

Received: 2018.02.28
Accepted: 2018.07.17
Published: 2018.10.08

Protective Effect and Mechanism of Alprostadil in Acute Respiratory Distress Syndrome Induced by Oleic Acid in Rats

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This work was supported by the National Natural Science Foundation of China (No. 81560679 and 31600094) and the Natural Science Research Foundation of Jilin Province for Sciences and Technology (grant No. 201601011851C)

Background: This study investigated the role and mechanism of alprostadil in acute respiratory distress syndrome (ARDS) induced by oleic acid (OA) in rats.





Material/Methods: Sprague-Dawley rats were randomly divided into control, OA model, and OA + Alprostadil (2.5, 5, and 10 µg/kg, respectively) groups. The ARDS model was induced by femoral vein injection of OA, and alprostadil was administered immediately. Lung injury was evaluated by lung wet-dry weight ratio (W/D) and histological analyses. Expressions of ACE, inflammatory mediators, apoptotic-related proteins, and proteins in the MAPKs and NF-κB signaling pathways were determined by Western blot or immunohistochemical staining.

Results: Compared with the control group, the OA model group had significantly increased W/D, lung injury score, and collagen deposition at 3 h after OA injection. However, alprostadil (10 µg/kg) treatment significantly reduced OA-induced elevation of these indicators. Additionally, OA-induced expression of TNF-α and IL-1β were suppressed by alprostadil. The OA-induced activation of nuclear factor (NF) κB p65 was also reduced by alprostadil. Furthermore, we found that Alprostadil had an inhibitory effect on the phosphorylation of JNK, ERK1/2, and p38 MAPKs. Alprostadil inhibited Bax but increased Bcl-2, indicating a suppressive role in apoptosis. Remarkably increased expression of ACE in the OA model group was observed, which was decreased by alprostadil.

Conclusions: Alprostadil has a protective effect on ARDS induced by OA in rats, possibly through inhibiting apoptosis, suppressing the activation of MAPKs and NF-κB signaling pathways, and decreasing ACE protein expression. Therefore, the use of alprostadil in clinical ARDS treatment is promising.

MeSH Keywords: **Alprostadil • Oleic Acid • Respiratory Distress Syndrome, Adult**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/909678>

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Background

Acute respiratory distress syndrome (ARDS), also known as acute lung injury (ALI), is one of the most challenging clinical conditions in critical care medicine. It is a severe, life-threatening medical condition characterized by acute and widespread inflammation in the lungs [1], which leads to protein-rich non-hydrostatic pulmonary edema and refractory hypoxemia [2]. It increases lung “stiffness” and impairs the ability of the lung to remove carbon dioxide [3]. Many pathogenic conditions can trigger ARDS, such as sepsis and pneumonia [4]. Although the outcome of ARDS has improved with advances in ventilator strategies, mortality is still high. The LUNG SAFE study reports a 40% hospital mortality rate, with a significant increase across the ARDS severity categories, in line with the Berlin definition (34.9% in mild ARDS, 40.3% in moderate ARDS, and 46.1% in severe ARDS) [5]. No specific and effective pharmacological intervention for ARDS is currently available [3]; therefore, it is critical to develop new clinical drugs for the treatment of ARDS.

Alprostadi, a lipid microsphere-incorporated prostaglandin E1, is reported to promote pulmonary vasodilation [6]. It also has anti-inflammatory and anti-platelet aggregation properties [7]. A meta-analysis demonstrated that inhaled prostaglandins could reduce pulmonary artery pressure and increase oxygenation [8]. Prostaglandin E1 is also reported to decrease lung vascular permeability [9]. In addition, Hasegawa et al. reported that prostaglandin E1 improved microcirculation [10]. Thus, alprostadi may be useful in prevention and treatment of ARDS. However, the underlying mechanism by which alprostadi prevents ARDS remains unknown.

A recent study showed that activation of the mitogen-activated protein kinases (MAPKs) pathway is involved in ARDS [11]. The phosphorylation levels of p38 MAPK, extracellular signal-regulated kinase (ERK), and Jun N-terminal kinase (JNK) are all significantly increased in LPS-induced lung injury [12]. Consequently, inhibition of these proteins efficiently attenuates LPS-induced pulmonary inflammatory response [13,14]. Additionally, the possible mechanism by which oleic acid (OA) induces acute lung injury (ALI)/ARDS in rats is that OA activates the NF- κ B signaling pathway to increase the expressions of many pro-inflammatory cytokines, including TNF- α and IL-1 β [15]. Therefore, we hypothesized that alprostadi can protect against OA-induced ARDS by inhibiting the MAPKs and NF- κ B pathways.

The renin-angiotensin system (RAS) plays a key role in maintaining blood pressure homeostasis, as well as fluid and salt balance [16]. For many years, angiotensin-converting enzyme (ACE) has been known as the key enzyme in the regulation of RAS [17]. The pulmonary circulation is an important target for the renin-angiotensin system in the lungs. ACE activity

triggers vasoconstriction by generating angiotensin II from angiotensin [18]. Previous reports indicate that the RAS plays a critical role in ARDS [19]. For instance, angiotensin II (Ang II) and AT1 receptor (AT1R) induces pulmonary vasoconstriction, suggesting vital roles of Ang II and AT1R in elevating pulmonary vascular tone and causing pulmonary edema [20]. In addition, accumulating data suggest that Ang II also increases vascular permeability via AT1R [21]. Several mediators have been implicated in Ang II-regulated vascular permeability changes, including the eicosanoids leukotriene C4, prostaglandin E2 and prostaglandin I2, and vascular permeability factor [22]. Therefore, ACE functions as a lung-injury-promoting factor in ALI, which needs to be controlled during the treatment and prevention of ARDS.

Dysregulation of apoptosis pathways can result in epithelial injury, which is the characteristic of ALI [23,24]. The Bcl-2 family, including an anti-apoptotic gene (Bcl-2) and a pro-apoptotic gene (Bax), is also closely associated with apoptosis [25]. The sensitivity to apoptotic signals mainly depends on the process of competitive dimerization of Bcl-2/Bax in cells. When Bcl-2 is expressed at a high level, Bcl-2/Bcl-2 homodimer and Bcl-2/Bax heterodimer are created and the DNA cleavage activity of endonuclease is inhibited to suppress apoptosis. When Bax is overexpressed, Bax/Bax homodimer is formed to accelerate apoptosis. Finally, it has been demonstrated that the ratio of Bcl-2 to Bax is a crucial index in the process of apoptosis, indicating the severity of apoptosis [26]. Strategies to block apoptosis pathways could be effective for limiting ALI.

