

# Dexmedetomidine Mitigates LPS-Induced Acute Lung Injury in Rats Through HMGB1-Mediated Anti-Inflammatory and Antioxidant Mechanisms

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## ABSTRACT

**Purpose:** To investigate the effect of dexmedetomidine on lipopolysaccharide (LPS)-induced acute lung injury in rats, and the underlying mechanism.

**Methods:** Healthy male SD rats (n=54) were randomly divided into three groups: normal, model and dexmedetomidine groups, with 18 rats in each group. Rats in the model and dexmedetomidine groups were given LPS at a dose of 8 mg/kg, to establish a model of acute lung injury. Rats in the dexmedetomidine group were injected intraperitoneally with dexmedetomidine at a dose of 50 µg/kg prior to establishment of the model, while rats in the normal group received intraperitoneal injection of normal saline in place of dexmedetomidine. Hematoxylin and eosin (H&E) staining was used to observe changes in lung tissue in each group. Changes in wet/dry weight ratio of lung tissue were compared among the groups. Enzyme-linked immunosorbent assay was used to determine the expressions of inflammation indices i.e. interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1β (L-1β)] in lung tissue. Levels of MDA were measured with thiobarbituric acid method. Superoxide dismutase (SOD) activity was assayed through enzyme rate method, while nitric oxide was measured using nitrate reductase assay. The expression levels of high mobility group protein B1 (HMGB1), p-PI3K, p-Akt, p-IκB, p-NF-κB, and Toll-like receptor 4 (TLR4) in lung tissue were determined with Western blotting.

**Results:** In the normal group, lung tissue structure was intact, with clear alveolar cavity, non-edematous alveolar space, and absence of inflammatory infiltration. In the model group, lung tissue was disordered, with thinner alveolar cavity, widened and thicker alveolar space, and inflammatory cell infiltration. Lung tissue of the dexmedetomidine group was significantly improved, relative to the model group. Compared with the normal group, lung wet/dry ratio, and levels of inflammatory indices (MDA, NO and HMGB1, p-PI3K, p-Akt, p-IκB, p-NF-κB, and TLR4) were significantly increased in the model group, while the SOD level was significantly reduced (p<0.05). However, compared with the model group, wet/dry ratio of lung, MDA, NO and HMGB1, p-PI3K, p-Akt, p-IκB, p-NF-κB, and TLR4 levels were significantly decreased, while SOD levels increased significantly (p<0.05).

**Conclusion:** Dexmedetomidine mitigates LPS-induced acute lung injury in rats through HMGB1-mediated anti-inflammatory antioxidant effect, which may be closely related to the TLR4/NF-κB and PI3K/Akt pathways.

**Keywords:** Dexmedetomidine, HMGB1, anti-inflammatory, antioxidant effects, LPS, acute lung injury.

## INTRODUCTION

Acute lung injury is an acute, progressive and

aggravated dyspnea and refractory hypoxemia due to a variety of intra-pulmonary and external pathogenic factors, and it results in acute respiratory distress syndrome. Lipopolysaccharide (LPS)-induced endotoxemia is the most common cause of acute lung injury. However, the pathogenesis of acute lung injury is complicated.

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Currently, it is thought to be closely related to inflammatory mediators and oxidative stress [1]. Accumulation of reactive oxygen free radicals enhances the expressions of inflammatory factors and exacerbates inflammatory response. Inflammatory factors kill harmful microorganisms, but at the same time, excess levels of inflammatory factors damage lung tissue [2]. High-mobility protein B1 (HMGB1), a closely conserved nuclear protein that is commonly dispersed in mammalian cells, facilitates aggressive immune cell secretion and often stimulates the development of inflammatory factors. [3]. Outside sedation and respiratory support which serve as mere palliatives, there are no effective treatments for acute lung injury.

