

Oridonin alleviates lipopolysaccharide-induced acute lung injury in mice through inhibiting apoptosis, oxidative stress, and inflammation by modulating VIP/cAMP/PKA/AQPs signaling pathway

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Abstract

Oridonin (ORI) are a traditional Chinese herbal medicine extracted from *Isodon rubescens* (Hemsl.) H.Ha. There are few studies on the protective effect of ORI ameliorates acute lung injury (ALI) in mice. This result showed that compared with the ALI group, ORI significantly reduced the total protein content, Wet-to-dry (W/D) ratio, the number of neutrophils, lymphocytes, and monocytes. Moreover, ORI was able to reduce cell apoptosis, the levels of Macrophage inflammatory protein-2 (MIP-2), Intercellular adhesion molecule-1 (ICAM-1), Tumor necrosis factor- α (TNF- α), Malondialdehyde (MDA) and Myeloperoxidase (MPO) and increase the level of Superoxide (SOD). In addition, ORI could up-regulate the levels of VIP, cAMP, p-PKA/PKA and AQP1. In conclusion, our findings indicate that ORI can modulate the inflammatory injury of lung tissue caused by LPS through the VIP/cAMP/PKA/AQPs signaling pathway, suggesting that ORI can be a candidate for the therapeutic or preventive intervention in acute lung inflammation.

INTRODUCTION

ALI is mainly caused by inflammation, oxidative stress and pulmonary dysfunction, and is characterized by refractory hypoxemia and non-cardiac pulmonary edema[1]. Although there are recognized methods for the prevention or treatment of ALI, it is accompanied by many adverse reactions, with a mortality rate of 40%[2]. Over the past 20 years, the integrity of the pathogenesis of ALI has progressed. ALI is an acute inflammatory disorder that causes the destruction of the pulmonary endothelial and epithelial cell barriers[3]. LPS causes the loss of alveolar-capillary membrane integrity, activates peripheral neutrophils and releases pro-inflammatory agent [4, 5].

According to the report, natural drugs play an key role in the diseases [6]. *Isodon rubescens* (Hemsl.) H.Ha is widely distributed in China[7]. It has been used in traditional Chinese medicine as an anti-inflammatory agent for a long time[8, 9]. The plant name was checked on <https://wfoplantlist.org/plant-list>. ORI is a natural diterpenoid compound that can be isolated from *Isodon rubescens* (Hemsl.) H.Ha [10, 11]. ORI is a natural compound of tetracycline diterpenoids (structure shown in Fig. 1). ORI has significant efficacy in the treatment and prevention of respiratory inflammation[12]. Studies have found that oridonin can reduce the mortality of mice with ALI, improve the pathological characteristics of the lung, and reduce the concentrations of inflammatory cytokines in serum[13]. It has also been reported that oridonin can improve ALI by regulating the nuclear factor Nrf2-mediated oxidative stress[14]. And oridonin can alleviate ALI by increasing the level of Nrf2, and inhibiting the activation of proinflammatory pathway [14]. Although the protective effects of ORI on ALI have been reported in the literature, the regulatory mechanism of ORI affecting ALI through VIP/cAMP/PKA/AQPs pathway has not been studied. Therefore, this paper explored the protective effect of ORI on ALI by building a mouse model of LPS, using VIP/cAMP/PKA/AQPs pathway as the research mechanism. It will provide experimental evidence for the subsequent lung protection pharmacodynamics study of oridonin.

MATERIALS AND METHODS

Reagent

Oridonin (ORI, PHL89745) used in the experiment were all purchased from the Sigma-aldrich company (USA). The purity of the Ori was affirmed to be more than 99.81%. The ORI was dissolved in 1% dimethyl sulfoxide (DMSO).

Experimental animals

Fifty healthy male SPF Kunming mice, weighing 30–35 g, were obtained by the Laboratory Animal Center of Chinese Academy of Medical Sciences. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of “The Second Affiliated Hospital of Shandong First Medical University” (approval no. 2022-091).

Establishment of mouse acute lung injury model

The rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium. The subcutaneous tissue was separated from the middle incision of the neck, the trachea was fully exposed, and the trachea was punctured along the proximal end of the heart, and 5 mg/kg LPS was injected to construct the ALI model. 60 mice were randomly divided into six groups: healthy control group (Control group), Model group (ALI group), low dose ORI group (L-ORI group, 10 mg/kg), medium dose ORI group (M-ORI group, 20 mg/kg), low dose ORI group (H-ORI group, 40 mg/kg) and Dexamethasone group (Dex group, 5 mg/kg), 10 rats in each group. After successful modeling, the model + ORI group was given 10, 20, and 40 mg/kg ORI by gavage once a day for 14 consecutive days, and the Control group and the ALI group were replaced with normal saline of the same volume. After the administration, the rats were anesthetized. Blood was centrifuged, and the upper serum was separated. The mice were killed by neck breaking method, and the lung tissues were quickly separated.

Sample acquisition and processing

12 h after model establishment, the mice were sacrificed by intraperitoneal injection of pentobarbital sodium (75 mg/kg) anesthesia. After the mice were cut along the direction from the xiphoid process to the sternal stalk, the lungs were exposed and the right hilar lung was ligated. The upper lobe of the right lung was cut and placed into EP tube. The middle lobe of the right lung was fixed in 4% paraformaldehyde for the preparation of paraffin specimens and sections. After the trachea in the neck was separated, a "V" incision was made above the cricoid cartilage, and an indwelling needle was inserted onto the bulge. The suture was fixed, and 1 mL syringe was connected. Then 0.3 mL sterile normal saline was gently injected, and the lavage fluid was recovered after repeated washing for three times. The lavage fluid collected after lavage for three times was mixed evenly to obtain bronchoalveolar lavage fluid (BALF).

Lung function test

24 h after the last dose, the total lung resistance (RL), pulmonary outlet resistance (Re), and pulmonary dynamic compliance (C_{dyn}) were measured using the RES3050 animal ventilator and the AniRes2005 software analysis system.

Determination of wet W/D ratio

The middle lobe tissue of the right lung was washed with saline, and the surface water and blood were dried after weighing. The tissue was placed in an 80 °C drying box for drying for 72 h to a constant weight. The W/D ratio was counted by weighing. The supernatant of venous anticoagulation and BALF was taken, and the protein concentrations in plasma and BALF were measured by BCA protein detection.

The number of white blood cell count (WBC), the proportion and number of each inflammatory cell in bronchoalveolar lavage fluid

PBS buffer was added into the prepared alveolar lavage fluid sediment for re spinning and mixing, and then dropped onto the slide to make smear. After natural drying, add 1–3 drops of Reich dye solution to evenly cover the smear. After dyeing for 1 min, add 3–6 drops of Giemsa dye solution, and fully mix it with Swiss dye solution for 5–8 min. The number of WBC was inspected and counted, and the proportion and total number of various types of white blood cells were analyzed.

Flow cytometry

BALF was gathered and the cells was counted. For neutrophil examination, cells were stained with anti-Ly6G-FITC and tested by flow cytometry.

Determination of apoptosis

Take the right middle lobe lung tissue, add 1 mL of 4 °C precooled PBS solution, slowly grind the cell suspension, filter it with a 200-mesh filter screen, discard the supernatant, wash it twice with 4 °C precooled PBS solution, and adjust the cell count to 1×10⁶, 5 μL AnnexinV and 5 μL PI was incubated for 15 min, the apoptosis of lung tissue was tested by flow cytometry.

Nitric oxide measurement

The concentration of NO in BALF and serum was tested by Griess reaction with nitrate reductase methods (Beyotime biotechnology, Shanghai, China).