Oleic acid (OA) is a fatty acid derived from animal and plant oils [27]. When administered intravenously, OA induces acute diffuse lung injury, which is similar to the initial phase of ALI and ARDS in humans [28]. Following OA administration, pulmonary vascular endothelial cell injury, local hemorrhage, edema, and inflammation can occur [29,30]. Thus, use of OA to mimic ARDS in animal models is well established.

In this study, an OA-induced ARDS model was used to investigate the effects of alprostadi on lung injury. Moreover, the possible mechanisms involved in alprostadi's role in ARDS were also examined in MAPKs and NF- κ B pathways, ACE expression, and apoptosis. Our study thus may provide experimental evidence for the clinical use of alprostadi for treatment of ARDS.

Material and Methods

Animals

Thirty male Sprague-Dawley rats (200–300 g weight and 7–8 weeks of age) were purchased from the Animal Center of Yanbian University, Jilin, China (License No.: SCXK

[Jilin]-2014-007). The animals were housed at 22°C with a 12-h light/dark cycle and free access to food and water. The experimental protocol was approved by the Animal Care Committee of the Ethics Committee on Animal Research at Yanbian University. All procedures were performed strictly according to the *National Institute of Health Guide for the Care and Use of Laboratory Animals*.

Animal grouping and treatment

The rats were randomly divided into 5 groups (n=6): control, OA (Bai Hui Biological Technology Co., Ltd, Shenyang, China) model, OA + Alprostadil (2.5 µg/kg), OA + Alprostadil (5 µg/kg), and OA + Alprostadil (10 µg/kg) groups. The dose of alprostadil was determined according to previous reports [31,32]. After the rats were anesthetized with 20% urethane (Bai Hui Biological Technology Co., Ltd.) at a dose of 1 g/kg, a dose of 0.1 ml/kg OA and an equal amount of saline were injected slowly into the femoral vein at to induce ARDS. Rats in the control group were administered an equal volume of saline. Immediately after OA administration, alprostadil injection (Beijing Tide Pharmaceutical Co., Ltd., China national medicine permission number: H01980024) at the dose of 2.5, 5, or 10 µg/kg and an equal amount of saline were given administered to rats in the OA + Alprostadil (2.5 µg/kg), OA + Alprostadil (5 µg/kg), OA + Alprostadil (10 µg/kg) groups, respectively, by femoral vein injection. Three hours after OA administration, all animals were sacrificed and the lungs were excised.

Lung wet/dry (W/D) ratio

The wet weight of the middle lobe of the right lungs was determined immediately after removal. After being dried in an oven at 60°C for 48 h, the dry weight of the right lung was measured. Then, the lung W/D ratio was calculated.

Hematoxylin-eosin (H&E) staining

The left lung tissue samples were fixed in 10% buffered formalin for 48 h and then dehydrated by graded ethanol. After that, samples were embedded in paraffin and cut into 5-µm sections. The sections were stained with hematoxylin and eosin according to routine procedure and then observed under a light microscope (VANOX, Olympus, Japan). The lung injury score was evaluated based on the following: a) alveolar congestion, b) hemorrhage, c) infiltration or aggregation of neutrophils in airspace or vessel wall, and d) thickness of alveolar wall/hyaline membrane formation. Each item was scored on a 5-point scale as follows: 0, minimal damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximal damage [33].

Masson trichrome staining

Fibrosis was evaluated using Masson's trichrome-stained sections. The sections were observed under 200× magnification with a microscope (VANOX, Olympus, Japan). A blue signal indicated positive staining for collagen. The extent of fibrosis in these sections was graded using the following criteria: none (0), no evidence of fibrosis; mild (1+), focal regions of fibrosis involving less than 20% of the lung. Fibrosis involved the pleura and the interstitium of the sub-pleural parenchyma with some distortion of alveolar architecture; moderate (2+), more extensive fibrosis involving 20%–50% of the lung and fibrotic regions mostly extending inward from the pleura and still focal; and severe (3+), widespread fibrosis, involving more than 50% of the lung. Confluent lesions with extensive derangement of parenchymal architecture included cystic air spaces lined by cuboidal epithelium [34].

Immunohistochemistry

Lung sections of 5 µm were kept in an oven at 60°C for 24 h to increase section adherence to the slide. After deparaffinization and rehydration, the sections were treated with 10 mM citrate buffer (Cat #ZLI-9064, ZSGB-BIO, Beijing, China) for 5 min for antigen retrieval. Then, the sections were incubated with 3% H₂O₂ for 10 min to eliminate endogenous peroxidase activity, followed washing 3 times with PBS or TBS for 2 min each time. Sections were then washed with PBS 3 times for 2 min each time. We then added rabbit anti-rat anti-ACE antibody (1: 300 dilution, Elabscience Biotechnology Co., Ltd, China), followed by incubation at 4°C overnight. Sections were then washed 3 times with PBS or TBS for 2 min each time. We added reagent 1, then incubated sections at room temperature for 20 min, followed by washing 2 times with PBS for 2 min each time. Reagent 2 was then added, followed by incubation at room temperature for 20–30 min and washing 3 times with PBS for 2 min each time. Color development was performed with DAB (Cat # ZLI-9019, ZSGB-BIO, Beijing, China). We washed sections with deionized water to make the section counterstained, dewatered, and transparent, then sealed the section and viewed it under the microscope. Under 200× magnification, 5 different microscopic fields were randomly chosen. Quantitative analysis of ACE expression was performed by calculating the average optical density of ACE-positive staining.

Western blot analysis

Total proteins were isolated from the rest of the right lung tissue homogenate. Protein concentration was determined using the BCA Protein Assay Kit (Beyotime, China). Equal quantities of protein samples were separated by SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked with 5% skimmed milk, and incubated with anti-ACE

(1: 3000), anti-TNF- α (1: 3000), anti-IL-1 β (1: 1500), anti-Bcl-2 (1: 1500), anti-Bax (1: 1500), anti-NF- κ B p65 (1: 1500), anti-p-p38MAPK (1: 1500), anti-p38MAPK (1: 1500), anti-p-ERK1/2 (1: 1500), anti-ERK1/2 (1: 1500), anti-p-JNK (1: 1500), anti-JNK (1: 1500), and anti- β -actin (1: 1500) antibodies at 4°C overnight. The goat anti-rabbit (or mouse) secondary antibody conjugated with horseradish peroxidase (1: 5000) was added as secondary antibody. All the antibodies used were purchased from Elabscience Biotechnology Co., Ltd. (China). Immunodetection was carried out with ECL detection reagent (Beyotime, China). β -actin was used as a loading control. Results of all protein expression by Western blot are expressed as ratios of the optical density value of the target bands to those of internal controls by using Quantity One software (Bio-Rad Technical Service Department).

