α7nAChR Agonist Activates the Nrf2/HO-1 Signaling Pathway to Reduce Acute Lung Injury Induced by Endotoxic Shock

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Abstract

Objective: Alpha 7 nicotinic acetylcholine receptors (α7nAChRs) can inhibit the activation of macrophages and the production of pro-inflammatory cytokines and exert inhibitory effects on systemic and local inflammatory responses. The objective of this study was to observe the protective effect of α7nAChR agonist against acute lung injury (ALI) caused by endotoxic shock, and to explore the regulatory mechanism of the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling pathway.

Methods: A total of 40 Sprague-Dawley rats were randomly divided into sham operation group (Sham group, n=10), endotoxic shock-induced ALI model group (ALI group, n=10), ALI + α7nAChR agonist (PHA568487, 3134, Tocris Bioscience, USA) group (PHA group, n=10) and ALI + α7nAChR agonist + Nrf2 inhibitor (ML385, HY-100523, MCE, USA) group (ML group, n=10). The rats received a tail vein injection of LPS to initiate ALI. Six hrs after injection, arterial blood was analyzed for blood gases and lung wet weight/dry weight (W/D) was determined. Lung histopathology was determined by H&E staining and apoptosis quantified by TUNEL. Levels of intercellular adhesion molecule-1 (ICAM-1), TNF-α, IL-1β, malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), choline acetyltransferase (ChAT) and acetylcholine esterase (AchE) in bronchoalveolar lavage fluid were measured via ELISA. Western blotting revealed levels of nuclear factor kappa-B (NF-κB), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), phosphatidylin-ositol-3-kinase (PI3K), protein kinase B (Akt), phosphorylated Akt (p-Akt), HO-1, Nrf2, thioredoxin reductase 1 (Trx-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Results: It was found that α7nAChR agonist increased the partial pressure of oxygen (PaO_2) and pH, reduced the partial pressure of carbon dioxide ($PaCO_2$) and W/D ratio, alleviated pulmonary edema and oxidative stress injury, and suppressed inflammatory responses. At the same time, it activated PI3K to phosphorylate Akt, inhibited cell apoptosis, and protected the lung tissues of ALI rats. Moreover, α7nAChR agonist facilitated nuclear translocation of Nrf-2 and up-regulated HO-1 and Trx-1 expression. Nrf-2 activity was required for the protective effect of α7nAChR .

Conclusion: α7nAChR agonist can improve endotoxic shock-induced ALI by activating the cholinergic anti-inflammatory pathway and the Nrf2/HO-1 signaling pathway.

Background

Endotoxin-induced acute lung injury (ALI) is a common complication occurring after septic shock or systemic inflammatory responses, with high morbidity and mortality rates[1]. It is pathologically manifested as increased permeability of pulmonary capillary endothelium and alveolar epithelium, which leads to non-cardiogenic pulmonary edema and ventilation/perfusion mismatch, thus causing acute respiratory distress syndrome (ARDS)[2, 3]. ALI/ARDS is more serious and complicated than the original disease. In the development process of ALI/ARDS, the lung tissues suffer from the activation and

aggregation of inflammatory cells and oxidative free radical injury, and the synthesis and excessive release of inflammatory mediators can lead to uncontrolled inflammatory responses, immune dysfunction, pulmonary microcirculation disturbance, etc[4-6]. Therefore, determining the mechanisms of inflammatory pathogenesis will be clinically significant for improving the prognosis of ALI/ARDS and reducing its mortality rate.

Alpha 7 nicotinic acetylcholine receptors (α7nAChRs) play a vital role in the regulation of the body's peripheral inflammation. Receptor activation inhibits macrophage activation through intracellular signal transduction, thus suppressing the production of the pro-inflammatory cytokines, TNF and ILs, and exerts inhibitory effects on local and systemic inflammatory responses in the body[7]. In mouse models of *Escherichia coli*-induced pneumonia, applying an α7nAChR agonist can reduce the level of the chemokine monocyte chemotactic protein-2 (MIP-2) in alveolar lavage fluid, the extravascular exudation of neutrophils, and the mortality rate of these rats[8]. Moreover, acetylcholine additionis able to remarkably reduce the release of various pro-inflammatory cytokines by LPS-stimulated human macrophages cultured *in vitro*., but this effect disappears after the knockout of α7nAChRs in rats. The above findings suggest that α7nAChR agonists may play an anti-inflammatory role in ALI, whose mechanism is correlated with anti-oxidative stress and inflammatory stimulation.

Studies have revealed that nuclear factor erythroid 2-related factor 2 (Nrf2) dissociates from the Nrf2/kelch-like ECH-associated protein 1 (Keapl) complex under oxidative stress, which facilitates the nuclear translocation of Nrf2 and activates antioxidant response element (ARE)-mediated protein kinase pathways such as protein kinase C (PKC), phosphatidyl-inositol 3-kinase (PI3K)/protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) pathways[9]. The target genes regulated by Nrf2 include stress genes and antioxidant genes, mainly including catalase (CAT), superoxide dismutase (SOD) and heme oxygenase-1 (HO-1). Among them, HO-1 is considered to promote adaptive cytoprotective responses against oxidative stress and inflammatory stimulation[10]. It can alleviate endotoxic shock-induced lung injury through resisting inflammation and oxidative stress and inhibiting cytokine release. As such, it was speculated that α7nAChR agonist could activate the Nrf2/HO-1 signaling pathway to relieve endotoxic shock-induced ALI.

Materials And Methods

Laboratory animals and grouping

A total of 40 male Sprague-Dawley (SD) rats weighing 220-260g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. [Laboratory Animal Production License No.: SCXK (Beijing) 20120007]. Animal experiments were conducted in a barrier system provided by the Department of Laboratory Animals, China Medical University [Laboratory Animal Using License: SYXK (Liaoning) 2013001]. This study was approved by the Laboratory Animal Welfare and Ethics Committee of China Medical University (2019104). Using the random number table method, the rats were divided into 4 groups, namely, sham operation group (Sham group, n=10), endotoxic shock-induced ALI model group

(ALI group, n=10), ALI + α7nAChR agonist (PHA568487, 3134, Tocris Bioscience, USA) group (PHA group, n=10), and ALI + α7nAChR agonist + Nrf2 inhibitor (ML385, HY-100523, MCE, USA) group (ML group, n=10).

