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Dexmedetomidine ameliorates LPS induced acute lung injury via GSK-3β/STAT3-NF-κB signaling pathway in rats

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ABSTRACT

Acute lung injury (ALI) is a serious complication of sepsis and an important cause of death in intensive care. Studies have shown that DEX can inhibit inflammation. However, the anti-inflammatory effect and protective mechanism of DEX in lipopolysaccharide (LPS) induced ALI are still unclear. ALI model was established by intraperitoneal injection of LPS (10 mg/kg) in Sprague-Dawley (SD) male rats. Firstly, at 4, 6, 8, 12 and 24 h after LPS treatment, lung injury including pathologic histology, lung edema, and inflammation were detected. The optimal time point for lung injury was determined to be 12 h, at which time DEX was added to further test. Furthermore, STAT3 inhibitor (NSC74859) and GSK-3β inhibitor (SB216763) were added to verify the role of STAT3, GSK-3β and NF-κB in ameliorated ALI. Our results show that DEX pretreatment significantly decreased lung Wet-to-Dry weight (W/D) ratio and MPO activity and ameliorated LPS induced lung histopathological alterations. In addition, we confirmed that DEX can increased the phosphorylation of STAT3 and GSK-3β, and inhibit the phosphorylation of nuclear factor-κB (NF-κB) p65 in the inflammatory response induced by LPS. What's more, NSC74859 inhibited the phosphorylation of STAT3 and reversed the protect effect of DEX on LPS. SB216763 inhibited the phosphorylation of NF-κB and reversed the damage effect of LPS and plays the same anti-inflammatory effect as DEX. In summary, our data demonstrated that DEX can ameliorate ALI induced by LPS through GSK-3β/STAT3-NF-κB pathway.

1. Introduction

The mortality of sepsis and septic shock ranged from 30% to 85% [1]. The ultimate cause of death is usually secondary to multiple organ dysfunction, and the lung is one of the most vulnerable target organs [2]. Diffuse inflammation of lung parenchyma and severe pulmonary dysfunction are important causes of death from sepsis. Nearly 50% of patients with severe sepsis develop acute lung injury (ALI) and more severe form of acute respiratory distress syndrome (ARDS) [3]. ALI is a severe disease syndrome and the pathogenesis is complicated with high morbidity and mortality [4,5]. It is usually caused by apoptosis, autophagy, oxidative stress and inflammatory reactions, and the deregulation of inflammatory responses during lung injury has been recognized as a key role in reducing acute lung injury [6].

It has been identified that lipopolysaccharide (LPS) as an effective inducer of ALI induced by bacterial septicemia [7,8]. When LPS is exposed to the lungs, many macrophages are activated and inflammatory cells leakage, resulting in the release of uncontrolled inflammatory cytokines and the activated or inhibited of multiple signal pathways [9]. LPS induces neutrophil infiltration and activation of transcription factors, such as nuclear factor kappa B (NF-κB), which is thought to lead to pulmonary dysfunction and the development of ALI [10].

NF-κB is a significant transcription factor, which plays an important role in the regulation of inflammation [11]. It is essential for transcription and production of various important pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6 [12]. Studies have shown that GSK-3β can regulate the stability and activity of NF-κB by affecting the interaction of NF-κB subunit p65 and CREB with coactivator CBP [13,14]. Furthermore, it is involved in signaling pathways that control innate immune response, including pro-inflammatory cytokine and interleukin production [15]. Although originally described as a regulator of glycogen synthase involved in the development of cell metabolism, proliferation and apoptosis, GSK-3β plays a significant role in modulating the development of sepsis and shock [16]. Recent studies have shown that GSK-3β inhibitor can effectively inhibit the pro-inflammatory response of LPS to mice and mediated the protective effect

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of endotoxin shock [17]. Previous studies have shown that the activation of STAT3 occurs before severe lung injury, which is related to production of the pro-inflammatory molecules TNF-α, IL-1β, CCL2, and MHC class II, indicating that STAT3 may play a critical role in initiating pulmonary inflammation in ALI [18]. With the development of STAT3 research, its role in immunity and inflammation has been paid more and more attention [19–21].

Dexmedetomidine (DEX) is a potent and selective α2-adrenoceptor agonist with an imidazole structure that has sedation, analgesia, as well as hemodynamic stabilization effects [22]. In recent years, it has been reported that DEX has good anti-inflammatory effects on lung, kidney, heart and other important organs in different models such as spinal cord injury, myocardial ischemia-reperfusion, and sepsis [22–24]. Recent studies have shown that DEX plays an anti-inflammatory role by blocking the TLR-4/NF-κB pathway in LPS-induced ALI [25]. Furthermore, DEX also lessened ketamine induced apoptosis of neural stem cells by increasing the phosphorylation level of GSK-3β [26]. In addition, DEX attenuated neurocognitive deficits induced by isoflurane in senile mice by increasing the level of P-STAT3 [27]. These effects indicate that DEX may have beneficial effects on the pathophysiology of inflammation, which involves decreased activation of NF-κB.

However, the specific anti-inflammatory mechanism of DEX ameliorates ALI induced by LPS is not clear. Therefore, we investigated the regulatory effect of DEX on the NF-κB pathway through STAT3 and GSK-3β and the potential protective mechanism of DEX on ALI. The results provide a theoretical basis for future clinical research and the development of new anti-inflammatory drugs.

