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Original Article

Dexmedetomidine protects against LPS-induced lung injuries in mice through alleviation of inflammation and oxidative stress

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Abstract: Objective: The aim of this study was to assess the protective effects of dexmedetomidine on acute lung injuries (ALI), examining its underlying mechanisms. Methods: A mouse ALI model was reproduced by intra-tracheal administration of lipopolysaccharide (LPS 2 mg/kg). Mice were randomly divided into four groups: control group; LPS group, LPS+dex 20 μg/kg group, and LPS+dex 40 μg/kg group. Lung wet/dry weight ratios were examined, as well as myeloperoxidase (MPO) activity. The content of malondialdehyde (MDA) and levels of superoxide dismutase (SOD) and glutathione (GSH) in lung tissues were also determined. In addition, levels of tumor necrosis factor-alpha (TNF-α) and IL-6, as well as total protein concentrations in bronchoalveolar lavage fluid (BALF), were assayed. Results: The present study found that lung tissues from the LPS group were significantly damaged, while single-dose dexmedetomidine treatment significantly and dose-dependently attenuated LPS-induced ALI with decreased histopathological damage, lung wet/dry weight ratios, and MPO activity. Levels of IL-6 and TNF-α, as well as total protein concentrations in BALF, were elevated by LPS challenge and dose-dependently alleviated by dexmedetomidine treatment. The content of MDA was increased, while levels of SOD and GSH were decreased in LPS challenged lung tissues. These effects were partially reversed by dexmedetomidine treatment. Furthermore, dexmedetomidine inhibited TLR4 expression and activation of NF-κB in lung tissues. Conclusion: Present findings suggest that dexmedetomidine may exert protective effects against LPS-induced ALI in mice by reducing inflammatory response and oxidative stress. TLR4/NF-κB pathway suppression may play a role in the underlying mechanisms.

Keywords: Dexmedetomidine, acute lung injury, lipopolysaccharide, toll like receptor 4, NF-κB, inflammation

Introduction

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), characterized by refractory hypoxia, inflammation, increased vascular permeability, and decreased lung compliance, has been associated with high mortality despite major treatment efforts made over the past several decades [1]. A variety of clinical situations, such as hemorrhagic shock, multiple transfusions, inhalation of toxic gas, severe sepsis, and major trauma, may lead to the pathogenesis of ALI. However, gram-negative bacteria-induced sepsis is the most common contributor to ALI/ARDS.

Lipopolysaccahrides (LPS), the primary component of the outer membrane of gram-negative bacteria, has been frequently employed to establish animal models of sepsis for experimental studies. At present, lungs are regarded as one of the most insulted organs during the development of multiple organ dysfunction syndrome caused by LPS-induced sepsis [2].

Data has revealed that excessive production of pro-inflammatory cytokines, resulting from activation of a number of signal transduction cascades in lung cells, contributes to LPS-induced ALI [3]. Data has also shown that oxidative stress, due to the imbalance between exposed oxidants and endogenous antioxidants, plays a role in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [4, 5]. It has been established that oxidative stress and inflammation are interrelated biological

responses to detrimental stimulation [6-9]. In ALI patients, inflammation from lung tissue has a vicious impact on immune response, including potential oxidative stress [10]. On the other hand, increased oxidant levels could aggravate lung inflammation [11]. It was reported that LPS decreased SOD activities and GSH levels with a significant increase of MDA content in lung tissues. This has been considered to be related with the condition of oxidative stress [12].To date, several studies have presented measures to relieve LPS-induced ALI, indicating that agents acting against either inflammation or oxidative stress may be beneficial for LPS-induced ALI [3, 13].

Dexmedetomidine is known as a potent selective α_2-adrenergic agonist with sedative, analgesic, sympatholytic, and anxiolytic effects [14]. It protects against hemorrhagic shock or resuscitation relevant lung injuries by suppressing inflammatory gene expression in lung tissues [15]. It reduces pro-inflammatory cytokine production in septic patients and in experimental animal models of endotoxemia and sepsis [16, 17]. It provides protection against oxidative stress caused by ionizing radiation [18]. It alleviates pulmonary edema and improves oxygenation of mice with ALI induced by LPS [19]. Recently, an *in vitro* study showed that dexmedetomidine attenuated oxidative stress in lung alveolar epithelial cells [20].

