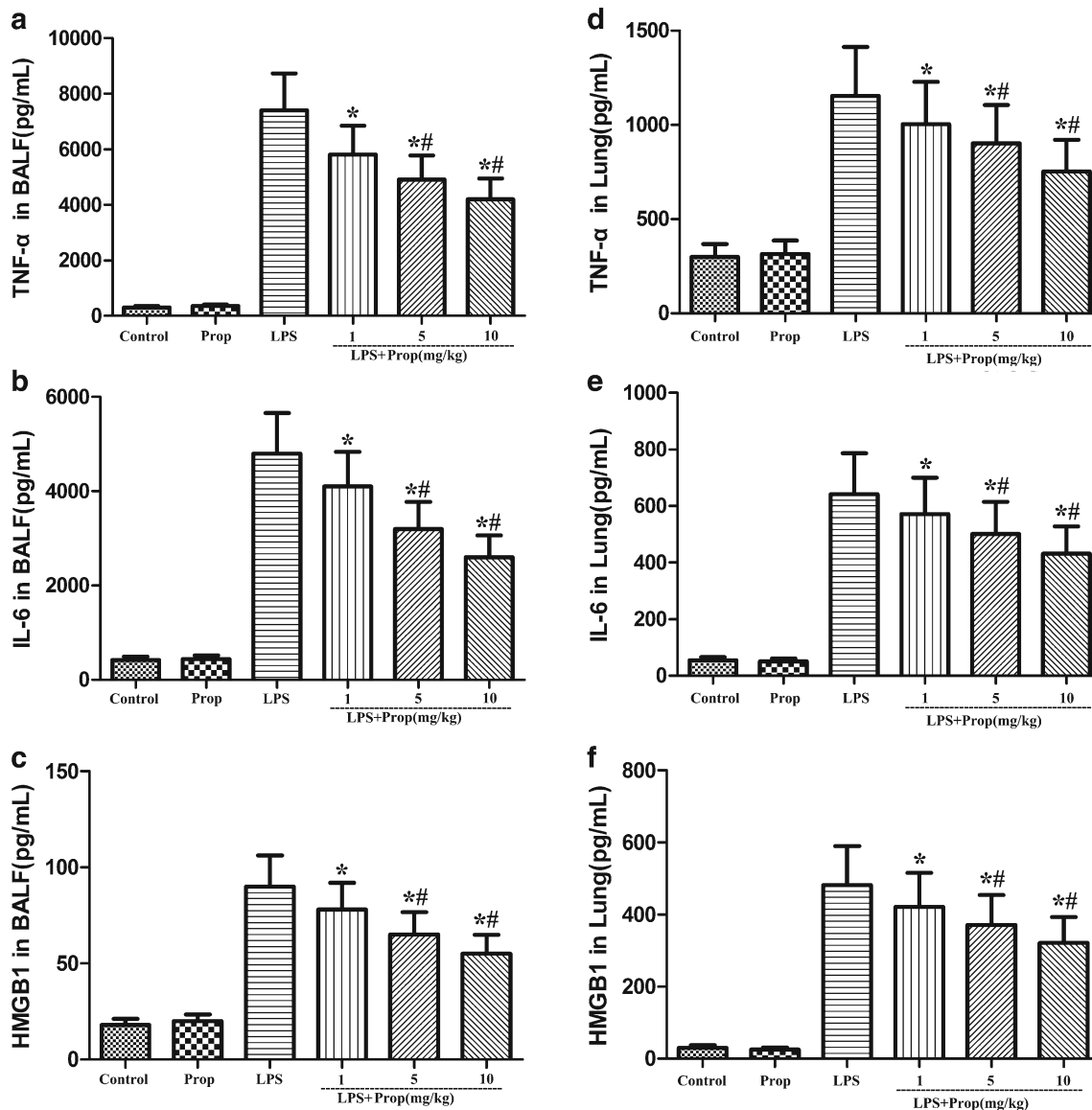

Fig. 4. Propofol inhibited LPS-induced expressions of TNF-α, IL-6, and HMGB1 in BALF and lung tissues. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg · h) through the left femoral vein cannula. BALF and lung tissues were collected at 24 h after LPS administration to analyze the inflammatory cytokines TNF-α (**a**, **d**), IL-6 (**b**, **e**), and HMGB1 (**c**, **f**). The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with LPS group; #$P < 0.01$ compared with LPS group. *Prop* propofol.