Preparation of endotoxic shock model

The rat model of endotoxic shock was established according to Martin A's method[11]. Rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (3 mg/kg) and placed on a fixed rack. Next, the ALI model was established by tail vein injection of LPS (*Escherichia coli* O55: B5, Sigma, USA). Reduction of MAP by 50% represents successful modeling. Sham group received a 200 μL tail vein injection of the vehicle, 10% dimethyl sulfoxide (DMSO). At 30 min after modeling, the PHA group received a tail vein injection of 0.4 mg/kg PHA568487, while MLA group underwent tail vein injection of 0.4 mg/kg PHA568487 and 6 mg/kg ML385.

Sample collection and detection

Six hours after LPS injection, rats were anesthetized with 2% pentobarbital sodium and fixed on the operating table in the supine position. The abdominal cavity was cut open along the abdomen midline to separate tissues and expose abdominal aorta. Then the rats were killed by cardiac puncture and bloodletting, and blood was placed in GEM Premier 3000 blood gas analyzer to detect pH and partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$). Thereafter, the chest skin was separated to fully expose the thoracic cavity and trachea. Next, right and left lung tissues were separated from 3 rats in each group to measure the wet weight/dry weight (W/D) ratio. For the remaining rats, the bronchus was ligated, and 22-G needle was inserted and connected with a syringe containing 1 mL of saline. Subsequently, the cold saline was slowly injected into the lung, and bronchoalveolar lavage fluid (BALF) was collected after repeated suction and centrifuged at 3000 rpm for 10 min. Supernatant was stored at -80°C until analysis. Following lavage, the whole lung tissues were placed on the surface of ice, followed by isolation of right and left lung tissues, with sections of lung tissue fixed in 10% neutral formalin solution for hematoxylin and eosin (H&E) staining, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and immunofluorescence (IF) detection. The remaining lung lobes were stored frozen under liquid nitrogen.

Determination of W/D ratio of lung tissues

The blood and impurities covering the surface of the whole lung tissues of rats were removed, and then the tissues were weighed using an electronic balance to obtain the wet weight. Next, the tissues were dried in a 65°C oven for 24 h, and dry weight determined. Finally, the ratio of W to D was calculated to quantify the edema of the lung tissues.

H&E staining

Formalin-fixed lung tissue samples were placed sequentially into 70% alcohol for 12 h, 80% ethyl alcohol for 12 h, then 90% alcohol for 30 min, 95% alcohol for 30 min (repeated once), absolute alcohol for 30 min (repeated once), and finally xylene for 5 min. Subsequently, samples were soaked in paraffin. Paraffin-embedded samples were sliced, subjected to H&E staining, and sealed with neutral gum, followed by observation under an optical microscope (CX33, Olympus Corporation, Japan).

Lung tissue slices were observed microscopically according to Smith's scoring standard[12]. Zero to four points were given, respectively, according to the extent of localized necrosis of the lung, thickening of the alveolar septum, enlargement of the alveolar cavity, and formation of pulmonary hyaline membranes according to the scale: 0 point for no damage, 1 point for lesion range <25%, 2 points for lesion range = 25-50%, 3 points for lesion range = 50-75 %, and 4 points for lesion range >75%. The average score was recorded from two pathologists who independently observed and analyzed 10 high power fields in each sample slice.

TUNEL staining

A TUNEL staining kit (C1086, Beyotime, China) was used to detect apoptosis in lung tissue cells. Lung tissue slices were deparaffinized by two cycles of xylene, for 5 min each time. After gradient alcohol soaking, these slices were dropwise added with 20 μg/mL DNase-free proteinase K (20 mg/mL) for 30 min of incubation at 37°C, and washed by phosphate-buffered saline (PBS) twice. Each slice was incubated in 50 μL of TUNEL test solution for 1 hr at 37°C in the dark, followed by PBS washing 3 times. At last, the slices were observed under a fluorescence microscope after mounting using anti-fluorescence quenching mounting solution, and pictures were taken.

Enzyme-linked immunosorbent assay (ELISA)

ELISA kit (USCN, China) was utilized to detect the content of ICAM-1、TNF-α、IL-1β、IL-6、MDA、MPO、SOD、ChAT and AchE in BALF according to instructions. Specifically, 50 μL of appropriate standard was added to the first and second wells of the first row, respectively, on the ELISA plate, and diluted at multiple ratios in sequence. Then 40 μL of sample diluent was added to each sample well, and 10 μL of samples were added and incubated at 37°C for 30 min. Washing liquid was added to each well, incubated for30 s, then the liquid was discarded, which was repeated 5 times. Thereafter, 50 μL of enzyme-labeled reagents were added for 30 min of incubation at 37°C, and after washing, 50 μL of chromogenic reagent A and 50 μL of chromogenic reagent B were added, respectively, for 15 min of color development at 37°C in the dark. Finally, 50 μL of stop buffer was added to each well, the absorbance at 450 nm was detected using a microplate reader, and the sample content was calculated according to the standard curve.

Detection of reactive oxygen species (ROS) via IF

Frozen lung tissues were sliced on a freezing microtome. Slices were incubated in antigen retrieval buffer for 10 min. Next, the normal goat serum was added for 15 min incubation at room temperature, and then decanted. After that, Mito-Tracker Red CMXRos working solution (C1049, Beyotime, China) was added,

and then slices were washed by PBS thoroughly. DAPI solution was added after the slices were slightly dried for incubation at room temperature for 10 min away from light, followed by PBS washing 3 times. Finally, anti-fluorescence quenching mounting medium was applied for mounting, and photos were taken using the fluorescence microscope.