Morphological change of lung tissue

The lung tissues were dehydrated with ethanol, removed with xylene, and embedded in paraffin. Sliced and stained with Hematoxylin and eosin (H&E). Two histopathological experts assessed the morphological changes of the lung sections under microscope in a blinded manner. Parameters evaluated included erosion, edema, hemorrhage, fibrin-like necrosis, inflammatory infiltration, and ulceration.

Determination of the biochemical indexes of lung tissue

The lower lobe of the right lung was homogenized in a 250 mg ice bath and prepared into a 10% solution, which was then centrifuged. The supernatant was gathered for the determination of MIP-2, ICAM-1, TNF- α , MDA, SOD and MPO contents.

Western blot analysis

The lung tissue was weighed, the protein concentration was quantified by BCA Protein As-Say Kit. Then the primary antibody was used: rabbit anti-VIP (1:1000; Abcam), Camp (1:1000; Abcam), p-PKA (1:1000; Abcam), PKA (1:1000; Abcam), AQP1(1:1000; Abcam) and GAPDH (1:5,000; Abcam). Horseradish peroxidase-conjugated second antibody (1: 5,000; Abcam) was added and incubated for 1 h.

Statistical method

All the data were referred to as the mean $\pm$ SEM. One-way analysis of variance (ANOVA) was executed followed by Tukey multiple comparison using GraphPad Prism 8.4.2. A P value < 0.05 was considered significant.

RESULTS

Effect of ORI on pulmonary function

As shown in Table 1, compared with the Control group, the levels of RL and Re in the ALI group were significantly raised ($P < 0.01$), and the level of Cdyn was significantly reduced ($P < 0.01$). Compared with the ALI group, the levels of RL and Re in the M-ORI, H-ORI and Dex groups were significantly reduced ($P < 0.05$ or $P < 0.01$), and the levels of Cdyn in the M-ORI, H-ORI and Dex groups were significantly raised ($P < 0.05$ or $P < 0.01$).

Table 1
Effect of ORI on pulmonary function. The data were expressed as means $\pm$ SD, n = 10. # $P < 0.05$, ## $P < 0.01$ vs the Control group, * $P < 0.05$, ** $P < 0.01$ vs the ALI group, respectively. Same as followed.

Group	RL	Re	Cdyn
Control group	51.59 $\pm$ 2.64	60.03 $\pm$ 2.99	192.59 $\pm$ 6.31
ALI group	107.58 $\pm$ 8.93##	102.42 $\pm$ 4.53##	89.69 $\pm$ 4.95##
L-ORI group	92.52 $\pm$ 9.74	90.52 $\pm$ 4.53	108.58 $\pm$ 6.07
M-ORI group	76.43 $\pm$ 5.69*	73.85 $\pm$ 3.92*	137.69 $\pm$ 5.09**
H-ORI group	62.31 $\pm$ 6.39**	65.36 $\pm$ 5.64**	169.24 $\pm$ 4.92**
Dex group	59.52 $\pm$ 9.74**	64.52 $\pm$ 4.53**	178.58 $\pm$ 6.07**

The W/D lung weight ratio and protein concentration in BALF of mice

As shown in Fig. 2A, compared with the Control group, the W/D ratio in the lung tissue of mice in the ALI group was significantly raised ($P < 0.01$). Compared with the ALI group, the W/D ratios of the lung tissue in the M-ORI group, the H-ORI group, and the Dex group were significantly reduced ($P < 0.01$). As shown in Fig. 2B, compared with the Control group, the protein concentration in BALF of mice in the ALI group was significantly raised ($P < 0.01$). Compared with the ALI group, the protein concentrations in BALF of the M-ORI group, the H-ORI group and the Dex group were significantly reduced ($P < 0.01$).

Number of associated inflammatory cells in BALF

As shown in Fig. 3, the number of neutrophils, lymphocytes and monocytes in BALF of the ALI group was significantly higher than the Control group ($P < 0.01$). Compared with the ALI group, the number of neutrophils, lymphocytes and monocytes in the M-ORI group, H-ORI group and Dex group were significantly reduced ($P < 0.05$ or $P < 0.01$).

Proportion of relevant inflammatory cells in BALF

As shown in Fig. 4A, the total count of WBC of BALF in the ALI group was significantly higher than Control group ($P < 0.01$), while the counts of WBC in the M-ORI group, H-ORI group, and Dex group were significantly lower than ALI group ($P < 0.01$). As shown in Fig. 4B, the percentage of neutrophils in BALF of the ALI group was significantly higher than Control group ($P < 0.01$), while the percentages of neutrophils in the M-ORI group, H-ORI group and Dex group were significantly reduced ($P < 0.01$). As shown in Fig. 4C, the percentage of monocytes in BALF of the ALI group was significantly higher than that of the Control group ($P < 0.01$), and the percentages of monocytes in the M-ORI group, H-ORI group and Dex group were significantly reduced ($P < 0.01$). The results indicated that ORI could improve the proportion of various types of inflammatory cells in BALF, reduce the accumulation of inflammatory cells in the lung, protect lung and reduce injury.

ORI attenuates LPS-induced inflammatory response

As shown in Fig. 5A, the ratio of neutrophils accumulation was significantly reduced ($P < 0.01$). Furthermore, as shown in Fig. 5B and 5C, ORI could reduce the induction of NO in BALF and serum. Therefore, these results indicated that ORI could restrain the pulmonary inflammation.

Effect of ORI on pathological changes of lung tissue

As shown in Fig. 6A, the alveoli in the Control group were arranged neatly, and the structure was complete. There was no significant edema and inflammatory cell infiltration, and the structures of bronchi and bronchioles were intact. Compared with the Control group, a wider alveolar septum, much inflammatory cell infiltration and some fresh bleedings were examined in the lung tissue in the ALI group. Comparison with the ALI group, wider alveolar septum and infiltration of peribronchial inflammatory cells were observed in the lung tissue in the ORI low-dose group. In the M-ORI group, inflammatory cell

infiltration was examined, and the structures of pulmonary alveoli and bronchi at all levels were intact. A small amount of inflammatory cell infiltration was examined in the ORI high dose group, and the alveolar structure and bronchi of all levels were intact. The alveolar structure and bronchi of mice in the Dex group were intact, and there was no significant edema and inflammatory cell infiltration. The results of comparison among different groups showed that the lung histopathological observations in the H-ORI group and Dex group were more similar. Importantly, as shown in Fig. 6B, LPS-induced inflammatory response decreased with the increase in ORI concentration.

Effect of ORI on apoptosis rate of lung cells in ALI mice

As shown in Fig. 7A and 7B, compared with the Control group, the apoptosis rate in the ALI group were significantly raised ($P < 0.01$). Compared with the ALI group, the apoptosis rate in the ORI and Dex group were significantly reduced ($P < 0.05$ or $P < 0.01$).

Changes of MIP-2, ICAM-1 and TNF- α contents in lung tissue in ALI mice

As shown in Fig. 8, compared with the Control group, the levels of MIP-2, ICAM-1 and TNF- α in the ALI group were significantly raised ($P < 0.01$). Compared with the ALI group, the levels of MIP-2, ICAM-1 and TNF- α in the M-ORI group, H-ORI group and Dex group were significantly reduced ($P < 0.05$ or $P < 0.01$).

Change of MDA, SOD and MPO in lung tissue of mice

As shown in Fig. 9, compared with the Control group, the levels of MDA and MPO in the ALI group were significantly raised, and the level of SOD was significantly reduced ($P < 0.01$). However, compared with the ALI group, the levels of MDA and MPO in the M-ORI group, H-ORI group, and Dex group were significantly reduced ($P < 0.05$ or $P < 0.01$), and the SOD level was significantly raised ($P < 0.01$).

