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Propofol reduces acute lung injury by up-regulating gamma-aminobutyric acid type a receptors

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ABSTRACT

Background: We used a two-hit lung injury rat model that involves mechanical ventilation (MV) following lipopolysaccharide exposure to investigate the effects of propofol on the expression of $GABA_A$ receptors ($GABA_AR$) and cytokine responses, and we then determined the specific effects of GABA on cytokine responses *in vitro* in alveolar epithelial cells (AECs).

Methods: Forty-eight adult male Wister rats were equally and randomly divided into the following 4 groups ($n = 12$) using a random number table: sham group, sham + propofol group, lipopolysaccharide (LPS) + VILI group, and LPS + VILI + propofol group. All animals were anesthetized, and the animals received a 3.75 mg/kg intratracheal instillation of endotoxins or phosphate-buffered saline (PBS) as the control, as described previously. After 30 min, rats were ventilated for 5 h in a volume-controlled ventilation mode. In the LPS + VILI group, animals were ventilated with a tidal volume (Vt) of 22 ml/kg and zero positive end-expiratory pressure (PEEP) at a respiratory rate of 16–18 breaths/min, whereas control (sham) rats were ventilated with a Vt of 6 ml/kg and PEEP of 5 cmH_2O at a rate of 45–55 breaths/min. The FiO_2 remained constant as 0.4, propofol was administered intravenously in the LPS + VILI + propofol and sham + propofol groups at a rate of 10 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ while normal saline at the same rate was intravenously administered in the LPS + VILI and sham groups during the entire mechanical ventilation period. Five hours after mechanical ventilation, the rats were killed. Survival rates, histopathology, concentrations of inflammatory mediators in bronchoalveolar lavage fluid (BALF), wet weight/dry weight (W/D) ratio of the lung, myeloperoxidase (MPO) activity in lung tissues, and expression of GAD and $GABA_AR$ by immunohistochemical detection and Western blotting were assessed. Then, human type II-like alveolar epithelial cells (A549 cells) were cultured to full confluence and incubated with GABA (100 nM) alone, picrotoxin alone, a $GABA_AR$ antagonist (PTX, 50 nM), or GABA + PTX for 10 min, followed by stimulation with LPS (control) at 100 ng/ml for 4 h. The concentrations of IL-1β, IL-2, IL-8, and IL-10 were then measured.

Results: Administration of propofol in a two-hit lung injury rat model can increase survival rates and the expression of GAD and $GABA_AR$ ($P < .05$). The administration of propofol can attenuate the release of pro-inflammatory cytokines both *in vivo* and *in vitro*, and the administration of propofol can attenuate histopathological changes, the W/D ratio, and MPO activity ($P < .05$).

Conclusions: In this study, we found that the administration of propofol improved lung function, alleviated lung injury, and up-regulated the GAD and $GABA_AR$ expressions in a two-hit model of acute lung injury (ALI) characterized by intratracheal instillation of an endotoxin and prolonged MV. Therefore, the protective effects of propofol may be associated with the up-regulation of $GABA_A$ receptors in AECs.

1. Introduction

It is well known that acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS),are common complications with high morbidity and mortality in critically ill patients (Xu et al., 2014; Ware and Matthay, 2000).ALI and ARDS often occur in

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serious diseases, such as severe infection, shock, trauma, and burns, and are clinically characterized by progressive hypoxemia and respiratory distress syndrome, contributing to diffuse pulmonary interstitial and alveolar edema caused by pulmonary capillary endothelial cells and alveolar epithelial cell injury (Brun-Buisson et al., 2004; Stapleton et al., 2005). The majority of patients suffering from ALI/ARDS require mechanical ventilation (MV) for life support (Brun-Buisson et al., 2004; Lopez-Cuenca et al., 2012; Bouferrache and Vieillard-Baron, 2011). However, MV can also exacerbate preexisting lung injuries; the resulting damage has been termed ventilator-induced lung injury (VILI) (Tremblay et al., 1997; Tremblay and Slutsky, 2006).Thus, an important therapeutic method to manage patients with ALI/ARDS is to reduce pulmonary and systemic inflammatory responses that may have played a critical role in inducing multiple distal organ dysfunction (Phua et al., 2009).

Patients undergoing MV, including patients in the intensive care unit (ICU) or the operating room, require medications for general anesthesia or sedation. Although they are often necessary to support critically ill patients receiving MV, these medications can also have adverse effects, including prolonged MV and an increased ICU length of stay (Hartman et al., 2009; Ostermann et al., 2000).

Propofol (2,6-di-isopropylphenol) is one of the most commonly used intravenous anesthetics and is mainly used for the induction and maintenance of anesthesia, as well as ICU sedation. In recent years, propofol has been used for the treatment of insomnia (Vasileiou et al., 2012; Marik, 2004; Hutchens et al., 2006). Apart from its use as an anesthetic, propofol exerts several non-anesthetic effects (Vasileiou et al., 2009; Wang et al., 2013). Additionally, several studies have shown that propofol has anti-inflammatory effects, such as suppression of the biosynthesis of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-activated macrophages (Chen et al., 2005; Takemoto, 2005) and reduction of cluster of differentiation 14 (CD14) and toll-like receptor 4 (TLR4) expression in endotoxin-induced lung injury (Wu et al., 2009; Ma et al., 2010),which may be beneficial in the context of VILI. However, the mechanisms by which propofol exerts these anti-inflammatory effects remain unknown.

Propofol is an anesthetic which can activate gamma-aminobutyric acid (GABA) A receptor activity directly through the specific binding subunits of $GABA_AR$ (Trapani et al., 2000), and also propofol plays an important role in the inhibition of neuronal death (Ito et al., 1999; Hollrigel et al., 1996). GABA synthesized from glutamate by decarboxylation *via* the enzymatic activity of glutamic acid decarboxylase (GAD) produces fast synaptic inhibition in neurons by activating $GABA_A$ receptors through a GABA-gated anion channel. Recently, the $GABA_A$ receptor has been demonstrated to exert anti-inflammatory properties in addition to its traditional role of synaptic transmission (Bhat et al., 2010; Duthey et al., 2010; Prud'homme et al., 2015).