Western blotting

Lung tissues were taken out from the liquid nitrogen, added with radioimmunoprecipitation assay (RIPA) lysate containing protease inhibitor for 30 min of lysis and centrifuged. Then the supernatant was taken to measure protein concentration, and SDS-PAGE was performed. Following proteintransfer to PVDF membranes and blocking, nuclear factor kappa B (NF-κB), B-cell lymphoma-2 (Bcl-2), Bcl-2-associated X protein (Bax), PI3K, Akt, phosphorylated (p)-Akt, HO-1, Nrf2, thioredoxin reductase 1 (Trx-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies diluted at 1:1000 were added for incubation at 4°C overnight. Then membranes were washed by Tris-buffered saline with Tween 20 (TBST), and horseradish peroxidase-labeled secondary antibodies were added for incubation at room temperature for 2 h. Subsequently, enhanced chemiluminescence (ECL) kit and gel imaging system were employed for color development of proteins, and Image Tools was used for analysis.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

A total of 1 mL of TRIzol (15596026, Invitrogen, USA) was added to the lung tissues in liquid nitrogen, and ribonucleic acids (RNAs) were extracted by chloroform method and reverse transcribed (K1622, Invitrogen, USA). Then HO-1, Nrf2, PKC-α and GAPDH primers were added separately, and qRT-PCR kit (204057, Qiagen, USA) was used to prepare the reaction system. Reaction conditions: pre-denaturation at 95°C for 30 s, and PCR at 95°C for 5 s and 60°C for 20 s for 40 cycles. The results were expressed via the $2^{-\triangle\triangle Ct}$method. Primer sequences are listed in Table 1.

Table 1: Primer sequences

Gene	Primer
HO-1	Forward: AGTAGGCCACATTACACTGCT
	Reverse: GACCCACACCTCACAAATTGA
Nrf2	Forward: TCATGTGGTCAAGACGAGAT
	Reverse: GGAGGAAGGAAGGCATGA
Trx-1	Forward: AGAATGAGGACTGGGTGAGAAAC
	Reverse: CAACGGCTCTGGATAAAGTGTCTA
GAPDH	Forward: GCACACAGTACATCCGTCA
	Reverse: TTCTCCGAACGTGTCACGT

Statistical analysis

Data were expressed as mean ± standard deviation using GraphPad Prism 8.0 (GraphPad software, Inc., San Diego, CA, USA). Student's *t*-test was performed to compare if means were significantly ($p<0.05$) different.

Results

α7nAChR agonist reduced endotoxic shock-induced ALI

Due to the stimulation of toxins during septic shock, catecholamines in the body are increased, and blood flow perfusion in microcirculation, cardiac output, and blood pressure are decreased. As time goes on, there is constriction of capillary sphincters, tissue perfusion is reduced, tissue ischemia and hypoxia appear, and acidic products of anaerobic metabolism build up, resulting in a drop in blood pH, pulmonary capillary exudation, pulmonary edema formation, and hypoxemia. In this experiment, it was found that an α7nAChR agonist could prevent the drop in PaO_2 and pH, reduce $PaCO_2$ (vs. ALI group, $p<0.05$) (Figure 1-A) and W/D ratio, and relieve pulmonary edema in ALI rats (Figure 1-B). Using H&E staining, such histopathological changes as inflammatory cell infiltration, interstitial edema, and obvious thickening of the alveolar septum in the lung of ALI rats were observed. After the α7nAChR agonist was given, the degree of inflammatory cell infiltration in the lung tissues was lowered, interstitial edema was alleviated, alveolar septum thickening was decreased (Figure 1-C), and the score of lung injury was reduced (vs. ALI group, $p<0.05$) (Figure 1-D). The above results indicate that α7nAChR agonist can effectively treat endotoxic shock-induced ALI.

α7nAChR agonist inhibited inflammatory responses in ALI rats

The activation and aggregation of inflammatory cells in the lung tissues are the main causes of ALI/ARDS. When nAChR on immune cells are activated, local or systemic immune responses can be downregulated, thus inhibiting inflammation. In this study, after ALI-induced rats were treated with an α7nAChR agonist, ChAT and AChE were released (Figure 2-A), and the expression of NF-κB was inhibited (Figure 2-B). However, the inhibition of NF-κB could down-regulate ICAM-1, and block the release of pro-inflammatory cytokines, TNF-□ and IL-1β. Rats in PHA group exhibited reduced content of ICAM-1□IL-6, TNF-□ and IL-1β in BALF (Figure 2-C), suggesting that α7nAChR is able reduce the inflammatory lung responses of ALI rats.

α7nAChR agonist suppressed oxidative stress injury in ALI rats

Under the action of endotoxins, the body produces a large amount of reactive oxygen species (ROS), which cause cellular and organ damage. Based on this, ELISA was firstly carried out to detect the content of MDA, MPO and SOD in BALF. The α7nAChR agonist promoted SOD release and inhibited MDA and MPO (vs. ALI group, $p<0.05$) (Figure 3-A). IF revealed that the ROS level, compared to control group, was

increased in ALI group and decreased in PHA group (Figure 3-B). It can be concluded from the above results that α7nAChR agonist inhibits oxidative stress injury in ALI rats.

α7nAChR agonist activated the PI3K/Akt signaling pathway to alleviate lung cell apoptosis in ALI rats

When ALI occurred, inflammatory cytokines were released, which elevated the expression of pro-apoptosis factor Bax in the lung, reduced the expression of anti-apoptosis factor Bcl-2, and thus increased the rate of apoptosis in lung tissue cells (Figure 4-A). Studies have shown that the activation of the PI3K/Akt signaling pathway can lower the apoptosis rate of alveolar type II cells and inhibit the expression of Bax, thus protecting the lung. TUNEL staining results in this study demonstrated that α7nAChR agonist reduced the LPS-triggered apoptosis of lung cells during ALI (Figure 4-B). Western blotting confirmed that α7nAChR agonist could stimulate PI3K to phosphorylate Akt, and the activated Akt acted on the downstream target proteins of the signaling pathway (Figure 4-C), blocked cell apoptosis, and protected lung tissues.

