ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2024; 23(3):330-338.
DOI: 10.18502/ijaai.v23i3.15642

Nebulized Dexmedetomidine Alleviates Oxidative Stress in Ventilator-induced Lung Injury via Keap1-Nrf2-ARE Pathway

Jun Zha[1,2], Youjia Yu[3], Ji Zhu[2], Guiru Li[4], Xiaolin Deng[4], and Hong Xie[1]

[1] Department of Anesthesiology, The Second Affiliated Hospital, Soochow University, Suzhou, China
[2] Department of Anesthesiology, The Second Affiliated Hospital, Wannan Medical College, Wuhu, China
[3] Department of Anesthesiology, Suzhou Xiangcheng People's Hospital, Suzhou, China
[4] Department of Anesthesiology, Suzhou Hospital, Affiliated Hospital of Medical School, Nanjing University, Suzhou, China

Received: 29 January 2024; Received in revised form: 5 March 2024; Accepted: 13 March 2024

ABSTRACT

This study aimed to explore the underlying mechanism of nebulized dexmedetomidine (DEX) in ameliorating ventilator-induced lung injury (VILI)-induced oxidative stress in rats.

Forty 7 to 8-week-old Sprague-Dawley rats at the specific pathogen-free level were randomized into the control group, model group, nebulized dexmedetomidine (WH-YM) group, and dexmedetomidine intravenous infusion (JM-YM) group, each containing 10 rats. Except for the control group, rats in the other groups underwent mechanical ventilation (tidal volume, 40 mL/kg; respiratory rate, 70 breaths per minute; inspiratory-to-expiratory ratio, 1:2; fraction of inspired oxygen, 21%; positive end-expiratory pressure, 0 cmH2O). Nebulized DEX (6.3 μg/kg), and isodose intravenous DEX were given to rats of WH-YM and JM-YM groups prior to ventilation. Post 4-hour ventilation, rats were euthanized. Lung tissue wet-to-dry weight ratio, H&E staining for assessing diffuse alveolar damage (DAD), and expression levels of Nrf2 and Keap1 detected by qRT-PCR and Western blot were compared. Inflammatory markers TNF-α, IL-2, and IL-6, and oxidative stress indices malondialdehyde (MDA) and superoxide dismutase (SOD), were quantified in lung tissues and serum samples using commercial kits.

Rats in the WH-YM and JM-YM groups demonstrated significant ameliorations in the wet-to-dry weight ratio and DAD score, decreased Keap1, TNF-α, IL-2, and IL-6 levels in lung tissues and serum samples, but increased Nrf2 and SOD level than those of controls. These changes were more pronounced in the WH-YM group than in the JM-YM group.

DEX effectively alleviates VILI-induced oxidative stress and inflammation via the Keap1-Nrf2-ARE signaling pathway., especially in the nebulized administration.

Keywords: Inflammation; Dexmedetomidine; Nebulization; Ventilator-induced lung injury

Corresponding Author: Hong Xie, PhD;
Department of Anesthesiology, The Second Affiliated Hospital, Soochow University, Suzhou, China.Tel:(+86 512) 6828 2030,
E-mail: hongx93044@126.com

INTRODUCTION

Mechanical ventilation is an important respiratory support that has been extensively applied to the

treatment of critical illnesses. However, it may induce and aggravate lung injury, resulting in ventilator-induced lung injury (VILI).[1] VILI is characterized by pulmonary interstitial edema, increased alveolar-capillary permeability, alveolar hemorrhage, local infiltration of numerous inflammatory cells, and cell apoptosis. The clinical manifestations of VILI are similar to those of acute respiratory distress syndrome (ARDS). It is generally believed that oxidative stress, inflammatory response, and chemokines are closely linked with the occurrence and progression of VILI.[2] Oxidative stress, serving as a vital event involved in VILI, has remained a research hotspot.