It is interesting to note that several recent studies have confirmed that $GABA_A$ receptors are also expressed in the lung airway and alveolar epithelial cells (AECs)(Zaidi et al., 2011; Xiang et al., 2013; Jin et al., 2006). Nevertheless, their role in response to propofol remains to be investigated in the therapy of ALI/ARDS. Thus, we hypothesized that the protective effects of propofol ameliorate the pulmonary inflammatory response as a result of up-regulating the expression of $GABA_A$ receptors. To test our hypothesis, we used a two-hit lung injury rat model that involves MV following lipopolysaccharide exposure to examine the effects of propofol on the expression of $GABA_A$ receptors and cytokine responses, and we then determined the specific effects of GABA on cytokine responses *in vitro* in AECs. Although most ALI animal studies involve a single injury to the lungs, critically ill patients in the ICU often suffer multiple injuries to the lungs and require MV and sedatives after the initial injury has ensued. We thus used a two-hit lung injury rat model that mirrors the progress of ICU patients with ALI/ARDS and to determine whether propofol conferred protection in this model.

2. Materials and methods

All animal experiments were performed with the approval of the Animal Care and Use Committee of Yangzhou University, Yangzhou, China.

2.1. Two-hit rat model of lung injury and in vivo propofol exposure

Forty-eight adult male Wister rats (weighing 200 to 230 g) were provided by the Comparative Medicine Center of Yangzhou University. Animals were housed in a temperature (22 ± 1 °C)-controlled room, under a 12:12-h light: dark cycle (lights on from 8:00 am to 8:00 pm), and with food and water provided *ad libitum*. Prior to the experiments, animals were allowed to habituate to the housing facilities for at least 1 week, and all efforts were made to minimize animal suffering. All animals were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), and the animals then received a 3.75-mg/kg intratracheal instillation of endotoxin (LPS, *Escherichia coli* serotype 055:B5, Sigma-Aldrich, St. Louis, MO, USA) or phosphate-buffered saline (PBS) as a control as described previously (Hoegl et al., 2015).After 30 min, rats underwent a tracheotomy and were ventilated for 5 h in a volume-controlled ventilation mode (DW 3000, Zhenghua Biologic, Anhui Province, China). In the LPS + ventilator-induced lung injury (LPS + VILI) group, animals were ventilated with a tidal volume (Vt) of 22 ml/kg and zero positive end-expiratory pressure (PEEP) at a respiratory rate of 16–18 breaths/min, whereas control (sham) rats were ventilated with a Vt of 6 ml/kg and PEEP of 5 cmH_2O at a rate of 45–55 breaths/min as described previously (Chen et al., 2014). The FiO_2 remained constant at 0.4. Before beginning MV, propofol (Fresenius Kabi, China) was intravenously administered *via* tail vein in the LPS + ventilator-induced lung injury + propofol (LPS + VILI + propofol) group and the control + propofol (sham + propofol) group at a rate of 10 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ while normal saline at the same rate was intravenously administered *via* tail vein in the LPS + VILI and sham groups during the entire mechanical ventilation period. Five hours after mechanical ventilation, the rats were killed, and lung injury was evaluated by the following parameters: (1) wet-to-dry (W/D) ratio, (2) GAD and $GABA_A$ receptor ($GABA_AR$) expression in the lung tissues, (3) GAD and $GABA_AR$ Western blot expression in the lung tissues, and (4) myeloperoxidase (MPO) activity in the lung tissues.

2.2. Survival rates

The survival rates of each group were assessed during the 5-h mechanical ventilation period following LPS exposure.

2.3. Bronchoalveolar lavage fluid (BALF) analysis

The left lung that was removed from each rat was infused with4 ml of PBS at room temperature, which was withdrawn and reinfused three times. There were no differences in the volume of saline recovered (3.5 ± 0.7 ml fluid) after the lung lavage process between the four groups. BALF was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated into aliquots and was stored at −70 °C for batch analysis by enzyme-linked immunosorbent assay (ELISA). The levels of TNF-α and IL-1β were measured using rat TNF-α and IL-1β ELISA kits (both from R&D Systems Inc., USA) according to the manufacturer's instructions. CXCL1 and IL-6 concentrations in the BALF were measured by ELISA using specific antibodies and standards (Duo Set, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.4. Histopathological examination

The inferior lobe of the right lung from each animal was fixed in 10% neutral formalin and prepared for routine paraffin embedding.

Tissues were cut into 4-μm-thick sections, mounted on slides, and stained with hematoxylin-eosin (H&E). Samples were examined and scored by a blinded observer using a Nikon Eclipse i5 photomicroscope, a light microscope with a Nikon DS-Fi1c camera, and the Nikon NIS Elements version 4.0 image analysis system (Nikon Instruments Inc., Tokyo, Japan). The main histopathological lung damage score was calculated as previously described (Takao et al., 2005), with a minor modification. Briefly, histopathological lung damage was examined by scoring alveolar congestion, hemorrhage, leukocyte infiltration or aggregation in air spaces/vessel walls, perivascular/interstitial edema, and alveolar wall/hyaline membrane formation thickness. The severity of each item was rated on a 4-point scale graded from 0 (minimal) to 3 (maximal), yielding a range of total scores from 0 to 15 (most severe).

2.5. Wet weight/dry weight (W/D) ratio of the lung

The superior lobe of the right lung was weighed and then dried and re-weighed at 60 °C for 48 h in an oven. To assess tissue edema, the W/D ratio was calculated.