α7nAChR agonist activated the Nrf2/HO-1 signaling pathway

When ALI caused lung oxidative stress, Nrf2 was activated with a decreased degradation rate, and the content of Nrf2 in the nucleus was increased significantly (Figure 5-A). In addition, α7nAChR agonist was found to facilitate the entry of Nrf-2 to the nucleus and initiate the expression of downstream antioxidant proteins, HO-1 and Trx-1 (Figure 5-B).

α7nAChR agonist relieved ALI through the Nrf2/HO-1 signaling pathway

To further explore involvement the Nrf2/HO-1 signaling pathway, the rats were co-administered Nrf2 inhibitor with α7nAChR agonist, and the protective effect of α7nAChR agonist against ALI was observed. The pulmonary alveolar wall was thickened, inflammatory cell infiltration was observed, and injury scores were increased in ML group (vs. PHA group, $p<0.05$) (Figure 6-A). Moreover, the content of IL-6, TNF-α and IL-1β in BALF was increased (Figure 6-B), the antioxidant factor SOD was decreased, and MDA and MPO were increased (Figure 6-C) (vs. PHA group, $p<0.05$). Since the PI3K/Akt pathway is involved in the activation of Nrf2-ARE and the regulation of related dependent gene expressions, apoptosis and Akt activation were examined. It was discovered that Akt phosphorylation was inhibited (Figure 6-D), and the apoptosis rate of lung tissue cells was raised in ML group (Figure 6-E). The above results suggest that α7nAChR agonist decreases the lung injury in ALI rats through the Nrf2/HO-1 signaling pathway.

Discussion

Imbalance in the regulatory function on immune inflammatory responses is a main cause of damage from sepsis. Under the action of endotoxins, the body produces a large amount of ROS, which leads to cellular damage and release of pro-inflammatory cytokines, thereby further causing the dysfunction of multiple organs, including the brain, lung and liver[13]. Therefore, in theory, the occurrence and mortality rate of ALI could be reduced by boosting intracellular anti-inflammatory and anti-oxidative stress

pathways. In this study, a rat model of endotoxic shock-induced ALI was established by tail vein injection of LPS, and the effects of treatment with an α7nAChR agonist observed on lung injury, inflammatory responses, oxidative stress injury, and apoptosis. The α7nAChR agonist reduced endotoxic shock-triggered ALI and reduced inflammatory responses and oxidative stress injury, and this effect was prevented by inhibition of the Nrf2/HO-1 signaling pathway.

Nerves can exerting local or systemic anti-inflammatory effects by releasing acetylcholine, which combines with α7nAChRs on the surface of various immune cells to block the synthesis and release of pro-inflammatory cytokines by inhibiting or up-regulating intracellular downstream signaling pathways[14], thus ultimately alleviating tissue damage from excessive inflammation. After interfering with the production of a7nAChRs or knocking out a7nAChRs in rats, neither electric stimulation nor a7nAchR can inhibit the release of inflammatory cytokines such as TNF-α[15]. In the present study, α7nAChR agonist elevated PaO_2 and pH in ALI rats, alleviated pulmonary edema, and effectively inhibited inflammatory cytokine release and oxidative stress injury, suggesting that α7nAChR stimulation can effectively reduce lung injury in ALI rats.

The cholinergic anti-inflammatory pathway (CAP) can protectively downregulate immune responses. It has been confirmed that α7nAChRs are core receptors mediating CAP. When α7nAChRs are activated, cholinergic anti-inflammatory signals can play anti-inflammatory roles in cells through NF-κB, Janus activated kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) and PI3K/Akt pathways[10, 14]. As a specific α7nChRA agonist, PHA568487 stimulates CAP activity through specific binding to α7nAChRs[16]. In this study PHA568487 was given to ALI-induced rats, which effectively stimulated the release of AChE and ChAT, and inhibited NF-κB. PHA568487 activated PI3K to promote Akt phosphorylation, thus inhibiting the expression of Bax and reducing the apoptosis rate of lung cells.

Under normal physiological conditions, Nrf2 binds to cytoplasmic linker protein Keap1, contributing to Nrf2 degradation[9]. Under oxidative stress, proteasomes degradation is blocked Nrf2, resulting in Nrf2 accumulation. In this study, Nrf2 levels in the lung of ALI rats were increased, consistent with the involvement of Nrf2 in oxidative stress injury. HO-1 is a Nrf2-regulated gene that prevents vascular inflammation, and plays a crucial role in anti-apoptosis, anti-tumor, anti-inflammation, and antioxidant effects[9, 17]. In the current research, it was found that both nuclear Nrf2 expression and HO-1 expression in the lung of ALI rats was up-regulated after PHA568487 intervention[18]. It has been illustrated in studies that the up-regulation of HO-1 protein expression and subsequent increase in CO concentration in the liver, kidney and lung tissues of rats with endotoxic shock can reduce the mortality rate of rats, thereby protecting the above organs. Correspondingly, Nrf2 inhibitor caused a rise in inflammatory cytokines, aggravation of oxidative stress injury, and increase in apoptosis by blocking the effect of α7nAChR agonist, implying that α7nAChR agonist may relieve the lung injury in ALI rats through the Nrf2/HO-1 signaling pathway.

To sum up, α7nAChR agonist activates the CAP and the Nrf2/HO-1 signaling pathway to inhibit inflammatory responses, relieve oxidative stress injury, lower the apoptosis rate and alleviate pulmonary

edema, and thus thus should be further investigated as a potential method for treatment of endotoxic shock-induced ALI.

Declarations

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Availability of data and materials

Data supporting the conclusions of this article are presented in the manuscript.

Authors' contributions

YM-Z and ZQ-H conceived and designed the experiments. YM-Z, MH-L, LF-W, and ZQ-H performed the experiments. YM-Z, MH-L, LF-W, and ZQ-H analyzed the data, and YM-Z and ZQ-H wrote the paper. All authors read and approved the final manuscript.