Dexmedetomidine is an  $\alpha_2$  adrenergic receptor agonist with good sedative and analgesic effects, and mild and easily-reversible depressive effect on respiration [4]. Studies have revealed that dexmedetomidine significantly reduces the expression of inflammatory factors, decreases pulmonary edema, and mitigates pathological changes associated with hyperoxia-induced acute lung injury, but its mechanism of action is unclear [5]. This research was performed to explore the impact of dexmedetomidine on acute LPS-induced lung injury in rats and the process involved.

## MATERIALS AND METHODS

### Experimental animals

A total of 54 stable male SD rats (mean weight =  $218 \pm 12$  g) were given by the Guangdong Medical Experimental Animal Center [producer authorization SCXK (Guangdong) 2018-0035]. Rats were acclimatised for 1 week at a laboratory temperature of  $24 \pm 1$  °C, a humidity of  $53 \pm 3$  per cent, and a bright 12-hour dark photoperiod of 12-hours.

This report was accepted by the Xi'an Xidian Community Hospital Animal Ethics Committee in compliance with the 'Principles of Experimental Animal Treatment' (NIH publication No. 85-23, updated 1985), approval number is 201903362[6].

### Main instruments and reagents

The major reagents and instruments used, and their sources (in brackets) were: electronic balance (Shanghai JingkeTianmei Trading Co. Ltd., model: FA2204B); low-temperature, high-speed centrifuge (Beckman Coulter Trading China Co.Ltd., model: Allegra X-15R); -80°C refrigerator (Company, model: MDF-C8V (N)); biological microscope (Shanghai Putan Optical Instrument Co. Ltd., model: MM-4XB); NO assay kit (Shanghai Qiyi Biological

Technology Co. Ltd., specification: 48T); MDA assaykit (Shanghai Future Industry Co. Ltd., specification: 48T);SOD assay kit (Beijing Beiren ChemicalTechnology Co. Ltd., specification: 100T); IL-6 detection kit (Shenzhen Kerunda Biological Engineering Co. Ltd., specification: 48T); TNF- $\alpha$  assay kit (Shenzhen Branch) Runda Biological Engineering Co. Ltd., specification: 96T); IL-1 $\beta$  assay kit (Shanghai Kanglang Biotechnology Co. Ltd., specification: 96T); dexmedetomidine (Jiangsu Hengrui Pharmaceutical Co.Ltd., production batch number: 20180248, specification: 2ml: 200g), and lipopolysaccharide (Beijing Huanyu Biotechnology Co. Ltd.).

### Rat groups and treatments

The rats were randomly divided into three groups: regular control, model and dexmedetomidine, with 18 rats in each category. Acute lung damage in rats in the model and dexmedetomidine groups was calculated by administration of LPS at a dosage of 8 mg / kg. Rats in the dexmedetomidine community were injected intraperitoneally with dexmedetomidine at a dose of 50  $\mu$ g / kg prior to administration of LPS at a dose of 8 mg / kg, while rats in the usual control group were injected intraperitoneally with normal saline.

At the conclusion of the test, both rats had been killed.

### Observation indicators

After 5 h of establishment of acute lung injury, the rats were placed on the operating table. Tissue samples were taken from the upper and lower lobes of the left lung, and were lysed with lysing solution on ice. The supernatant was centrifuged and kept in the refrigerator prior to use.

The superior lobe of right lung was fixed in formalin solution. Paraffin sections of the lung tissue were subjected to H & E staining and examined under a light microscope for changes in lung tissue.

The wet weight/dry weight ratio of lung was determined using the lower rightlobe of the lungs. After removal of surface moisture, the lower right lobe of the lung was weighed. Then, it was re-weighed after drying in a 65 °C oven for 48h, and the dry weight was recorded.

The amounts of interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\omega$  (TNF-5-007) and leukocytes in the lung tissue of each community were calculated using an enzyme-linked immunosorbent assay (ELISA). Malondialdehyde (MDA) level in each group was measured using the thiobarbituric acid method. The activity of SODS

was assayed using enzyme rate method, while nitric oxide (NO) level in each group was determined using nitrate reductase method. The expression levels of HMGB1, p-PI3K, p-Akt, p-I $\kappa$ B, p-NF- $\kappa$ B, and Toll like receptor 4 (TLR4) in lung tissue of each group were determined with Western blotting.