2.6. Immunohistochemical detection of GAD and $GABA_A$ receptors

Paraffin sections were dewaxed by the routine method and were incubated for 10 min with 3% hydrogen peroxide to block endogenous peroxide activity. Each section was incubated with blocking solution (normal goat serum) at room temperature for 15 min and then washed with distilled water and PBS. Each section was incubated overnight at 4 °C with a 1:150 dilution of primary anti-GAD antibodies (GAD65/67 antibody, Abcam, Inc., Cambridge, MA) or anti-$GABA_AR$ antibodies ($GABA_A$ receptor alpha 2 antibody, Abcam, Inc) then washed two more times with PBS. Each section was incubated with the goat anti-rabbit secondary antibody (1:500), followed by treatment with streptavidin-biotin-horseradish peroxide (Zhongshan Biological Technology, Beijing, China). Each section was stained with diaminobenzidine (DAB) and counterstained with hematoxylin. It was calculated using mean density and analyzed using Image-Pro Plus v6.0 (MediaCybernetics, Rockville, MD, USA).

2.7. Western blot analysis

The GAD and $GABA_AR$ proteins were detected by Western blot analysis. The lung tissues were finely homogenized in ice-cold radioimmunoprecipitation lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) using grinding rod, suspended in ice-cold lysis buffer (the recipe for the lysis buffer: sucrose 427.6 mg, 1 M pH 7.4 Tris 50 μl, 1 M $MgCl_2$ 25 μl, 0.25 M EGTA 100 μl, 0.25 M PMSF 20 μl,100 mM DTT 5 μl, 10 mM leupeptin 20 μl dissolved in 4 ml double distilled water), and centrifuged at 12,000*g* for 10 min at 4 °C. The supernatants were recovered for subsequent analysis. Protein concentrations were determined using a Bicinchoninic Acid Protein Assay kit. After measuring the protein concentrations of each sample, 50 μg of the protein sample was solubilized in sodium dodecyl sulfate (SDS) loading buffer. The samples were loaded into a 10% SDS polyacrylamide gel, electrophoresed, and then transferred to polyvinylidene difluoride (PVDF) membranes. Finally, the PVDF membranes were incubated with anti-GAD antibodies (GAD antibody, 1:1000, CST, Inc.) or anti-$GABA_AR$ antibodies ($GABA_A$ receptor alpha 2 antibody, 1:1000, Abcam, Inc.), followed by the corresponding secondary antibodies. Protein-antibody complexes were detected with an enhanced chemiluminescence (ECL) system (Bio-Rad). A quantitative assessment was performed using Image Lab software (Bio-Rad). Staining with anti-β-actin (1:1500, Merck Millipore, Germany) was used to normalize the protein levels of each sample. The blot density from the sham group was set as 100%. The relative density values from the other groups were determined by dividing the optical density values from these groups by the sham value after each was normalized to the corresponding β-actin.

2.8. Assay of MPO activity in lung tissues

In our study, MPO activity in the lung tissues, as an indicator of neutrophil accumulation, was used to evaluate pulmonary neutrophil infiltration. Briefly, lung tissues waere homogenized (10%, *w/v*) in ice-cold 1.15% KCl, 0.01 M sodium, and potassium phosphate buffer solution (pH 7.4) using a glass homogenizer. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatant was collected. The activities of myeloperoxidase (MPO) in the lung tissue were evaluated using assay kits (Nanjing Jiancheng Bioengineering Institute, China). All procedures were performed according to the manufacturer's instructions.

2.9. Cell culture and treatment

Human type II-like alveolar epithelial cells (A549 cells) were obtained from the Cellular Immunity Laboratory of Medical College, Yangzhou University. Cells were cultured to full confluence and were incubated with GABA (100 nM) alone, picrotoxin alone, a $GABA_A$ receptor antagonist (PTX, 50 nM), or GABA + PTX for 10 min, followed by stimulation with LPS (control) at 100 ng/ml (*Escherichia coli* serotype 055:B5, Sigma-Aldrich) for 4 h. This dose of LPS was previously used for the induction of inflammatory responses (Zhang et al., 1999).

2.10. Statistical analysis

All data are described as the means ± SD and analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). The differences between groups were analyzed by one-way ANOVA. Multiple comparisons were performed using the Bonferroni procedure. Correlation between different variables was assessed by Spearman's coefficient. Differences with $P < .05$ were considered to be statistically significant.

3. Results

3.1. Effect of propofol on the 5-h survival rate of rats

As shown in Fig. 1, the 5-h survival rate was 100, 100, 9, and 36% in the sham, sham + propofol, LPS + VILI, and LPS + VILI + propofol groups, respectively. Compared with the LPS + VILI group, the 5-h survival rate was significantly higher in the LPS + VILI + propofol group ($P < .05$).

3.2. Effect of propofol on lung W/D ratio and lung injury score

The W/D ratio and lung injury score were significantly higher in the

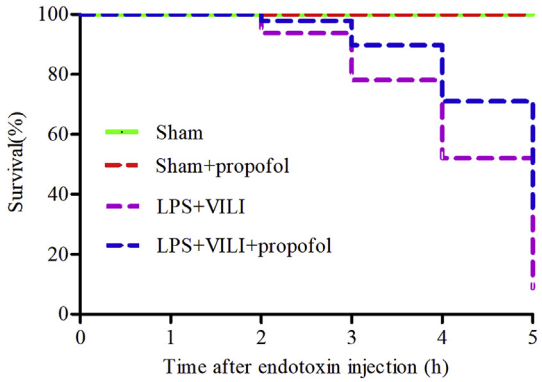

Fig. 1. The effect of propofol on survival rates in the two-hit lung injury rat model. The 5-h survival rates were 100, 100, 9, and 36% in the sham, sham + propofol, LPS + VILI, and LPS + VILI + propofol groups, respectively. The survival rate was significantly higher in the LPS + VILI + propofol group than in the LPS + VILI group ($P < .05$).