2. Materials and methods

2.1. Materials

DEX was obtained from Wuhan Belka Biomedical Co., Ltd. (Wuhan, China) and diluted with saline. LPS (*Escherichia coli* 055: B5) was purchased from Sigma Co., Ltd. (Beijing, China) and diluted with saline. STAT3 specific inhibitor NSC74859 was purchased from MCE (Shanghai, China) and GSK-3β specific inhibitor SB216763 was purchased from Selleck (Shanghai, China) and diluted with 1% DMSO. The kit of myeloperoxidase (MPO) activity was purchased from Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits of IL-1β and TNF-α were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animal and housing

Sprague-Dawley (SD) male rats (200 ± 20 g) were purchased from the Laboratory Animal Center of Harbin Medical University (Harbin, Heilongjiang, China). The rats were housed with a constant temperature of 22 ± 1 °C and a relative humidity of 55 ± 5%, a 12 h day/night cycles and free access to water and food. This study was approved by the Animal Ethics Committee of China Northeast Agricultural University and the guidelines for the care and use of laboratory animal from the National Institute of Health.

2.3. Experimental procedure and drug administration

This experiment is divided into two parts.

2.3.1. Experiment I

Forty-eight rats were randomly divided into two groups: control group (CON, n = 8), LPS group (LPS, 10 mg/kg, n = 40). Eight animals were anesthetized with isoflurane in LPS group at each time point (4, 6, 8, 12 and 24 h) after LPS intraperitoneal injection. The control group was intraperitoneally injected with the same volume of normal saline. Then the rats were euthanasia, and the lung tissues were removed quickly and stored in the −80 or fixed with 10% buffered formalin.

2.3.2. Experiment II

Fifty-six rats were randomly divided into seven groups (n = 8 in each group).

(i) CON group: saline was given intraperitoneally.
(ii) LPS group: LPS (10 mg/kg) was given intraperitoneally [28].
(iii) DEX + LPS group: DEX (30 μg/kg) was given intraperitoneally 30 min before the LPS treatments [29].
(iv) NSC74859 group: STAT3 specific inhibitor NSC74859 (5 mg/kg) was given intraperitoneally 30 min before the DEX and LPS treatments. The dosage of NSC74859 administered was based on previously reports [30].
(v) SB216763 group: GSK-3β specific inhibitor SB216763 (5 mg/kg) was given intraperitoneally 30 min before the LPS treatments [31].
(vi) DEX group: DEX (30 μg/kg) was given intraperitoneally.
(vii) DMSO group: inhibitor solvent was given intraperitoneally.

After (12 h) LPS administration, animals were anesthetized using isoflurane and euthanized, then lung tissue samples were collected.

2.4. Histopathological lung analysis

To evaluate the histological alterations, right lung upper lobe was fixed with 10% buffered formalin for 24 h, embedded in paraffin, and sectioned at 4–5 μm thickness. After deparaffinization and dehydration, the stained with hematoxylin and eosin (H&E). All sections were observed under a light microscope (TE2000, Nikon, Japan) and assessed by the same observer who was blinded to group assignment. The lung injury score was based on the average score of the following items: edema, hemorrhage, infiltration of neutrophils into airspace or the vessel wall, and thickness of the alveolar wall, which were scaled from 0, no injury; 1, slight injury (25%); 2, moderate injury (50%); 3, severe injury; and 4, very injury (almost 100%). The results were graded from 0 to 4 for each item, as described previously [32]. The lung injury scores were evaluated by two pathologists who were blinded to the experimental conditions.

2.5. Lung Wet-to-Dry (W/D) ratio

To quantify the degree of pulmonary edema, we measured the W/D weight ratio of right lung middle lobe tissues. The right lung middle lobe was removed, rinsed briefly in saline and then weighed to obtain the wet weight. The lung was then dried at 80 °C in an oven to get the dry weight. The W/D ratios were then calculated by dividing the wet weight by the dry weight [33].

2.6. MPO activity assay

The lungs tissues were frozen and homogenized in homogenate medium. Then the homogenate was done in accordance with the manufacturer's instruction. Finally, the MPO activity was quantized as absorbance at 460 nm.

2.7. Cytokine level measurements

The concentrations of proinflammatory cytokines IL-1β and TNF-α in serum were quantified by using commercially obtained enzyme-linked immunosorbent assay (ELISA) kits. Each assay was carried out according to the instructions provided by the manufacturer.

2.8. Immunohistochemistry analysis

Sections were dewaxed and then antigen retrieval, primary antibody incubation, and labeling with horseradish enzyme was performed according to the manufacturer's instructions, next the color reaction was

Table 1
The gene-specific primers used for Quantitative Real-time PCR.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
IL-1β	AAGGTCCTGAGGGCAAAGAG	AACTTGAGGGAACCACTCGG
TNF-α	GGCCACCACGCTCTTCTGTC	GGGCTACGGGCTTGTCACTC
GAPDH	AGTGCCAGCCTCGTCTCATA	GATGGTGATGGGTTTCCCGT

performed with a DNA coloring kit (Solarbio, Beijing, China). And then sections were counterstained with hematoxylin and transparent with xylene. Finally, samples were observed with a DP73 type microscope (Olympus, Japan).