Statistical analysis

SPSS 19.0 software was used for statistical analysis. Data are presented as mean \pm SD. Statistical significance of differences was assessed using one-way ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

Results

Alprostadiil reduces OA-induced lung edema in rats

To examine the effect of alprostadiil on OA-induced ARDS, we first investigated the W/D ratios of lungs in rats. The W/D ratios of lung tissues represent the lung water content, which can be used to indicate lung edema [35]. As shown in Figure 1, the W/D ratio in the OA model group was significantly increased ($P < 0.05$) compared with that in the control group; however, alprostadiil (2.5, 5, 10 μ g/kg) treatment decreased the OA-induced W/D ratio compared with the OA model group. Statistical significance was observed when 10 μ g/kg alprostadiil was administered ($P < 0.05$). These results suggest that alprostadiil has protective effects on OA-induced lung edema.

Alprostadiil ameliorates OA-induced lung tissue histopathological changes

Next, we investigated the effect of Alprostadiil on the histopathological changes of lungs by H&E staining. The structure of the alveoli was complete and there was no obvious pathological change in the control group (Figure 2A). OA challenge caused significant pathological changes, including infiltration of inflammatory cells, aggregation of neutrophils in the alveolus and alveolar fluid, thickening of the alveolar wall, and pulmonary congestion (Figure 2A). Alprostadiil treatment ameliorated the tissue damage in a dose-dependent manner. Importantly, in the OA + Alprostadiil (10 μ g/kg) group, the damaged structure

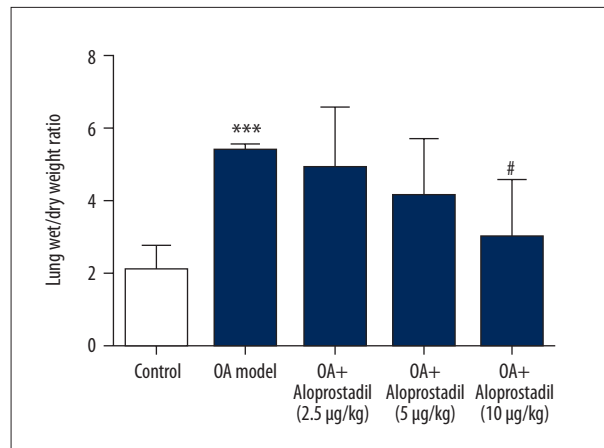


Figure 1. Effects of alprostadiil on the lung wet/dry weight ratio in OA-induced ARDS rats. OA (0.1 ml/kg) was injected from femoral vein of rats and the control group was injected with the same amount of vehicle. Alprostadiil (2.5, 5 or 10 μ g/kg) was injected into the femoral vein immediately after OA administration. Then, rats were sacrificed at 3 h after OA stimulation. Wet/dry (W/D) weight ratio of the lungs was measured in the 5 groups of rats. Data are expressed as mean \pm SD (n=6 in each group). *** $P < 0.005$ versus control group; # $P < 0.05$ versus OA model group.

of the alveoli was obviously restored compared with that in the OA model group. Moreover, the lung injury scores (Figure 2B) of the OA model group were significantly increased as compared with the control group. In contrast, compared with the OA model group, alprostadiil significantly decreased the lung injury score. Similar results were obtained by Masson trichrome staining. As shown in Figure 3, more collagen deposition and higher fibrosis scores were observed in OA model group compared with the control group. Alprostadiil treatment reduced collagen deposition and fibrosis scoring in a dose-dependent manner. These data suggest that Alprostadiil protects rat from OA-induced lung tissue damage.

Alprostadiil inhibits the OA-induced inflammatory responses in the lung tissues of rats

The effect of alprostadiil on the production of the pro-inflammatory mediators TNF- α and IL-1 β was also analyzed by Western blot at 3 h after administration of OA. OA administration caused a significant increase in TNF- α and IL-1 β expression in the lung tissues when compared with the control group (Figure 4A-4C; $p < 0.05$). Alprostadiil (2.5, 5, and 10 μ g/kg) administration caused an obviously decrease in the expression of TNF- α and IL-1 β in the lung tissue when compared with the OA model group. These results indicate that alprostadiil inhibits the production of pro-inflammatory mediators and protects rats from OA-induced lung inflammation.