NF-κB is the main transcription factor modulating inflammatory gene expression, exerting vital effects in the pathogenesis of ALI [21]. NF-κB has been reported to play a role in regulating cardiac oxidative stress [22]. Moreover, in LPS-induced ALI, the toll-like receptor 4 (TLR4), known as a transmembrane protein and signal transduction molecule, associating with innate and adaptive immune responses, is the upstream regulator of NF-κB [23]. Triggering of TLR4 leads to activation of NF-κB and subsequent regulation of pro-inflammatory factors [24]. However, whether the effects of dexmedetomidine on inflammation and oxidative stress in LPS-induced ALI are linked to TLR4/NF-κB pathways remains to be further elucidated. The present study investigated the effects of dexmedetomidine on mice with LPS-induced ALI. This study measured MPO activity, biomarkers of oxidative stress, and extent of lung edema and lung histopathological damage in lung tissues, as well as pro-inflammatory cytokines and protein concentrations in bronchoalveolar lavage fluid (BALF). In addition, this study explored the possible mechanisms of these effects by examining the activation of TLR4/NF-κB pathways.

Material and methods

Animals and grouping

Male C57BL/6 mice, weighing 20-25 g, were used in the present study. All mice were purchased from Wenzhou Medical University Animal Center and were housed in a temperature-controlled room (22-25°C) with relative humidity (55±5%) under a 12-hour light/dark environment. Animal care and experiments were approved by Wenzhou Medical University Ethics Committee and all procedures were performed according to the Declaration of Helsinki of the World Medical Association. Twelve hours prior to model establishment, all mice began fasting with free access to water.

There were two settings of experiment in our study. Intra-tracheal instillation of LPS was used to produce a mouse model of sepsis, as previous study described [25]. In the first set of experiments, forty-eight mice were randomly divided into four groups. In the control group, mice received intra-tracheal 50 μl phosphate-buffered saline (PBS, pH=7.4). In the LPS group, mice received intra-tracheal instillation of PBS 50 μl containing LPS 2 mg/kg to induce acute lung injury. In the LPS+dex 20 μg/kg group, mice received same dose of LPS as in LPS group and dex 20 μg/kg (i.p.) immediately after intra-tracheal LPS instillation. In the LPS+dex 40 μg/kg group, mice received same dose of LPS as in LPS group and dex 40 μg/kg (i.p.) immediately after intra-tracheal LPS instillation. If mice died during the observation period, data of the dead mice were excluded. Make-up experiments were done to keep the number of mice at 12 in each group. The dose of dexmedetomidine used was according to a pilot study and previous studies [26, 27]. Intraperitoneal (i.p.) pentobarbital sodium was used for anesthesia in all experimental mice.

Six hours after LPS administration, the right lung was excised and divided into 3 parts. The upper part was immersed in 10% formalin solution for histopathological examination. The middle part was for Western blot analysis of TLR4,

NF-κB p65, IκBα, and p-IκBα expression. The lower part was for analysis of MPO activity and oxidative stress biomarkers. The left lungs were used for calculation of wet/dry (W/D) weight ratios.

In the second set of experiments, forty mice were also divided into the same four groups as described as above (n=10 in each group). Six hours after intra-tracheal instillation, BALF were recruited and tested for TNF-α and IL-6 levels, as well as total protein concentrations. Mice were sacrificed at 6 hours after intra-tracheal instillation because a previous study showed that peak concentrations of cytokines occur in the BALF by that time point [25].

Chemicals and reagents

Dexmedetomidine was obtained from Xinchen Pharmacy (Jiangsu, China). LPS (Escherichia coli 055: B5) and ELISA kits for analysis of TNF-α and IL-6 were purchased from BioLegend (CA, USA). Antibodies for TLR4, NF-κB p65, IκBα, and p-IκBα and protein extraction kits were purchased from Cell Signaling (MA, USA). All materials used in the process of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (CA, USA). In addition, the MPO, MDA, SOD, and GSH testing kits and protein assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Histological evaluation

Six hours after intra-tracheal instillation, the upper right part of lungs was fixed in 4% formalin for 24 hours, dehydrated, and embedded in paraffin. Paraffin-embedded lung tissues were then serially sectioned to 4-5 μm thickness in each unit and stained with hematoxylin-eosin (HE). An examiner, blinded to the animal group, assessed the histological changes of lung tissues under a microscope and described the conditions of inflammatory cell infiltration, alveolar exudation, and alveolar hemorrhage [27].

WB analysis of TLR4 and NF-κB

Activation of TLR4/NF-κB pathways was examined. Using the nuclear and cytoplasmic proteins extraction kit, samples were extracted from homogenized lung tissues obtained from right middle lobe at 6 hours after intra-tracheal instillation. Western blot analysis was performed to test levels of NF-κB p65, p-IκBα, and IκBα. Levels of TLR4 in lung tissues were also tested. Equal amounts of protein with 40 μg were loaded onto a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes. Subsequently, the membranes were blocked with 5% fat-free milk for 2 hours, followed by overnight incubation with primary antibodies at 4°C. Afterward, the membranes were washed with TBS solution three times and then incubated with peroxidase-conjugated secondary antibody (1:1000 dilution) for 1 hour at room temperature. Following incubation with primary and secondary antibodies, bands on the membranes were visualized using an enhanced chemiluminescence (ECL) system. GelDoc-2000 Imaging System (Bio-Rad Company, USA) was used for quantitative analysis of density in each immunoblot bands.