Dexmedetomidine (DEX) is an α2-adrenergic receptor agonist with the properties of sympathetic nerve inhibition, analgesia, sedation, inhibition of catecholamine release, and mild respiratory depression. It is applied in the early stage of sedation in critically ill patients. Notably, the potent anti-inflammatory and anti-oxidative activities of DEX have been recently validated, exerting a protective effect on the heart, lungs, brain, and kidneys.[3-6] DEX contributes to alleviating sepsis-induced acute lung injury by downregulating the receptor for advanced glycation end products (RAGE).[7] Zhu et al,[8] proposed that DEX prevents tissue damage and VILI caused by endotracheal intubation, although the exact mechanism remains unclear.

Nebulized DEX offers more clinical benefits to patients with lung injury than that of an intravenous route.[9] The Keap1-Nrf2-ARE signaling pathway is a major signaling transduction involved in oxidative stress.[10] Nuclear factor erythroid 2-related factor 2 (Nrf2) is the main regulator for the oxidative stress that binds to Kelch-like ECH-associated protein 1 (Keap1) in the inactivated state under normal circumstances. Stimulated by oxidative stress, Nrf2 uncouples Keap1 via the conformational change in covalent modifications of amino acids in Keap1 and thus binds to the antioxidant-responsive element (ARE).[11,12] It further activates target genes, thereafter inducing the release of anti-inflammatory and anti-oxidative proteins. Hence, Nrf2 contributes to inhibiting oxidative stress, alleviating inflammatory response, and enhancing the anti-apoptotic capacity. Keap1 functions as an oxidative stress sensor that maintains intracellular homeostasis alongside Nrf2. Moreover, Nrf2 exerts inhibitory effects on oxidative stress and cell apoptosis by regulating heme oxygenase-1 (HO-1) and quinone oxidoreductase-1 (NQO1).[13] We speculated that the Nrf2-Keap1-ARE signaling pathway was involved in VILI-induced oxidative stress, which could be protected by nebulized DEX.

In the present study, we created an in vivo VILI model in rats managed by mechanical ventilation. Through pathological examination and a series of biological experiments, we explored the protective effect of nebulized DEX on VILI and the underlying mechanism.

MATERIALS AND METHODS

Experimental Animals and Grouping

A total of 40 male Sprague-Dawley (SD) rats (7 to 8 weeks old, 200±20 g) in the specific pathogen-free (SPF) level were provided by the Pizhou Dongfang Breeding Co., Ltd (No. SCXK, Jiangsu, 2022-0005, China). They were habituated for 7 days in a standard environment with a temperature of 26℃±1℃ and relative humidity of 85% and given free accesses to food and water. The experiment has been approved by the Animal Ethics Committee of the Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences. Great efforts have been made to minimize both the pain and the number of animals used (2022-B31). Rats were randomly divided into a control group, model group, nebulized dexmedetomidine group (WH-YM), and dexmedetomidine intravenous infusion group (JM-YM), with 10 rats per group.

After food fasting for 6 hours and fluid fasting for 2 hours, rats in the model, WH-YM, and JM-YM groups were intraperitoneally administrated with pentobarbital sodium 3% at the dose of 40 mg/kg and placed in the supine position. A 2-cm incision was made on the midline of the neck to bluntly separate subcutaneous tissues and muscles, thus exposing the trachea. Using the small animal ventilator (DW-3000S, Zhenghua Biologic Apparatus Facilities, Anhui, China) connected with an 18-gauge needle, mechanical ventilation was performed in rats of the model, WH-YM and JM-YM group with the following indexes: tidal volume, 40 mL/kg; respiratory rate, 70 breaths per minute; inspiratory-to-expiratory ratio, 1:2; fraction of inspired oxygen (FiO_2), 21%; positive end-expiratory pressure (PEEP), 0 cmH_2O.

Rats in the WH-YM group were given a single dose of nebulized DEX (Batch No. 22071631; Yangtze River Pharmaceutical Group) at the dose of 6.3 μg/kg prior to the mechanical ventilation using an intratracheal

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

nebulizer device (YAN-30012, Yuyan Instruments, Shanghai, China). Those in the JM-YM group were given an intravenous infusion of DEX at the dose of 6.3 μg/kg. The dosage of DEX in mice was conversed to human equivalent dose. Rats in the control group were anesthetized intubated for 4-h spontaneous breathing . All rats were sacrificed 4 hours later.