2.9. Quantitative Real-time PCR

Total RNA was extracted from the lung tissues using Trizol reagent (Invitrogen, Carlsbad, USA) as described in the instructions. cDNA synthesis was performed in 1 μg of total RNA using Prime Script™ RT reagent Kit with gDNA Eraser (Takara, Dalian, China) according to the instructions. Quantitative Real-time PCR was performed in Light Cycler® 480 II Detection System (Roche) using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China) as described in the instructions. The reaction system (10 μL) was composed of 5 μL 2 × SYBR Green PCR Master Mix (Roche, Basel, Switzerland), 0.3 μL forward primer, 0.3 μL reverse primer, 1 μL cDNA and 3.4 μL ddH_2O. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression mRNA against GAPDH. Each sample was tested in triplicate. Gene-specific primers are listed in Table 1 and synthesized by Sangon Co, Ltd. (Shanghai, China).

2.10. Western blot

Western blot analysis was performed as previously described [29]. Frozen lung tissues were homogenized in ice-cold RIPA Lysis Buffer supplemented with phenyl methane sulfonyl fluoride (PMSF) (Beyotime Biotechnology, Shanghai, China). The protein concentration was determined by the BCA Protein Assay kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of protein sample were loaded on SDS-PAGE Gel Quick Preparation Kit (Beyotime Biotechnology, Shanghai, China), and then transferred to PVDF membranes, which were blocked in 5% skim milk for 2 h at room temperature, and then incubated overnight with primary antibodies dilution Buffer (Leagene Biotechnology, Beijing, China) at 4 °C. Primary Antibodies and dilutions were as follows: 1:1000 for STAT3, P-STAT3, GSK-3β, P-GSK-3β (Cell Signaling Technology, USA); 1:1000 for NF-κB, 1:200 for P-NF-κB (Bioss, Beijing, China); 1:1000 for IL-1β, TNF-α, 1:2000 for GAPDH (Wanlei Biotechnology, Shenyang, China). After extensive washing with phosphate-buffered saline with Tween 20 (TBST) for 5 times of 5 min, the membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated antibody or goat anti-mouse secondary antibody (ZSGB-BIO, Beijing, China) for 2 h at room temperature, then washed with TBST. The signals were visualized using the ECL kits (Tanon Science & Technology Co, Shanghai, China). The blots were scanned using a Tanon 5200 Imaging System (Tanon Science & Technology Co., Shanghai, China). GAPDH was used as a loading control.

2.11. Statistical analysis

The statistical analyses of all the data were performed using GraphPad Prism (version 7.0, GraphPad Software Inc., San Diego, CA,

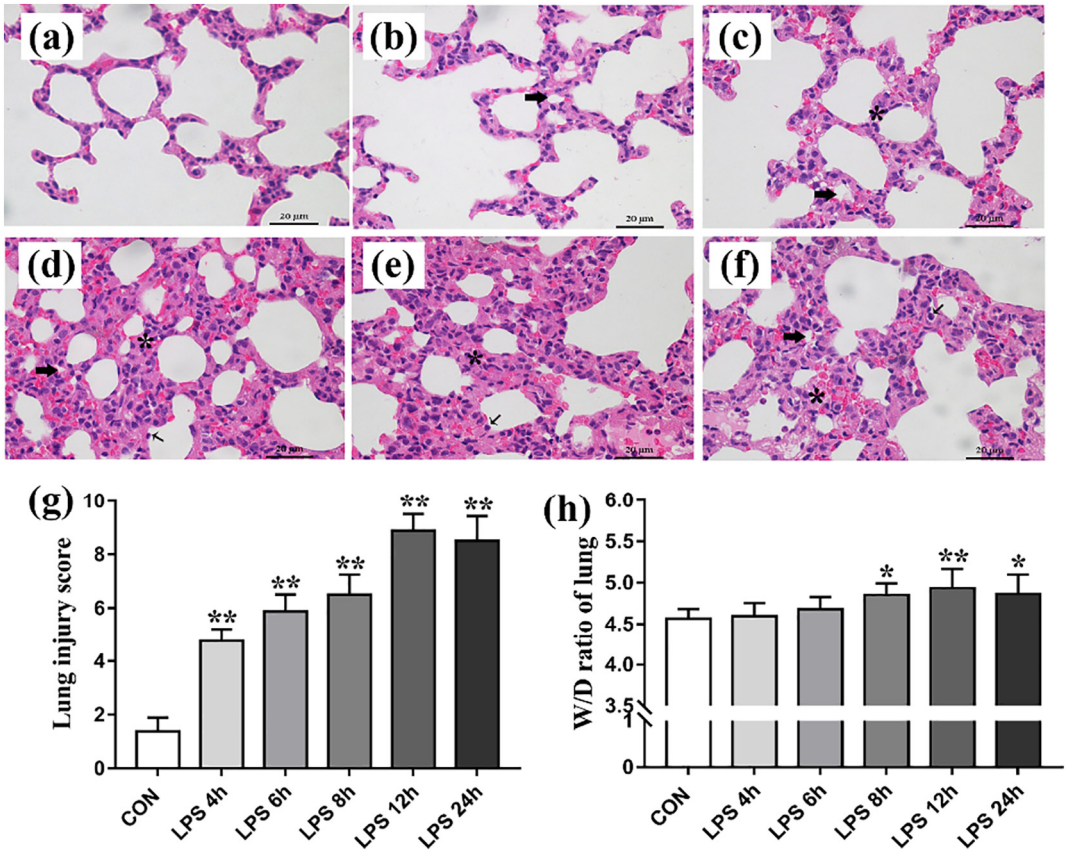

Fig. 1. Pathological changes of lung tissue induced by LPS. Microscopic photographs of morphologic changes in lung tissue (HE staining, original magnification ×400, n = 8): (a), (b), (c), (d), (e), (f) represents the CON, LPS 4, 6, 8, 12 and 24 h group, respectively; (g) ALI score; (h) lung W/D ratio. Data are expressed mean ± SD $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the CON group. Thin arrow: inflammatory cell infiltration; thick arrow: alveolar collapse; star: thickened alveolar wall.