Effects of Propofol on the Concentration of Total Protein and the Inflammatory Cell Counts in BALF

To examine the effects of propofol on LPS/HMGB1-induced pulmonary inflammation, the concentration of total protein and the numbers of inflammatory cells, such as neutrophils and macrophages, in BALF were analyzed at 24 h after LPS/HMGB1 injection. As shown in Figs. 3 and 6, after LPS/HMGB1 challenging, the concentration of total protein and the numbers of total cells, neutrophils, and macrophages significantly increased compared with control group. However, this increase was apparently attenuated by propofol treatment in a dose-dependent manner ($P<0.05$; Fig. 3 and 6).

Effects of Propofol on the Concentrations of Pro-Inflammatory Cytokines in BALF and Lung Tissues

To further evaluate the anti-inflammatory action of propofol, the concentrations of pro-inflammatory cytokines TNF-α, IL-6, and HMGB1 in BALF and lung tissues were analyzed at 24 h after LPS/HMGB1 administration by ELISA. As illustrated in Figs. 4, 5, 6, and 7, the concentrations of TNF-α, IL-6, and HMGB1 in BALF and lung tissues significantly increased in LPS/HMGB1 group. However, propofol treatment markedly decreased the levels of TNF-α, IL-6, and HMGB1 compared to those in LPS/HMGB1 group in a dose-dependent manner ($P<0.05$; Figs. 4 and 7).

Effects of Propofol on the Expressions of TLR2 and TLR4 in Lung Tissues

In this study, the expressions of TLR2 and TLR4 in lung tissues were detected by Western blotting. As shown in Fig. 8, the levels of TLR2 and TLR4 proteins showed significant increases in LPS group at 24 h after LPS administration. However, this increase was apparently attenuated by propofol treatment in a dose-dependent manner ($P<0.05$; Fig. 8a, b).

Effects of Propofol on LPS-Induced NF-κB Activation in Lung Tissues

Western blot analysis was used to determine the phosphorylation of NF-κB p65 and IκB-α, which reflected the activation of NF-κB in lung tissues. As shown in Fig. 8, LPS administration significantly increased the phosphorylation of NF-κB p65 and IκB-α, as compared with control group. However, propofol treatment markedly decreased
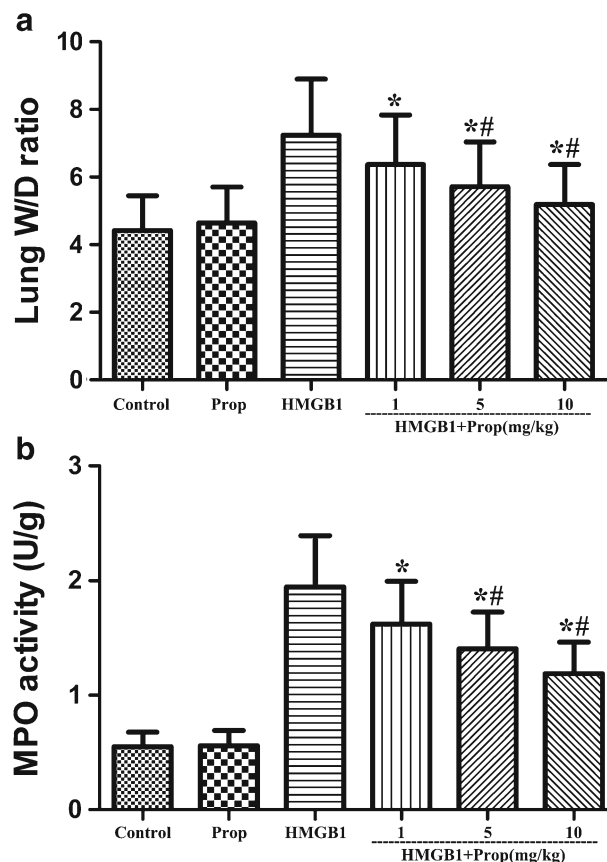

Fig. 5. Propofol reduced the lung wet/dry weight ratio and the MPO activity in HMGB1-induced ALI rats. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after HMGB1 injection, followed by infusion at 5 mg/(kg·h) through the left femoral vein cannula. The lung wet/dry weight ratio (a) and the MPO activity (b) were determined at 24 h after HMGB1 administration. The data are presented as means ± SD ($n=20$). *$P<0.05$ compared with HMGB1 group; #$P<0.01$ compared with HMGB1 group. *Prop* propofol.

the phosphorylation of NF-κB p65 and IκB-α induced by LPS in a dose-dependent manner ($P<0.05$; Fig. 8c, d). These results showed that propofol could inhibit NF-κB activation in LPS-induced ALI rats.