Hematoxylin and Eosin Staining

The upper lobe of the right lung in rats was collected, fixed in 10% paraformaldehyde, and prepared for tissue sections. They were stained in hematoxylin and eosin (H&E), and examined under a light microscope (CIC, XSP-C204, Olympus, Japan). Pathological changes in rat lung tissues were assessed by grading the diffuse alveolar damage (DAD) score, and a higher score indicated more severe damage.

Measurement of Wet-to-dry Weight Ratio of Lung Tissues

The middle lobe of the left lung in rats was collected for weighing the wet (W) weight. After drying at 60℃ for 24 hours, the dry (D) weight of lung tissues was weighed. Finally, the W/D weight ratio was calculated using the following formula: W/D weight ratio (%)=W (g)/D (g)×100%.

Enzyme-linked Immunosorbent Assay

Homogenates of rat lung tissues and serum samples of rats were collected for measuring the relative levels of tumor necrosis factor-alpha (TNF-α; ER20497M, ER20497H; Weiao Bio, Co., Ltd, China), interleukin (IL)-2 (ELK1151M, ELK1151H; ELK Biotechnology Co., Ltd, USA) and IL-6 (ER20298M, ER20298H; Weiao Bio, Co., Ltd, China) using commercial enzyme-linked immunosorbent assay (ELISA) kits.

Measurement of Superoxide Dismutase and Malondialdehyde MDA

Homogenates of rat lung tissues and serum samples of rats were collected for measuring the relative contents of superoxide dismutase (SOD) and malondialdehyde (MDA) using commercial kits (A001-3-2, A003-1-2; Nanjing Jiancheng Bioengineering Institute, China).

Western Blot

Total proteins were extracted from rat lung tissues using the bicinchoninic acid method. Protein samples were prepared, loaded on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Mini-Protein Tetra System (Bio-RAD), and transferred on the polyvinylidene difluoride membranes at 250 mA for 70 minutes. After blockage of nonspecific antigens in 5% skimmed milk at room temperature for 1 hour, membranes were incubated with primary antibodies of anti-Keap1 (60027-1-Ig; Proteintech, USA), anti-Nrf2 (ab92946; Abcam, USA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 60004-1-Ig; Proteintech, USA) at 4℃ overnight. On the next day, they were incubated with the secondary antibody of goat anti-rabbit immunoglobulin (Ig)G antibody (heavy and light chains) at room temperature for 1 hour. Band exposure was conducted on a chemiluminescence imaging system (SH-523; Shenhua Science Technology Co., Ltd, China).

Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from rat lung tissues using TRIzol. After measuring the optical density (OD) at 260 nm and 280 nm, qualified RNA samples (OD_{260}/OD_{280}, 1.7 to 2.0) were reversely transcribed into cDNAs. They were subjected to thermal amplification of 40 cycles at 95℃ for 30 seconds, 95℃ for 5 seconds, and 60℃ for 34 seconds on the 7500 Real-Time PCR System (Bio-Rad CFX Touch). Relative levels of Nrf2 and Keap1 were calculated by the $2^{-\Delta\Delta Ct}$ method. Sequences of primers used in qRT-PCR are listed in Supplementary Table 1.

Statistical Analysis

Statistical processing was performed using SPSS 25.0. Measurement data were expressed as mean±standard deviation. Differences among 3 or more groups were compared by one-way analysis of variance, followed by the least significant difference test for pairwise comparison. $p<0.05$ was considered statistically significant.

