Effects of Dexmedetomidine on Inflammatory Factors and Mitogen Activated Protein Kinase-Related Signaling Pathways of Alveolar Lavage Fluid in Mice with Lipopolysaccharide-Induced Acute Lung Injury

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To investigate the effects and mechanisms of Dexmedetomidine on inflammatory factors and mitogen-activated protein kinase related signaling pathways in alveolar lavage fluid of mice with acute lung injury induced by lipopolysaccharide. 36 clean-grade male Kunming mice were randomly divided into blank control group (normal control group), lipopolysaccharide-induced acute lung injury model group, and lipopolysaccharide+dexmedetomidine group, with 12 mice in each group. The lipopolysaccharide group mice were intraperitoneally injected with 0.1 ml 5 mg kg^{-1} Lipopolysaccharide, lipopolysaccharide+Dexmedetomidine mice were injected with 25 µg kg^{-1}. Dexmedetomidine intraperitoneally with 0.1 ml and 1 h later with 5 mg kg^{-1}. Lipopolysaccharide injected with 0.1 ml intraperitoneally and the blank control group mice were intraperitoneally injected with normal saline of the same volume as the lipopolysaccharide group. All mice in each group were sacrificed after intraperitoneal injection for 6 h, and their lung tissues were separated by thoracotomy and their bronchoalveolar lavage fluid was recovered for subsequent detection. Analysis groups of mice lung tissue pathology, compare groups of mice lung tissue pathology score, lung wet weight/dry weight (W)/(D), mitogen-activated protein kinase pathways (p38 lightning, extracellular signal regulating kinase, c-jun amino terminal kinase) and bronchoalveolar lavage fluid protein expression level of tumor necrosis factor alpha beta, interleukin-1β, myeloperoxidase, protein concentration. In the blank control group, lung tissue structure was basically normal and alveolar structure was intact. lipopolysaccharide group mice had obvious lung tissue damage, and inflammatory cells appeared in the alveoli, with significantly increased exudation of red blood cells and proteins. Lipopolysaccharide+Dexmedetomidine group mice lung tissue damage significantly improved, inflammatory cell infiltration decreased, and red blood cell, protein exudation significantly decreased. Lung histopathological scores and bronchoalveolar lavage fluid levels of tumor necrosis factor alpha, interleukin-1β and myeloperoxidase in lipopolysaccharide group were significantly higher than those in blank control group ($p<0.05$). The lung histopathological scores and the levels of tumor necrosis factor alpha, interleukin-1β and myeloperoxidase in bronchoalveolar lavage fluid of lipopolysaccharide+Dexmedetomidine group were significantly lower than those of lipopolysaccharide group ($p<0.05$). The protein concentration in W/D and bronchoalveolar lavage fluid in lipopolysaccharide group was significantly higher than that in blank control group ($p<0.05$). Protein concentration in W/D and bronchoalveolar lavage fluid of lipopolysaccharide+Dexmedetomidine group was significantly lower than that of lipopolysaccharide group ($p<0.05$). The protein expression levels of extracellular signal regulating kinase, c-jun amino terminal kinase and P-P38 in the lipopolysaccharide group were significantly higher than those in the blank control group ($p<0.05$). The protein expression levels of extracellular signal regulating kinase, c-jun amino terminal kinase and P-P38 in the lipopolysaccharide+Dexmedetomidine group were significantly lower than those in the lipopolysaccharide group ($p<0.05$). There was no significant difference in extracellular signal regulating kinase, c-jun amino terminal kinase and p38 protein expression levels in each group ($p>0.05$). Dexmedetomidine can significantly alleviate acute lung injury induced by lipopolysaccharide in mice, and its mechanism may be realized by blocking the activation of mitogen-activated protein kinase-related signaling pathway and thereby reducing inflammatory response, providing a new target for the treatment of sepsis induced acute lung injury in the futurey.

Key words: Acute lung injury, dexmedetomidine, mitogen-activated protein kinase, lipopolysaccharide, myeloperoxidase

