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The protective effect of dexmedetomidine on LPS-induced acute lung injury through the HMGB1-mediated TLR4/NF-κB and PI3K/Akt/mTOR pathways

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ABSTRACT

The aim of present study was to evaluate the protective effects of dexmedetomidine (DEX) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) and investigate its possible mechanisms mediated by HMGB1. In vivo, pulmonary pathology observation and myeloperoxidase (MPO) activity were also examined to evaluate the protective effect of DEX in the lungs. Tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) in bronchoalveolar lavage fluid (BALF), serum and lung tissues LPS-induced rats were detected. The oxidative indices including superoxide dismutase (SOD), Malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) in serum were also determined. Additionally, nitric oxide (NO), TNF-α, IL-6 and IL-1β, MDA, SOD and GSH-Px in the supernatants of LPS-induced BEAS-2B cells were measured. Furthermore, we detected the protein expression of high mobility group box-1 protein (HMGB1), Toll-like receptor 4 (TLR4), myeloid differentiating factor 88 (MyD88), inhibitor of NF-κB (IκBα), p-IκBα, nuclear factor kappa-B (NF-κB), p-NF-κB, phosphatidylinositol 3′-kinase (PI3K), p-PI3K, protein kinase B (Akt), p-Akt, mammalian target of rapamycin (mTOR) and p-mTOR in LPS-induced ALI rats and LPS-induced BEAS-2B cells. Immunohistochemical and immunofluorescence analyses of HMGB1 in lung tissues or BEAS-2B cells were also conducted to evaluate the mechanisms of DEX. DEX effectively attenuated pulmonary pathology, and ameliorated the levels of MPO, SOD, MDA, GSH-Px, TNF-α, IL-6, IL-1β and NO in LPS-stimulated rats and BEAS-2B cells. Additionally, treatment with DEX inhibited the expression of HMGB1, TLR4, MyD88, p-IκB, p-NF-κB, p-PI3K, p-Akt and p-mTOR in vivo and in vitro. Immunohistochemical and immunofluorescence analyses also showed that DEX suppressed HMGB1 levels in lung sections and BEAS-2B cells. Treatment with glycyrrhizin, an inhibitor of HMGB1, confirmed that HMGB1 was involved in the mechanism of DEX on LPS-induced ALI. The transfection of HGMB1 siRNA also confirmed these findings in vitro. In conclusion, the present study showed that DEX exerted a protective effect on LPS-induced ALI rats likely through the HMGB1-mediated TLR4/NF-κB and PI3K/Akt/mTOR pathways.

1. Introduction

Acute lung injury (ALI) is characterized by the pathogenesis of acute inflammation in the air spaces and lung parenchyma. Many factors, such as severe sepsis, trauma, shock and inhaling harmful gas could contribute to ALI. ALI is a typical clinical disease associated with significant morbidity and mortality (Chen et al., 2015a). Despite technical development and advanced supportive treatment in intensive care units, ALI has contributed to a high mortality rate of 30% to 40% (Matthay and Zemans, 2011; Rubenfeld et al., 2005). Unfortunately, there have been few effective drugs to treat acute lung injury. Lipopolysaccharide (LPS) has been recognized as a principal component causing ALI. LPS induces inflammatory reactions and conduces to the imbalance of the redox process. Upon stimulation with LPS, the pivotal inflammatory cytokines including tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), are initiated and participate in the progression of acute lung injury. LPS challenge also leads to oxidative stress, and an imbalance between oxidant and anti-oxidant systems is involved in the pathogenesis of ALI.

High-mobility group box 1 (HMGB1), identified as a nuclear non-histone DNA-binding protein, is involved in the stabilization of nucleosome structure and regulation of transcription. HMGB1 is reported to control inflammatory dysfunctions by binding to the cellular receptors including toll-like receptor (TLR)-2 and TLR-4. (Yang et al., 2010). Recently, several studies have shown that Toll-like receptors (TLRs) play an essential role in the innate immune system which is

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responsible for inflammation process in ALI (Zhang et al., 2015a). The NF-κB activation caused by bacterial endotoxins is required for the transcription and generation of pro-inflammatory mediators including tumour necrosis factor (TNF)-α, interleukin (IL)-1β and interleukin (IL)-6 in the early phase of acute lung injury. The phosphatidylinositol 3′-kinase (PI3K)/Akt signalling pathway regulates cell survival and oxidative stress in pulmonary inflammation. Akt regulates NF-κB activation and controls mammalian target of rapamycin (mTOR) activity (Jiang et al., 2017).

Dexmedetomidine (DEX), a highly selective agonist of the α2-adrenergic receptor, is clinically used for the sedation of ill patients. Numerous evidence has indicated that DEX exhibits anti-inflammatory properties in various disorders, such as cerebral infarction (Wu et al., 2016), cognitive dysfunction (Yamanaka et al., 2017), myocardium ischemia-reperfusion (Sun et al., 2017) and sepsis (Zhang et al., 2015b). Several studies have indicated that DEX exhibited a protective effect on pulmonary dysfunction (Heil et al., 2016; Hanci et al., 2012). However, the mechanism by which DEX exerts an anti-inflammatory effect on acute lung injury remains unknown. Therefore, the aim of the present study was to evaluate the pharmacological effect of DEX on LPS-induced acute lung injury and detect its potential mechanism by inhibiting HMGB1-mediated TLR4/NF-κB and PI3K/Akt/mTOR pathways.