Effects of Propofol on the Expressions of TLR2 and TLR4 in A549 Alveolar Epithelial Cells

To further examine the anti-inflammatory mechanistic basis of propofol, we investigated the effects of propofol on TLR2 and TLR4 expressions in A549 alveolar epithelial cells. Similar to what we observed in animal experiments, TLR2 and TLR4 protein upexpressions were dramatically increased in cells stimulated with LPS. However, these increases were markedly inhibited by propofol treatment ($P<0.05$; Fig. 9a, b).
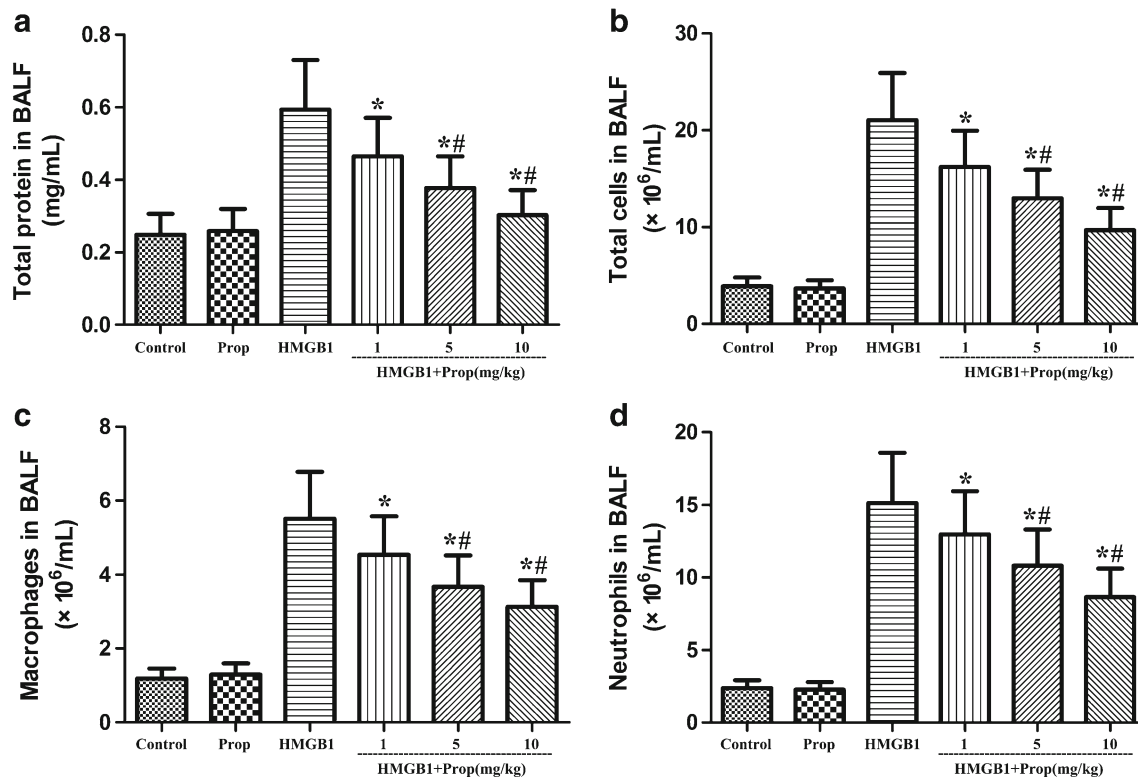
Fig. 6. Effects of propofol on the total protein concentration and the numbers of total cells, neutrophils, and macrophages in BALF. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after HMGB1 injection, followed by infusion at 5 mg/(kg · h) through the left femoral vein cannula. BALF was collected at 24 h after HMGB1 administration to measure the total protein concentration (**a**) and the numbers of total cells (**b**), neutrophils (**c**), and macrophages (**d**). The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with HMGB1 group; #$P < 0.01$ compared with HMGB1 group. *Prop* propofol.