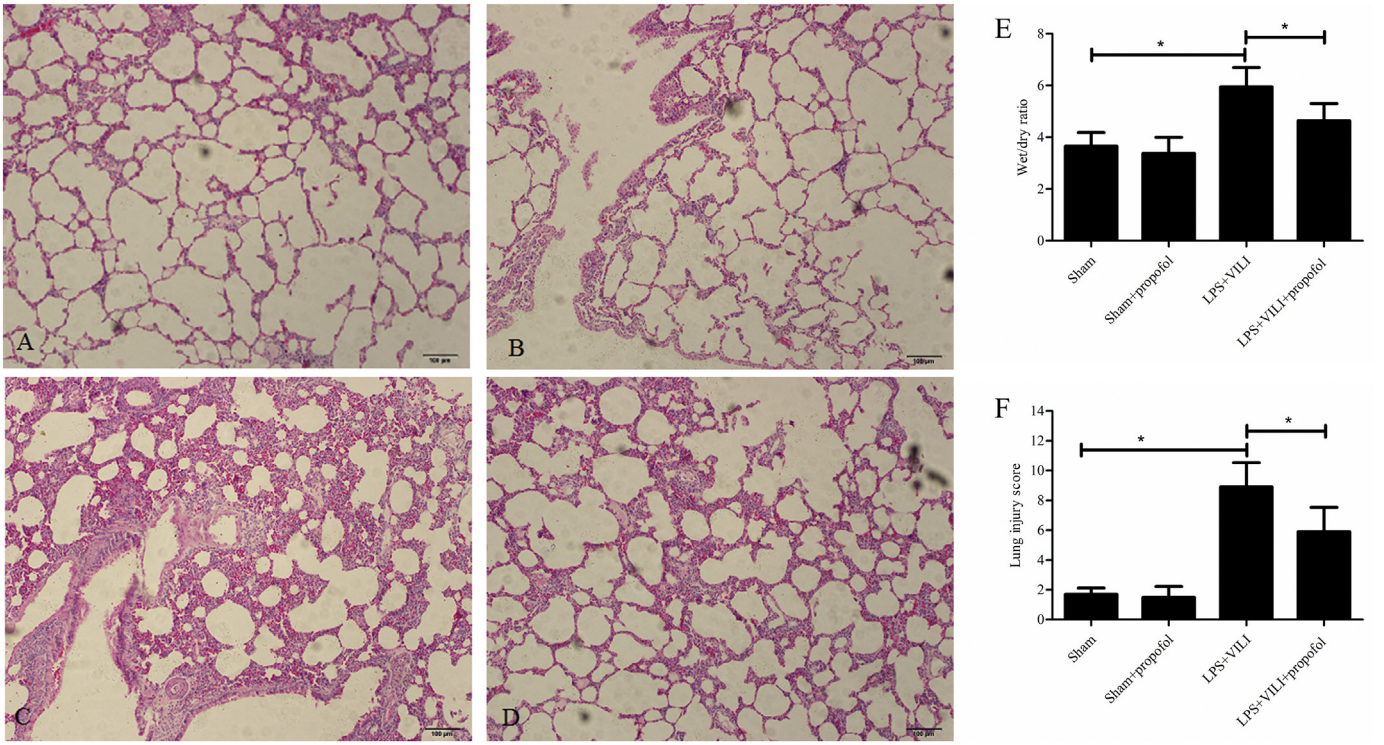

Fig. 2. Histopathology of representative lung sections and the effect of propofol on the lung wet/dry ratio, lung injury score. A, sham group. B, sham + propofol group: no differences were observed compared with the sham group. C, LPS + VILI group: edematous changes in alveolar walls, swelling of alveolar epithelial cells, and massive polymorphonuclear infiltration were observed. D, LPS + VILI + propofol group: less damage was observed compared with the LPS + VILI group(original magnification ×100). Bar indicates 100 μm. Data are expressed as the means ± SD from three independent experiments. *$P < .05$.

LPS + VILI group than in the sham group ($P < .05$). Compared with the LPS + VILI group, the W/D ratio and lung injury score were significantly lower in the LPS + VILI + propofol group ($P < .05$) (Fig. 2E, Fig. 2F).

3.3. Effect of propofol on pathological changes in the lungs

Five hours after MV, the lungs were harvested and subjected to H&E staining. Lung tissues from the sham and sham + propofol groups showed normal structures with no obvious histopathological changes were observed under a light microscope. In contrast, lung tissues from the LPS + VILI group showed obvious pathological changes, including lung edema, alveolar hemorrhage, alveolar wall thickening, and inflammatory cell infiltration. However, compared with the LPS + VILI group, pathological changes in lung tissues from the LPS + VILI + propofol group were significantly alleviated (Fig. 2A-2D) ($P < .05$).

3.4. Effect of propofol on MPO activity in lung tissues

As shown in Fig. 3A, MPO activity in lung tissues was significantly higher in the LPS + VILI group compared with the control group ($P < .05$). However, compared with the LPS + VILI group, MPO activity in lung tissues was significantly lower in the LPS + VILI + propofol group ($P < .05$).

3.5. Effect of propofol on TNF-α, IL-1β, IL-6, and CXCL1 concentration in BALF

TNF-α, IL-1β, IL-6, and CXCL1 levels in BALF were analyzed by ELISA to detect inflammation in the lungs of rats after the administration of LPS combined with VILI. Fig. 3B-3E show that very low levels of TNF-α, IL-1β, IL-6, and CXCL1 were detected in the sham and sham + propofol groups. Conversely, the administration of LPS combined with VILI resulted in a significant increase in TNF-α, IL-1β, IL-6, and CXCL1 levels ($P < .05$), while treatment with propofol partially decreased the secretion of TNF-α, IL-1β, IL-6, and CXCL1 ($P < .05$).