USA). The significant values were obtained using a *t*-test. All the data were expressed as mean ± SD, displayed a normal distribution and passed the test for equal variance. Quantitative analysis of integral optical density was performed using Image-Pro Plus software (Media Cybernetics, Maryland, USA). $P < 0.05$ was considered statistically significant. $P < 0.01$ was considered statistically extremely significant.

3. Results

3.1. Effect of LPS on lung tissue specimens

We examined the histology of lung tissues of all the rats in this study by HE staining (Fig. 1). The results demonstrated that there were no obvious pathological changes in lung tissue of CON group (Fig. 1a). When LPS was induced in rats for 4 h, pathological lung damage was minor (Fig. 1b). However, the pathological lung damage was apparent when rats were LPS for 12 h (Fig. 1e), characterized by inflammatory cell infiltration, thickening of alveolar wall thickness and clear congestion. The results of the lung W/D ratio (Fig. 1h) analyses indicated that 12 h ($P < 0.01$) and 24 h ($P < 0.05$) LPS group were significantly higher than that of the CON group.

3.2. Systemic and pulmonary inflammation induced by LPS in rats

To assess the neutrophil accumulation and the expression of inflammatory factors, the activity of MPO and the expression of IL-1β and TNF-α were measured at different time points (Fig. 2). The level of MPO activity in the LPS group was significantly higher than that in the CON group (Fig. 2a). Western blotting analysis showed that LPS-stimulation significantly enhanced the production of IL-1β and TNF-α in the lung of rat. The expression of IL-1β and TNF-α protein increased gradually at 4 h after LPS administration, and reached its peak at 12 h (Fig. 2d, e, f). In addition, we detected the levels of IL-1β and TNF-α in serum and found that the LPS group were significantly higher than those in the CON group at each time point (Fig. 2b, c). These results show that LPS could induce the production of sepsis and inflammatory cytokine production at 4 h, and the inflammation at 12 h was the most obvious.

3.3. Effect of DEX on LPS-mediated lung histopathologic changes

As shown in Fig. 3, the CON group rats had intact alveolar wall structure and no exudate in interstitial of lung. In the LPS group, large number of neutrophils accumulated in the alveoli, the alveolar wall thickened, and the alveolar space became smaller, mesenchymal hyperemia. DEX (Fig. 3c) or GSK-3β inhibitor (Fig. 3e) pretreatment significantly alleviated these changes. The protective effect of DEX was attenuated by STAT3 inhibitor (Fig. 3d), and the number of neutrophils and the thickness of alveolar wall increased slightly. There were no obvious pathological changes in the DEX (Fig. 3f) and DMSO (Fig. 3g) groups. The lung injury score is shown in Fig. 3h.

The results of lung W/D ratio are basically consistent with morphological change. As shown in (Fig. 3i), LPS group was significantly higher than that of the CON group ($P < 0.01$); NSC74859 group was significantly higher than that of the CON group ($P < 0.01$); DEX + LPS group was significantly lower than that of the LPS group ($P < 0.01$); There was no significant difference between SB216763 group, DEX group, DMSO group and CON group.

3.4. Effect of DEX on inflammatory response in the lung tissues

To explore the potential mechanism of DEX on acute lung injury in vivo, we assayed the inflammatory cytokines in LPS stimulated rat. Compared with CON and DEX group the MPO activity of lung tissue in LPS group was significantly increased. However, DEX or SB216763 application significantly inhibited the increased MPO activity of LPS, but the application of NSC74859 antagonized the inhibitory effect of DEX on LPS (Fig. 4b). As illustrated in Fig. 4, results showed that LPS-stimulation alone significantly enhanced the mRNA (Fig. 4e, f) and protein (Fig. 4a, c, d) expressions of IL-1β and TNF-α in the lung of rat, whereas DEX or SB216763 pre-treatment reduced the increase of IL-1β and TNF-α. But the application of NSC74859 antagonized the inhibitory effect of DEX on LPS.

3.5. Effect of DEX on GSK-3β/STAT3-NF-κB pathway

To clarify the mechanism of DEX inhibits pulmonary inflammation induced by LPS, we further studied the expression of STAT3, GSK-3β and NF-κB in lung tissues. As shown in Fig. 5, the expression of P-NF-κB