#### STATISTICAL ANALYSIS

Measurement data was matched between two groups using independent sample t-tests, while single-factor multi-sample mean analyses were used for analysis of different groups. Both statistical analyses have been conducted for the SPSS 20.0 software kit. Values with  $p < 0.05$  have been taken as representative with statistical importance.

#### RESULTS

##### Changes in lung tissue of rats in each group

In the usual test sample, the lung tissue framework remained unchanged. The alveolar cavity was open, the alveolar space was clean of the oedema, and there was no inflammatory cell infiltration. In the model community, lung tissue composition was disrupted, with narrower alveolar cavity, larger and thicker alveolar space and inflammatory cell infiltration. There has been a major change in the lung tissue design in the dexmedetomidine group relative to the control community. The findings are seen in the figure 1.

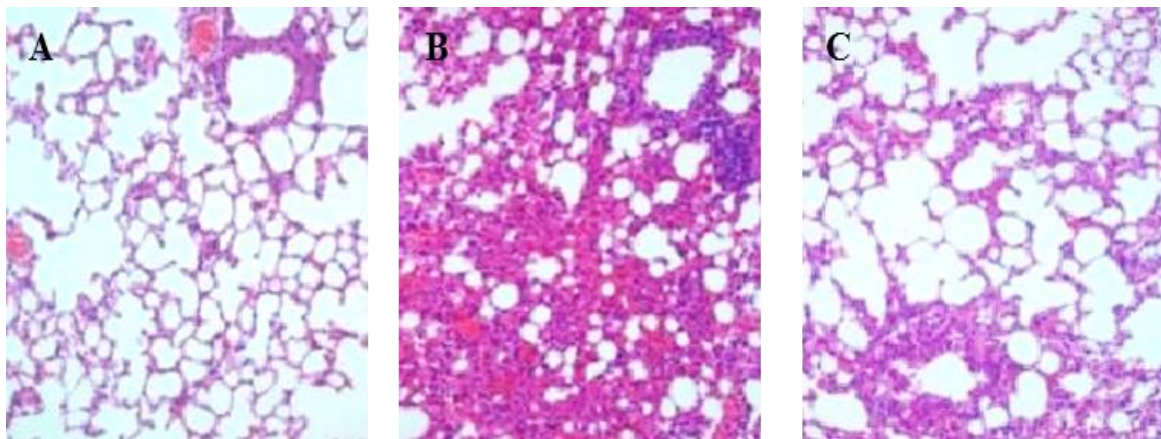


Figure 1. Changes in lung tissue of rats in each group.

A: normal control group; B: model group; C: dexmedetomidine group

##### Changes in lung wet weight/dry weight ratio of rats in each group

Compared with the standard population, there was a substantial rise in the wet / dry lung ratio in the test community ( $p < 0.05$ ). Compared to the test

population, the wet / dry lung ratio in the dexmedetomidine community was substantially diminished ( $p < 0.05$ ). The findings are seen in Table 1.

Table 1. Changes in wet/dry ratio of lung of each group of rats ( $\bar{x} \pm s$ )

Group	n	Wet weight/dry weight ratio of lung
Normal control	18	3.86 $\pm$ 1.25
Model	18	5.66 $\pm$ 1.41 <sup>a</sup>
Dexmedetomidine	18	4.02 $\pm$ 0.73 <sup>b</sup>
F		13.13
P		<0.001

<sup>a</sup> $P < 0.05$ , compared with the normal group, <sup>b</sup> $p < 0.05$ ; compared with the model group.

##### Comparison of inflammatory indices of rats in each group

As seen in Table 2, relative to the usual sample, the amounts of IL-6, TNF-5-007 and IL-1 $\beta$  in the lung tissues of the model community were substantially increased. In comparison, there was a substantial rise in the levels of IL-6 and TNF in the lung tissue of

the dexmedetomidine community compared to the sample group, whereas the levels of TNF-5-007 and IL-1 $\beta$  were slightly decreased ( $p < 0.05$ ).