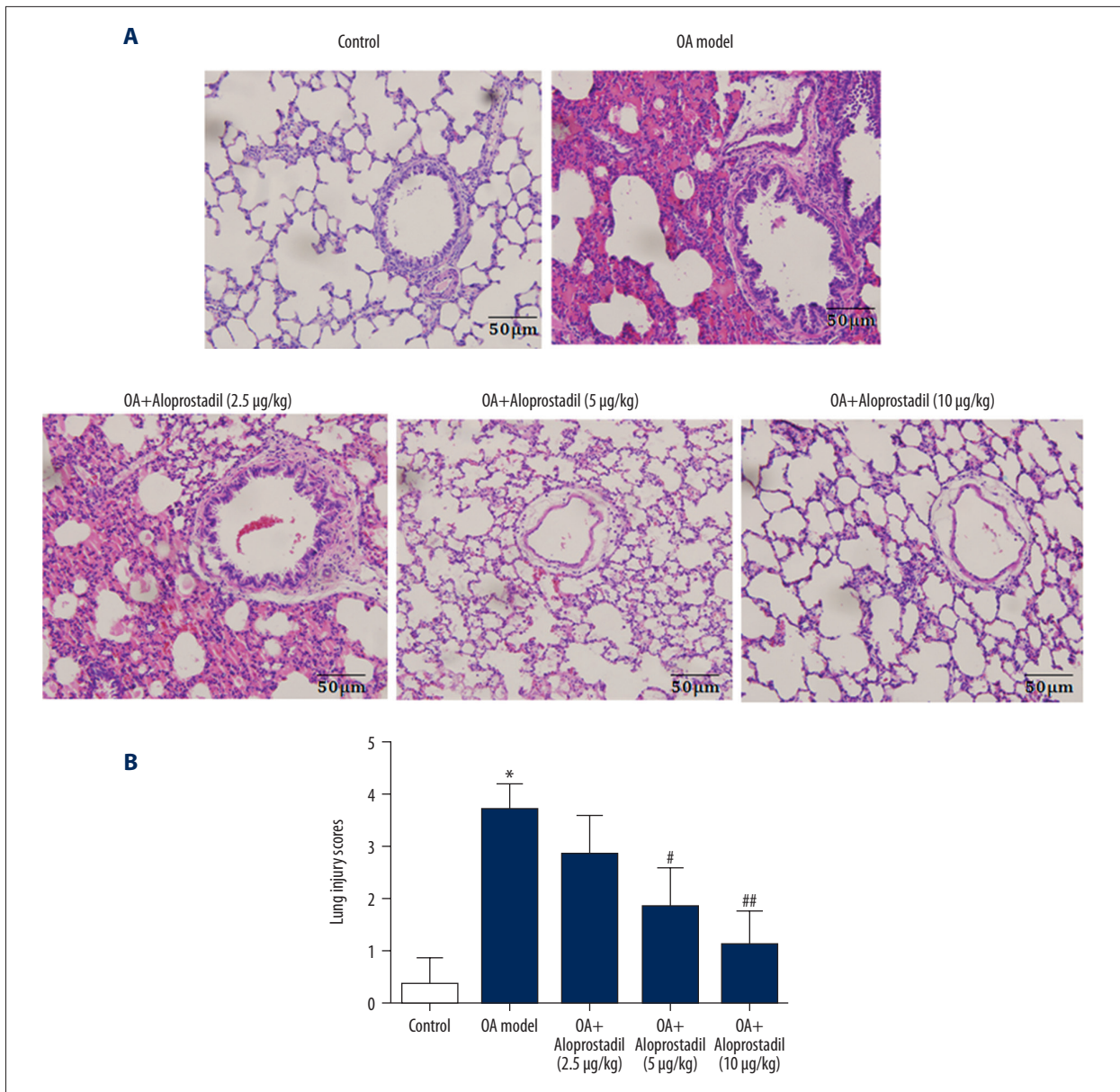


Figure 2. Effects of alprostadi on histopathological changes in lung tissues of OA-induced ARDS rats. Rats were injected with OA and alprostadi as described in Figure 1. **(A)** H&E staining of lung tissues from the 5 group of rats (200× magnification; scale bar, 50 μm). **(B)** The lung injury score was graded for each group, from 0 (no damage) to 4 (maximum damage) according to the criteria described in the ‘Materials and Methods’ section. Data are expressed as mean ±SD (n=6 in each group). * P<0.05 versus control group; # P<0.05 and ## P<0.01 versus OA model group.

Alprostadi suppresses MAPKs and NF-κB signaling pathways in OA-induced ARDS rats

NF-κB and MAPK signaling pathways play key roles in OA-induced ARDS pathogenesis [36,37]; therefore, we investigated whether alprostadi could affect these pathways. Western blot analysis showed that phosphorylation of MAPKs was significantly up-regulated by OA, including p-p38 MAPK (Figure 5A, 5B), p-ERK1/2 (Figure 5A, 5C), and p-JNK

(Figure 5A, 5D). Moreover, OA administration markedly increased the phosphorylation of p65 (Figure 5A, 5E). However, treatment with alprostadi significantly inhibited the phosphorylation of p38 MAPK, ERK1/2, JNK, and p65, indicating that alprostadi can alleviate ARDS by suppressing MAPKs and NF-κB signaling pathways.

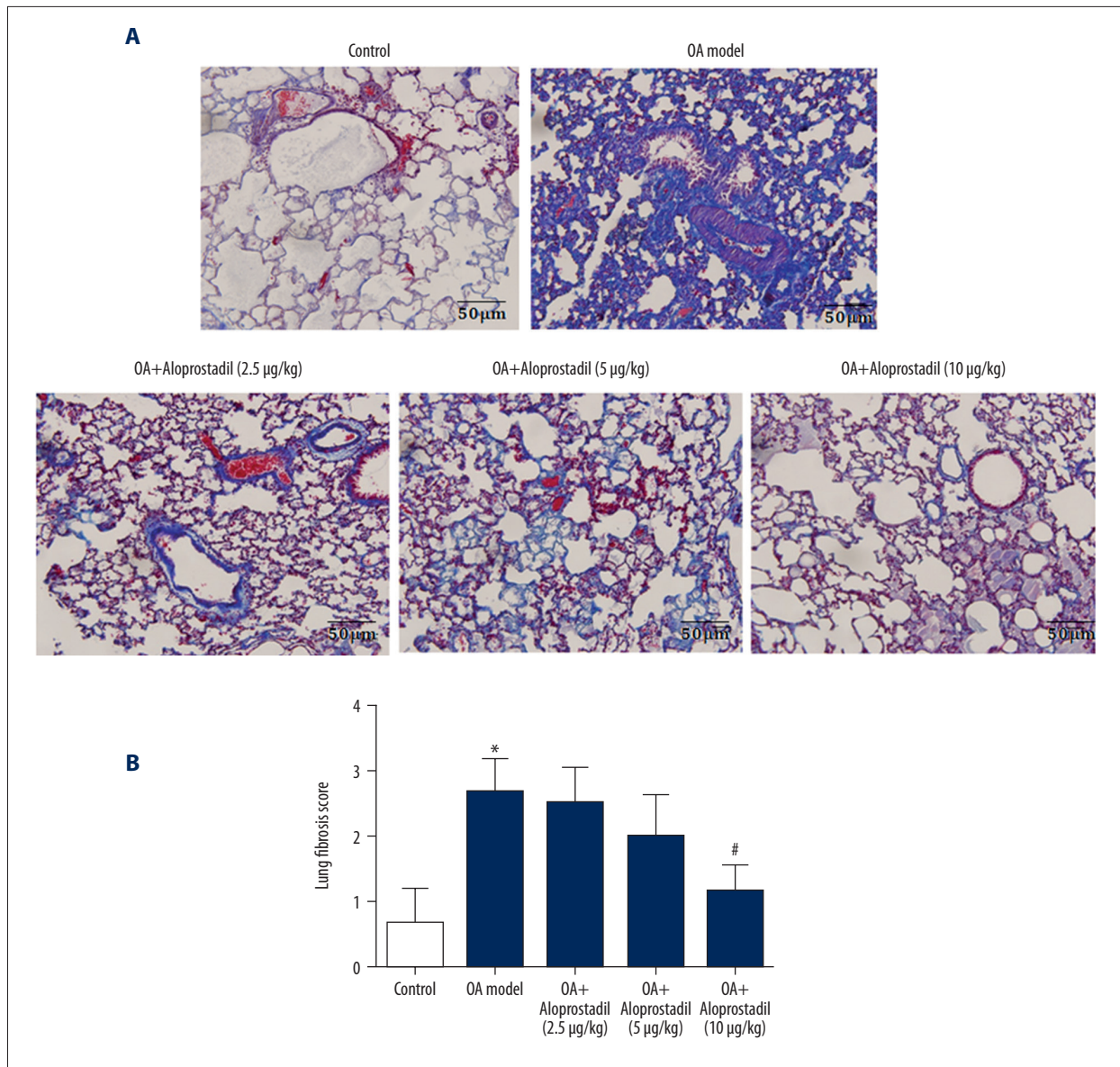


Figure 3. (A, B) Effects of alprostadil on collagen production in lung tissues of OA-induced ARDS rats. Rats were injected with OA and alprostadil as described in Figure 1. Masson trichrome stains of lung tissues are shown (200× magnification; scale bar, 50 µm). A blue signal indicated positive staining for collagen.