Effects of Propofol on LPS-Induced NF-κB Activation in A549 Alveolar Epithelial Cells

Propofol was found to inhibit LPS-induced NF-κB activation in our animal experiments. We also examine whether this inhibitory effect of propofol happens to alveolar epithelial cells. As shown in Fig. 9, we found that LPS greatly enhanced the phosphorylation of NF-κB p65 and IκB-α, which reflected the activation of NF-κB. However, treatment of propofol inhibited the LPS-induced NF-κB activation in A549 alveolar epithelial cells ($P < 0.05$; Fig. 9c, d).

DISCUSSION

In this study, we evaluated the protective effects of propofol in a model of LPS-induced ALI in rats. Consistent with previous studies [27], propofol administration attenuated lung inflammatory injury after LPS, as revealed by decreased elevation of the lung wet to dry weight ratio, total cells, neutrophils, macrophages, and MPO activity,

associated with reduced lung histological damage. In addition, we also found that propofol post-treatment markedly inhibited the release of HMGB1 and other pro-inflammatory cytokines in a model of LPS-induced ALI in rats. Furthermore, propofol significantly inhibited LPS-induced TLR2/4 protein upexpression and NF-κB activation in lung tissues and in LPS-stimulated A549 alveolar epithelial cells.

Previous studies have shown that propofol can inhibit the inflammatory responses and mitigate endotoxin-induced ALI [24, 28]. As is already known, the etiologies of ALI are complex, and additional mechanisms by which propofol affords protection in LPS-induced lung injury are needed to be further investigated. Evidence suggests that HMGB1 plays a critical role in the progression of ALI. HMGB1-based therapeutic strategy may be more meaningful in ALI, because HMGB1 is a late inflammatory mediator; this will give us a much broader intervention window. Therefore, the current study aimed to test the hypothesis that propofol can alleviate LPS-induced ALI through preventing the expression of HMGB1.
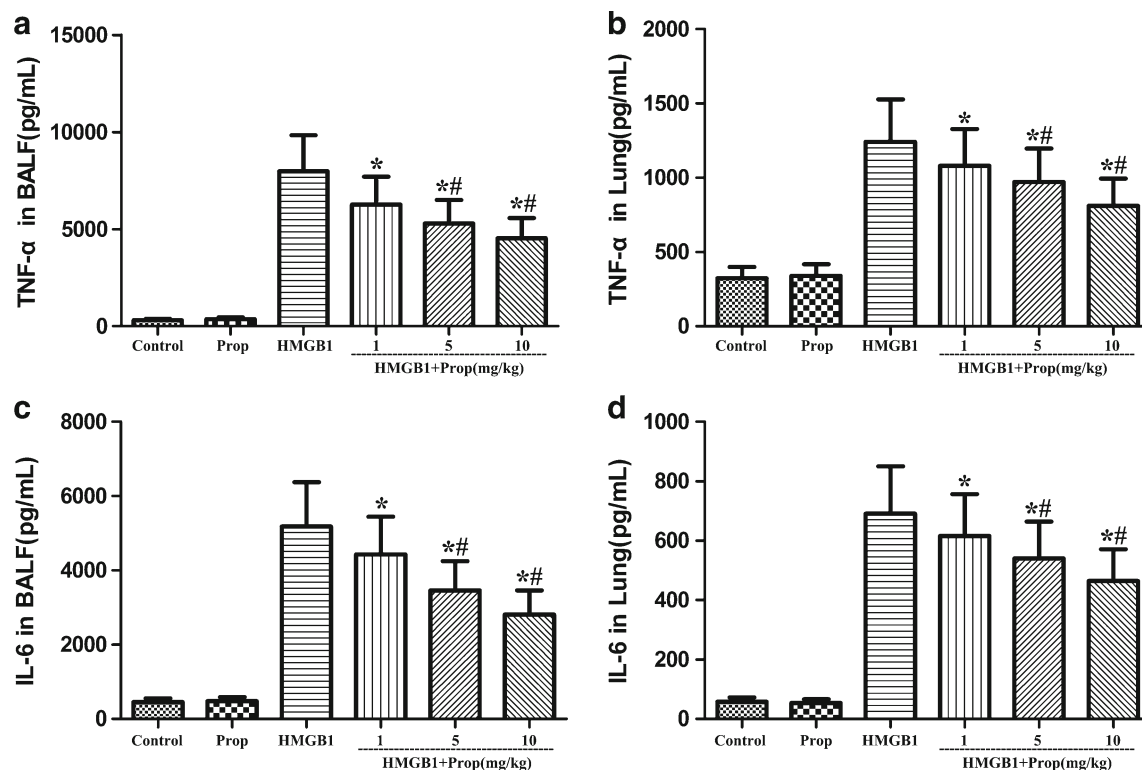
Fig. 7. Propofol inhibited HMGB1-induced expressions of TNF-α and IL-6 in BALF and lung tissues. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after HMGB1 injection, followed by infusion at 5 mg/(kg·h) through the left femoral vein cannula. BALF and lung tissues were collected at 24 h after HMGB1 administration to analyze the inflammatory cytokines TNF-α (**a, b**) and IL-6 (**c, d**). The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with HMGB1 group; #$P < 0.01$ compared with HMGB1 group. *Prop* propofol.