Effects of ORI on the VIP/cAMP/PKA/AQPs signaling pathway

As shown in Fig. 10, compared with the Control group, the protein expressions of VIP, cAMP, p-PKA/PKA and AQP1 in the ALI group were significantly reduced ($P < 0.01$). Compared with the ALI group, the levels of VIP, cAMP, p-PKA/PKA and AQP1 in the M-ORI group, H-ORI group and Dex group were raised ($P < 0.01$). In the H-ORI group, the levels of VIP, cAMP, p-PKA/PKA and AQP1 were close to those in the Dex group.

DISCUSSION

The pathogenesis of ALI is complex and has made rapid progress. Gram-negative bacteria infection is the main pathogenic element, which induces lung inflammatory cell infiltration and oxidative stress response, leading to pulmonary interstitial edema and alveolar cavity exudation[15]. After LPS injection, ALI model

will be established, which will lay the foundation for animal experiment[16, 17]. In this study, the mouse model of lung tissue injury induced by lipopolysaccharide showed destroyed alveolar structure, inflammatory infiltration of alveolar cavity. Therefore, the ALI mouse model could be successfully established by induction with lipopolysaccharide. The pathological score of lung tissue and the W/D ratio of lung tissue in the ORI treatment group and Dex group were lower than ALI group. The results confirmed that dexamethasone and ORI could reduce the pathological damage of lung tissue and alleviate alveolar interstitial edema. Therefore, ORI had a certain protective effect on lung tissue.

At the early stage of inflammatory reaction, the total quantity of inflammatory cells in peripheral blood decrease rapidly and then increase gradually[18]. Therefore, peripheral blood WBC becomes a key marker of acute infection. The quantity of WBC in the blood after LPS injection is decreased significantly[19]. This study showed that the total white cells in bronchoalveolar lavage fluid of ALI group were higher than those of other groups, and the proportion of various types of inflammatory cells was raised to different degrees. At the same time, it proved that ORI played an anti-inflammatory affect by regulating the ratio of white blood cells in peripheral blood to restrain the accumulation of inflammatory cells in lung tissue, thereby inhibiting the occurrence of ALI and protecting lung tissue.

ALI is an organ injury caused by systemic inflammatory response of the body[20]. The overexpression of TNF- α advance the secretion of MIP-2, enhances the accumulation of WBC and the activation and infiltration of neutrophils [21, 22]. The stimulation of inflammation up-regulates the level of ICAM-1 in pulmonary vascular endothelial cells, and increases the neutrophils infiltration to the inflammatory sites, resulting in aggravation of tissue damage. The immune dysfunction of the body further triggers the apoptosis of alveolar epithelial cells in the lung tissue[23]. This study showed that the levels of MIP-2, ICAM-1 and TNF- α in the lung tissue of the ALI group were higher than the Control group, indicating that the lung tissue produced a serious inflammatory response, which triggered an inflammatory chain reaction, local neutrophil infiltration, inflammatory exudation and the aggravation of inflammatory response. ALI often produces a great number of oxygen free radicals with the activation of macrophages and neutrophils and respiratory burst[24]. Excessive free radicals can make the unsaturated fatty acids in the biofilm peroxidation to form a lipid peroxidation product, MDA, which is increased[25]. The antioxidant SOD in the body is decreased, and the antioxidant capacity is decreased[26]. Among them, MPO is used as a marker enzyme for neutrophil activation, and the change in its activity reflects the degree of neutrophil activation[27]. SOD, as an important oxygen free radical scavenger in the body, has extremely strong antioxidant effect, capable of scavenging superoxide anion and free radicals to defend cells from damage[28]. This study showed that ORI had good antioxidant and anti-inflammatory effects, reduced the expression of cytokine-induced neutrophil chemokine MIP-2, inhibited the migration of neutrophils and the release of leukocytes, and reduced the expression of ICAM-1, thereby inhibiting the adhesion of neutrophils to tissues and improving the degree of pulmonary edema. It could advance the expression of SOD, ameliorate the ability of scavenging free radicals in mice, reduce the release of lipid metabolites such as MDA and MPO, reduce the generation of pro-inflammatory factor TNF- α , and control the cascade reaction of inflammation, thereby improving the lung tissue function.

In the research on the treatment of ALI, VIP not only inhibits inflammation of lung tissue, but also improves pulmonary edema, and it is considered one of the potential therapeutic targets[29]. Study have shown that VIP, as an immune neuropeptide, can bind to G protein-coupled transmembrane receptors, activate adenylate cyclase (ACs) and promote cAMP release. cAMP is a critical material for histiocytes to maintain physiological function[30]. PKA is one of that main target proteins of cAMP. cAMP can bind to the regulatory subunit of PKA to promote phosphorylation of PKA, and a complete VIP/cAMP/PKA activation pathway is formed[31]. AQPs, which are closely related to the water and liquid metabolism of tissue cells, are one of the main downstream target proteins in this pathway[32]. VIP/cAMP/PKA/AQPs play a critical role in the regulation of water and liquid metabolism of lung histiocytes[33]. Thus, VIP can dominate the level of AQPs through cAMP/PKA, thereby affecting the water and liquid metabolism of lung histiocytes and improving pulmonary edema. AQPs are a family of receptor proteins distributed on cell membranes that control the transmembrane transport of water molecules[34]. AQP1 is confirmed to be closely related to ALI pulmonary edema. AQP1 is mainly distributed in the capillaries, lymphatic vessel and mesothelial cells beside the respiratory tract, and is mainly involved in the reabsorption of water and liquid in the pulmonary interstitium[35]. AQP1 is highly dependent on the phosphorylated PKA regulatory pathway. p-PKA regulates AQP1 by binding to phosphorylation sites in AQP protein, affecting water-liquid transport and cell endocytosis and exocytosis[32]. VIP/cAMP is also the activator of p-PKA, which will increase the water and liquid transport capacity of AQP1, and enhance the reflux absorption of the lymphatic and venous system, thereby reducing the interstitial lung and alveolar edema[36]. Therefore, the activation of VIP/cAMP/PKA pathway could promote the activated expression of AQP1. This study suggested that the level of tissue AQP1 protein in the lung in the ALI group was reduced during the development process of ALI. After treatment with oridonin, the expression level of AQP1 in the lung tissue in each group was significantly higher than that in the ALI group, and each protein in the VIP/cAMP/PKA signaling pathway was in a high expression state. This result further indicated that the VIP/cAMP/PKA/AQPs signaling pathway might be restrain in the lung tissue with acute lung injury, and oridonin could exert its protective effect on the lung of mice with ALI by activating the VIP/cAMP/PKA/AQPs signaling pathway.

In summary, oridonin reduces LPS-induced ALI by targeting VIP/cAMP/PKA/AQPs signaling pathway, as shown in the flow chart illustrated in Fig. 11. This study will provide a theoretical basis for the clinical application of oridonin in the treatment of acute lung injury.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR CONTRIBUTIONS

Qinghua Liu and Wenli Shang: Conceptualization, Methodology, Investigation, Writing - original draft, Writing-review & editing. Junli Zhang and Ran Chen: Conceptualization, Methodology, Investigation. Li Wei: Investigation, Resources. HaiDong Wang: Supervision. Min Zhang and Maokui Yue: Conceptualization, Methodology, Investigation, Supervision, Project administration.