Alprostadil reduces expression of the pro-apoptotic protein Bax but increases expression of the anti-apoptotic protein Bcl-2

Activation of apoptosis-related proteins plays a central role in the process of cellular apoptosis. To determine whether alprostadil affects OA-induced lung tissue apoptosis, we investigated Bcl-2 and Bax expression by Western blot. OA stimulation resulted in an obvious increase of Bax (Figure 6A, 6B) but decrease of Bcl-2 (Figure 6A, 6C) expression in lung tissues. However, 10 µg/kg alprostadil treatment significantly prevented

the increase of Bax expression and the decrease of Bcl-2 expression in lung tissues. Therefore, alprostadil can inhibit apoptosis in lung tissues induced by OA.

Alprostadil inhibits the production of ACE in the lung tissue of OA-induced ARDS rats

Clinical and experimental studies have shown that lung injury induced by ARDS is associated with abnormal activation of the RAS [38]. Activity of angiotensin-converting enzyme (ACE) is most prominent in pulmonary circulation under physiological

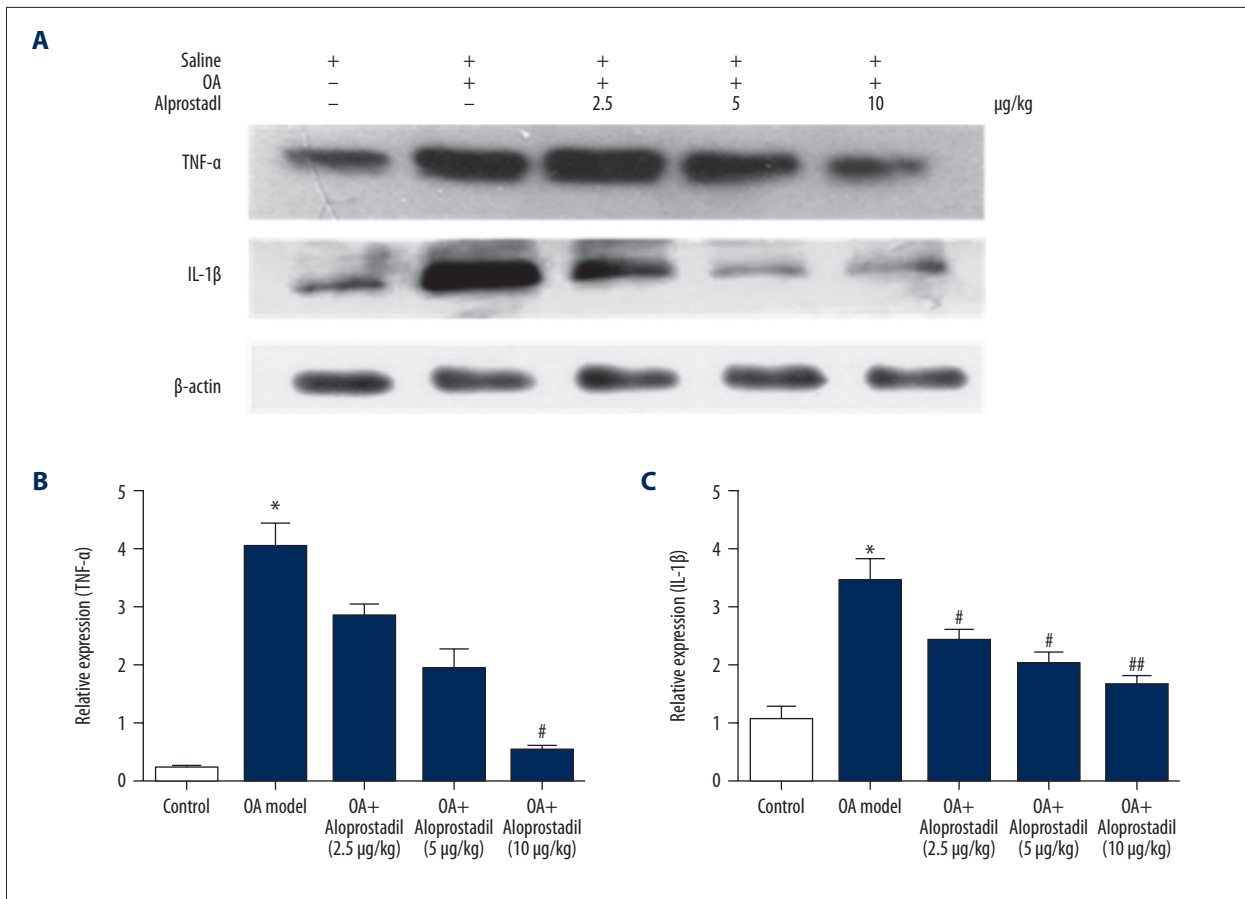


Figure 4. Effects of alprostadiil on TNF- α and IL-1 β expression in lung tissues of rats with OA-induced ARDS. Rats were injected with OA and alprostadiil as described in Figure 1. **(A)** Proteins were extracted from the lungs and analyzed by Western blot with anti-TNF- α and anti-IL-1 β antibodies. β -actin was used as internal control. **(B, C)** Bar graphs represents semi-quantitative densitometry from Western blot analysis. Data are expressed as mean \pm SD (n=6 in each group). * P<0.05 versus control group; # P<0.05 and ## P<0.01 versus OA model group.

conditions. It has been confirmed that serum ACE activity is significantly elevated in active ARDS patients, and that the measurement of serum ACE activity is useful in following the clinical course of the disease [39]. Several studies have suggested that monitoring serum ACE is useful as an indicator of endothelial cell integrity [40]. Staining results showed that the expression of ACE protein in the lung tissue from the OA model group was obviously higher than that from the control group (Figure 7A), suggesting that ACE is involved in the development of ARDS. In contrast, in the OA + Alprostadiil (5 and 10 μ g/kg) group, the expression of ACE was partially reduced compared with that in the OA model group (Figure 7A, 7B).

The effect of Alprostadiil on ACE production was also analyzed by Western blot. OA administration caused a significant increase in ACE expression in the lung tissue when compared with the control group (Figure 7C, 7D p<0.05). As expected, alprostadiil prevented the increase of ACE in OA-induced ARDS rats in a dose-dependent manner, and a significant difference was found

when 10 μ g/kg alprostadiil was administered (Figure 7B, 7C). These results suggest that alprostadiil inhibits the production of ACE in the lung tissue of OA-induced ARDS rats.