Ethics approval

All animal procedures performed in this study were reviewed and approved by the animal experiment was approved by the Ethics Committee of Experimental Animal Welfare of China Medical University (IACUC. No. 2019104).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

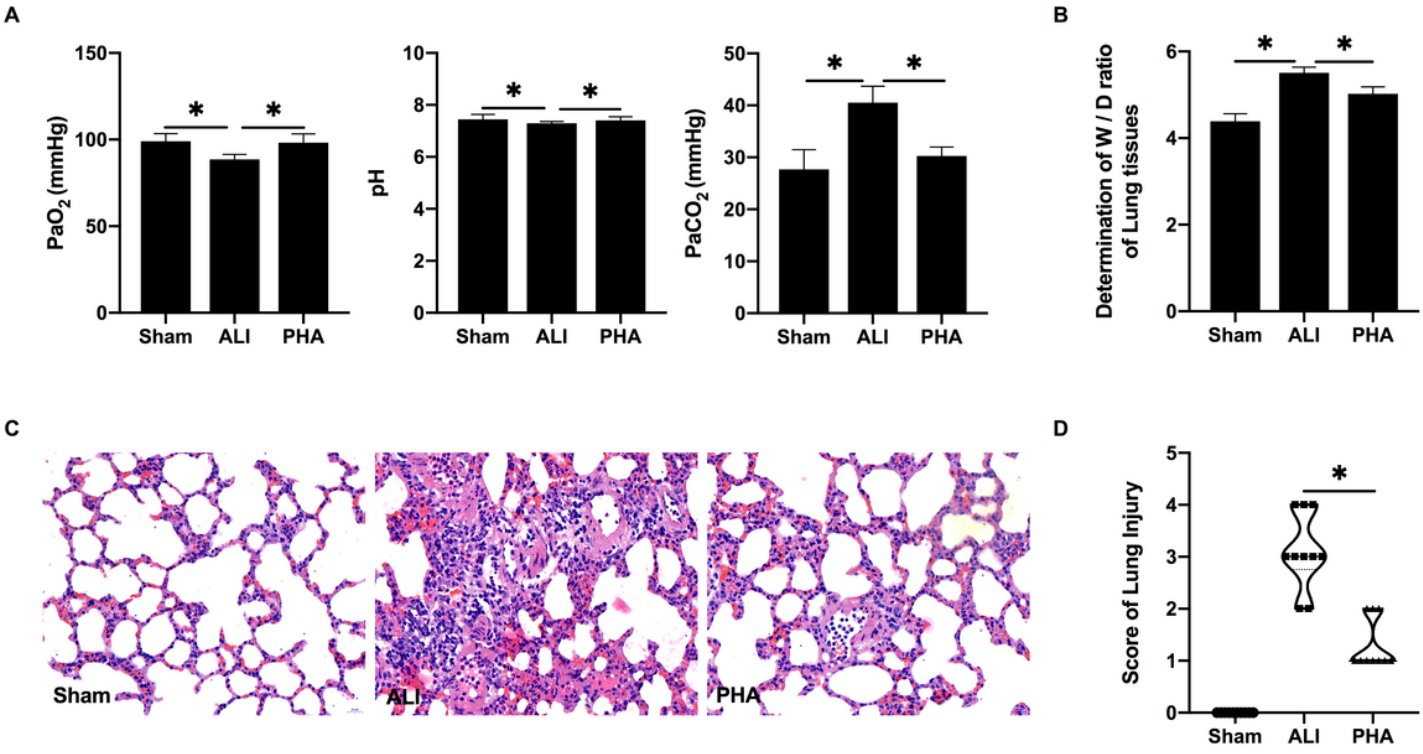

Figure 1

α7nAChR agonist reduced endotoxic shock-induced ALI A:Blood-gas results；B：Determination of W/D ratio of lung tissues；C：H&E staining；D：Lung tissue slices；* indicates P＜0.05.