Clinical and experimental studies have shown that ALI is an early and fatal complication of septic shock [1] and that the TLR2/4-mediated NF-κB activation signaling pathway could be an early molecular event leading to ALI during septic shock [29, 30]. Previous studies have shown that propofol significantly increased survival in CLP rat and the mechanisms involved in down-regulating the expression of TLR2/4 and blunting NF-κB activation in the lung [17, 19–21]. To evaluate our hypothesis, we examined pulmonary inflammation in LPS-induced ALI with or without propofol treatment. We observed that the pulmonary inflammation was significantly increased in a rat LPS model. However, propofol administration significantly inhibited pulmonary inflammation by inhibiting the TLR2/4 expressions in a rat LPS model. Previous studies have shown that propofol administration significantly blunted NF-κB activation in septic rat [18, 21]. NF-κB is a critical transcription factor in TLR-mediated signaling

pathways [31] and plays a critical role in regulation of the expression of a number of genes, including inflammatory cytokines such as HMGB1, TNF-α, and IL-1β [32–35]. In this study, we demonstrated that propofol would down-regulate HMGB1 expression in LPS-induced ALI model and that the inhibition of HMGB1 expression would be associated with the inhibition of TLR2/4 and NF-κB activation by propofol during sepsis. An important limitation of the current study is that LPS is not equivalent to sepsis. In this study, we only investigated the effects of propofol on LPS-induced ALI in a rat model; a further study to investigate the protective effects of propofol on sepsis-induced ALI in clinical studies is needed.