Analysis of myeloperoxidase (MPO) activity

Six hours after intra-tracheal instillation, tissues from the right lower part of the lungs were collected, homogenized, and centrifuged (4°C, 12,000 × g, 30 minutes). MPO activity was assayed using the myeloperoxidase fluorometric activity assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Absorbance of the samples was measured with a spectrophotometer at 460 nm. MPO activity of the lung tissues was expressed as units per gram of protein (U/g).

Biomarkers of oxidative stress induced by LPS in lung tissues

Six hours after intra-tracheal instillation, tissues were obtained from the right lower part of the lungs. They were homogenized and dissolved in extraction buffer. To evaluate the oxidative status of LPS-challenged lung tissues, malondialdehyde (MDA) content and levels of super-oxide dismutase (SOD) and glutathione (GSH) in lung tissues were detected by corresponding test kits, according to manufacturer instructions.

W/D ratio

Six hours after intra-tracheal instillation, the left lung was excised and rinsed briefly in phos-

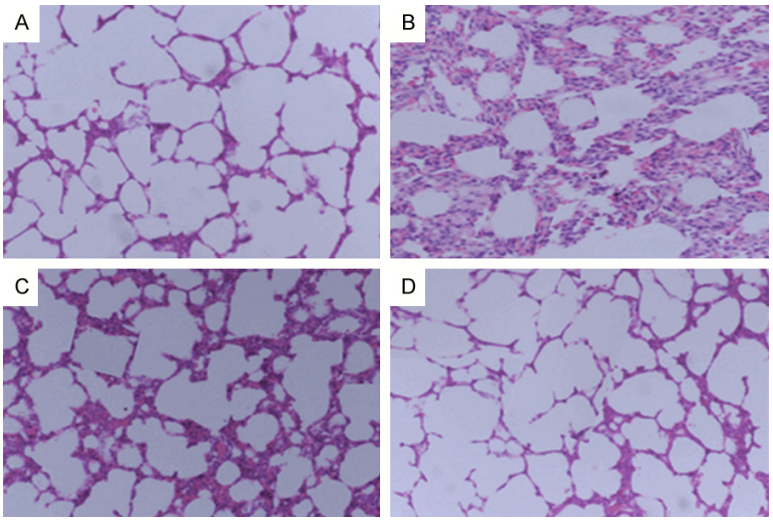

Figure 1. Effects of dexmedetomidine on LPS-induced histopathological changes of the lung tissue. LPS induced marked lung tissue damage, represented with substantial inflammatory cell infiltration and abundant alveolar exudation, as well as hemorrhages. However, these histopathological changes induced by LPS were ameliorated by dexmedetomidine in lung tissues. Lung sections in 5μm were obtained and stained with hematoxylin eosin in each group 6 hours after LPS challenge. Images (magnification, ×200) of representative sections by light microscopy from mouse lungs stained with hematoxylin eosin after harvest from the four groups are shown. (A) Control group; (B) LPS group; (C) LPS+dex 20 μg/kg group; (D) LPS+dex 40 μg/kg group.

phate-buffered saline (PBS). It was blotted, then the "wet" weight was obtained. The lungs were then placed in an incubator at 80°C for 72 hours to obtain the "dry" weight. The wet/dry weight ratio (wet weight divided by the dry weight) of the lungs was calculated, representing the extent of lung edema.

Cytokines and protein measurement in BALF

In the second set of experiments, bronchoalveolar lavage (BAL) was performed by intra-tracheal instillation of 0.5 mL ice-cold phosphate-buffered saline (PBS) followed by gentle aspiration from the lungs. BAL fluid (BALF) was collected three times and the recovery ratio of the fluid was about 90%. The recruited fluid was then pooled and centrifuged (3000 rpm, 10 minutes, 4°C). Supernatants were collected and levels of TNF-α and IL-6 were measured using enzyme-linked immuno-sorbent assay (ELISA) kits, following manufacturer instructions. The supernatants were also measured for total protein concentrations using the bicinchoninic acid (BCA) protein assay kit. Total protein content was expressed as μg/mL.

Statistical analysis

SPSS 15.0 (Chicago, IL, USA) was used for data analysis. Data were tested for normality and homogeneity of variances. Normally distributed quantitative data are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test, were used to compare data among the different groups. Statistical significance is indicated by $P < 0.05$.