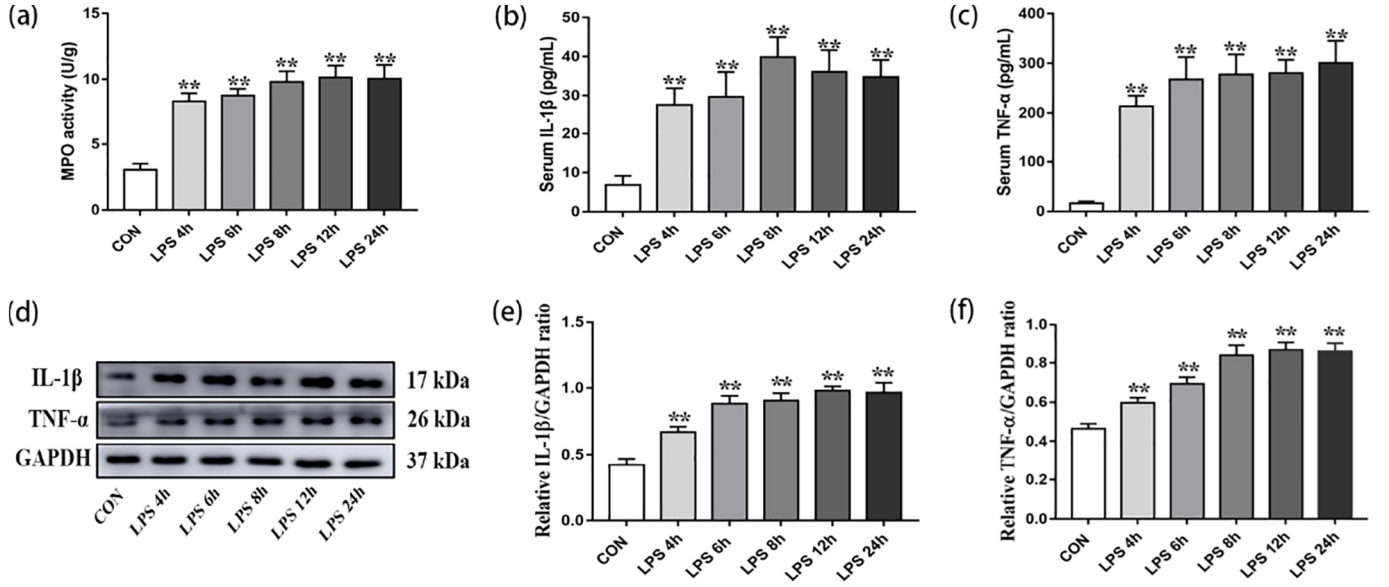

Fig. 2. Effect of LPS on inflammatory factors in serum and lung tissue (n = 4). The activity of MPO (a) in lung tissue and contents of IL-1β (b) and TNF-α (c) in serum were detected. The protein expression (d) of IL-1β (e) and TNF-α (f) in rat lung were normalized to GAPDH. Data are expressed mean ± SD. $^{**}P < 0.01$ compared to the CON group.

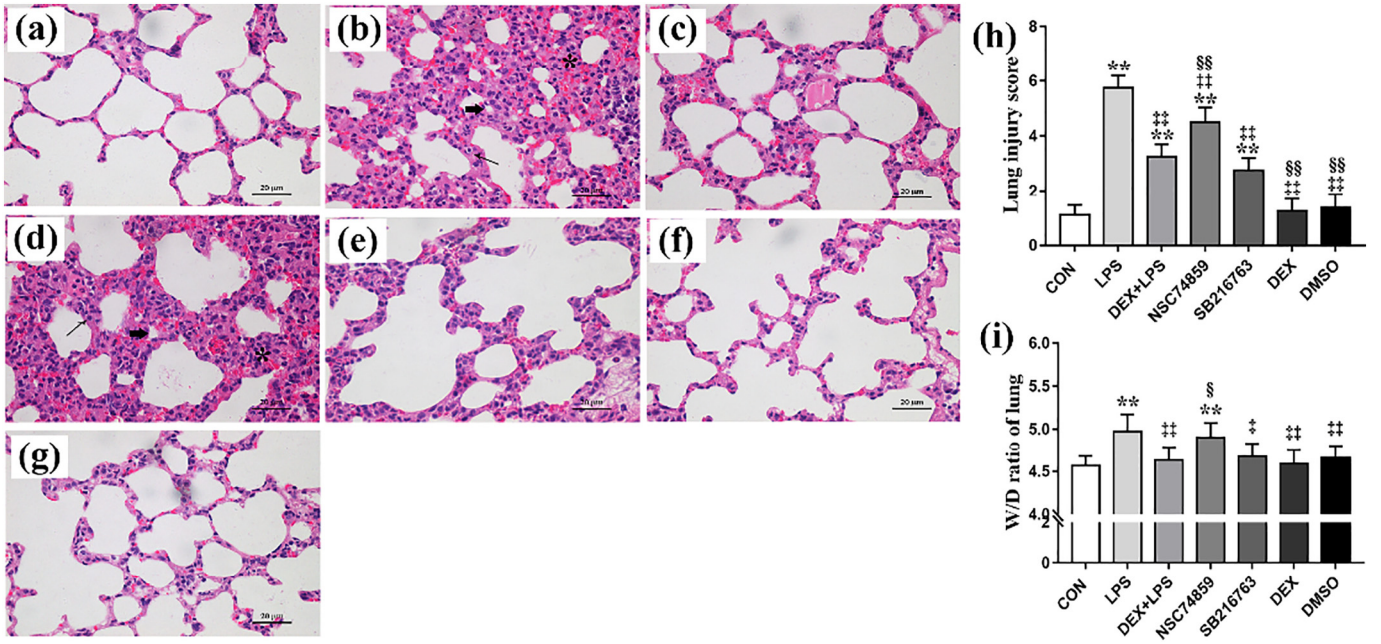

Fig. 3. Effect of DEX on histological alternations in LPS induced ALI in the different groups (HE staining, original magnification ×400, n = 8): (a), (b), (c), (d), (e), (f), and (g) represents the CON, LPS, DEX + LPS, NSC74859, SB216763, DEX, and DMSO group, respectively; (h) ALI score. (i) Lung W/D ratio. Data are expressed mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the CON group; $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$ compared to the LPS group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ compared to the DEX + LPS group. Thin arrow: inflammatory cell infiltration; thick arrow: alveolar collapse; star: thickened alveolar wall.