##### Comparison of indices of oxidative stress among the rat groups

Table 3 indicates that the amounts of MDA and NO in the lung tissue of the model group were

substantially higher than those in the normal community, yet the development of SOD in the model group was significantly lower than in the normal group ( $p < 0.05$ ). In comparison, the amounts of MDA and NO in the lung tissues of the

dexmedetomidine community were significantly lower than in the control group, although the activity of SOD was significantly higher than in the model group ( $p < 0.05$ ).

**Table 2. Comparison of inflammatory indices of rats in each group ( $\bar{x} \pm s$ )**

Group	n	IL-6 (pg/mg)	TNF- $\alpha$ (pg/mg)	IL-1 $\beta$ (pg/mg)
Normal	18	27.32 $\pm$ 5.36	199.99 $\pm$ 52.63	15.33 $\pm$ 3.69
Model	18	281.72 $\pm$ 14.29 <sup>a</sup>	926.51 $\pm$ 88.62 <sup>a</sup>	53.59 $\pm$ 5.56 <sup>a</sup>
Dexmedetomidine	18	171.56 $\pm$ 29.84 <sup>b</sup>	396.87 $\pm$ 76.48 <sup>b</sup>	35.77 $\pm$ 5.29 <sup>b</sup>
<i>F</i>		782.42	462.83	272.95
<i>P</i>		<0.001	<0.001	<0.001

<sup>a</sup> $P < 0.05$ , compared with the normal group, <sup>b</sup> $p < 0.05$ ; compared with the model group.

**Table 3. Comparison of oxidation indexes of rats in each group ( $\bar{x} \pm s$ )**

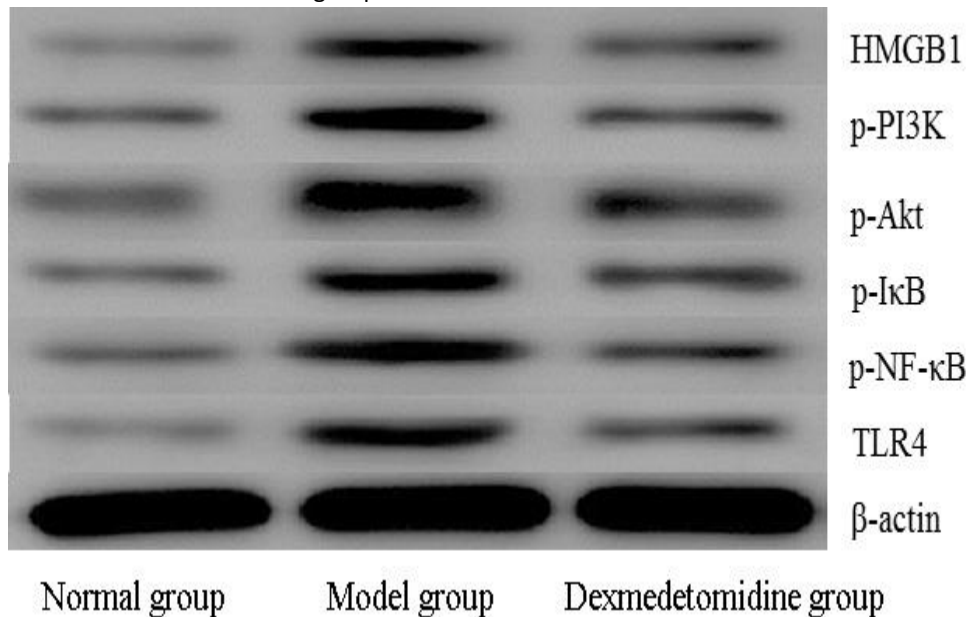
Group	n	MDA (nmol/mg)	SOD (U/mg)	NO ( $\mu$ mol/L)
Normal	18	1.62 $\pm$ 0.14	26.78 $\pm$ 4.33	16.47 $\pm$ 1.52
Model	18	6.59 $\pm$ 0.98 <sup>a</sup>	15.66 $\pm$ 3.27 <sup>a</sup>	45.36 $\pm$ 2.33 <sup>a</sup>
Dexmedetomidine	18	3.37 $\pm$ 0.41 <sup>b</sup>	21.67 $\pm$ 3.37 <sup>b</sup>	32.23 $\pm$ 1.72 <sup>b</sup>
<i>F</i>		298.92	41.01	106.18
<i>P</i>		<0.001	<0.001	<0.001

