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The effects of dexmedetomidine administered at various times on acute lung injury in rats

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Abstract. – OBJECTIVE: To investigate the effects of dexmedetomidine (Dex) treatment administered at various times on acute lung injury (ALI).

MATERIALS AND METHODS: Thirty Wistar rats were randomly divided into five groups (n = 6/group). Lipopolysaccharide (LPS) was intraperitoneally injected into the rats in the LPS, Dex1, Dex2, and Dex3 groups to induce ALI, while the control group (C) was left untreated. Rats in the Dex1 group were intraperitoneally administered with 50 μg/kg Dex 30 minutes before modeling. Rats in the Dex2 group were injected with 25 μg/kg Dex 30 minutes before modeling and two hours after. Rats in the Dex3 group received 50 μg/kg Dex two hours after modeling. The animals in the C and LPS groups were given an equal volume of saline. The wet-to-dry (W/D) weight ratio of the rats' lungs was calculated, and pathological alterations in lung tissues were observed. The concentrations of inflammation-related factors and the expression of Janus kinase 1 (JAK1), signal transducer and activator of transcription 3 (STAT3), and matrix metallopeptidase 9 (MMP9) were measured.

RESULTS: The W/D ratio, expression of inflammatory factors, and expression of JAK1, STAT3, and MMP9 were significantly increased in the ALI rats ($p < 0.05$) compared with the C group. The level of anti-inflammatory factors in the Dex-treated groups was also significantly increased compared with the LPS group ($p < 0.05$). The concentration of anti-inflammatory factors in the Dex2 group was significantly higher than that recorded in the Dex1 and Dex3 groups ($p < 0.05$).

CONCLUSIONS: Dex treatment administered at different times protects rats against LPS-induced ALI to varying degrees. The protective effects of Dex were most robust when administered both before and after LPS stimulation.

Key Words:
Acute lung injury, Dexmedetomidine, Inflammatory cytokines, Lipopolysaccharide, Matrix metallopeptidase 9, Janus kinase 1, Signal transducer and activator of transcription 3.

Introduction

The clinical features of acute lung injury (ALI) are progressive dyspnea and refractory hypoxemia. Late-stage ALI often leads to acute respiratory distress syndrome (ARDS), which is a critical illness with poor prognosis that has high morbidity and mortality in critically ill patients[1]. Dexmedetomidine (Dex) has been used in clinical settings due to its sedative, analgesic, diuretic, and anti-sympathetic effects. Dex alleviates the inflammatory response in septic mice, and its anti-inflammatory effect may be related to the cholinergic anti-inflammatory pathway[2]. Similar to the α_2 adrenergic receptor agonist, Dex has an imidazoline structure, and activation of the imidazoline receptor can exert an anti-inflammatory effect. Studies have shown that Dex not only inhibits the inflammatory response but also protects important organs, such as the heart, lung, brain, kidney, and liver[3-5]. In addition, recent evidence[6-8] suggests that the protective effects of Dex on the tissues may vary depending on the time at which it is administered.

Lipopolysaccharide (LPS)-induced lung injury models are widely used to study the pathogenesis and treatment of ALI and ARDS[9,10]. Research has suggested that Dex exerts its pre-treatment effect 30 minutes before modeling[11]. Two hours after LPS modeling, inflammatory cells have been shown to start increasing and infiltrating the

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lung tissues. In light of these findings, Dex was administered 30 minutes before and two hours after modeling to evaluate its protective effects on ALI. A rat ALI model was established via intraperitoneal injection of LPS. The effects of Dex treatment at three different times (pre-treatment, pre-treatment plus post-treatment, and post-treatment) on inflammatory responses and JAK/STAT signaling pathways in ALI rats were investigated.