Acute lung injury is mainly manifested as intractable hypoxemia, respiratory distress and other symptoms. Although the assessment and treatment techniques of acute lung injury have been improved, the incidence of acute lung injury is still increasing year by year in recent years, and no effective drugs have been found to treat acute lung injury[1]. Sepsis and other causes can all induce the onset of acute lung injury, especially sepsis, which is the main cause of acute lung injury[2]. However, the pathological process of acute lung injury is extremely complex, and its pathogenesis has not been fully revealed. Therefore, it is of great significance to explore the pathogenesis of acute lung injury in order to improve the clinical symptoms and prognosis of patients. Before clinical study showed that excessive inflammatory cytokines secretion can participate in the pathological process of acute lung injury and play an important role in the Tumor necrosis factor-α (TNF-α), Interleukin 1β (IL-1β), Interleukin-6 (IL-6), Myeloperoxidase, Myeloperoxidase (MPO) all belong to former inflammatory factor, can activate or enhance body inflammatory reaction[3,4]. Mitogen activated protein kinase (MAPK), which is widely present in many types of cells, can be activated under the induction of many factors such as ultraviolet light, inflammatory mediators and so on, and transfer the extracellular information to the nucleus, finally leading to a series of biological changes[5]. Dexmedetomidine (Dex) is currently widely used in critically ill patients, with ideal sedative and analgesic effects, and can be used as one of the adjuvant clinical anesthesia drugs[6]. Clinical studies have confirmed that dexmedetomidine plays an important role in resisting inflammatory response and protecting organs[7]. However, the mechanism of the anti-inflammatory effect of dex and sepsis induced acute lung injury is not clear, so the purpose of this study is to explore the effect of DEX on alveolar lavage fluid inflammatory factor and MAPK-related signaling pathway in mice with acute lung injury induced by lipopolysaccharide (LPS). 36 clean grade male Kunming mice were purchased from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences with production license No.: SCXY (Shanghai) 2019-0005 and body mass (23±2) g. All the 36 mice were raised in cages, 9 in each cage, feeding and drinking freely, changing day and night every 12 h, the temperature was 25~30°, the humidity was 55~60°, and they were raised according to SPF grade. Dexmedetomi was ordered from Nanjing Saihongrui Biotechnology

Co., LTD. Lipopolysaccharide was purchased from Beijing Keriiji Biotechnology Co., LTD. Normal saline was purchased from Youkang Hengye Biotechnology (Beijing) Co., LTD. ELISA kit was purchased from Guangzhou Jianlun Biotechnology Co., LTD. BCA protein detection kit was purchased from Shanghai Jingke Chemical Technology Co., LTD. Inverted microscope was purchased from Guangzhou Minmei Photoelectric Technology Co., LTD. Electronic balance purchased from Sedris Group, Germany; The marker was purchased from Meigu Molecular Instruments (Shanghai) Co., LTD. The cryogenic high speed centrifuge was purchased from Beijing Ganming Gene Technology Co. LTD. 36 mice were randomly divided into the blank control group, LPS group and LPS+Dex group, with 12 mice in each group. Among them, the LPS group mice were intraperitoneally injected with 5 mg kg^{-1} LPS, the LPS+Dex group mice were intraperitoneally injected with 0.1 ml 25 mg kg^{-1} LPS, and the blank control group mice were intraperitoneally injected with normal saline of the same volume as the LPS group. Each group was intraperitoneal injection followed by 6 h, followed by thoracotomy for lung tissue separation and recovery of Bronchoalveolar lavage fluid (BALF). He staining was used to detect and analyze the pathological changes of lung tissues of mice in each group. According to relevant literature[8], the pulmonary histopathological scores of mice in each group were analyzed, mainly including alveolar wall thickening, inflammatory cell infiltration, and hemorrhage. The scores were 0~4 points, 0 points: no damage occurred. 1 point: slight damage occurs;2 points: moderately impaired; Three points were poisoned and damaged, and the total score ranged from 0 to 12. The contents of TNF-α, IL-1β and MPO in BALF of mice in each group were detected by ELISA, and the protein level in BALF of mice in each group was detected by BCA protein kit. The middle lobe of the right lung of mice in each group was selected, and the exudate and blood stains on the surface were sucked dry and weighed on an electronic balance, which was the wet weight; After baking the middle lobe of the

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right lung in the oven for 84 h, it was taken out and weighed, which was the dry weight. The wet lung tissue weight (W)/dry lung tissue weight (D) was used to determine the degree of pulmonary tissue edema in each group. Western blot was used to detect the protein expression levels of MAPK-related pathways (P38, ERK and C-Jun n-terminal kinase (JNK)) in lung tissues of mice in each group. All data on this study adopts SPSS19.0 were analyzed, and the type of histopathological grading and BALF of TNF-α, IL-1β, MPO content measurement data such as adopt ($\bar{X}\pm s$) said, comparison between the two groups using t test, multiple sets of comparison between using single factor analysis of variance, $p<0.05$ as difference is significant. In the blank control group, lung tissue structure was basically normal and alveolar structure was intact. LPS group mice had obvious lung tissue damage, and inflammatory cells appeared in the alveoli, with significantly increased exudation of red blood cells and proteins. LPS+Dex group mice lung tissue damage significantly improved, inflammatory cell infiltration decreased, and red blood cell, protein exudation significantly decreased. ABC is shown in fig. 1. Lung histopathological scores and BALF levels of TNF-α, IL-1β and MPO in LPS group were significantly higher than those in blank control group ($p<0.05$). The lung histopathological scores and the levels of TNF-α, IL-1β and MPO in BALF of LPS+Dex group were significantly lower than those of LPS group ($p<0.05$) (Table 1). The protein concentration in W/D and BALF in LPS group