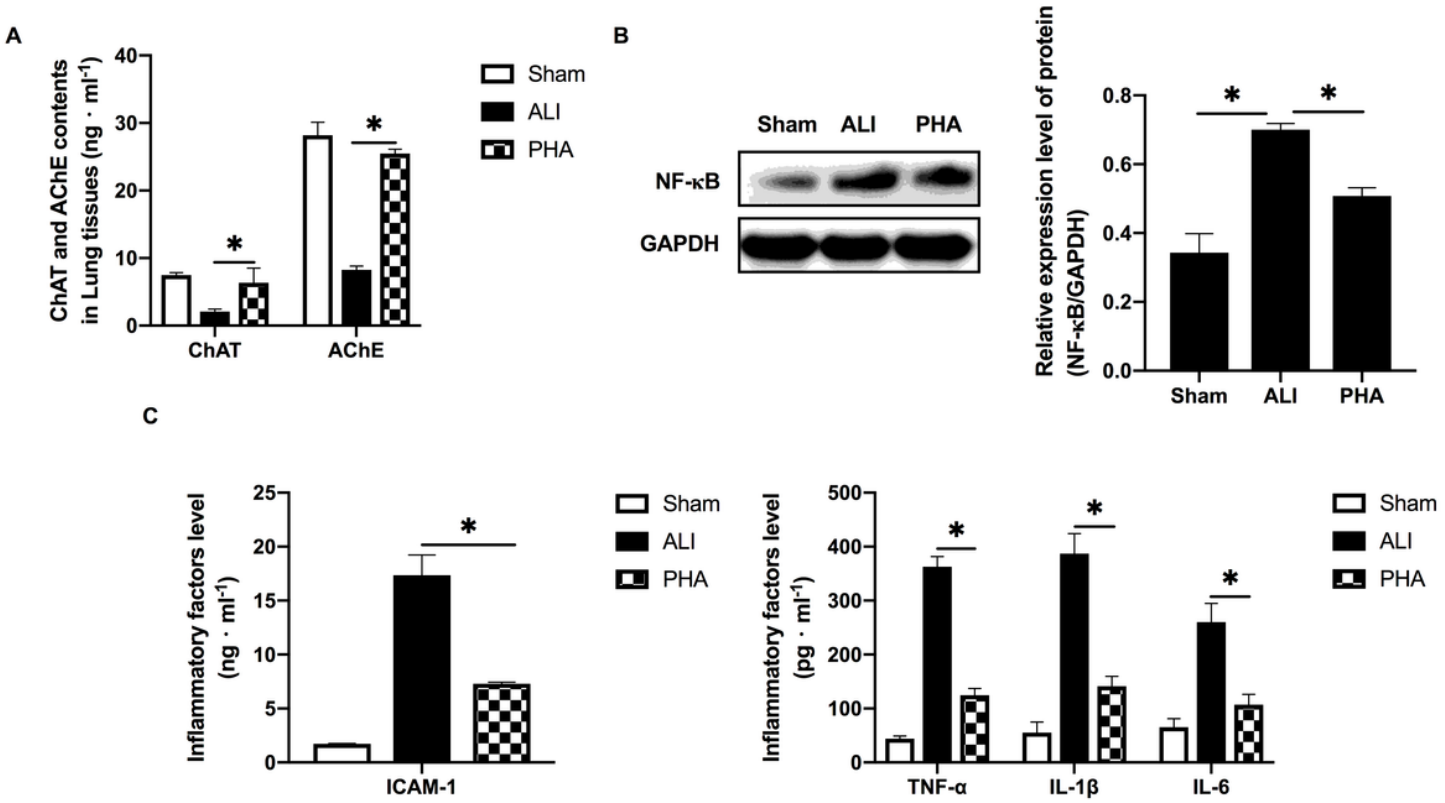

Figure 2

α7nAChR agonist inhibited inflammatory responses in ALI rats A：ChAT and AChE were detected by ELISA; B:The expression of NF-κB in the lung detect by Western blot;C:Inflammatory factors ICAM-1，TNF-α，IL-1β，IL-6 in BALF detect by ELISA; * indicates P＜0.05.

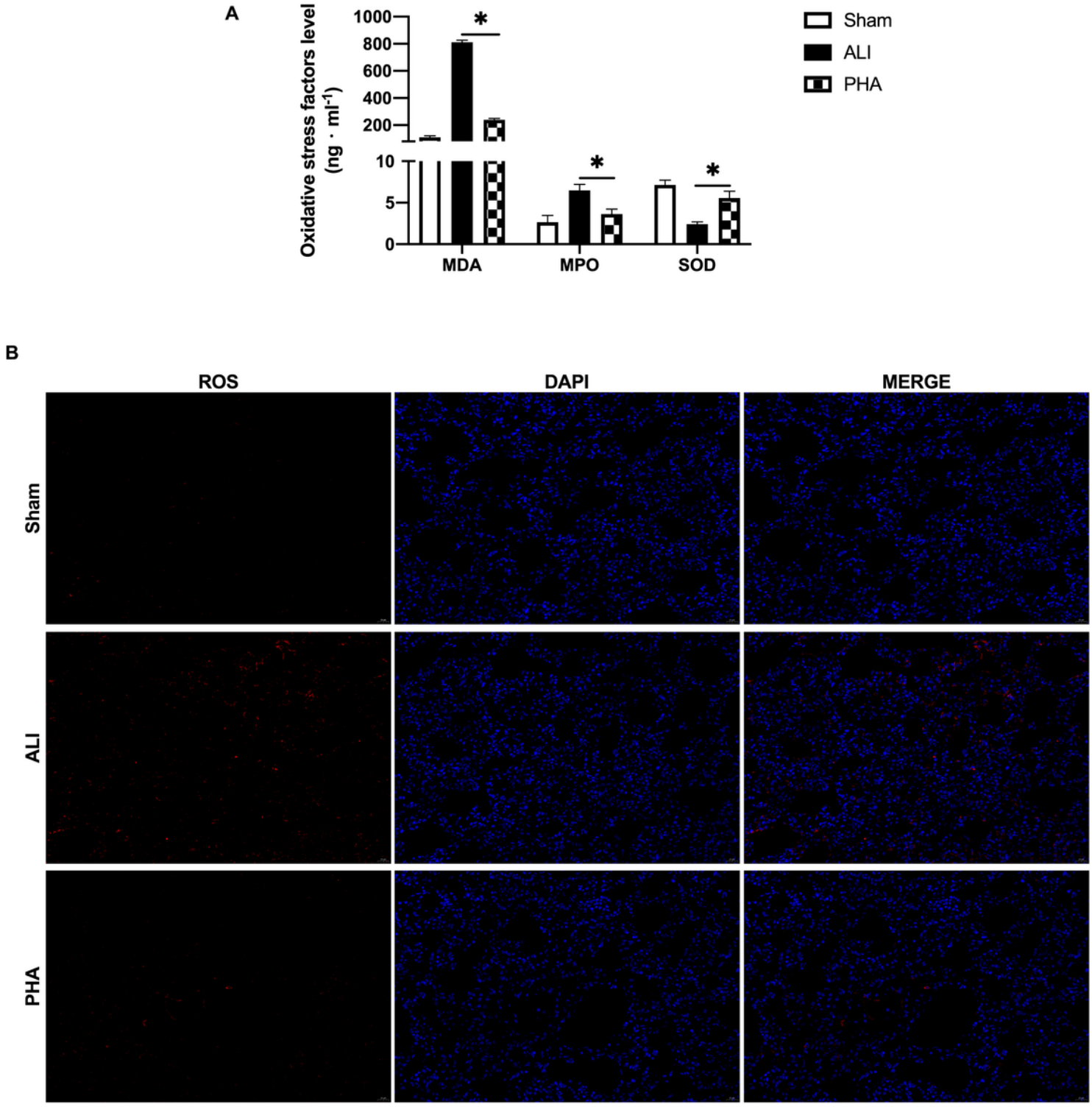

Figure 3

α7nAChR agonist suppressed oxidative stress injury in ALI rats A: Oxidative stress factors MDA, MPO and SOD in BALF detect by ELISA; ROS in the lung detect by IF.* indicates P□0.05.