The anesthetic concentration of propofol used for clinical medication is less than 5 mg/kg/h to provide satisfactory sedation [36]. In addition to its anesthetic properties, a safe range of doses of propofol is neuroprotective against ischemia
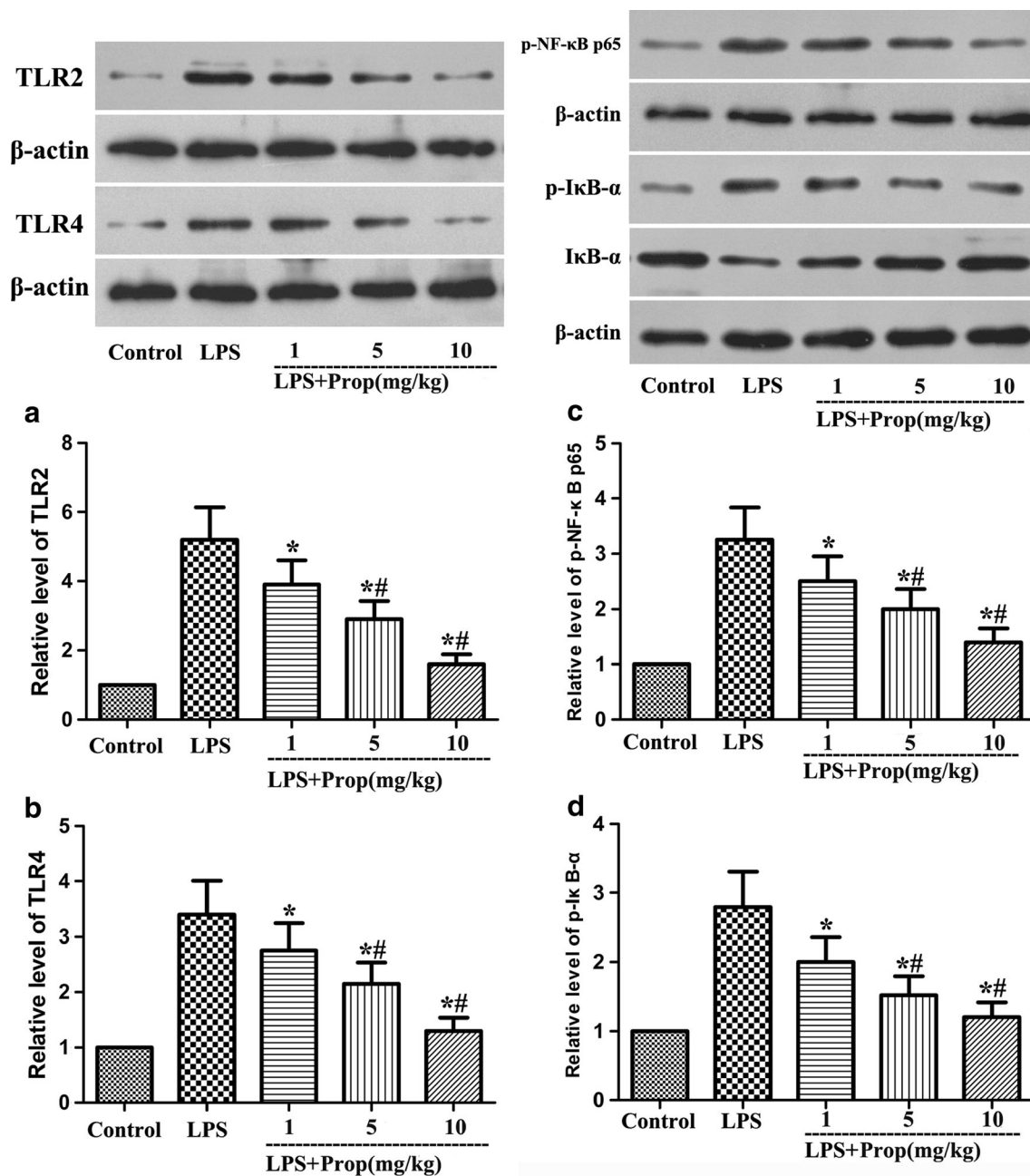
Fig. 8. Propofol inhibited the expressions of TLR2 and TLR4 and the phosphorylation of NF-κB p65 and IκB-α in lung tissues. Propofol bolus (1, 5, or 10 mg/kg) was infused continuously 30 min after LPS injection, followed by infusion at 5 mg/(kg·h) through the left femoral vein cannula. At 24 h after LPS administration, the expressions of TLR2 (**a**) and TLR4 (**b**) and the phosphorylation of NF-κB p65 (**c**) and IκB-α (**d**) were detected by Western blotting. The data are presented as means ± SD ($n = 20$). *$P < 0.05$ compared with LPS group; #$P < 0.01$ compared with LPS group. *Prop* propofol.