Results

Effects of dexmedetomidine on LPS-induced histopathological changes of lung tissue

Six hours after intra-tracheal instillation, histopathological changes of the lung tissues were examined. In the control group, H&E staining (200 × original magnification) showed integrated structures of lung tissues (a). However, the lung tissues of the mice administered with LPS alone showed marked damage, including substantial inflammatory cell infiltration, abundant alveolar exudation, and hemorrhages, indicating the occurrence of ALI (b). After treatment with dexmedetomidine 20 μg/kg (i.p.), inflammatory cells infiltration with mild exudation were visualized (c). After treatment with dexmedetomidine 40 μg/kg (i.p.), only mild inflammatory infiltration was visualized without alveolar exudation (d) (**Figure 1**).

Effects of dexmedetomidine on LPS-induced expression of TLR4, phosphorylation of IκB-α, and NF-κB p65 nucleus translocation

Expression of TLR4, IκBα phosphorylation, and NF-κB p65 nucleus translocation at 6 hours after intra-tracheal LPS challenge were assayed by Western blot. It was found that expression of TLR4, phosphorylation levels of IκBα, and translocation of NF-κB p65 into the nucleus were increased 6 hours after the LPS challenge, compared to the control group ($P < 0.05$).

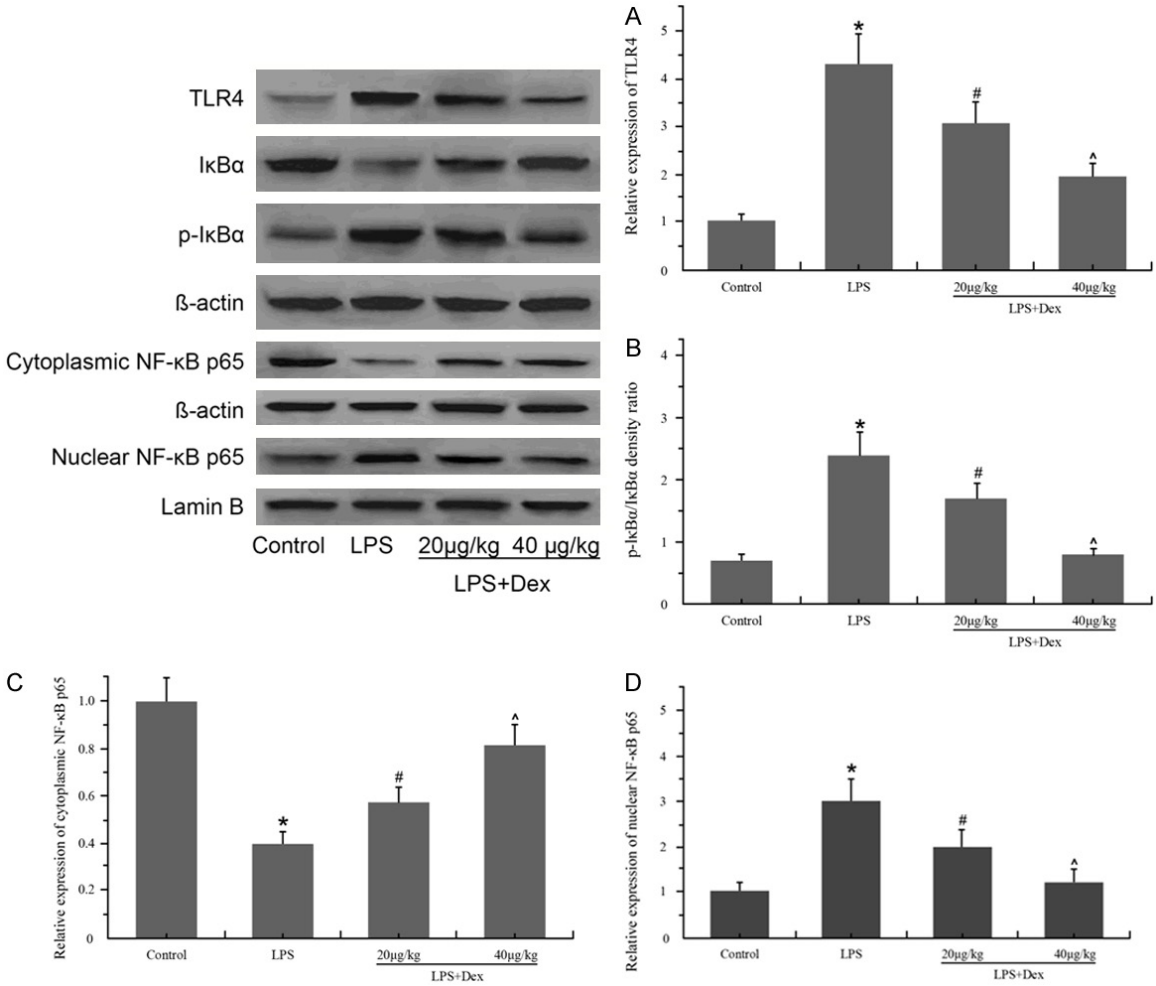

Figure 2. Dexmedetomidine dose-dependently affected LPS-induced expression of TLR4, phosphorylation of IκB-α, and NF-κB p65 nucleus translocation. Expression of TLR4 (A), phosphorylation of IκB-α (B), expression of cytoplasmic (C) and nuclear NF-κB p65 (D) were detected by Western blotting. Data are shown as mean ± SD (n=12). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