Materials and Methods

Clean-grade healthy adult male Wistar rats (Beijing Vital River Laboratory Animal Technology Co., Beijing, China) weighing 200-250 g were randomly divided into five groups (n = 6/group): a blank control (C) group, a lipopolysaccharide (LPS) group, a dexmedetomidine pre-treatment (Dex1) group, a dexmedetomidine combined treatment (Dex2) group, and a dexmedetomidine post-treatment (Dex3) group. The LPS group was administered an ALI model of 12.5 ml/kg LPS (Beijing Solarbio Science Technology Co., Beijing, China) by intraperitoneal injection. The Dex1 group underwent intraperitoneal injection of 12.5 ml/kg Dex (Jiangsu Hengrui Medicine Co., Lianyungang, China), intraperitoneal injection of 12.5 ml/kg LPS after 30 minutes, and an intraperitoneal injection of 12.5 ml/kg NS after two hours of modeling. Dex2 was administered at 6.25 ml/kg Dex and 6.25 ml/kg NS intraperitoneally, with 12.5 ml/kg LPS injected intraperitoneally 30 minutes later, and 6.25 ml/kg Dex and 6.25 ml/kg NS administered two hours after modeling. The Dex3 group was administered 12.5 ml/kg NS intraperitoneally, with 12.5 ml/kg LPS injected intraperitoneally 30 minutes later, and 12.5 ml/kg Dex administered two hours after modeling. The C and LPS groups were given the same amount of normal saline at the same time. The rats were anesthetized using 20% urethane (1 g/kg intraperitoneal injection) and euthanized six hours after the establishment of the model. Tail arteriovenous blood was collected, and lung tissue was retained. This study was conducted with approval from the Ethics Committee of Hebei University (2018020).

Wet-to-Dry (W/D) Weight Ratio of Lung Tissue

The right upper lobe tissue was removed, washed with normal saline, wiped, and weighed. Following this, the lung tissues were dried in a drying oven for 48 hours, after which they were weighed again to obtain the dry weight. The W/D ratio was calculated according to the formula W/D = wet weight (g)/dry weight (g).

Histological Analysis

The left lower lobe of the lung tissues was fixed with 4% paraformaldehyde, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and observed under a microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

The expression of inflammatory and anti-inflammatory factors in plasma was detected using ELISA kits (NEOBIOSCIENCE Biotechnology Co., Shenzhen, China) according to the manufacturer's instructions.

Immunohistochemical Detection of Janus Kinase 1 (JAK1), Signal Transducer and Activator of Transcription 3 (STAT3), and Matrix Metallopeptidase 9 (MMP9) in Lung Tissues

The right middle lobe tissue was harvested for tissue sectioning. The sections were dewaxed into water, incubated with 3% H_2O_2 for ten minutes, and washed with phosphate-buffered saline (PBS) four times for five minutes. They were then microwaved, washed with PBS three times, then incubated with serum, washed again with PBS, and incubated with designated primary antibodies. They were washed for a final time with PBS. The anti-JAK1, anti-STAT3, and anti-MM9 antibodies were purchased from Abcam (Cambridge, UK). Horseradish enzyme was then incubated, and tissue pieces were washed with PBS. DAB was added dropwise to avoid light, and hematoxylin was counterstained and dehydrated. Neutral gum seals were then performed and observed under a microscope. The optical density of the positive cells was analyzed using IPP software (version 5.0, Sydney, Australia).

Detection of mRNA Expression Levels of JAK1, STAT3, and MMP9 by Real-Time PCR

A total of 100 mg was taken from the left upper lobe tissue, added to the homogenate tube to grind the tissue, and centrifuged. The Ribonucleic Acid (RNA) was extracted (Servicebio, Wuhan, China). Following this, reverse transcription was performed using the RevertAid First Strand

cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) on a Polymerase Chain Reaction (PCR) instrument. Finally, a PCR amplification experiment was conducted using the FastStart Universal SYBR Green Master (Roche, Basel, Switzerland). The ΔΔCT method involves the A=CT value of the target gene-CT value of the reference gene, the B=A value of the experimental group-A value of the blank group, and the expression multiple=2^{-B}. The sequences of primers (Servicebio) were as follows: β-actin forward: CGTTGACATCCGTAAAGACC; β reverse: GCTAGGAGCCAGGGCAGTA. MMP-9 forward: CGTTGACATCCGTAAAGACC; MMP-9 reverse: GTTGTGGAAACTCACACGCC. JAK1 forward: ATGGAGTTTCTGCCTTCGGG, JAK1 reverse: CTCCGGAGCGTACCAAAACA. STAT3 forward: AAAGTATTGTCGCCCCGAGA, STAT3 reverse: CAGGTCGTTGGTGTCACACAG.

Statistical Analysis

Data were presented as mean ± standard deviation (`x ± s). One-way analysis of variance was used for comparing W/D in lung tissue, the concentrations of IL-10, TNF-α and IFN-γ, the protein expression of JAK1, STAT3, and MMP9, and the mRNA expression levels of JAK1, STAT3, and MMP9 among different groups. When $p <$ 0.05, Least Significant Difference was further used for testing. Data analysis was performed using SPSS19.0 s software.