reperfusion [37, 38] and has cardiovascular benefits against oxidative stress [39, 40]. However, abuse of propofol treatment causes severe complications in patients with critical illness and is called propofol infusion syndrome [41]. In this study, we found that propofol treatment (1, 5, and 10 mg/kg) attenuated
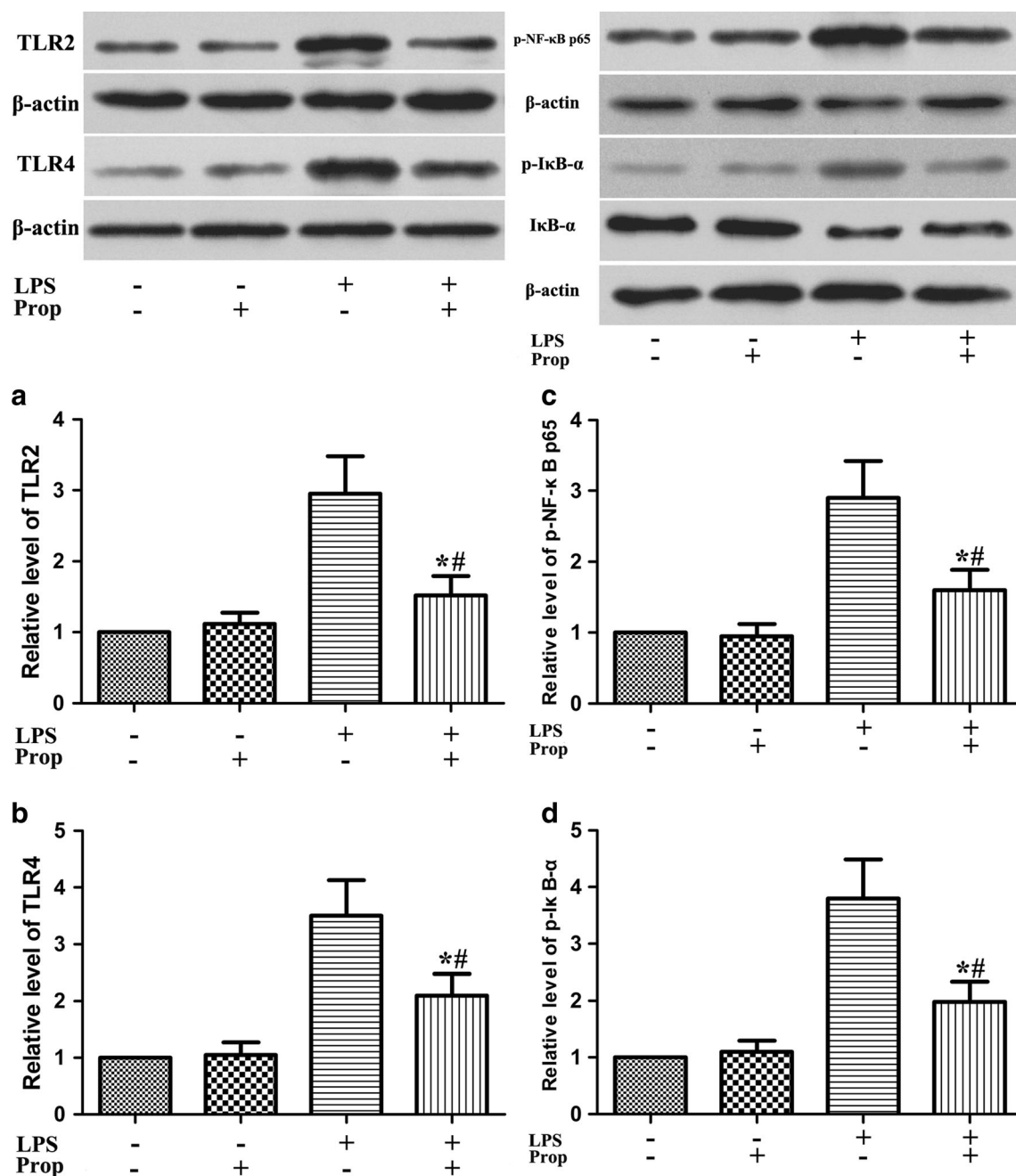
Fig. 9. Propofol inhibited the expressions of TLR2 and TLR4 and the phosphorylation of NF-κB p65 and IκB-α in LPS-stimulated A549 alveolar epithelial cells. A549 cells were treated with propofol (100 μM) 24 h after LPS (1 μg/mL) stimulation. The expressions of TLR2 (**a**) and TLR4 (**b**) and the phosphorylation of NF-κB p65 (**c**) and IκB-α (**d**) were detected by Western blotting. Data are presented as means ± SD from three independent experiments. *$P < 0.05$ compared with LPS group; [#]$P < 0.01$ compared with LPS group. *Prop* propofol.

LPS-induced ALI in dose-dependent manner. Because of toxicity of systemic propofol, we think that propofol treatment (5 mg/kg) was better than other treatment groups and should be explored further in larger animals and more relevant sepsis models in a path to human trials.

In summary, the present study provided evidence that propofol improves LPS-induced ALI accompanied with decreased HMGB1 expression. The mechanisms are, at least in part, through the down-regulation of TLR2/4 expressions and inhibition of NF-κB pathway.

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Author Contributions. Xiaoyan Wang: conception, design, analysis, and interpretation of data; writing the manuscript.

Gongming Wang: conception, design, analysis, and interpretation of data; writing the manuscript.

Chengxiao Liu: analysis and interpretation of data; writing the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures were performed in accordance with the Declaration of Helsinki of the World Medical Association. The study was approved by the ethics committee of Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China.

Conflicts of Interest. The authors report no proprietary or commercial interest in any product mentioned, or concept discussed, in this article.

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