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Figures

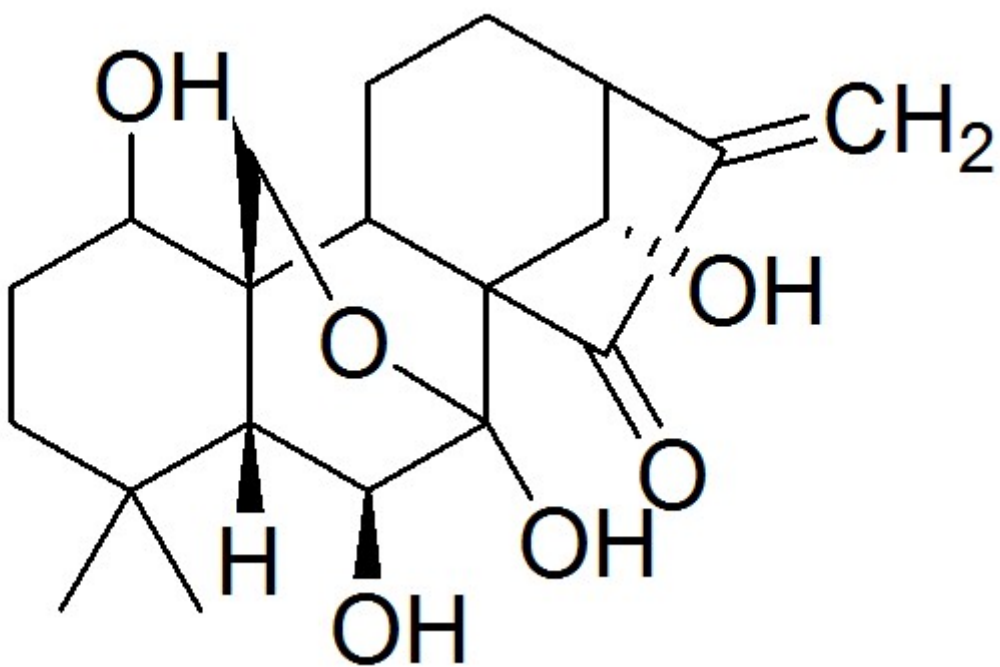
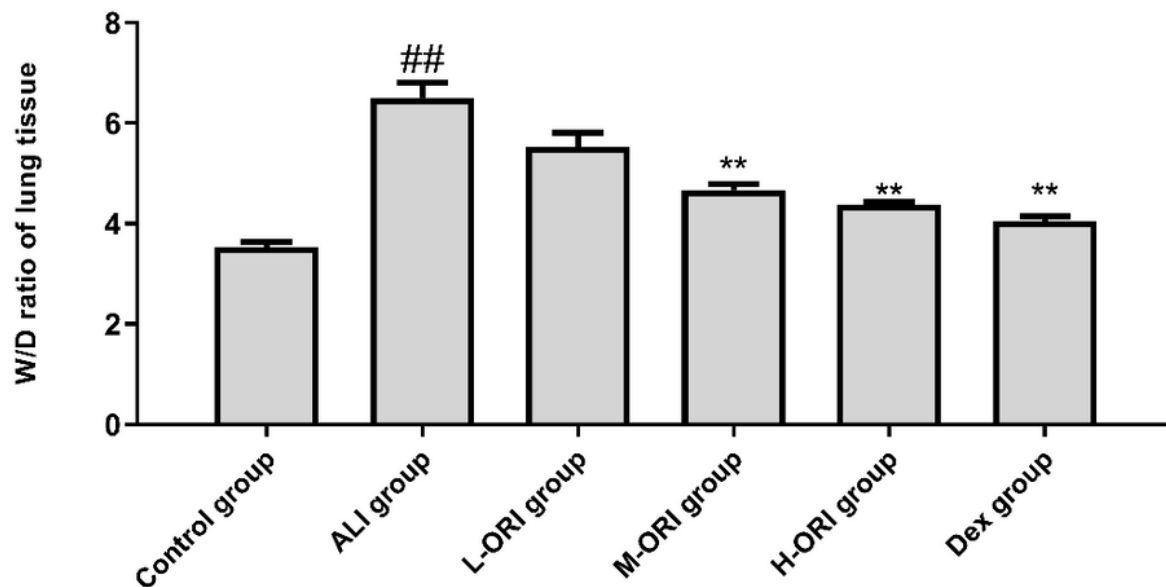
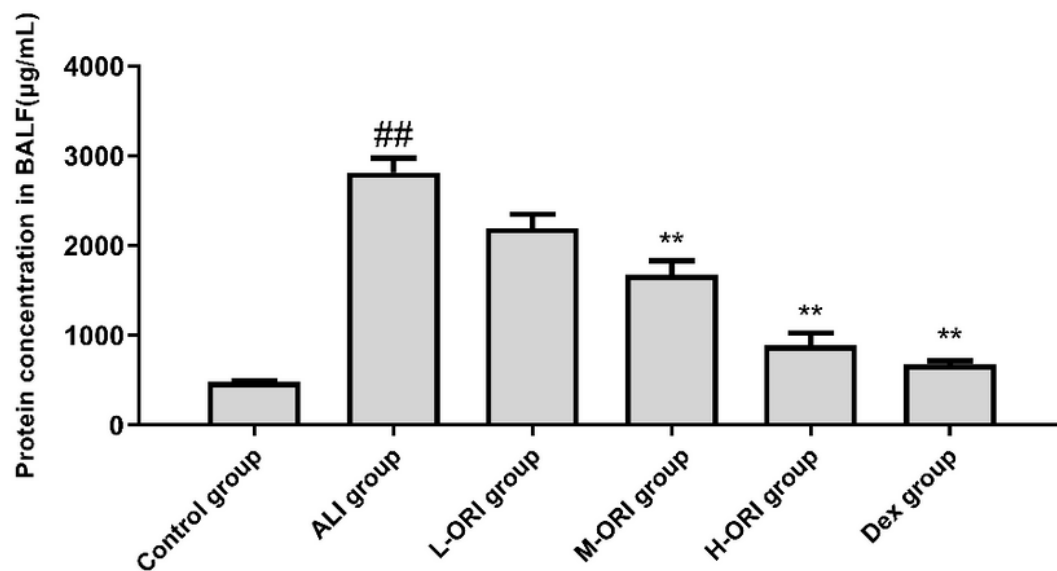
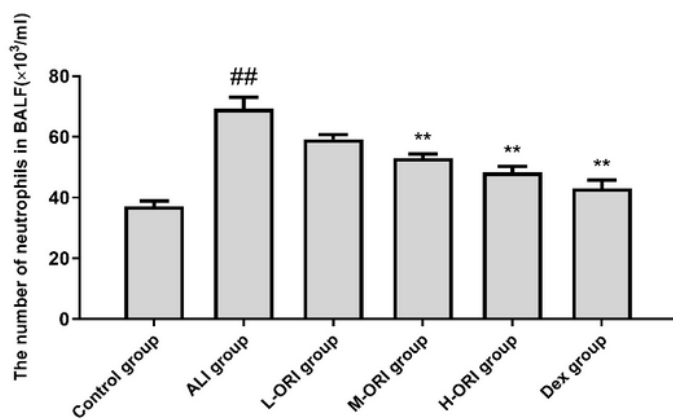
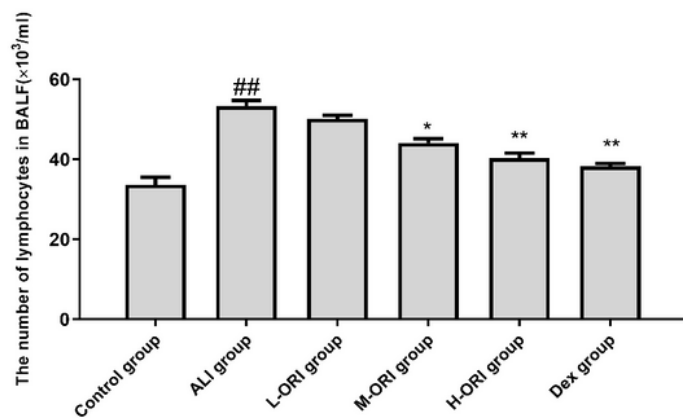
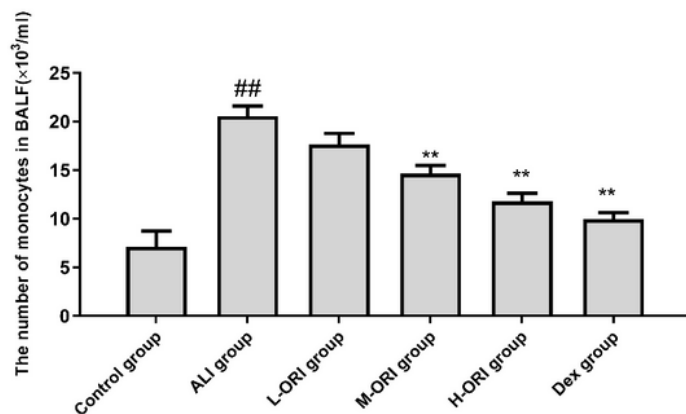