RESULTS

Pathological Changes in Lung Tissues of VILI Rats

Compared with those of the control group, H&E staining in rats of the model group presented alveolar wall thickening, dilation, and bleeding of capillaries lining in the alveolar wall, and infiltration of inflammatory cells. Pathological changes in the lung tissues were significantly relieved in rats of the WH-YM group and JM-YM group, which were significantly milder in the

former group (Figure 1, upper panel). Consistently, the DAD score was significantly lower in the WH-YM group and JM-YM group than in the M group, suggesting a milder diffuse alveolar damage. A significantly lower DAD score was detected in the WH-YM group compared with that of the JM-YM group (p<0.05) (Figure 1, lower panel), suggesting the protective effect of nebulized DEX on rat lung tissues.

In addition, the W/D weight ratio of rat lung tissues was significantly higher in the model, WH-YM group, and JM-YM groups than that of the control group, but lower in the WH-YM and JM-YM groups than that of the model group (p<0.05). Besides, a significantly lower W/D weight ratio was detected in rats of the WH-YM group than that of the JM-YM group (p<0.05) (Figure 2).

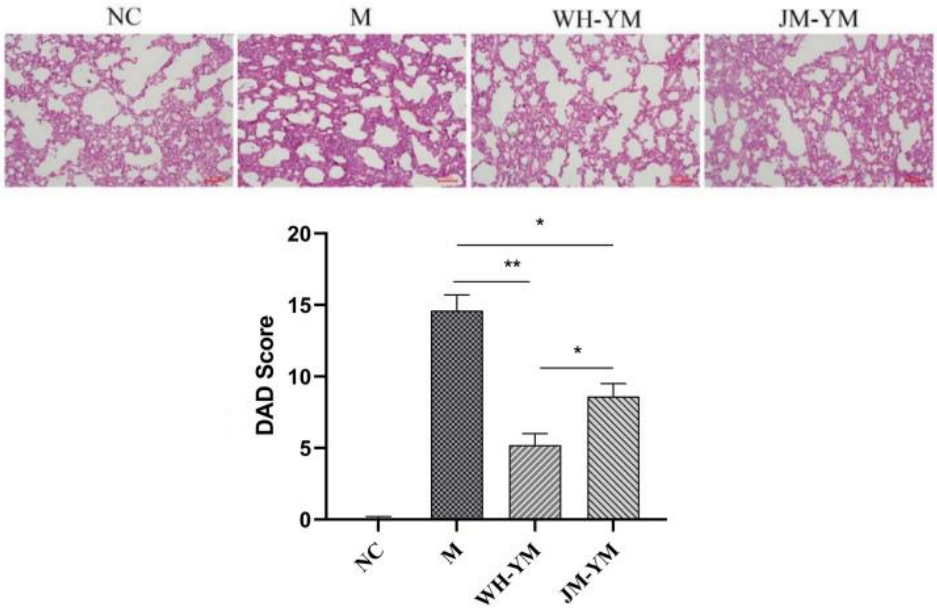

Figure 1. Hematoxylin and eosin staining of rat lung sections (upper panel) and the diffuse alveolar damage (DAD) score (lower panel). *p<0.05, **p<0.01.
NC: negative control, M: model group, WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine

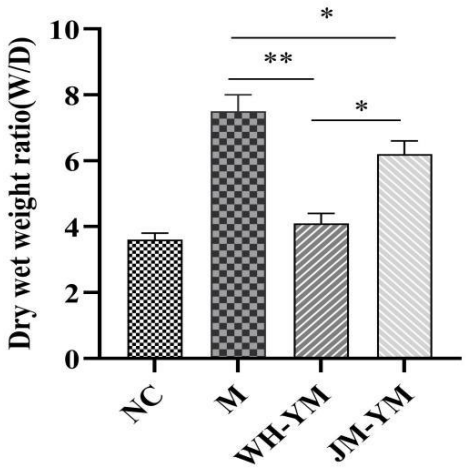

Figure 2. The wet-to-dry (W/D) weight ratio of rat lung tissues. n=3. *p<0.05, **p<0.01.
NC: negative control, M: model group, WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine

Inflammatory Response and Oxidative Stress in VILI Rats

We detected inflammatory factors in rat lung tissues by ELISA. Relative levels of IL-2, IL-6, and TNF-α were significantly higher in rats of the model group, WH-YM group, and JM-YM group than that of the control group, but lower in the WH-YM and JM-YM groups than that of the model group (p<0.05). Their levels were significantly lower in the WH-YM group compared with those of the JM-YM group, indicating the role of nebulized DEX in inhibiting inflammatory response (p<0.05; Figure 3A). Consistent changes in serum inflammatory factors were observed in rats of the control group, model group, WH-YM group and JM-YM group (Figure 3B).