However, these increases were attenuated by dexmedetomidine treatment at 20 μg/kg (i.p.) and 40 μg/kg (i.p.), respectively (P < 0.05). The higher-dose dexmedetomidine was more effective (P < 0.05) (**Figure 2**).

Effects of dexmedetomidine on MPO activity

MPO is a peroxidase enzyme, abundantly existing in neutrophil granulocytes, which plays an important role in ALI. The activity of MPO was evaluated to reflect the neutrophil accumulation within pulmonary tissues 6 hours after intra-tracheal LPS challenge (**Figure 3**). Present results showed that MPO activity increased significantly in the LPS group, compared with the control group (P < 0.05). However, the increased MPO activity was significantly attenuated by dexmedetomidine treatment at 20 μg/kg (i.p.) or 40 μg/kg (i.p.) (P < 0.05). The higher-dose dexmedetomidine was more effective (P < 0.05, n=12 per group) (**Figure 3**).

Effects of dexmedetomidine on stress-related biomarkers

To evaluate the effects of dexmedetomidine on oxidative stress induced by LPS challenge in lung tissues, MDA content, levels of SOD, and levels of GSH were evaluated, following manufacturer instructions. Present data showed that MDA content increased significantly in the LPS group (a), while levels of SOD (b) and GSH (c) significantly declined in the LPS group, com-

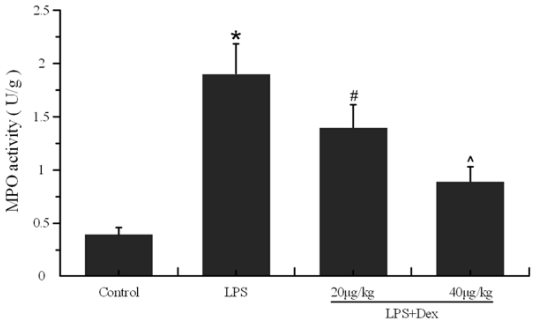

Figure 3. Dexmedetomidine alleviated MPO activity in mouse lung tissues 6 hours after LPS challenge. LPS induced elevated activity of MPO. However, it was dose-dependently ameliorated by dexmedetomidine in lung tissues. MPO activity is expressed as U/g. All data are presented as mean ± SD (n=12 per group). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

pared with the control group. However, these changes were significantly attenuated by dexmedetomidine treatment at 20 μg/kg (i.p.) or 40 μg/kg (i.p.). The higher-dose dexmedetomidine was more effective (P < 0.05) (**Figure 4**). All data are presented as mean ± SD (n=12 per group).

Effects of dexmedetomidine on lung wet/dry ratios

Six hours after intra-tracheal LPS instillation, to evaluate the changes of lung water accumulation, W/D weight ratios of the left lungs were analyzed. It was found that the mean W/D ratio in the LPS group was significantly increased, compared to the control group (P < 0.05). However, treatment with dexmedetomidine 20 μg/kg (i.p.) or 40 μg/kg (i.p.) effectively attenuated the increased water content of lungs (P < 0.05). The higher-dose dexmedetomidine was more effective. (P < 0.05, n=12 per group) (**Figure 5**).

Effects of dexmedetomidine on change of pro-inflammatory cytokine levels in BALF

Six hours after intra-tracheal LPS instillation, to evaluate the condition of inflammation induced by LPS challenge, pro-inflammatory cytokine levels of TNF-α and IL-6 in BALF were measured by ELISA assays. It was found that the cytokine production in LPS group was significantly increased, compared to the control group. It was also found that dexmedetomidine significantly reduced levels of TNF-α (a) and IL-6 (b) in BALF in the LPS+dex 20 μg/kg group or LPS+dex 40 μg/group in a dose-dependent manner, compared to the LPS group (P < 0.05) (**Figure 6**).

Effects of dexmedetomidine on protein concentrations in BALF

Six hours after intra-tracheal LPS instillation, to evaluate the reaction from LPS challenge, this study measured total protein concentrations in BALF. It was found that there were significantly increased protein concentrations in BALF 6 hours after the LPS challenge, compared to the control group. However, treatment with 20 μg/kg (i.p.) or 40 μg/kg (i.p.) dexmedetomidine dose-dependently reduced protein concentrations in BALF (P < 0.05) (**Figure 7**).