Figure 1

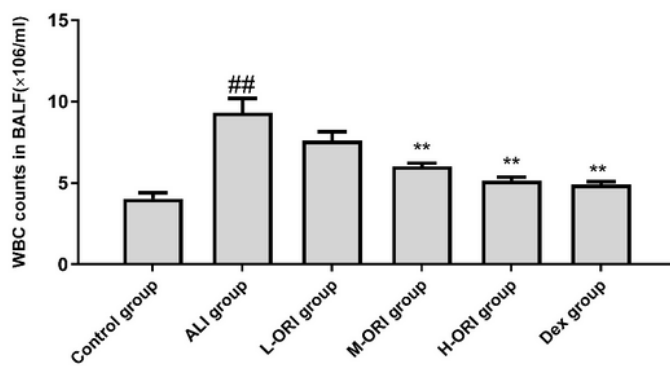
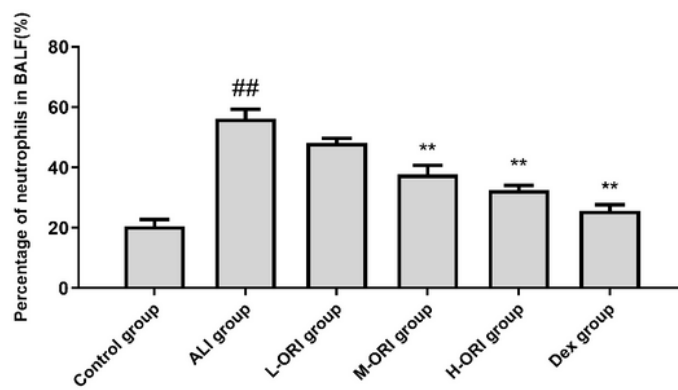
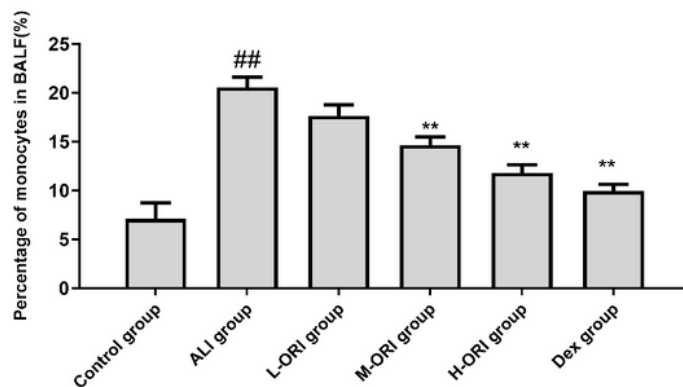
Chemical structures of oridonin.

A**B****Figure 2**

The effect of ORI on the W/D ratio of the lung tissues and the protein concentration in BALF. (A) The wet and dry weights of the lung tissues were tested to count the W/D ratio. (B) The protein concentration in BALF.

A**B****C****Figure 3**

The number of related infectious cells in alveolar lavage fluid of each group. (A). Number of neutrophils in BALF. (B). The number of lymphocytes in BALF. (C). The number of monocytes in BALF.

A**B****C****Figure 4**

The proportion of related infectious cells and the total number of white blood cells in the alveolar lavage fluid of each group. (A). The total number of white blood cells in BALF. (B). The percentage of neutrophils in BALF. (C). The percentage of macrophages in BALF.

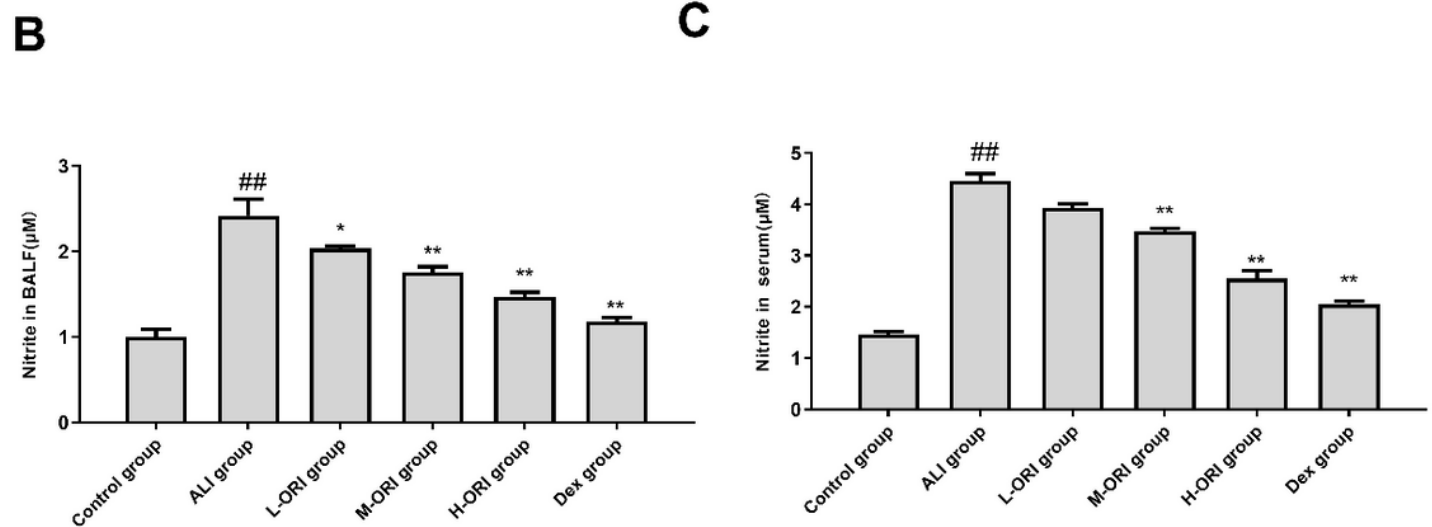
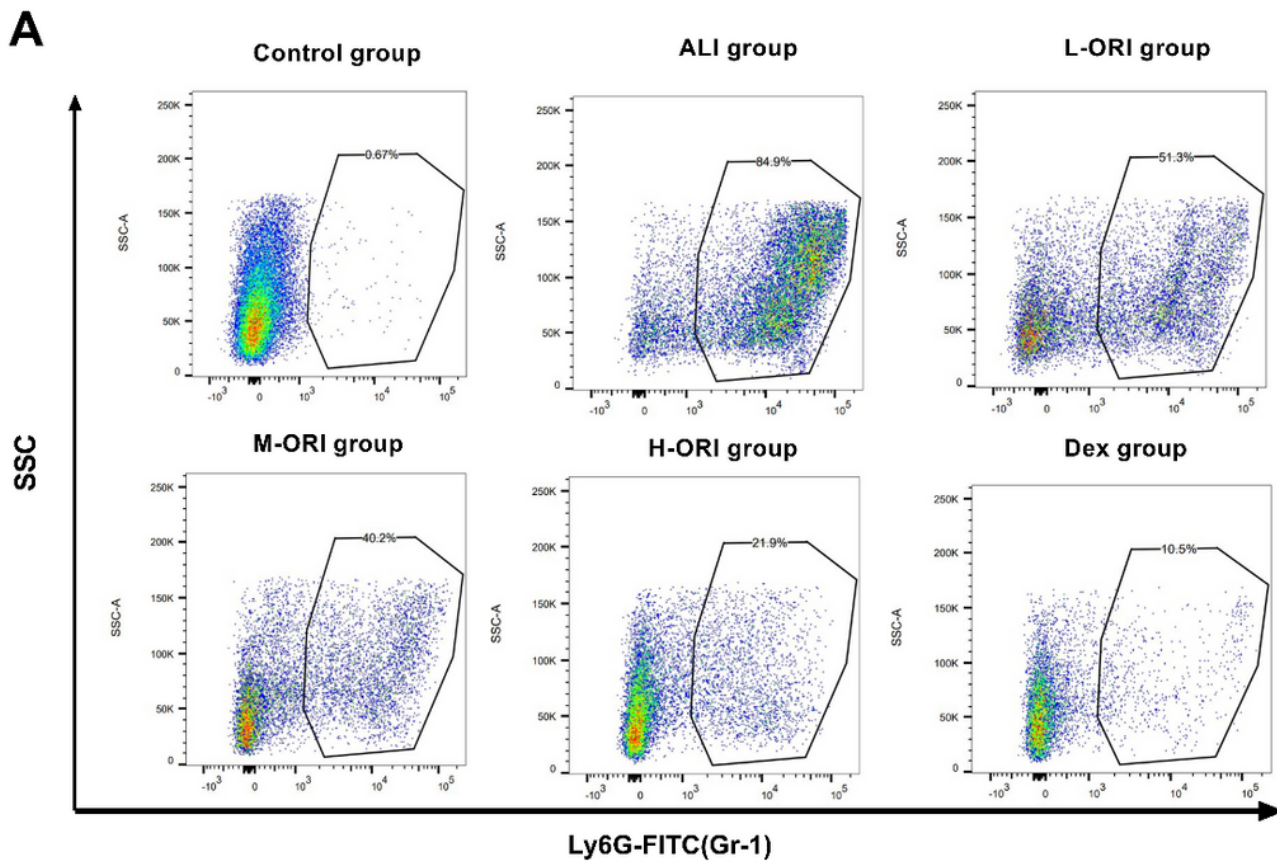