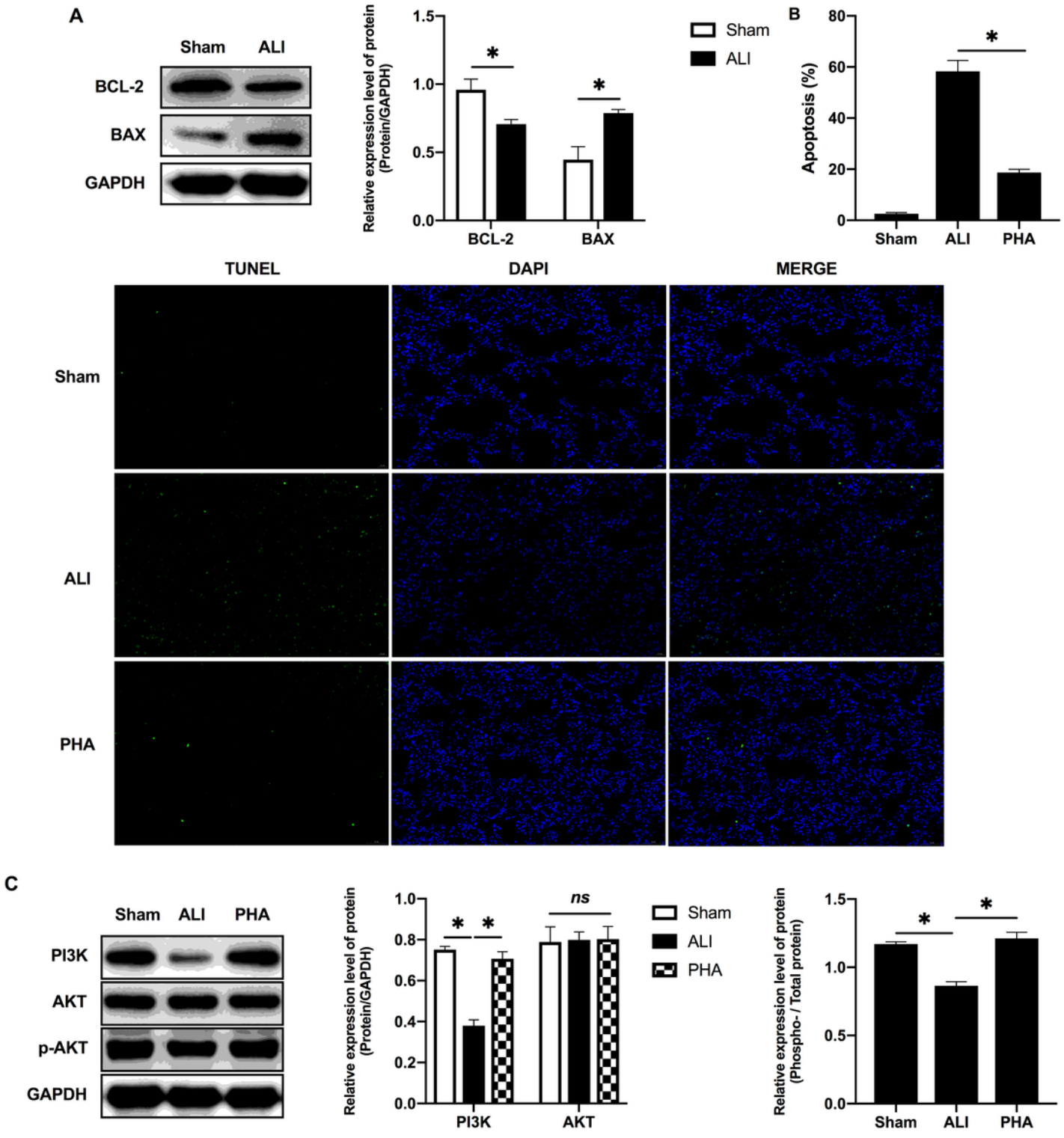

Figure 4

α7nAChR agonist activated the PI3K/Akt signaling pathway to alleviate lung cell apoptosis in ALI rats A:Bax/Bcl2 in the lung detect by Western blot；B：TUNEL stain were used to detect apoptosis of cell；C： PI3K/Akt signaling pathway were detected by Western blot;* indicates P＜0.05.