Results

Dex Treatment Alleviated LPS-Induced Pathological Alterations in Lung Tissue

In the control group, the lung tissue structure was considered normal. The alveolar wall was smooth, no evident thickening was observed, and no significant abnormal changes were observed in the structure of the alveolar space. In the LPS group, the lung tissue structure was clearly damaged. The alveolar wall showed evident thickening, the pulmonary interstitial capillaries showed clear expansion and congestion, and inflammatory cell aggregation was observed. The rats in the Dex1 and Dex3 groups evidenced clear lung tissue structure. However, there was still damage observed, such as thickening of the alveolar wall, an accumulation of red blood cells, and inflammatory cells in the interstitial lung. The degree of structural damage to the lung tissue in the Dex2 group was less than that observed in the Dex1 and Dex3 groups. In the Dex2 group, the alveolar wall was slightly thickened. Only a small number of red blood cells and the level of inflammatory cell infiltration were observed in the interstitial lung (Figure 1).

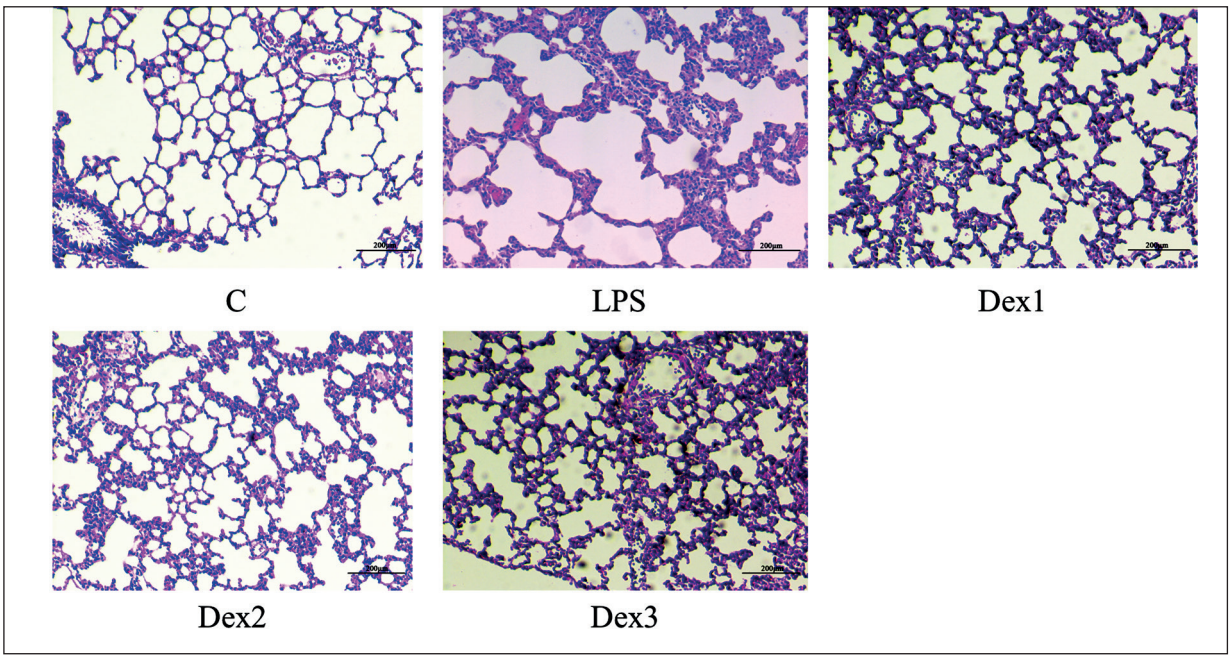

Figure 1. Pathological analysis of rat lung tissue samples using H&E staining (100× magnification).

Dex Treatment Decreased the W/D Ratio and Suppressed Inflammatory Responses in ALI Rats

The W/D ratio and the concentrations of IL-4, IL-10, TNF-α, and IFN-γ in the LPS group were significantly increased compared with group C ($p < 0.05$). The W/D ratio, TNF-α level, and IFN-γ concentration in the Dex-treated rats significantly decreased, while the concentrations of IL-4 and IL-10 significantly increased compared with rats in the LPS group ($p < 0.05$). The W/D ratio and levels of TNF-α and IFN-γ in the Dex2 group were significantly lower than those in the Dex1 and Dex3 groups, while the concentrations of IL-4 and IL-10 were significantly higher ($p < 0.05$; Tables I and II).