Discussion

In this study, we demonstrated that OA administration evoked severe lung injury, which was characterized by increased inflammatory cell infiltration, edema, and hemorrhage in the interstitium and alveolus, as well as collagen deposition. In addition, OA administration significantly increased lung W/D ration, the expression of ACE, pro-inflammatory factors, pro-apoptotic proteins, and activation of MAPKs and NF- κ B signaling pathways in the rat lung tissue. Importantly, alprostadiil treatment significantly attenuated OA-induced ARDS symptoms, as well as suppressing the elevation of these ARDS-related proteins and activation of the MAPKs and NF- κ B pathways. Taken together, these findings suggest that alprostadiil administration

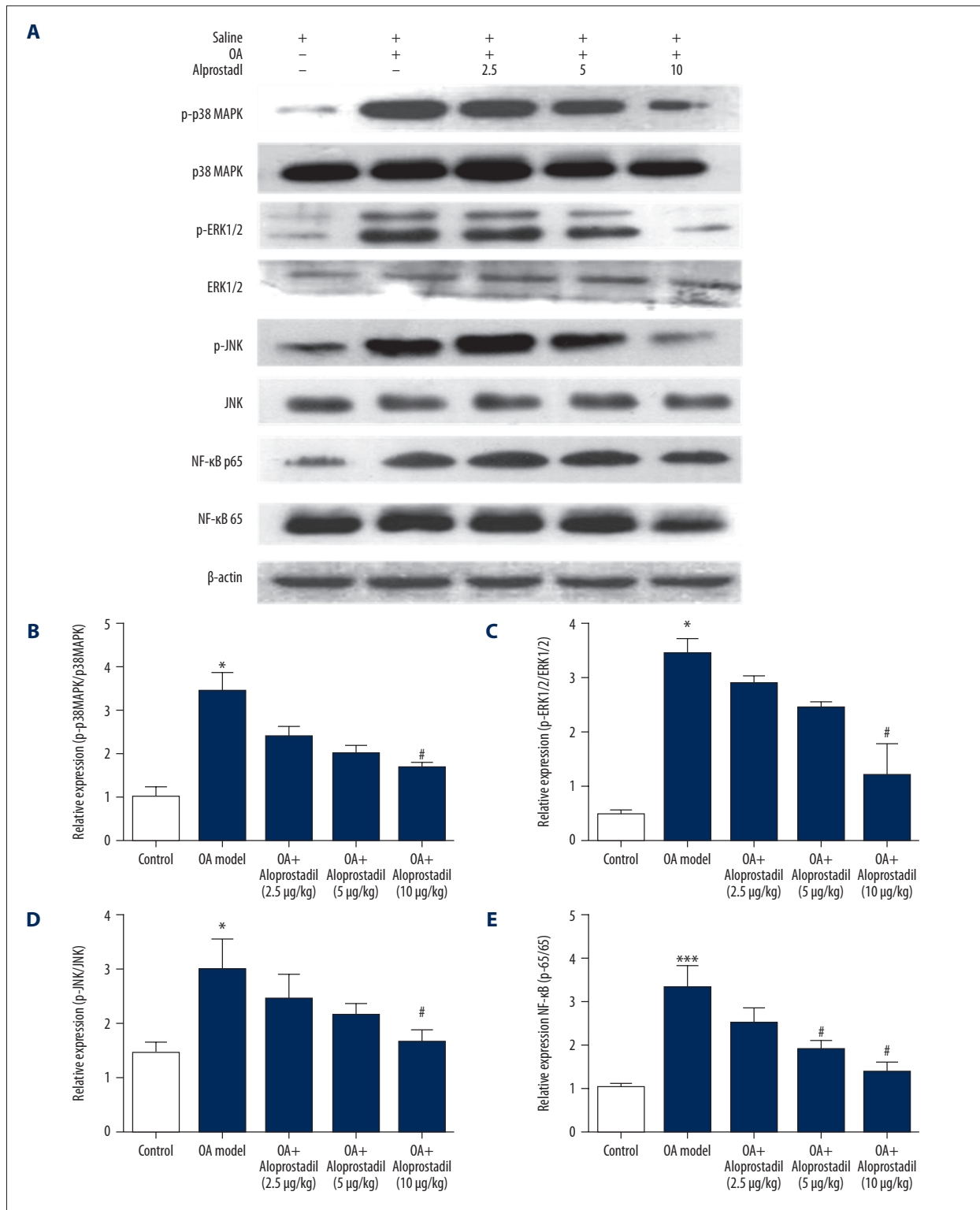


Figure 5. Effects of alprostadiil on the activities of MAPKs and NF-κB signaling pathways in lung tissues of rats with OA-induced ARDS. Rats were injected with OA and alprostadiil as described in Figure 1. **(A)** Proteins were extracted from the lungs and analyzed by Western blot with anti-p-p38-MAPK, anti-p-ERK1/2, anti-p-JNK, and anti-NF-κB-p65 antibodies. β-actin was used as internal control. **(B-E)** Bar graphs represents semi-quantitative densitometry from Western blot analysis. Data are expressed as mean ±SD (n=6 in each group). * P<0.05 and *** P<0.005 versus control group; # P<0.05 versus OA model group.