Discussion

Sepsis is a life-threatening disorder that commonly results in respiratory and cardiovascular compromise, as well as central nervous system dysfunction. The lungs are the most vulnerable organs during sepsis-induced multiple organ dysfunction [26]. Dexmedetomidine, the highly selective α_2 agonist, widely used as a sedative for critical ill patients, has been reported to modulate immune reaction following detrimental stimulation and reduce mortality for both patients and animals with sepsis [28, 29]. Dexmedetomidine has also been shown to possess pleiotropic biological properties, including anti-inflammatory and antioxidant effects [18, 20, 26]. In the present study, a mouse sepsis model was produced by intra-tracheal instillation of LPS. It was used to investigate whether dexmedetomidine treatment has protective effects against LPS-induced ALI. Possible mechanisms were also explored.

Present results demonstrate that dexmedetomidine could effectively alleviate histopathological lung damage, pulmonary edema, MPO activity, oxidative stress, and inflammatory response in LPS-induced ALI. Moreover, results show that dexmedetomidine administration inhibited activation of TLR4/NF-κB pathways in the pathogenesis of LPS-induced ALI.

Pro-inflammatory cytokines appear in the early development of an inflammatory response process, playing a critical role in the pathophysiol-

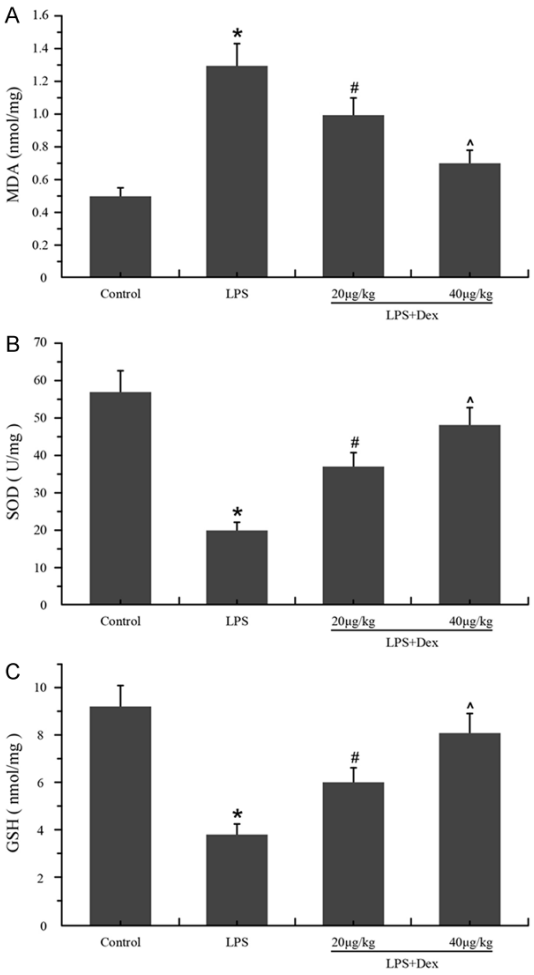

Figure 4. Effects of dexmedetomidine on MDA (A), SOD (B), and GSH (C) in lung tissues of LPS-challenges mice. Lung tissues were homogenized 6 hours after LPS challenge. LPS induced increased content of MDA (A) and declined levels of SOD (B) and GSH (C). However, these changes were dose-dependently attenuated by dexmedetomidine treatment. Data are presented as mean ± SD (n=12 per group). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

ogy of inflammation, contributing to the severity of lung injuries [30]. TNF-α and IL-6, the two characterized pro-inflammatory cytokines, are involved in the pathogenesis of ALI [30, 31]. A previous study showed that elevated levels of TNF-α and IL-6 have been presented in the bronchoalveolar lavage by LPS challenge [26]. Clinically, it has been noted that persistent elevation of these pro-inflammatory cytokines in patients with ALI is associated with more severe outcomes [32].