<sup>a</sup> $P < 0.05$ , compared with the normal group, <sup>b</sup> $p < 0.05$ ; compared with the model group.

#### Comparison of the expression levels of HMGB1, p-PI3K, p-Akt, and p-I $\kappa$ B, p-NF- $\kappa$ B, and TLR4 in lung tissue among the rat groups

Expression amounts of HMGB1, p-PI3 K, p-Akt, and p-I-USB, p-NF-USB, and TLR4 in the lung tissues of the model community were substantially higher than those of the usual control group

( $p < 0.05$ ). On the other hand, the expression levels of HMGB1, p-PI3 K, p-Akt, and p-I-CNB, p-NF-CNB, and TLR4 in the lung tissue of rats in the dexmedetomidine group were substantially lower than in the sample community ( $p < 0.05$ ). These effects are seen in both Figure 2 and Table 4.



**Figure 2. Comparison of the expression levels of HMGB1, p-PI3K, p-Akt, and p-I $\kappa$ B, p-NF- $\kappa$ B, and TLR4 in rat lung tissue among the groups**

**Table 4. Comparison of the expression levels of HMGB1, p-PI3K, p-Akt, and p-IkB, p-NF-κB and TLR4 in rat lung tissue among the groups**

Group	n	HMGB1	p-PI3K	p-Akt
Normal	18	1.01±0.02	1.00±0.21	0.99±0.03
Model	18	2.13±0.13 <sup>a</sup>	1.68±0.33 <sup>a</sup>	1.73±0.01 <sup>a</sup>
Dexmedetomidine	18	1.74±0.08 <sup>b</sup>	1.26±0.23 <sup>b</sup>	1.48±0.02 <sup>b</sup>
<i>F</i>		736.48	30.88	547.57
<i>P</i>		<0.001	<0.001	<0.001

Group	n	p-IkB	p-NF-κB	TLR4
Normal	18	1.00±0.01	1.01±0.02	0.99±0.03
Model	18	1.71±0.29 <sup>a</sup>	1.72±0.01 <sup>a</sup>	0.88±0.12 <sup>a</sup>
Dexmedetomidine	18	1.27±0.12 <sup>b</sup>	1.48±0.02 <sup>b</sup>	1.49±0.02 <sup>b</sup>
<i>F</i>		70.13	783.01	684.57
<i>P</i>		<0.001	<0.001	<0.001

<sup>a</sup>*P*<0.05, compared with the normal group, <sup>b</sup>*p*<0.05; compared with the model group.

## DISCUSSION

Acute lung injury, a clinical syndrome caused by a variety of factors is an early stage of acute respiratory distress syndrome. It is common in severe infections, traumas, shocks, and poisoning, with damaged alveolar capillary endothelial as the main pathological features [7]. Acute lung injury seriously affects the patient's respiratory system, especially lung tissue which does not return to normal even after effective treatment, thereby severely affecting the patient's quality of life. According to reports, infection is an important cause of acute lung injury, and infections caused by Gram-negative bacilli account for most infectious diseases [8]. Lipopolysaccharide (LPS) is a major component of the cell wall of Gram-negative bacilli. Studies have shown that LPS specifically recognizes and activates inflammatory mediators such as monocytes/macrophages and neutrophils, thereby inducing acute lung injury [9]. Dexmedetomidine is a highly selective receptor agonist which reduces the secretion and release of central norepinephrine by stimulating α<sub>2</sub> adrenergic receptors, thereby exerting a calming effect. It has been reported that dexmedetomidine regulates pathological processes in acute lung injury through multiple signaling pathways [10]. The present study investigated the effect of dexmedetomidine on LPS-induced acute lung injury in rats, and the mechanism(s) involved.