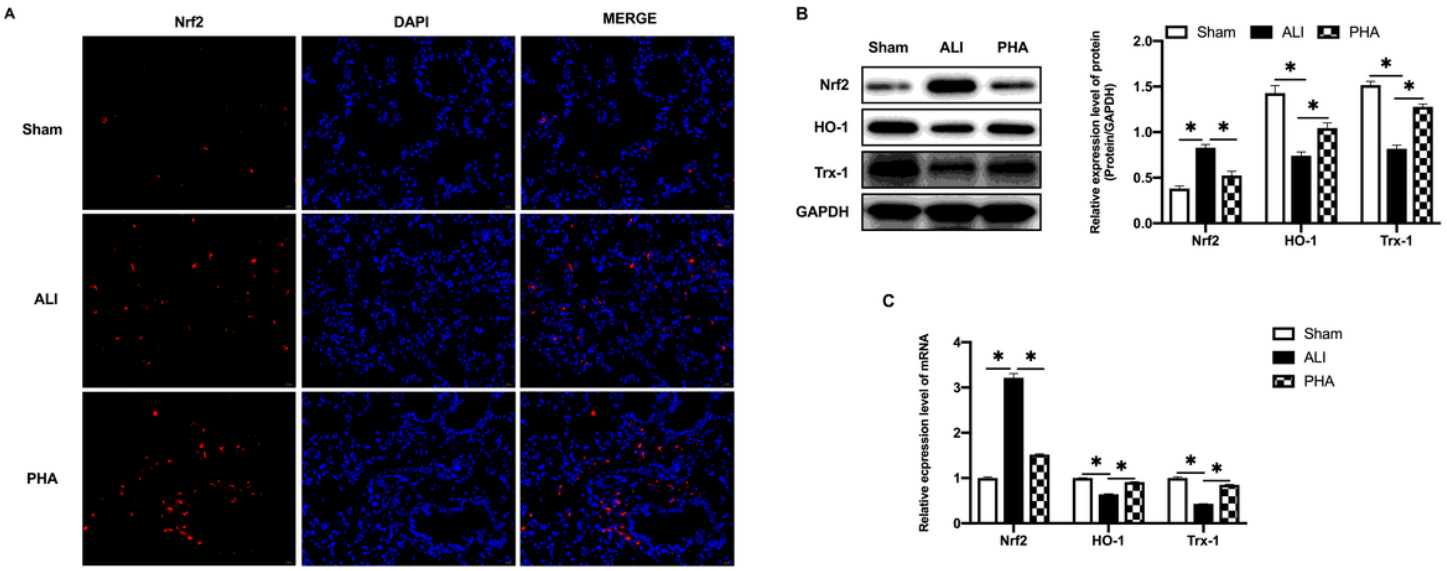

Figure 5

α7nAChR agonist activated the Nrf2/HO-1 signaling pathway A:The Nrf2 in the lung detect by IF; B: The expression of Nrf2、HO-1 and Trx-1 in the lung detect by Western blot;C、The mRNA expression of Nrf2、HO-1 and Trx-1 in the lung detect by qRT-PCR.* indicates P＜0.05.

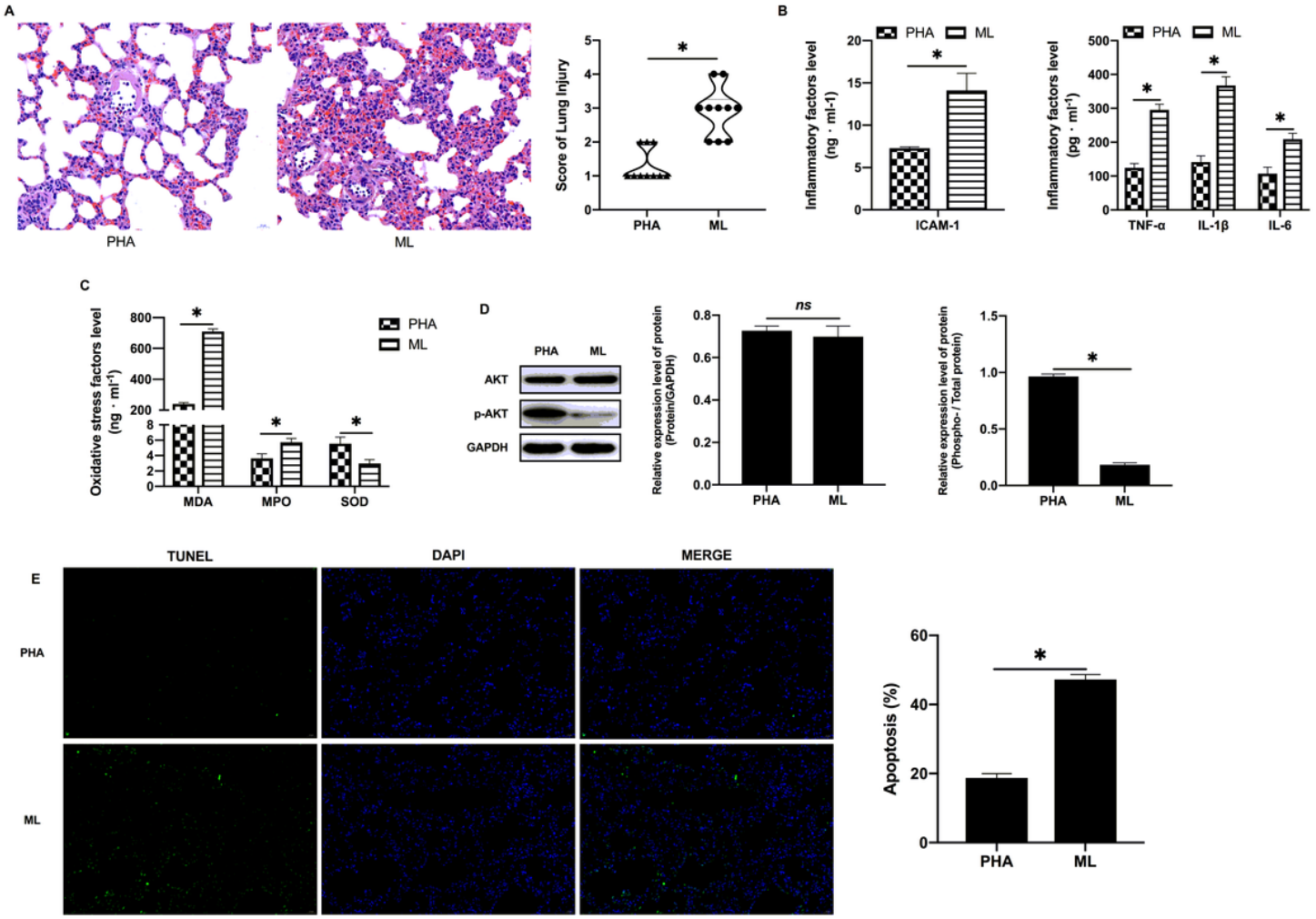

Figure 6

α7nAChR agonist relieved ALI through the Nrf2/HO-1 signaling pathway A□H&E staining and Lung tissue slices□B: Inflammatory factors ICAM-1□TNF-α□IL-1β□IL-6 in BALF detect by ELISA;C: Oxidative stress factors MDA, MPO and SOD in BALF detect by ELISA; D:PI3K/Akt signaling pathway were detected by Western blot; E: TUNEL stain were used to detect apoptosis of cell;* indicates P□0.05.