In LPS-induced acute lung injuries, LPS initiates the production of pro-inflammatory cyto-

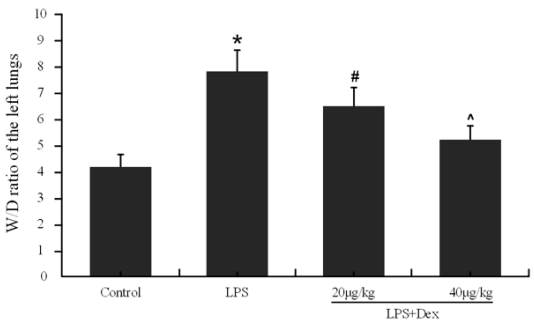

Figure 5. Effects of dexmedetomidine on Wet/Dry weight ratios. Left lungs of mice were excised 6 hours after LPS challenge and Wet/Dry weight ratios were calculated. LPS increased the Wet/Dry weight ratio of the lungs. However, this change was dose-dependently attenuated by dexmedetomidine treatment. All data are presented as mean ± SD (n=12 per group). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

kines, such as TNF-α and IL-6, from damage of endothelium, epithelium, macrophages, and neutrophil activation [29, 33]. LPS increases vessel permeability and disrupts the alveolar-capillary barrier, leading to lung edema. This is characterized by increased water content in the extracellular spaces of the lung tissue [34]. Furthermore, protein extravasation is a typical symptom of increased pulmonary vascular permeability due to both systemic and local inflammation after LPS challenge [35]. In the present study, LPS caused significant increased levels of IL-6 and TNF-α in the BALF. This result is in agreement with previous studies [36, 37]. In addition, dexmedetomidine decreased the production of both IL-6 and TNF-α in the BALF, suggesting that it possesses potential anti-inflammatory effects against LPS-induced ALI. Accordingly, dexmedetomidine reduced W/D ratios and effectively attenuated total protein concentrations in BALF. This was in parallel with histological damage of lung tissues, revealing that there was inflammatory cell infiltration and abundant alveolar exudation in lung tissues of mice with LPS challenge. These were dose-dependently reduced by dexmedetomidine treatment.

LPS-induced ALI is featured by bilateral neutrophils infiltration in the lungs caused by pro-inflammatory cytokines [29]. Myeloperoxidase (MPO) is the most abundant enzyme in the granulocytes of neutrophils. It has been associated with tissue damage in pulmonary inflammation. Hence, increased MPO activity is regar-

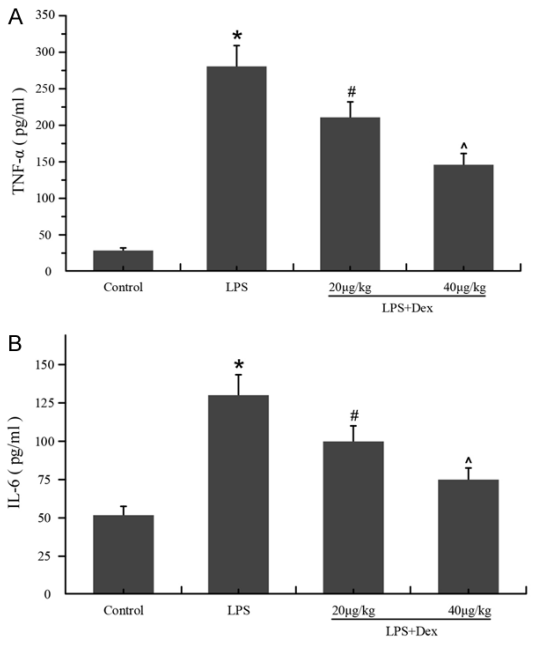

Figure 6. Dexmedetomidine ameliorated the production of pro-inflammatory cytokines in BALF. LPS increased the levels of pro-inflammatory cytokine levels of TNF-α and IL-6 in BALF 6 hours after LPS challenge. However, these changes were dose-dependently attenuated by dexmedetomidine treatment. Pro-inflammatory cytokine levels of TNF-α and IL-6 were measured by ELISA assays 6 hours after LPS challenge. All the data are shown as mean ± SD (n=10 per group). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

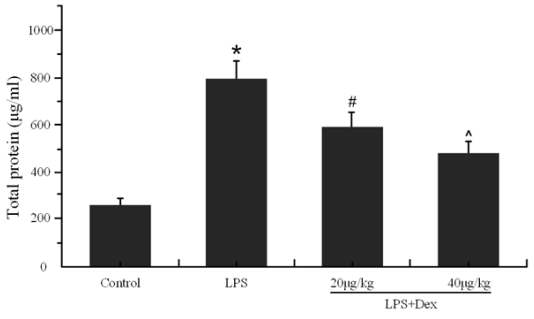

Figure 7. Dexmedetomidine decreased protein concentrations in BALF. LPS increased total protein concentrations in BALF 6 hours after LPS change. However, this change was dose-dependently attenuated by dexmedetomidine treatment. Data are shown as mean ± SD (n=10 per group). *P < 0.05 vs. control group, #P < 0.05 vs. LPS group, ^P < 0.05 vs. LPS+dex 20 μg/kg group.

ded as a major marker of neutrophil activation which releases inflammatory mediators, leading to further damage in lung tissues [38]. The present study found that dexmedetomidine decreased LPS-triggered MPO activity in lung tissues, implying that dexmedetomidine could inhibit the infiltration of neutrophils in injured lung tissues.