($P < 0.01$) was significantly increased after LPS administration. When pretreatment with DEX, the phosphorylated levels of STAT3 and GSK-3β were markedly upregulated ($P < 0.01$) and the phosphorylated levels of NF-κB markedly decreased ($P < 0.01$) compared to the LPS group, whereas NSC74859, an antagonist of STAT3, reversed the effect of DEX. SB216763 pretreatment could increase the phosphorylation of GSK-3β and decrease the activity of NF-κB, which resulted in the same protective effect as DEX pretreatment.

3.6. Immunohistochemistry for NF-κB activity

Immunohistochemical observation provides further evidence for DEX mediated lung protection pathway. First, we found that P-NF-κB was more expressed in alveolar epithelial cells and macrophages.

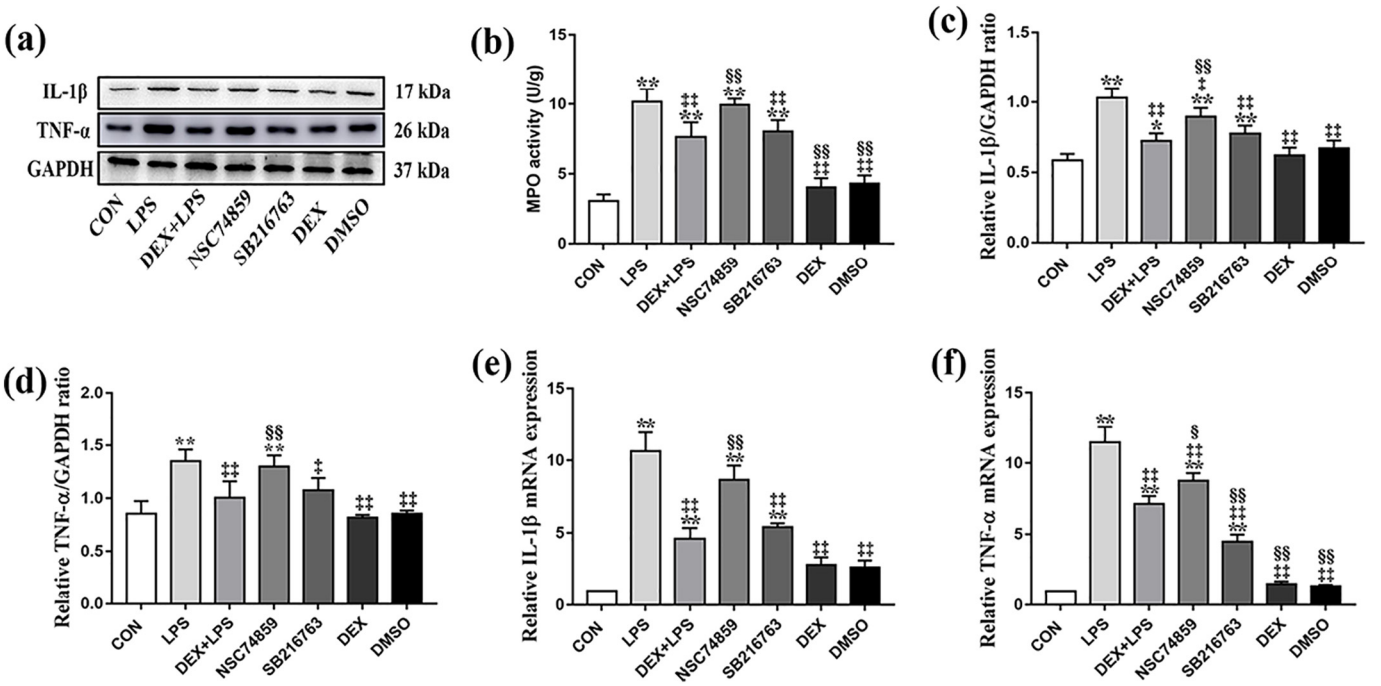

Fig. 4. IL-1β and TNF-α protein and mRNA expression and MPO activity (n = 4). The protein expression (a) of IL-1β (c) and TNF-α (d) in rat lung were normalized to GAPDH. The activity of MPO (b) in lung tissue was detected. The mRNA expression of IL-1β (e) and TNF-α (f) in rat lung were normalized to GAPDH. Data are expressed mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the CON group. $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$ compared to the LPS group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ compared to the DEX + LPS group.