3.6. Immunohistochemical detection of GAD and $GABA_AR$

In the LPS + VILI + propofol group, an intense positive immunohistochemical staining of GAD and $GABA_AR$ was observed in the bronchiolar epithelial cells. Additionally, a slightly positive immunohistochemical staining of GAD and $GABA_AR$ in the lung tissues was observed in the LPS + VILI group. In the sham and sham + propofol groups, however, a weaker immunohistochemical staining of GAD and $GABA_AR$ was detected in the bronchiolar epithelial cells compared with the LPS + VILI and LPS + VILI + propofol groups (Fig. 4A-4H). Fig. 4I-4J compares the semiquantitative results of GAD and $GABA_AR$ detected by immunohistochemical staining between the four groups. The GAD and $GABA_AR$ integral densities were significantly higher in the LPS + VILI and LPS + VILI + propofol groups than in the sham group ($P < .05$). Compared with the LPS + VILI group, the GAD and $GABA_AR$ integral densities were significantly higher in the LPS + VILI + propofol group ($P < .05$).

3.7. Western blot analysis of GAD and $GABA_AR$

We also examined the protein expression of GAD and $GABA_AR$ in rat lung tissues by Western blot analysis of lung homogenates. GAD and $GABA_AR$ protein expression was detected in all lung tissue samples from the four groups, as shown in Fig. 5. Compared with the sham group, the GAD and $GABA_AR$ protein expression levels were significantly increased in the LPS + VILI and LPS + VILI + propofol groups ($P < .05$). In addition, compared with the LPS + VILI group, the GAD and $GABA_AR$ protein expression levels were significantly increased in the LPS + VILI + propofol group ($P < .05$).

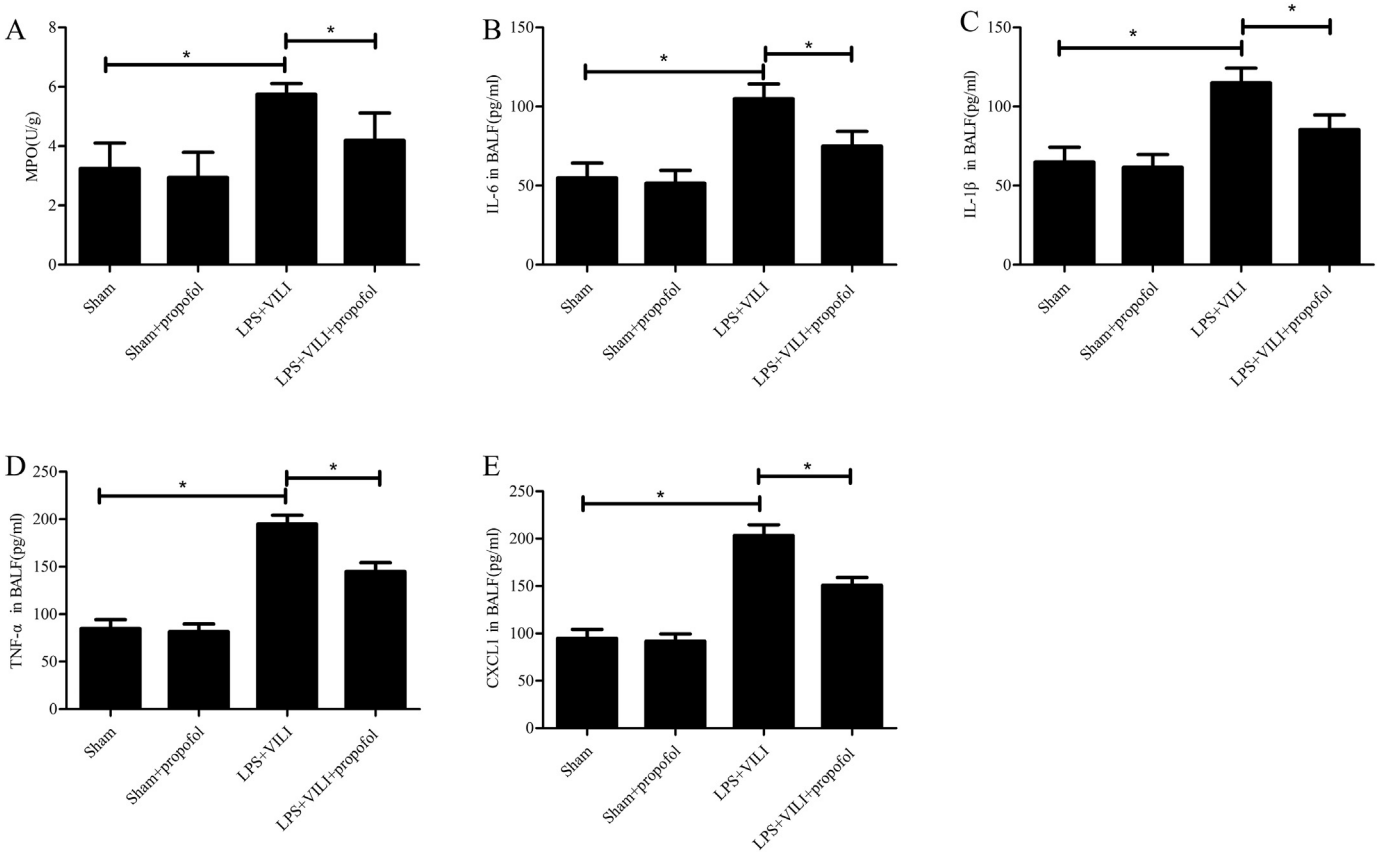

Fig. 3. The effect of propofol on MPO activity in lung tissues, and concentrations of inflammatory mediators in BALF(IL-6, IL-1β, TNF-α, and CXCL1). Data are expressed as the means ± SD from three independent experiments. *$P < .05$.