Another important aspect of ALI is oxidative stress, which generates from activated alveolar epithelial cells, endothelial cells, macrophages, and recruited neutrophils following airway stimulation [9, 39, 40]. It has been revealed that oxidative stress can not only cause direct tissue damage, but also affects molecular mechanisms regulating the process of lung inflammation [41]. Oxidative stress may turn on NF-κB activation, resulting in a large production of pro-inflammatory cytokines which promptly propagate to further inflammation [42]. Oxidative stress is characterized by increased content of melondialdehyde (MDA) and reduced production of anti-oxidative enzymes, including superoxide dismutase (SOD) and glutathione (GSH) [11].

MDA is known as a marker of oxidative stress, produced from the breakdown of polyunsaturated fatty acids by oxidation and an increase in free radicals, reflecting lipid peroxidation [43]. Therefore, it has served to indicate cell membrane impairment [44]. Under unstressed conditions, the activities of SOD and GSH contribute to the amelioration of oxidative injury and maintain a balance of oxidants and antioxidants in the organs, including the lungs [45]. SOD is one of the antioxidant enzymes that converts superoxide anions into hydrogen peroxide, while GSH acts as a major cellular antioxidant defense system by scavenging free radicals and other intermediate product from reactive oxygen species (ROS) [46]. LPS-induced oxidative stress, which is associated with an exaggerated production of cell damage, can lead to formation of lipid peroxidation related MDA and depletion of GSH and SOD, aggravating LPS toxicity via diminution of the antioxidant defense [47]. In a previous study, anti-oxidant agent treatment was shown to reduce pulmonary hypertension, hypoxia, and microvascular permeability in an animal model [48].

In the present study, LPS increased MDA content, accompanied by decreased activity of SOD and GSH content in lung tissues. Furthermore, this study demonstrated that treatment

with dexmedetomidine dose-dependently reduced MDA content and increased SOD activity and GSH content in LPS-challenged lung tissues of mice. Present data suggests that dexmedetomidine reduces LPS-induced ALI, not only by attenuating inflammation but also oxidative stress in lung tissues.

Based on the above findings, to further illuminate possible mechanisms of the protective effects of dexmedetomidine in LPS-induced ALI, this study investigated whether the anti-inflammatory and anti-oxidant characteristics of dexmedetomidine were associated with TLR4/NF-κB signaling pathway activation. TLR4 is known as an essential signaling receptor to LPS and exhibits immune responses to detrimental stimulation [49].TLR4 has been reported as the upregulator of NF-κB, which is an important transcription factor associated with expression of several inflammatory genes and inducible enzymes, playing an important role in the development of ALI [50]. In physiological conditions, NF-κB exists in the cytosol and binds to the inhibitory part IκBα. Under certain stimulation, NF-κB is activated by phosphorylation with ubiquitination of the inhibitory subunit IκBα, enabling NF-κB p65 to translocate into the nucleus and promote the subsequent transcription of immune and inflammatory genes/factors, including tumor necrosis factor (TNF)-α and IL-6 [51]. In response to LPS challenge, TLR4 binds to LPS, triggering the subsequent activation of NF-κB, promoting the release of TNF-α and IL-6 and both the two pro-inflammatory cytokines feedback to activate NF-κB. This positively amplifies the production of pro-inflammatory cytokines [52, 53]. It has been reported that, in an LPS-induced ALI murine model, gene expression levels of TNF-α and IL-6 in lung tissue were significantly increased in wild-type mice, compared with TLR4-deficient mice [22]. Correspondingly, another study found that active LPS elicited no response in TLR4-deficient murine macrophage cells [54]. The present study examined expression of TLR4 and activation of NF-κB in mouse lung tissues. Results showed increased levels of IL-6, TNF-α, TLR4, IκBα phosphorylation, and NF-κB p65 nucleus translocation by LPS challenge. However, these effects were partially reversed following treatment with dexmedetomidine. These findings further indicate that dexmedetomidine could protect against LPS-induced ALI by inhibition of both inflammation and oxidative stress. This effect was associated with the suppression of TLR4/NF-κB pathways.

Conclusion

In conclusion, the present study demonstrated that dexmedetomidine treatment reduced the severity of LPS-induced ALI in mice. The beneficial effects of dexmedetomidine on LPS-induced ALI may be attributed to the inhibition of both inflammation and oxidative stress, which have been associated with the suppression of LTR4/NF-κB signaling pathways. Present findings suggest that dexmedetomidine may be a promising choice of sedatives for ALI patients under attack from inflammation and oxidative stress.

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Disclosure of conflict of interest

None.

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