Figure 5

ORI could attenuate LPS-induced inflammatory response. (A) The percentage of neutrophils in the BALF was tested by flow cytometry. The concentration of nitrite in BALF (E) and serum (F) was tested.

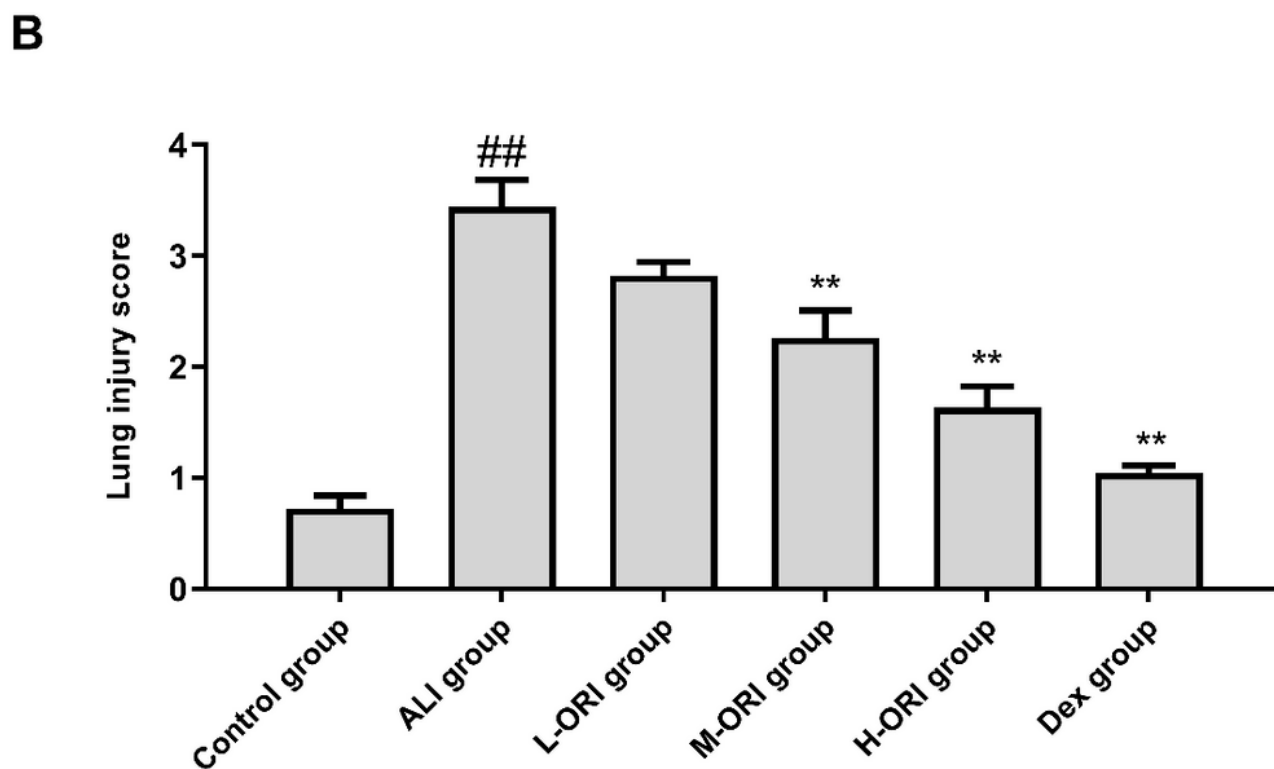
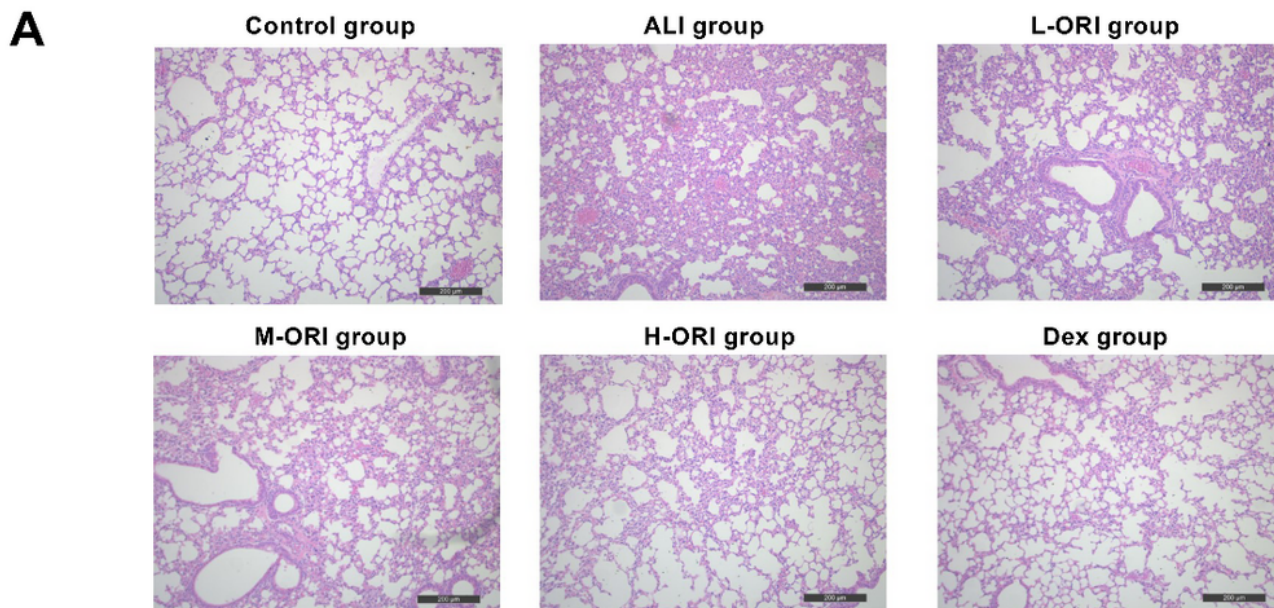
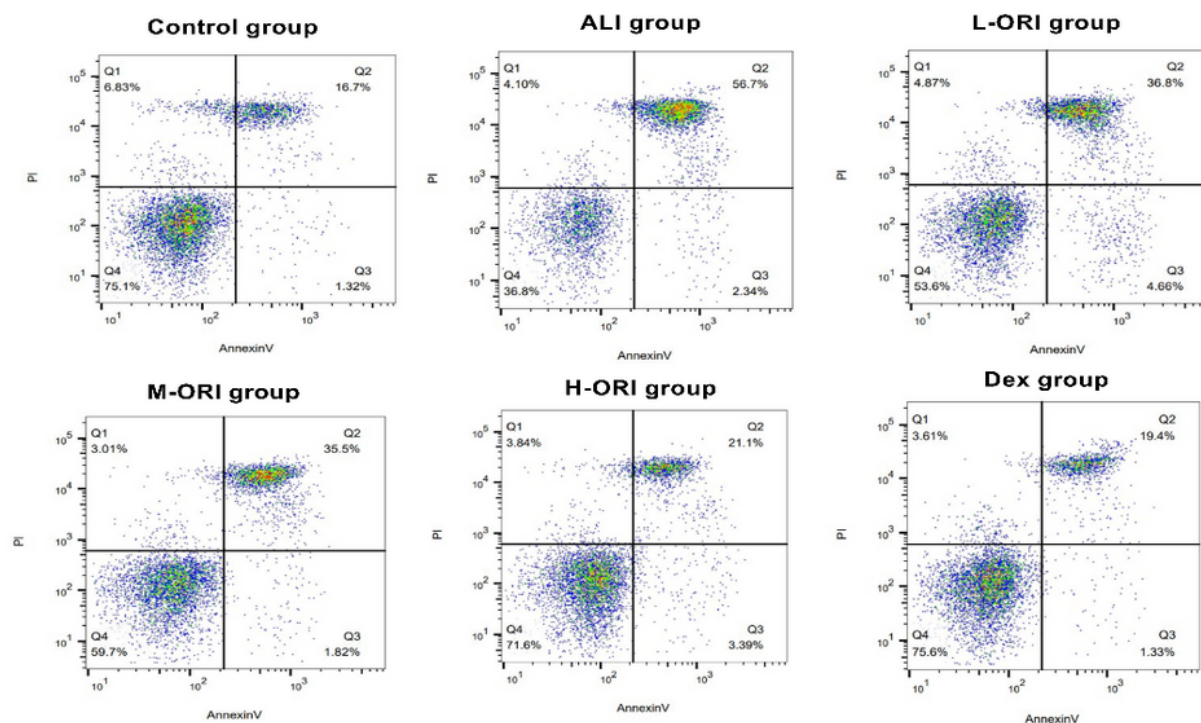