was significantly higher than that in blank control group ($p<0.05$). Protein concentration in W/D and BALF of LPS+Dex group was significantly lower than that of LPS group ($p<0.05$) is shown in Table 2. The protein expression levels of P-ERK, P-JNK and P-P38 in THE LPS group were significantly higher than those in the blank control group ($p<0.05$). The protein expression levels of P-ERK, P-JNK and P-P38 in the LPS+Dex group were significantly lower than those in the LPS group ($p<0.05$). There was no significant difference in ERK, JNK and p38 protein expression levels in each group ($p>0.05$) is shown in Table 3 and fig. 2. Sepsis caused by cell infection can induce acute lung injury, and its morbidity and mortality are extremely high. Clinical research proves, systemic inflammatory response, anti inflammation disorders in the pathogenesis of acute lung injury, however the specific mechanism is not fully revealed, and the clinical effective interventions have not been discovered, so to reveal the pathogenesis of acute lung injury in order to improve patients is the mainstream of the current clinical staff pursuit direction[9]. Dexmedetomidine, as a short-term sedative, has little effect on respiratory function[10]. The patient is easily aroused. Clinical literature has shown that dexmedetomidine can significantly inhibit sepsis and increase the expression level of inflammatory mediators in patients, which provides reference value for clinical treatment of sepsis[11]. It has also been reported that dexmedetomidine was used to intervene in acute lung injury caused by
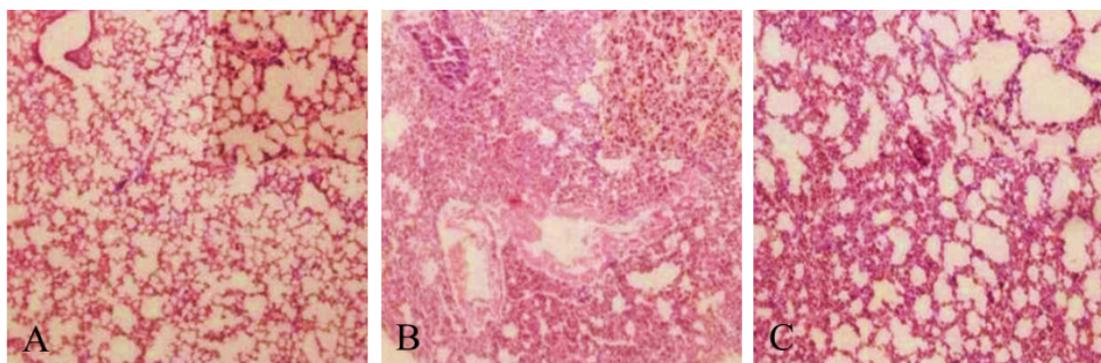

Fig. 1: Analysis of pathological changes in lung tissues of mice in each group
Note: A: Blank control group; B: LPS group; C: LPS+ Dex group

TABLE 1: NONHISTOPATHOLOGICAL SCORES AND BALF LEVELS OF TNF-A, IL-1B AND MPO IN EACH GROUP ($\bar{X}\pm S$)

Group	n	Lung histopathological Scores (score)	TNF-α (nmol/L)	IL-6 (μmol/L)	MPO (U/gprot)
Control group	12	1.05±0.02	181.11±15.12	31.36±4.31	17.49±3.62
LPS group	12	10.18±0.24*	302.33±23.66*	68.48±5.91*	31.36±2.49*
LPS+ Dex group	12	5.88±0.11#	265.08±30.38#	51.02±10.63#	20.47±3.96#

Note: * indicates compared with the blank control group $p<0.05$; # indicates compared to the LPS group $p<0.05$

TABLE 2: COMPARISON OF PROTEIN LEVELS IN W/D AND BALF IN EACH GROUP

Group	n	W/D	Protein concentration (%)
Control group	12	2.40±0.22	108.70±20.21
LPS group	12	5.99±0.24*	360.60±11.01*
LPS+Dex group	12	3.51±0.37#	10.82±15.70*