3.8. In vitro A549 cell experiments

To explore the specific effects of GABA in lung epithelial cells, human type II-like alveolar epithelial cells (A549 cells) were stimulated with LPS, followed by treatment with GABA, PTX, or GABA + PTX. First, we confirmed that the dose of PTX that was used did not induce cytotoxicity as reflected by a constant LDH level before and after PTX administration (Racher et al., 1990). The concentrations of IL-1β, IL-2, IL-8, and IL-10 were significantly reduced with GABA compared with the control, PTX alone, and GABA + PTX groups (Fig. 6)($P < .05$).

4. Discussion

ALI and ARDS are syndromes of acute respiratory failure that result from a disturbance of the alveolar-capillary barrier that is associated with several clinical disorders. ALI is a severe inflammatory disease with high morbidity and mortality rates; however, there are no effective ALI drugs for clinical use. Therefore, prevention of ALI is an important therapeutic goal. Propofol, a widely used intravenous anesthetic and sedative agent used to sedate intubated, mechanically ventilated ALI and ARDS patients, has a rapid onset and short duration of action and rapid elimination. Several studies have found that propofol attenuates ALI in various murine models (Zhao et al., 2014a, 2014b; Chen et al., 2008) and inhibits LPS-induced biosynthesis of the inflammatory cytokines TNF-α, IL-1β, and IL-6 (Zhao et al., 2014a; Cavalcanti et al., 2014). The anti-inflammatory effects of propofol have attracted increasing attention, however, the molecular mechanisms of these effects are still unclear. Although propofol has been studied in models using a single lung injury, our study is, to the best of our knowledge, the first to examine the effects of propofol in a clinically relevant two-hit lung injury model where propofol was administered after the lung injury was established. This particular experimental model mimics the in homogeneity that is typical of clinical disease.

In the present study, the data indicated that propofol treatment significantly improved 5-h survival rates, the pulmonary inflammatory reaction, and histopathological changes in addition to relieving pulmonary edema and lung vascular leakage. Moreover, propofol treatment increased the protein levels of GAD and $GABA_AR$ in the lungs in the two-hit rat model. These results demonstrated that the administration of propofol protected the lung from the two-hit injury in rats, and these protective effects of propofol may be associated with the up-regulation of $GABA_AR$.

Various inflammatory mediators, including the cytokines TNF-α and IL-1β and chemokines, have been shown to act as effector molecules in the disruption of the epithelial and endothelial barriers during MV. The release of TNF-α, IL-1β, IL-6, and the CXC chemokine family is associated with neutrophil recruitment and is correlated with the severity and mortality of ARDS (Moldoveanu et al., 2009). CXCL1, the homolog of human IL-8 in rats, plays a pivotal role as a chemotactic factor in neutrophil infiltration. IL-8 has been shown to be released by lung epithelial cells in response to mechanical stress. In our study, we observed that the administration of propofol attenuated the production of the inflammatory mediators in the lung and reduced lung permeability. Our results are in agreement with other studies showing anti-inflammatory effects of propofol in a variety of *in vivo* and *in vitro* models (Zhao et al., 2014a; Zhao et al., 2014b; Chen et al., 2008; Wei et al., 2013).

Interleukin-10 was originally discovered to be a cytokine synthesis inhibiting factor on the basis of its biological activity. It down-regulates the expression of pro-inflammatory cytokines, such as IL-1β, IL-6, MHC class II antigens, and co-stimulatory molecules, by up-regulating itself. In the present study, the administration of GABA attenuated the LPS-