Dex Treatment Regulated the Expressions of JAK1, STAT3, and MMP9 in ALI Rats

The mRNA and protein expression of JAK1, STAT3, and MMP9 in the LPS-injured rats were significantly higher than those in the control group ($p < 0.05$). The expression levels of JAK1, STAT3, and MMP9 in the Dex-treated rats significantly decreased compared with the LPS group ($p < 0.05$). The expression of JAK1, STAT3, and MMP9 in the Dex2 group were significantly lower than those in the Dex1 and Dex3 groups at both protein and mRNA levels ($p < 0.05$). In addition, there was no significant difference between the Dex1 and Dex3 groups ($p > 0.05$; Tables III and IV).

Discussion

In animal studies, the lung tissue W/D ratio has been shown to directly affect pulmonary vascular permeability[12]. In the present study, the W/D ratio of lung tissue in the LPS group significantly increased. Pathological changes observed under a light microscope showed that the lung tissue structure had clear damage, with thickening of the alveolar wall, pulmonary interstitial capillary congestion and edema, and inflammatory cell aggregation, all of which revealed ALI. Therefore, the model was successful[13]. Although the lung tissues of rats in the Dex1, Dex2, and Dex3 groups showed varying degrees of damage, the lung tissue damage was alleviated compared with the LPS group. This suggests that Dex exerts a protective effect and can alleviate damage to the lung tissue.

The ELISA results showed that the levels of TNF-α and IFN-γ were significantly higher in the LPS group compared with the control group. In comparison, the levels of IL-4 and IL-10 in the Dex pre-treatment, combined treatment, and post-treatment groups were significantly lower than those in the LPS group. These results suggest that Dex can exert a protective effect on the lungs by inhibiting the release of inflammatory factors and promoting anti-inflammatory factors *in vivo*. Elsewhere, it has been shown that Dex can increase the expression of IL-4 and IL-10 through the Th2 cell pathway, thereby exerting an inhibitory effect on inflammatory bowel disease[14]. In the present study, the expressions of TNF-α, IFN-γ, IL-4, and IL-10 in the lung tissue of the Dex combined-administration group indicated that the protective effect of Dex combined-adminis-

Table I. The W/D ratio of lung tissue in five groups (n=6/group, $\bar{x} \pm s$).

Group	W/D
C	3.32 ± 0.06
LPS	$5.10 \pm 0.15^{\Delta}$
Dex1	$4.31 \pm 0.07^{\Delta\#\star}$
Dex2	$3.84 \pm 0.05^{\Delta\#}$
Dex3	$4.38 \pm 0.09^{\Delta\#\star}$

$^{\Delta}p < 0.05$ compared with group C; $^{\#}p < 0.05$ compared with LPS group; $^{\star}p < 0.05$ compared with Dex2 group.

Table II. Concentrations of IL-4, IL-10, TNF-α and IFN-γ in five groups (n=6/group).

Group	IL-4 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	IFN-γ (pg/ml)
C	38.67 ± 1.46	106.39 ± 9.89	162.72 ± 2.05	21.22 ± 7.01
LPS	$46.87 \pm 0.89^{\Delta}$	$147.91 \pm 8.73^{\Delta}$	$335.20 \pm 17.39^{\Delta}$	$211.87 \pm 19.47^{\Delta}$
Dex1	$51.78 \pm 1.52^{\Delta\#\star}$	$176.91 \pm 7.44^{\Delta}\#^{\star}$	$257.99 \pm 2.53^{\Delta\#\star}$	$139.12 \pm 9.13^{\Delta\#\star}$
Dex2	$57.43 \pm 1.47^{\Delta\#}$	$202.03 \pm 8.49^{\Delta\#}$	$237.11 \pm 10.93^{\Delta\#}$	$96.14 \pm 7.81^{\Delta\#}$
Dex3	$50.70 \pm 1.30^{\Delta\#\star}$	$170.18 \pm 5.49^{\Delta\#\star}$	$268.14 \pm 7.42^{\Delta\#\star}$	$157.80 \pm 26.74^{\Delta\#\star}$

$^{\Delta}p < 0.05$ compared with group C; $^{\#}p < 0.05$ compared with LPS group; $^{\star}p < 0.05$ compared with Dex2 group.