2. Materials and methods

2.1. Reagents

Dexmedetomidine (DEX) was obtained from the Jiangsu Hengrui Medicine Co., Ltd. (Lianyungang, Jiangsu, China). LPS was supplied from Beyotime Institute of Biotechnology (Nanjing, Jiangsu, China). Dexamethasone (DXM) was purchased from the National Institutes for Food and Drug Control (Beijing, China). IL-1β, IL-6, TNF-α and IgE ELISA kits were obtained from Elabscience Biotech. Co. Ltd. (Wuhan, Hubei, China). Myeloperoxidase (MPO), Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) commercial kits were provided by the Jiancheng Institute of Biotechnology (Nanjing, Jiangsu, China). The NO detection kit was obtained from the Beyotime Institute of Biotechnology (Nanjing, Jiangsu, China). All antibodies were purchased from Cell Signalling Technology (Danvers, MA, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies (Carlsbad, CA, USA).

2.2. Animals

Male Sprague-Dawley rats (7–8 weeks, 180–220 g body weight) were obtained from Experimental Animal Centre of Medical College, Jilin University (Changchun, Jilin, China). The rats were maintained in an animal facility under standard laboratory conditions for 1 week prior to the experiments. The animals were provided with water and standard chow ad libitum. All experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.3. Experimental protocol for acute lung injury model

According to the weight, all rats were divided into six groups using the stratified randomized grouping method (n = 12): control group, LPS group, LPS + DXM (2 mg/kg) group, LPS + DEX (12.5 μg/kg), LPS + DEX (50 μg/kg) group, LPS + DEX (50 μg/kg) + glycyrrhizin group. The drugs were dissolved in 0.5% Sodium carboxymethylcellulose (CMC-Na), and then further diluted in vehicle (CMC-Na: PBS = 1:19, v/v). DEX and DXM were intraperitoneally administered once daily for three days. At 1 h after the drug treatment, the rats were intraperitoneally treated with glycyrrhizin (50 mg/kg, Sigma, Aldrich) for three continuous days. Simultaneously, the control and model group were administered with vehicle at equal volumes. At 1 h after the last treatment of glycyrrhizin treatment, the rats were acutely intratracheally instilled with 8 mg/kg LPS in 200 μl sterile PBS. Briefly, the LPS solution was instilled into the nose of rats with a pipette. Subsequently, the rats were gently shaken horizontally for 30 s to proportionately distribute the LPS solution in the lung tissues. Animals in control group were intratracheally injected with 200 μl of PBS without LPS. All rats were sacrificed at 6 h post LPS stimulation. Blood was collected from the abdominal aorta and subsequently centrifuged at 3000*g* for 10 min. Thereafter the serum was maintained at −80 °C for pending tests.

2.4. Collection of bronchoalveolar lavage fluid (BALF)

The lungs were lavaged three times with 500 μl of sterile PBS (total volume 1.5 ml) to collect BALF at 6 h after LPS or PBS administration. A total volume of 1.3 ml was recovered. The BALF samples were centrifuged at 3000*g* for 10 min at 4 °C and the cell-free supernatants were maintained at −80 °C to determine the cytokine concentrations.

2.5. Myeloperoxidase (MPO) activity assay

The MPO activities in lung homogenates were evaluated by using commercial test kits according to manufacturer's instructions. Lung samples were homogenized with cold normal saline and centrifuged at 12,000*g* (4 °C) for 20 min. Subsequently, the supernatants were collected and stored at −80 °C. The protein concentrations were calculated by using a BCA protein assay kit. Thereafter, 100 mg of the lung tissues was homogenized to obtain 5% homogenates. Then, the sample was placed in a 37 °C water bath for 15 min. The enzymatic activity was detected at 460 nm using a 96-well plate reader.

2.6. Pulmonary histopathology

The lung specimens were excised at 6 h after LPS exposure. The pulmonary samples were fixed in 4% neutral-buffered formalin for 48 h, embedded in paraffin and sectioned at 5-μm thickness. Subsequently, haematoxylin-eosin staining was conducted following a standard protocol, and the pathological alterations in the pathological samples were observed under a light microscope in a blinded manner. Hyperaemia, haemorrhage, oedema in the alveolar wall and inflammatory cell infiltration was evaluated by a semi-quantitative scoring system : 0 : normal, 0.5 : mild or very small amounts, 1 : mild or small amounts, 2 : moderate or more amount, 3 : severe or large amount, and 4 : extremely severe or extremely large amount. All the scores were accumulated and calculated for statistical analysis.

2.7. Experimental protocol for cell culture

Human bronchial epithelial BEAS-2B cells (ATCC) were cultured in DMEM medium supplemented with 10% heat-inactivated foetal bovine serum (FBS, PAA, Australia), 100 IU/ml streptomycin and 100 IU/ml penicillin (Amresco, Solon, OH, U.S.) at 37 °C in humidified atmosphere with 5% CO_2. The cells were divided into seven groups: control group, LPS group, LPS + glycyrrhizin group, LPS + DEX (5 μM), LPS + DEX (10 μM) group, LPS + DEX (20 μM) group, and LPS + DEX (20 μM) + glycyrrhizin group. Briefly, BEAS-2B cells in log-phase were seeded onto 96-well culture plates at a density of 1×10^5 cells/ml for 24 h. The cells were pretreated with DEX (5, 10, and 20 μM). After 1 h, the cells were incubated with glycyrrhizin (20 μM) for another 1 h and stimulated with LPS (4 μg/ml). After incubation for 24 h, the cells or the supernatants were harvested for detection.

2.8. Small-interfering RNAs (siRNAs) treatment

The siRNA sequences were designed at the Shanghai GenePharma