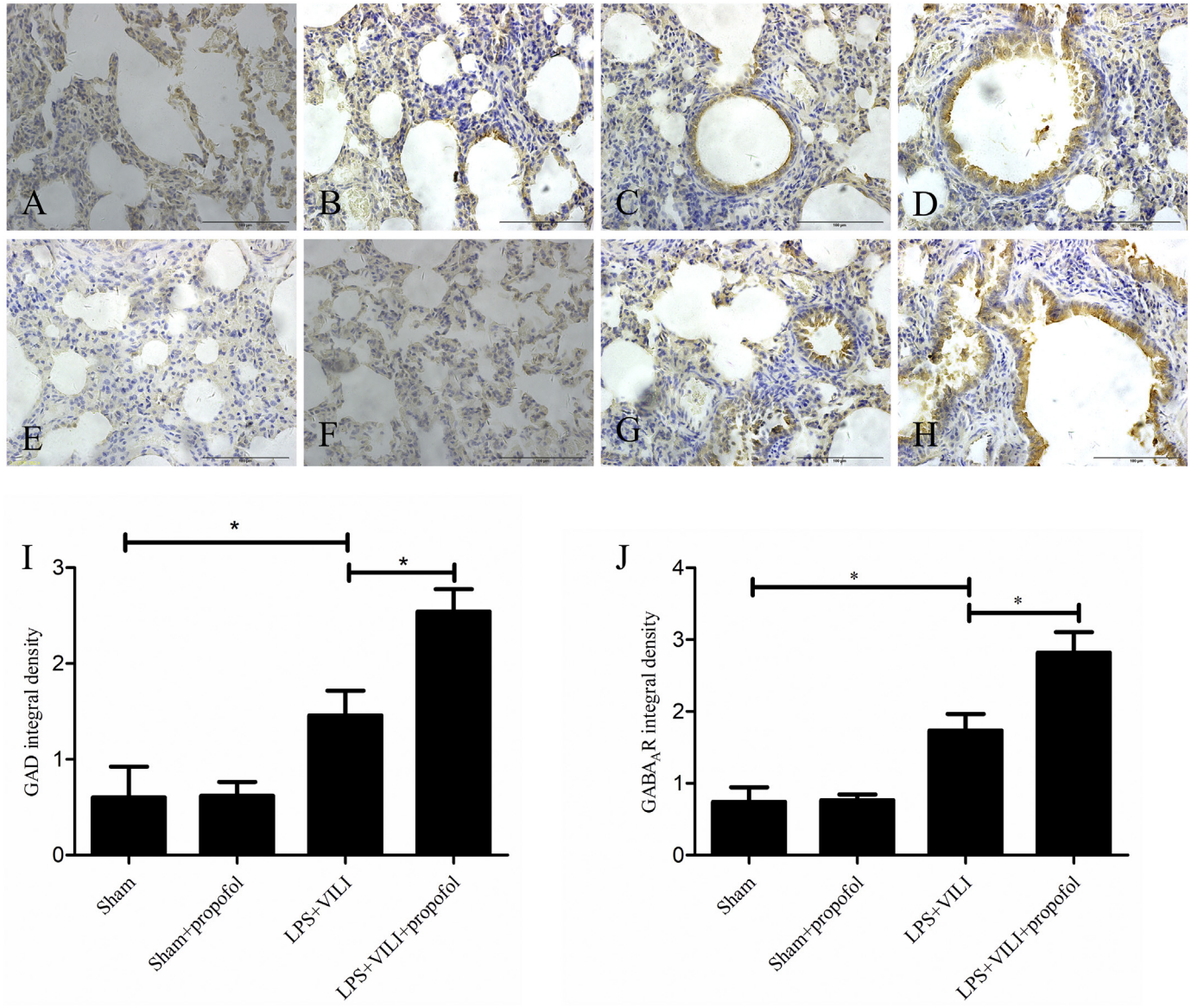

Fig. 4. Effect of propofol treatment on the expression of GAD (A-D) and GABA$_A$R (*E*-H) in a two-hit lung injury rat model: the sham group(A, E), the sham + propofol group(B, F), the LPS + VILI group(C, G), and the LPS + VILI + propofol group(D, H)(original magnification ×100). I: semiquantitative results of GAD detected by immunohistochemical staining, J: semiquantitative results of GABA$_A$R detected by immunohistochemical staining. Bar indicates 100 μm. *$P < .05$.

induced production of IL-1β and IL-10 in A549 cells. This phenomenon is consistent with previous studies showing that concentrations of IL-1β and IL-10 increased in response to the LPS challenge to counterbalance the pro- and anti-inflammatory responses (Erickson and Banks, 2011; Ward et al., 2011).

Recent studies have shown that the administration of propofol resulted in a reduction in lung damage, inflammation, and stress protein expression in various murine models (Zhao et al., 2014a; Zhao et al., 2014b; Chen et al., 2008). Our study is consistent with their observations even though we used a two-hit model of ALI. Thus, our results support the concept that propofol exerts anti-inflammatory effects that protect the lung from injury.

Previous studies have shown that propofol produces an anesthetic action primarily by binding to GABA$_A$ receptors in neurons of the central nervous system (CNS) (Ito et al., 1999; Hollrigel et al., 1996). A recent study by Xiang and colleagues reported the existence of GAD and GABA receptors in human airway epithelial cells (Xiang et al., 2007), and other investigators discovered GABA receptors in alveolar epithelial type II cells (Jin et al., 2005). In our initial experiments, we also discovered that propofol enhanced the GABA$_A$ receptor-mediated anion current in pulmonary epithelial cells (data not shown). In the present study, we further identified the presence of GAD and GABA$_A$ receptors both in rat airways and alveolar epithelial cells. We also demonstrated that the administration of propofol results in an increase in GAD and GABA$_A$ receptor expression in the lung tissue. We thus speculate that the increase in GAD expression after propofol administration may be the result of a positive feedback mechanism resulting from the binding between propofol and GABA$_A$ receptors.

In the present study, we found that the concentrations of cytokines were lower and the GAD and GABA$_A$R expressions were higher in the LPS + VILI + propofol group than in the LPS + VILI group under *in vivo* conditions. These observations support the hypothesis that propofol exerts anti-inflammatory properties partially through the up-regulation of GABA receptors. This concept was further supported by *in vitro* lung epithelial cell studies where LPS-induced cytokine responses were attenuated in the presence of GABA, which was reversed by the administration of picrotoxin. Furthermore, it has been reported that an increase in surface levels of GABA$_A$ receptors requires the activity of

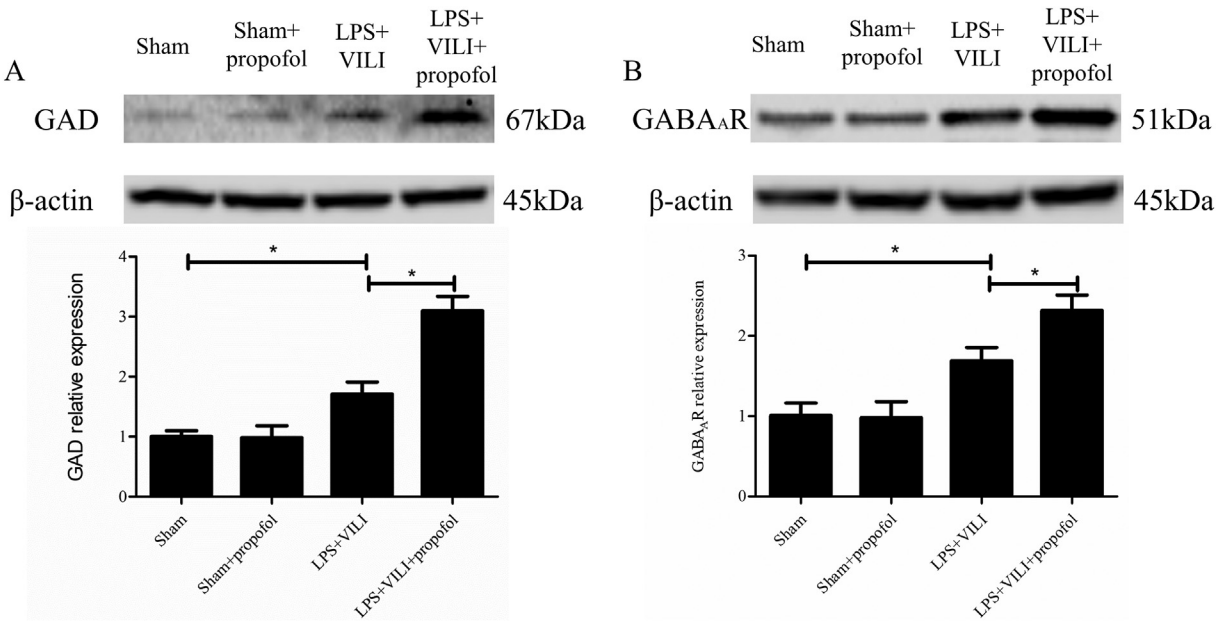

Fig. 5. Propofol increased GAD and $GABA_AR$ activation in lung tissues. The levels of GAD and $GABA_AR$ activation were assessed by Western blot analysis. Data are expressed as the means ± SD from three independent experiments. *$P < .05$.