Figure 6

Lipopolysaccharide-induced histopathological changes in the lung of mice induced by ORI. (A) H&E staining was used to observe the histopathological effect of ORI on lung tissue of acute lung injury in mice. (B) Assessment score of the degree of lung tissue damage.

A



B

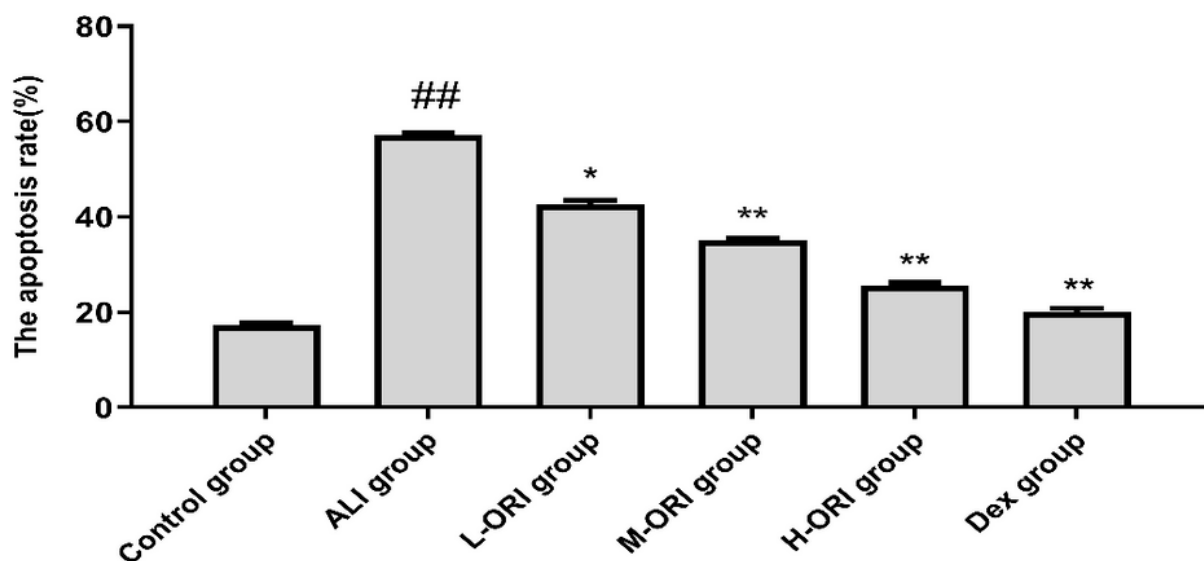
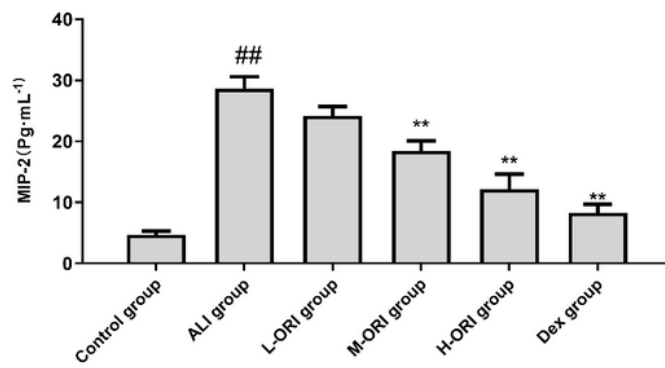
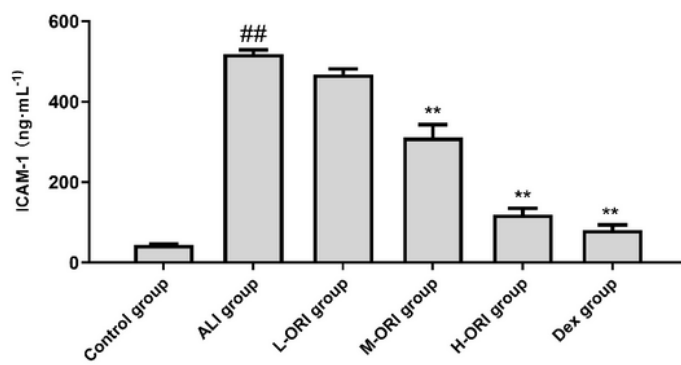
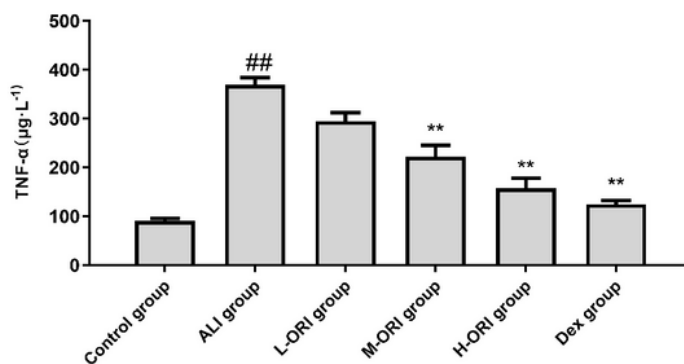
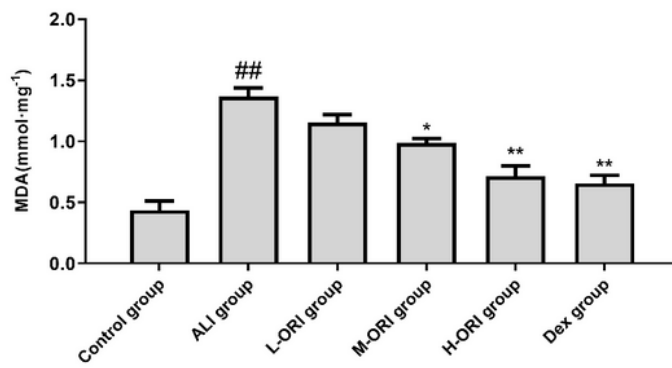
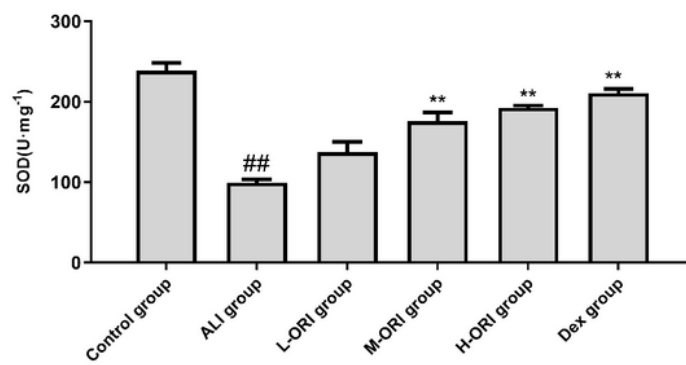
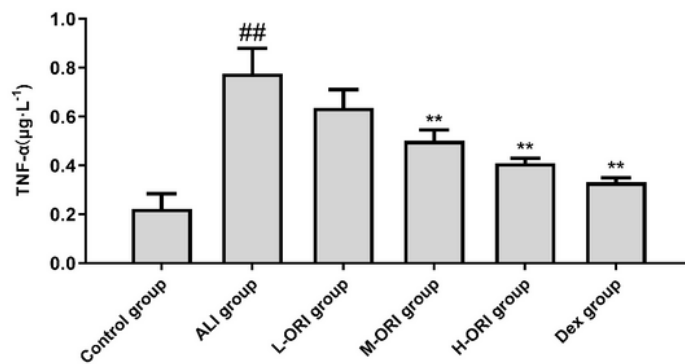