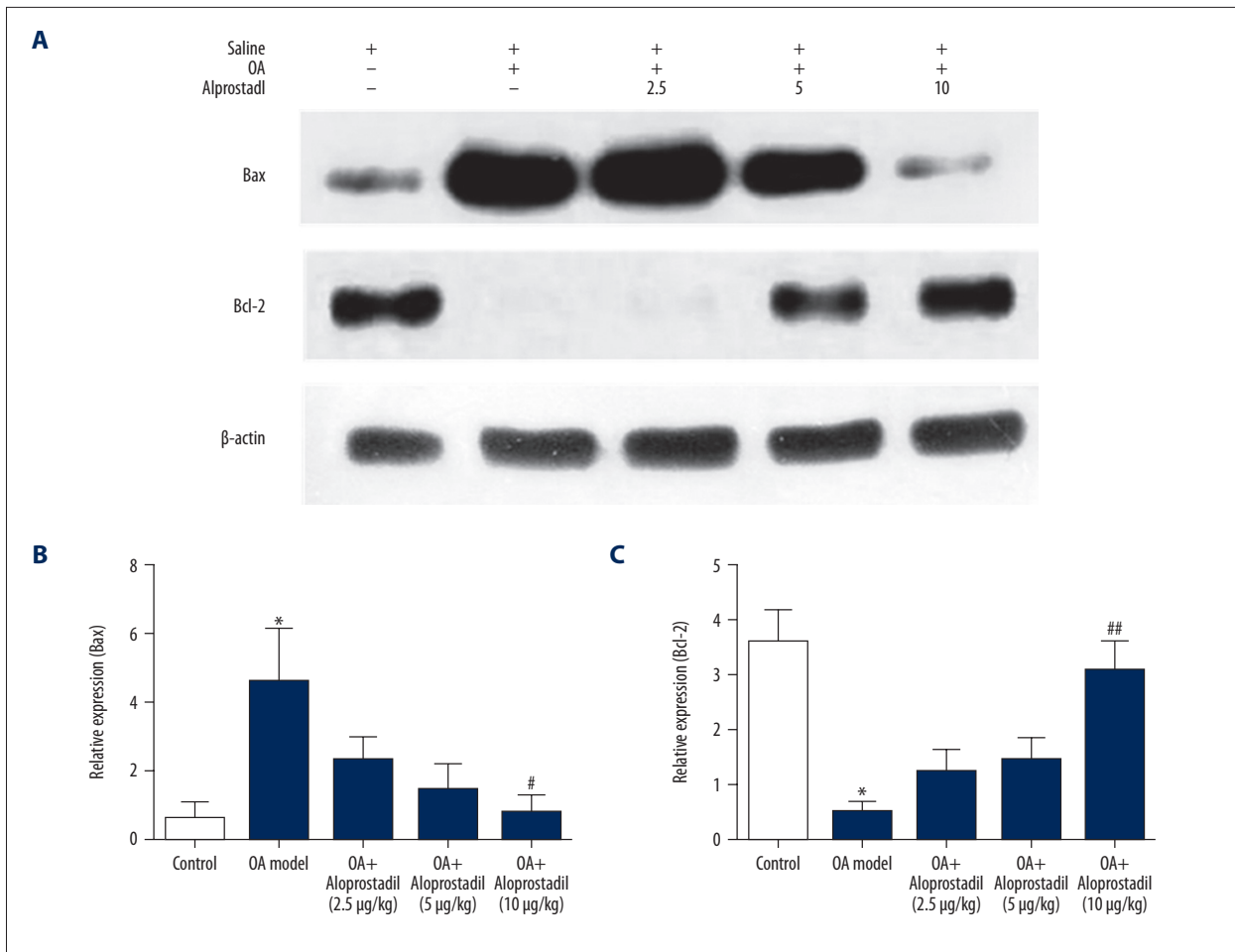


Figure 6. Effect of alprostadi on expression of Bax and Bcl-2 in lung tissues of rats with OA-induced ARDS. Rats were injected with OA and alprostadi as described in Figure 1. **(A)** Proteins were extracted from the lungs and analyzed by Western blot with anti-Bax and anti-Bcl-2 antibodies. β -actin was used as internal control. **(B, C)** Bar graphs represents semi-quantitative densitometry from Western blot analysis. Data are expressed as mean \pm SD (n=6 in each group). * P<0.05 versus control group; # P<0.05 and ## P<0.01 versus OA model group.

prevents OA-induced ARDS and inflammation and may be useful as a therapeutic agent for ARDS treatment.

Alprostadi is extensively used clinically for the treatment of multiple-system diseases [31–43]. However, the therapeutic effect of alprostadi in ARDS remains unclear. It was reported that Alprostadi exerts its effect through protecting the pulmonary vascular endothelial cells [8–10]. However, the OA-induced ARDS model features injury to pulmonary vascular endothelial cells [29,30]. Thus, in the present study, the OA-induced ARDS model was used to investigate the role of Alprostadi in ARDS. The lung W/D ratio, cytokine levels, and H&E and Masson trichrome staining of lung tissues were evaluated. The W/D ratio is considered as an indicator of lung edema [44], and OA has been reported to be able to augment edema formation and cytokine productions in the bronchoalveolar fluids of Swiss Webster mice [45]. TNF- α is known as a pro-inflammatory cytokine

and can stimulate the production of other cytokines [46,47]. Its production has been reported to be significantly increased in ALI/ARDS patients [48,49]. Masson trichrome staining was performed to observe the collagen formation in bronchial airways. Our results showed typical pathological changes in the lungs of OA-administrated rats, such as marked alveolar fluid, thickening of the alveolar wall and lungs, alveolar wall disruption and leukocyte infiltration, and collagen deposition in the lungs. The expression of TNF- α and IL-1 β in the lung tissues were significantly increased, and the W/D ratio was increased in ARDS rats. The OA + Alprostadi group showed clearly reduced lung damage, the W/D ratio of rat lung tissues, and the production of TNF- α and IL-1 β , as well as collagen formation, as compared with the OA model group. These data indicate the protective role of alprostadi in ARDS.