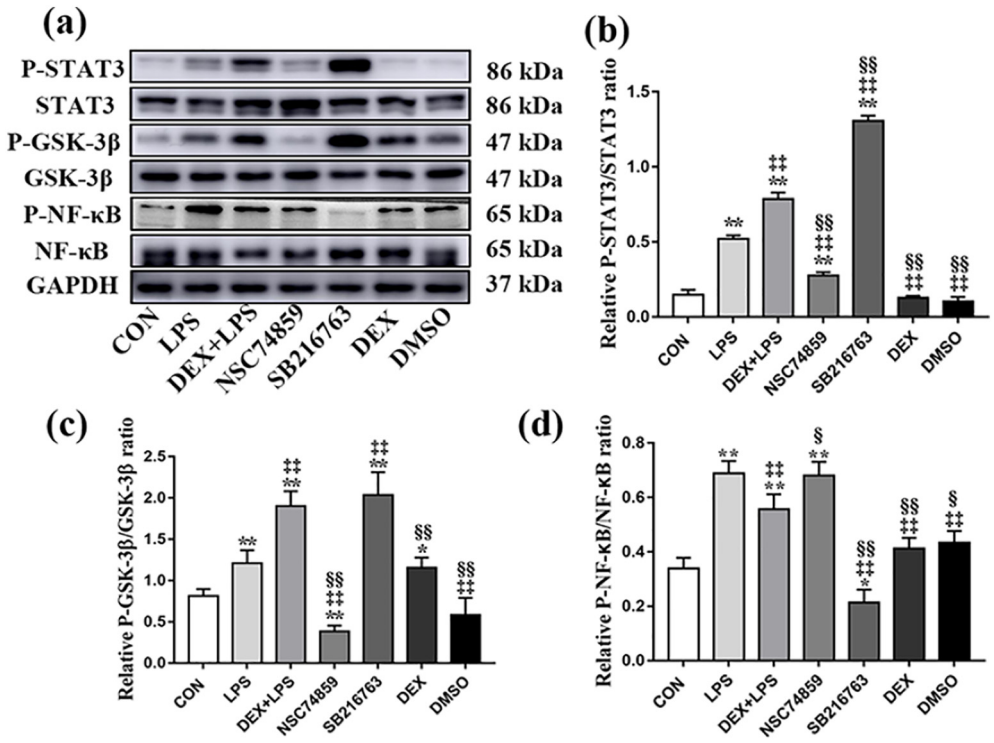

Fig. 5. Expression of all proteins in signal pathway (n = 4). The protein expression (a) of P-STAT3/STAT3 (b) and P-GSK-3β/GSK-3β (c) and P-NF-κB/NF-κB (d) in rat lung were normalized to GAPDH. Data are expressed mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the CON group; $^{‡‡}P < 0.01$ compared to the LPS group; $^{§}P < 0.05$, $^{§§}P < 0.01$ compared to the DEX + LPS group.

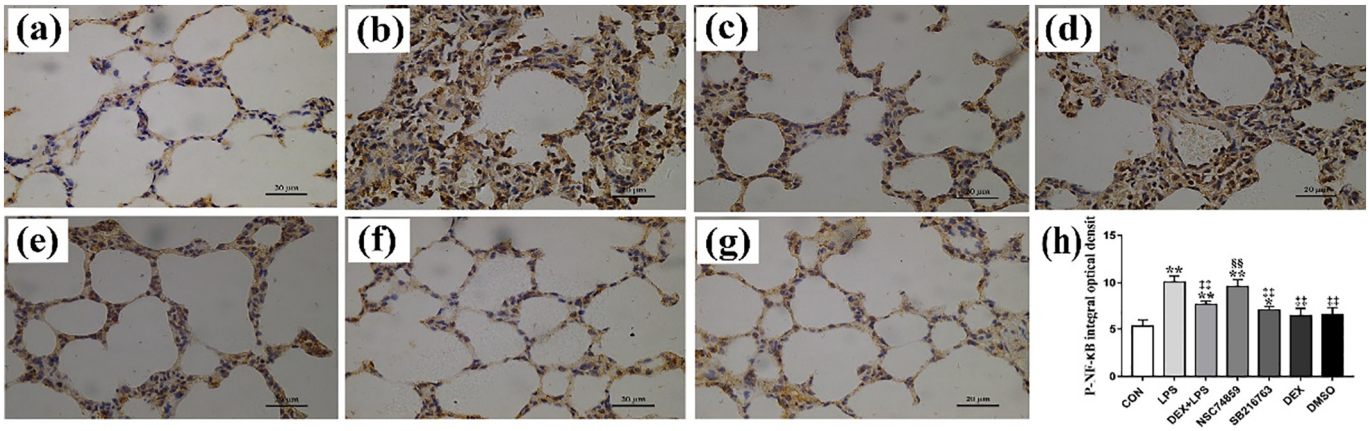

Fig. 6. Activation of NF-κB in rat lung tissues analyzed by immunohistochemical staining in different groups (original magnification × 400, n = 4): (a), (b), (c), (d), (e), (f), (g) represents the CON, LPS, DEX + LPS, NSC74859, SB216763, DEX, DMSO group, respectively; (h) Immunohistochemical score analysis. Data are expressed mean ± SD. $^{**}P < 0.01$ compared to the CON group. $^{‡‡}P < 0.01$ compared to the LPS group; $^{§}P < 0.05$ compared to the DEX + LPS group.

Second, the results demonstrated that the expression of P-NF-κB in LPS group was significantly higher than that in CON group, whereas DEX or SB216763 pretreatment could significantly reduce the expression of P-NF-κB. In addition, NSC74859 could antagonize the protective effect of DEX (Fig. 6).

4. Discussion

It has been reported that ALI is a serious inflammatory disease, and its important biological medium is LPS [34,35]. Intraperitoneal injection is a common method of applying drugs to rodents [36], and it is easy to operate. Therefore, we chose an animal model of ALI induced by intraperitoneal injection of LPS to simulate septicemia in vivo.

The typical feature of sepsis is systemic inflammation, so we detected the changes of inflammatory factors in serum at different time points. To verify the success of the ALI model, we observed the pathological and inflammatory changes of LPS injected intraperitoneally at different time points. Lung W/D ratio as an index of pulmonary edema, which is not only a typical symptom of systemic inflammation, but also a typical manifestation of local inflammation [37]. The critical feature of ALI is the lung parenchyma injury and acute inflammatory process, including the release of inflammatory mediators such as TNF-α, IL-1β and IL-6 [38]. Proinflammatory cytokines appear in the early stages of inflammation, which indicate the severity of ALI in a certain sense [39]. In this study, we found that the lung W/D ratio increased with the prolongation of LPS, especially at 12 h. The incidence of pulmonary edema and the degree of pulmonary edema were the highest at 12 h. The inflammatory factors gradually increased at 4 h after LPS administration, which was significantly higher than that in control group at 12 h. These results suggest that the ALI model was successfully established after intraperitoneal injection of LPS 12 h and the lung injury was the most obvious.