phosphoinositide 3-kinase (PI3K) and protein kinase C (PKC) (Porcher et al., 2011; Li et al., 2015). This finding is in accordance with a recent study reporting that a mechanism by which propofol pretreatment reduced LPS-induced ALI in rats was through increasing phosphorylation of Akt protein, as the inhibition of the phosphoinositide 3-kinase/Akt signaling pathway could partially reverse the lung-protective effects of propofol pretreatment on LPS-induced ALI in rats (Zhao et al., 2014a).

There are several potential limitations in our study. (1)Our model used MV with a tidal volume of 22 ml/kg. Although lower tidal volumes (*i.e.*, 6 ml/kg) are used in patients with ARDS, lung injury is heterogeneous with regional variation in lung mechanics that leads to local areas of high levels of stretch. To mirror the injury that occurs in these

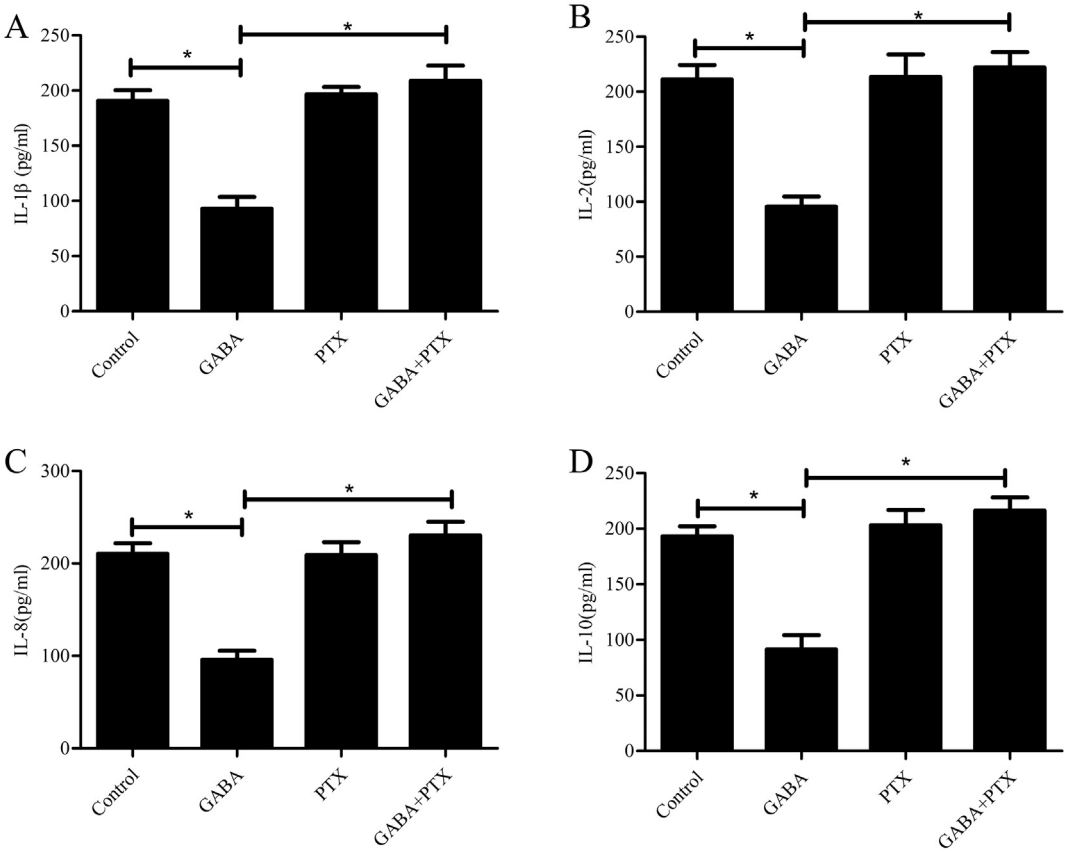

Fig. 6. Administration of GABA *in vitro* in A549 cell experiments suppressed the LPS-induced release of inflammatory mediators (IL-1β, IL-2, IL-8, IL-10) in lung epithelial cells. Data are expressed as the means ± SD from three independent experiments. *$P < .05$.

areas, the standard approach is to use MV with higher tidal volumes in preclinical models. (2) Our study only examined the effect of propofol on inflammation, and it is possible that propofol also affects other factors that contribute to noncardiogenic pulmonary edema in ARDS, including changes in epithelial barrier integrity and the active transport of salt and water across the epithelial barrier. (3) The involvement of $GABA_A$ receptors was the focus of the study; whether other mechanisms are also responsible for the protective effects of propofol remains to be elucidated. (4) We focused the investigation on propofol in the present study, but further studies using other sedatives (*e.g.*, midazolam) are needed.

In conclusion, in this study, we found that the administration of propofol improved lung function, alleviated lung injury, and up-regulated GAD and $GABA_AR$ expressions in a two-hit model of ALI characterized by the intratracheal instillation of an endotoxin and prolonged MV. Therefore, the protective effects of propofol may be associated with the up-regulation of $GABA_A$ receptors in alveolar epithelial cells.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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