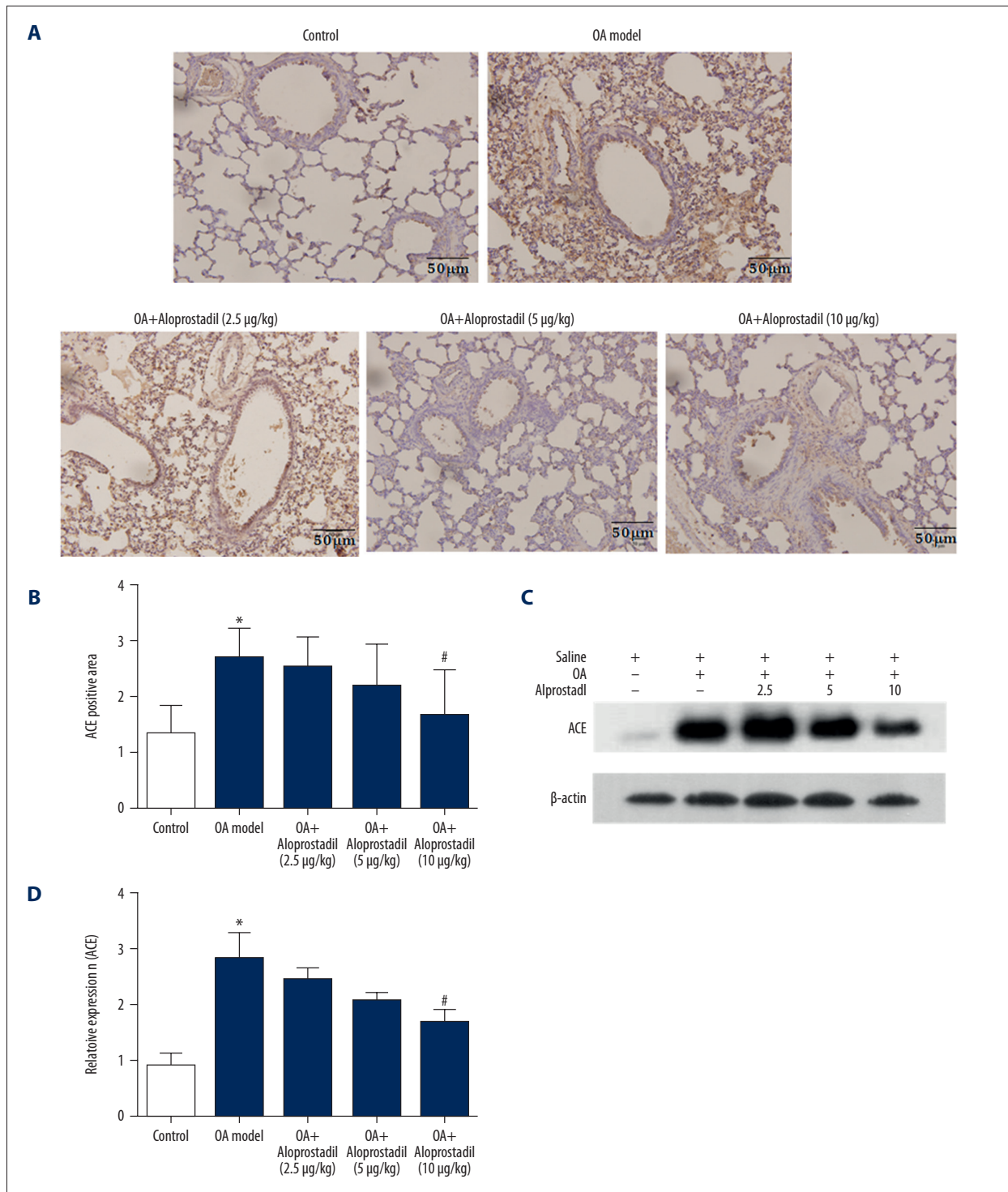


Figure 7. Effect of alprostadil on ACE expression in lung tissues of rat with OA-induced ARDS. Rats were injected with OA and Alprostadil as described in Figure 1. **(A)** Expression of ACE in lung tissues was observed by immunohistochemistry (200× magnification; Scale bar, 50 µm). Brown cells represent positive cells. DAB was used to stain the cells. **(B)** Bar graphs represents ACE-positive area from immunohistochemistry. **(C)** Proteins were extracted from the lungs and analyzed by Western blot with anti-ACE antibody. β-actin was used as internal control. **(D)** Bar graphs represent semi-quantitative densitometry from Western blot analysis. Data are expressed as mean ±SD (n=6 in each group). * P<0.05 versus control group; # P<0.05 versus OA model group.

NF- κ B is implicated in the regulation of inflammatory and immune responses, which play an important role in the development of ARDS. MAPKs also have critical roles in the regulation of cell growth, differentiation, and control of cellular responses to cytokines and stress [50]. NF- κ B and MAPKs signaling pathways can be activated by OA to regulate the release of pro-inflammatory cytokines, such as TNF- α and IL-1 β [51]. More recent studies have suggested that alprostadil reduced LPS-induced ALI through down-regulating NF- κ B activity and inhibiting expression of harmful inflammatory cytokines [52]. Our results showed that OA administration markedly increased the phosphorylation of NF- κ B p65 and MAPKs, including p38 MAPK, ERK1/2, and JNK. However, alprostadil can completely reverse the increased activation of MAPKs and NF- κ B signaling pathways induced by OA. Thus, we demonstrated that Alprostadil can protect against OA-induced ARDS through inhibiting the MAPKs and NF- κ B signaling pathways.

In addition, recent studies have shown that apoptosis is a crucial mechanism for the development of ARDS [53,54]. The balance between pro-apoptotic and anti-apoptotic factors in the lungs may play an important role in determining the fate of the epithelium. The Bcl-2 family, including an anti-apoptotic gene (Bcl-2) and a pro-apoptotic gene (Bax), is also closely associated with apoptosis [25]. The sensitivity to apoptotic signals mainly depends on the process of competitive dimerization of Bcl-2/Bax in cells. When Bcl-2 is expressed at a high level, Bcl-2/Bcl-2 homodimer and Bcl-2/Bax heterodimer are created and the DNA cleavage activity of endonuclease is inhibited to suppress apoptosis. When Bax is overexpressed, Bax/Bax homodimer is formed to accelerate apoptosis. Finally, it has been demonstrated that the ratio of Bcl-2 to Bax is a crucial index in the process of apoptosis, indicating the severity of apoptosis [26]. The effects of alprostadil on the regulation of Bcl-2 and Bax have previously been reported [55–57]. To confirm these findings, Western blot analysis was used to detect the protein expression levels of Bax and Bcl-2. In our study, significant apoptosis was seen in the OA model group lung tissue, while alprostadil downregulated the protein expression levels of Bax and upregulated the expression levels of Bcl-2 in lung tissue, which caused a decreased rate of lung tissue apoptosis in the OA+Alprostadil group. This supports that alprostadil protects against lung injury in OA-induced ARDS.

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In recent decades, RAS has been thought to be essential in maintaining blood pressure homeostasis, as well as fluid and salt balance [16]. The latest evidence suggested that activation of the pulmonary RAS can influence the pathogenesis of ARDS via such mechanisms as inflammation, vascular permeability, vascular tone, and fibroblast activity [58]. ACE is a key member of the classical renin-angiotensin system. The lungs are major organs that express ACE in humans and rats. The importance of RAS in ARDS has been widely emphasized. Idell et al. [58] found that ARDS patients had elevated ACE levels in bronchoalveolar lavage. Orfanos [59] also reported that pulmonary capillary endothelium-bound ACE activity was correlated with the severity of lung injury in patients with acute respiratory distress syndrome. In the present study, we used Western blot and immunohistochemistry to examine the expression of ACE, a lung-injury-promoting factor in ALI, in lung tissues to assess the degree of lung injury. We found that OA administration caused a significant increase in the expression of ACE in the lung tissues when compared with the control group, suggesting that ACE and ARDS have a significant correlation. It could be speculated that injection of OA aggravates lung injury, and the unceasingly increased ACE in the lungs would increase the RAS activity in the lungs. ACE may cause pulmonary vasoconstriction and pulmonary arterial pressure upgrading, as well as increasing pneumoangiogram permeability via increased RAS activity. In addition, ACE may promote the infiltration of monocytes/macrophages and promote inflammatory factor secretion, leading to more severe lung injury and the formation of pulmonary hypertension and fibrosis [60]. However, the upregulated ACE expression was significantly reversed by alprostadil treatment. These results suggest that the lung RAS participates in this injury process.

Conclusions

Alprostadil has protective effects against lung injury in OA-induced ARDS. This protective effect might be related to its ability to prevent the release of pro-inflammatory mediators, suppressing the activation of MAPKs and NF- κ B signaling pathways, down-regulating ACE expression, and inhibiting lung cell apoptosis. Our data thus suggest that alprostadil is a promising drug for use in the treatment of ARDS.

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