Figure 7

The effect of ORI on apoptosis rate of lung cells in ALI mice. (A) The apoptosis rate of lung cells in ALI mice tested by flow cytometry. (B) The apoptosis rate in ALI mice.

A**B****C****Figure 8**

Comparison of the contents of (A) MIP-2, (B) ICAM-1 and (C) TNF-α in the lung tissue of mice in each group.

A**B****C****Figure 9**

Comparison of (A) MDA, (B) SOD and (C) MPO levels in the lung tissue of mice.

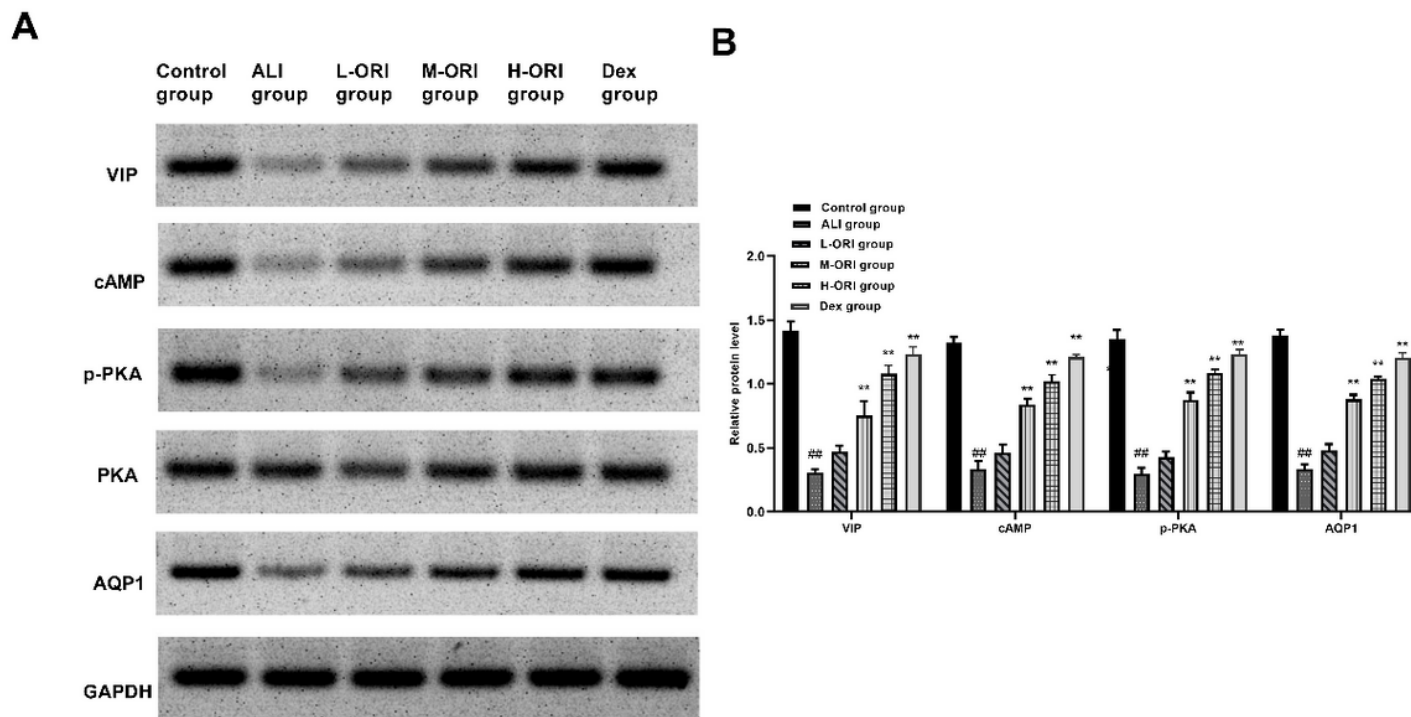


Figure 10

(A) Effect of ORI on the levels of VIP, cAMP, p-PKA, PKA and AQP1 in LPS-induced ALI mice. (B) Effect of ORI on the protein expression level of VIP, cAMP, p-PKA, PKA and AQP1 in LPS-induced ALI mice. GAPDH was used as an equal loading control.