Note: * indicates compared with the blank control group p<0.05; # indicates compared to the LPS group p<0.05

TABLE 3: COMPARISON OF PROTEIN EXPRESSION LEVELS IN MAPK-RELATED PATHWAYS OF MICE IN EACH GROUP

Group	n	p-ERK/ERK	p-JNK/JNK	p-p38/p38
Control group	12	100.02±10.11	100.00±2.15	103.05±3.15
LPS group	12	762.59±15.89*	126.05±2.00*	184.13±3.01*
LPS+Dex group	12	180.05±14.05#	104.05±2.15#	108.98±3.27#

Note: * indicates p<0.05 compared with the blank control group; # indicates p<0.05 compared to the LPS group
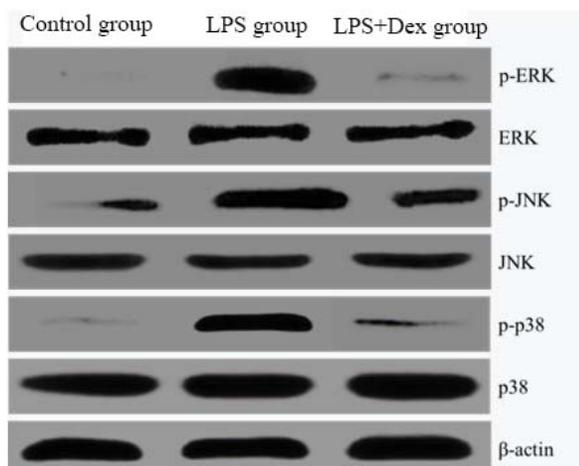

Fig. 2: Comparison of protein expression levels in MAPK-related pathways of mice in each group

limb ischemia-reperfusion in rats, and it was found that the inflammatory response in rats was significantly alleviated[12]. Clinical studies have confirmed that dexmedetomidine can significantly reduce the degree of ovarian tissue ischemia reperfusion injury. Studies on liver ischemia-reperfusion injury have shown that dexmedetomidine can play a protective role against erythrocyte deformability changes. In addition, dexmedetomidine can alleviate lung injury caused by pulmonary ischemia reperfusion[13]. Many foreign literatures have shown that in clinical practice, the use of dexmedetomidine in sedative sepsis patients can significantly reduce the production of inflammatory mediators, which is conducive to the alleviation of inflammatory responses and the extension of patient survival time[14]. All the above studies have confirmed that dexmedetomidine can play an anti-inflammatory and organ protective role. In this study, dexmedetomidine was used to intervene in mice with acute lung injury induced by LPS, and it was found that dexmedetomidine could significantly alleviate histopathological changes in mice with acute lung injury, reduce inflammatory cell infiltration, and significantly reduce erythrocyte and protein exudation. Clinical studies have confirmed that TNF-α and IL-1β can mediate the pneumonia-mediated response in acute lung injury/acute respiratory distress syndrome. The lung hiathological scores and BALF levels of TNF-α, IL-1β, MPO, protein concentration and W/D in LPS+ Dex group were significantly lower than those in LPS group (p<0.05). The results showed that dexmedetomidine could significantly reduce the release of TNF-α and other pre-inflammatory factors in bronchoalveolar lavage fluid of mice with acute lung injury, and improve the state of pulmonary edema. MAPK signaling pathways mainly include ERK, JNK and P38 signaling pathways, which are of great significance for maintaining normal physiological functions of cells, and the three pathways are interrelated to form a complex network[15]. Clinical studies have shown that LPS can participate in the pathogenesis of acute lung injury in sepsis and induce inflammatory responses. LPS can bind to cell membrane receptors and initiate signal transduction to the system, thereby activating the MAPK signaling pathway and ultimately accelerating the transcription of related genes to release many inflammatory mediators. The results of this study showed that the protein expression levels of P-ERK, P-JNK and P-P38 in the LPS+Dex group were significantly lower than those in the LPS group (p<0.05). This suggests that dexmedetomidine may reduce inflammatory responses in mice with acute lung injury by inhibiting MAPK-related signaling pathways. In summary, Dex can significantly alleviate acute lung injury induced by LPS in mice, and its mechanism may be realized by blocking the activation of MAPK-related signaling pathway and thereby reducing inflammatory response, providing a new target for the treatment of sepsis induced acute lung injury in the future.

Author's contribution:

Hui Xiao and S. Li contributed equally to this work as co-first author.

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