Table III. Protein expressions of JAK1, STAT3 and MMP9 in five groups (n=6/group, $\bar{x} \pm s$).

Group	JAK1	STAT3	MMP9
C	12.51 ± 1.74	9.34 ± 0.54	24.67 ± 3.61
LPS	28.02 ± 1.44$^{\Delta}$	18.94 ± 1.41$^{\Delta}$	98.17 ± 6.08$^{\Delta}$
Dex1	19.05 ± 1.52$^{\Delta\#\star}$	14.01 ± 1.16$^{\Delta\#\star}$	65.33 ± 4.63$^{\Delta\#\star}$
Dex2	15.78 ± 2.60$^{\Delta\#}$	11.11 ± 1.43$^{\Delta\#}$	52.67 ± 2.73$^{\Delta\#}$
Dex3	21.04 ± 1.82$^{\Delta\#\star}$	15.37 ± 1.84$^{\Delta\#\star}$	70.67 ± 4.84$^{\Delta\#\star}$

$^{\Delta}p < 0.05$ compared with group C; $^{\#}p < 0.05$ compared with LPS group; $^{\star}p < 0.05$ compared with Dex2 group.

Table IV. mRNA expression levels of JAK1, STAT3 and MMP9 in five groups (n=6/group, $\bar{x} \pm s$).

Group	JAK1	STAT3	MMP9
C	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
LPS	1.79 ± 0.11$^{\Delta}$	3.53 ± 0.06$^{\Delta}$	25.29 ± 1.87$^{\Delta}$
Dex1	1.54 ± 0.09$^{\Delta\#\star}$	2.52 ± 0.08$^{\Delta\#\star}$	15.25 ± 2.03$^{\Delta\#\star}$
Dex2	1.31 ± 0.09$^{\Delta\#}$	1.77 ± 0.09$^{\Delta\#}$	5.98 ± 1.08$^{\Delta\#}$
Dex3	1.56 ± 0.08$^{\Delta\#\star}$	2.60 ± 0.08$^{\Delta\#\star}$	16.33±2.06$^{\Delta\#\star}$

$^{\Delta}p < 0.05$ compared with group C; $^{\#}p < 0.05$ compared with LPS group; $^{\star}p < 0.05$ compared with Dex2 group.

tration on inflammatory reactions in ALI lung tissue was more significant than those in the LPS group.

The JAK/STAT signaling pathway is considered the major cytokine signaling pathway and plays a key role in cell cycle progression. Activation of the JAK/STAT signaling pathway may be inextricably linked to LPS, and inhibition of the JAK/STAT signaling pathway has indicated a certain protective effect on organs in animal sepsis models[15]. In the present experiment, the expressions of JAK1 and STAT3 in the LPS group increased significantly, while the expressions of JAK1 and STAT3 in Dex-treated rats decreased significantly. This suggests that Dex may inhibit JAK1/STAT3 signal transduction pathways by inhibiting the expression of JAK1 and STAT3, thereby resulting in lung protection.

MMP9 is an advanced protease in the MMP family. In the physiological state, only a small amount of MMP9 is expressed in lung tissue. In a pathological state, lung parenchymal cells, such as bronchial epithelial cells, fibroblasts, smooth muscle cells, endothelial cells, type II alveolar cells, and various inflammatory cells, such as neutrophils, eosinophils, lymphocytes, and mast cells, can produce MMP9. The expression of MMP9 in the Dex treatment group was significantly lower than that in the LPS group, and it was shown that reducing the expression of MMP9 reduced the degree of tissue damage[16]. The combination of Dex pre-treatment and post-treatment showed a significant decrease in the expression of MMP9 in lung tissue, while the lung protection was more pronounced.

Conclusions

In summary, our study showed that pre-treatment, combined treatment, and post-treatment with Dex all protected rats against LPS-induced lung injury. This protective effect may inhibit the release of inflammatory factors, increase the level of anti-inflammatory factors, and down-regulate the expressions of MMP9, JAK1, and STAT3 in lung tissue. We showed for the first time that the most robust protective effect of Dex was observed when administered both before and after LPS stimulation, which could be due to the pharmacokinetics of Dex and the pathophysiological processes of lung injury. Therefore, combined Dex administration not only plays a preventive role in pre-treatment but also exerts a therapeutic effect in post-treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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