It is well known that the NF-κB signaling pathway is key to accounting for the expressions of proinflammatory cytokines induced by

LPS [40]. The mechanism of ALI injury may be due to bacterial endotoxin LPS activation promoting the interaction of TLR4 and MYD88, resulting in activation of NF-κB and release of TNF-α and IL-6 [41]. A lot of experiments have proved that DEX has protective effect by inhibiting the activity of NF-κB in different models. For example, Wang et al. [42] reported that pretreatment with DEX inhibits the TLR4/NF-κB pathway in cerebral ischemia-reperfusion and produces neuroprotective effects. In addition, Yao et al. [43] demonstrated that DEX inhibits TLR4/NF-κB activation and reduces acute kidney injury after orthotropic autologous liver transplantation. Therefore, inhibiting the activation of NF-κB is an effective way to protect ALI.

In addition, we suspected that activated STAT3 may have an anti-inflammatory effect because we and others have demonstrated that the activation of STAT3 inhibited the activation of NF-κB [44]. Previous studies have reported that STAT3 is activated in lung during IgG IC-induced acute lung injury, and have macrophage dependent and neutrophil dependent [45]. However, studies have also shown that STAT3 activates lung protection in endotoxemia/pneumonia models and ALI models induced by zymosan [46,47]. We propose that there are two reasons for this phenomenon, one is due to the self-protection produced by the body, and the other is due to the diversity of STAT3 functions. The activation of STAT3 may produce pro-inflammatory or anti-inflammatory effects, which may be related to the experimental model, experimental object, experimental conditions and degree of injury [48,49]. Our results showed that NSC74859 inhibited the activation of STAT3, increased the activation of NF-κB, and ultimately reversed the protective effect of DEX on lung, which indicated that DEX could regulated the activity of NF-κB through STAT3 to protect lung injury.

GSK-3β is a serine threonine protein kinase, which is involved in many signal transduction pathways such as cell metabolism, proliferation and apoptosis [50]. Recent studies have found that GSK-3β is a potential therapeutic target for sepsis [16]. For example, transactivation activity of NF-κB is decreased by inactivation of GSK-3β, which inhibits LPS-induced inflammatory reaction [51]. Many evidences showed that the addition of GSK-3β inhibitor or knockout of GSK-3β gene decreased the activity of NF-κB and the expression of target gene IL-6 or TNF-α [52]. Previous studies have demonstrated the key role of GSK-3β in regulating microvascular barrier damage associated with ALI in mice, and provided a theoretical basis for regulating pulmonary edema under septic conditions [16]. In addition, GSK-3β regulated the inflammatory response by differentially affecting the nuclear amounts of transcription factors NF-κB subunit p65 and CREB interacting with the coactivator CBP [17]. In this study, DEX and SB216763 had direct inhibitory effects on LPS-induced ALI. In addition, specific inhibitors inhibited the activation of GSK-3β and NF-κB and reduced the expression of inflammatory factors. Our data suggest that GSK-3β, as an upstream effector, was essential to inhibit the activation of NF-κB after exposure to LPS.

An interesting phenomenon was found in our experiments, the association between STAT3 and GSK-3β. We found that NSC74859 not only affected the activity of STAT3, but also affected the activity of GSK-3β and SB216763 also produced the same effect. On the one hand, the results showed that STAT3 is related to the negative regulation of GSK-3β [53–55]. Inhibition of GSK-3β by leptin-stimulated STAT3 signaling pathway leads to the increase of β-catenin signaling and thus promotes neurogenesis [53]. On the other hand, the result also showed that GSK-3β inhibited STAT3 activation [56]. We speculate that STAT3 and GSK-3β regulate each other in ALI model, and the specific mechanism is not clear, which needs to be further verified in future studies. As far as we know, this is the first time describe that STAT3 and GSK-3β have mutually regulating effects in the study of DEX alleviating ALI. This study provides a theoretical basis for the development of new anti-inflammatory drugs and provides guidance for the clinical application of DEX as anti-inflammatory drugs.

5. Conclusion

In conclusion, our study demonstrate that lung injury was the most obvious after intraperitoneal injection of 10 mg/kg LPS for 12 h and inflammation is one of the important causes of ALI, and DEX can produce anti-inflammatory effects via GSK-3β/STAT3-NF-κB pathway.

Abbreviations

ALI	acute lung injury
DEX	dexmedetomidine
LPS	lipopolysaccharide
H&E	hematoxylin and eosin
mRNA	messenger ribonucleic acid
PCR	polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
STAT3	signal transducer and activator of transcription 3
GSK-3β	glycogen synthase kinase-3β
SD	Sprague-Dawley
CON	control group
PMSF	phenyl methane sulfonyl fluoride
TBST	phosphate-buffered saline with Tween 20

Data availability

The data used to support the findings of this study are included within the article.

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Author contributions

Author Z-HY conceived the strategy for this paper. F-XJ and S-JC performed the ALI-model. Z-HY and H-XY analyzed the data and helped in manuscript preparations. All authors participated in discussion and editing, and approved the final manuscript.

Declaration of Competing Interest

The authors declare they have no conflict of interests.

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