Cytokines are essential effector molecules and signalling molecules for acute lung damage and are implicated in cancer pathogenesis. Studies have shown that interruptions of homeostasis between pro-inflammatory and anti-inflammatory influences result of acute lung injury [11]. Cytokine

homeostasis is greatly compromised when severe lung damage happens. Interleukin-6 (IL-6) is a significant regulator of inflammatory cell differentiation; it is used as an indicator of inflammatory activity and disease intensity of the patient and may be used as a marker for the answer of the cytokine cascade to activation [12]. In addition, TNF-5-007 is a pro-inflammatory agent that induces cytokine signals such as IL-6 and IL-1 and promotes the production of a significant number of reactive oxygen species (ROS). The findings of this analysis show that dexmedetomidine greatly decreased levels of inflammatory factors and the degree of inflammatory cell infiltration.

It has been reported that oxidative damage is an important cause of acute lung injury due to imbalance between oxidative and antioxidant systems [13]. Malondialdehyde (MDA) is a product of lipid peroxidation in cell membrane lipid bilayers. The level of MDA is closely related to the amount of ROS. Superoxide dismutase (SOD) is an important physiological and endogenous antioxidant enzyme in all tissues, including the lungs. Nitric oxide (NO) reacts with various antioxidant enzymes in the body to maintain the stability of the internal environment. It protects lung tissue by removing superoxide anion under normal circumstances. When stimulated by LPS, the level of NO increases significantly, causing severe damage to alveolar capillary endothelial cells [14]. The findings of this research revealed that dexmedetomidine mitigated acute LPS-induced lung damage in rats due to antioxidant impact.

The high-mobility protein B1 (HMGB1) community is a non-histone chromosome-binding protein found in endothelial cells, epidermal cells, and alveolar macrophages in lung tissue. Studies have found that HMGB1 specifically binds to advanced glycation end-product protein receptors, and amplifies the cascade response in cells through NF- $\kappa$ B which in turn causes upregulations of IL-6 and other inflammatory factors [15]. The newly discovered transmembrane signaling receptor i.e. TLR4 links innate immunity and specific immunity. It mediates transmembrane signaling induced by LPS stimulation of Gram-negative bacilli. High mobility group protein B1 (HMGB1) activates the NF- $\kappa$ B pathway by binding to TLR4. It is known that Phosphatidylinositol-3 kinase / protein kinase (PI3K / Akt) signalling pathway plays an important role in the control of TLR4-mediated inflammation and suppression of undue immune response. Activation of the PI3K/Akt signaling pathway has significant effect on patients with acute lung injury: the expression level of p-Akt is significantly and positively correlated with the degree of cell damage [16]. In this study, dexmedetomidine significantly inhibited the expression of HMGB1 and the activation of the TLR4/NF- $\kappa$ B and PI3K/Akt pathways.

#### CONCLUSION

Dexmedetomidine mitigates LPS-induced acute lung injury in rats through HMGB1-mediated anti-inflammatory and antioxidant effects. The beneficial effect of dexmedetomidine may be closely associated with the TLR4/NF- $\kappa$ B and PI3K/Akt pathways.

#### DECLARATIONS

##### Acknowledgement

None.

##### Conflict of interest

No conflict of interest associated with this work.

##### Contribution of authors

This research was carried out by the authors mentioned in this paper, and the authors acknowledge any liability arising from allegations related to this report and its contents. The analysis was conceived and conceived by Congli Zhang; Yanhui Bai, Aijun Li, Congli Zhang collected and analysed the data; Yanhui Bai and Aijun Li wrote the text. The manuscript for publication was read and accepted by both contributors.

Yanhui Bai and Aijun Li have both contributed to this work and can be called co-authors.

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