Oxidative stress indexes, including MDA and SOD, were measured in the rats as well. The relative contents of MDA in lung tissues and serum samples were significantly higher in the model, WH-YM, and JM-YM groups compared to those of the NC group, but lower in the WH-YM group and JM-YM group compared to those of the model group (p<0.05; Figure 4A). The opposite changes were measured in the relative contents of SOD (p<0.05; Figure 4B). It is indicated that nebulized DEX protected VILI-induced oxidative stress.

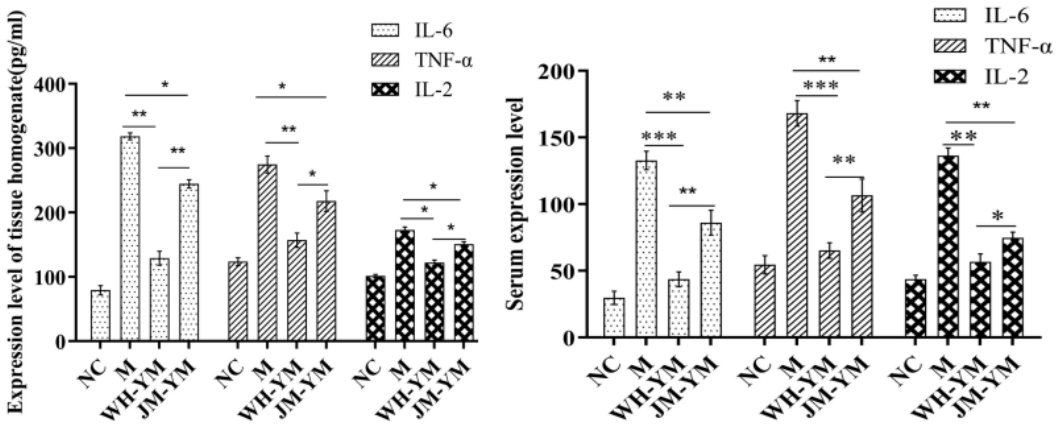

Figure 3. Relative levels of IL-2, IL-6, and TNF-α in rats. n=3. *p<0.05, **p<0.01, ***p<0.001. NC: negative control, M: model group, WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine

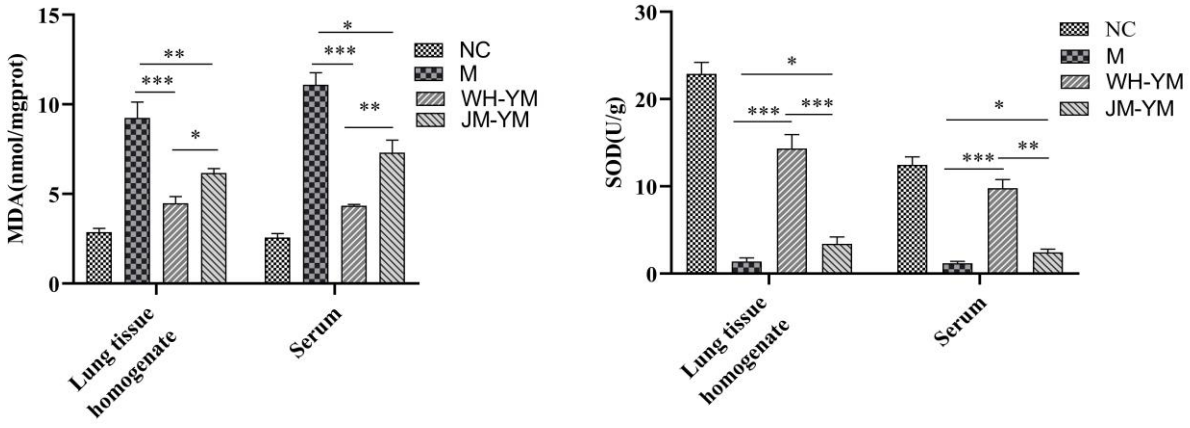

Figure 4. Relative contents of malondialdehyde (MDA) and superoxide dismutase (SOD) in rats. n=3. *p<0.05, **p<0.01, ***p<0.001. NC: negative control, M: model group, WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine

The Keap1-Nrf2 Signaling Pathway Involved in VILI *in vivo*

The Keap1-Nrf2 signaling pathway is a classic signal transduction involved in oxidative stress. We measured the protein and mRNA levels of Keap1 and Nrf2 in rat lung tissues. Compared with that of the control group, significantly upregulated Keap1 was detected in the remaining three groups (p<0.05). It was significantly downregulated in rats of the WH-YM group and JM-YM group than that of the model group, especially in the WH-YM group (p<0.05, Figure 5). The protein level of Nrf2 was found significantly downregulated in rats of the M group, WH-YM group, and JM-YM group than that of the control group, but higher in the WH-YM group and JM-YM group than that of the model group (p<0.05). Nrf2 was significantly upregulated in the WH-YM group compared with those of the JM-YM group (p<0.05, Figure 5). Changes in the mRNA levels of Keap1 and Nrf2 in rats were consistent with their protein levels (Figure 6).

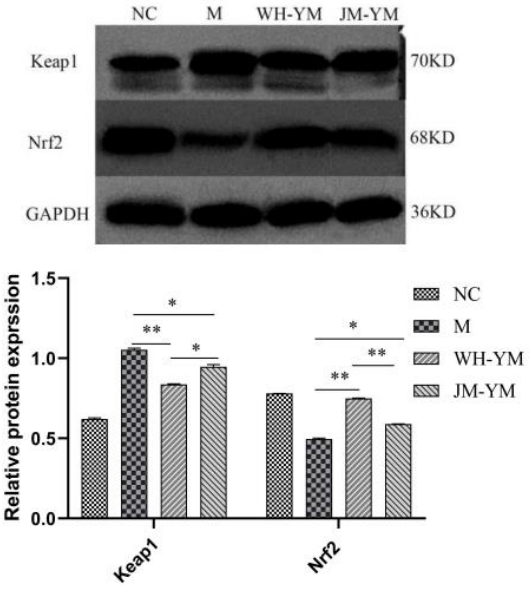

Figure 5. Protein levels of Kelch-like ECH associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2) in rats, and their quantitative analyses. n=3. *p<0.05, **p<0.01. NC: negative control, M: model group; WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine, GAPDH: glyceraldehyde-3-phosphate dehydrogenase

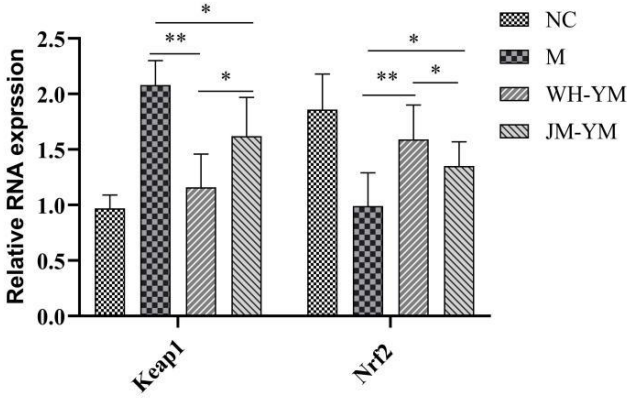

Figure 6. The mRNA levels of Kelch-like ECH associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2) in rat lung tissues . n=3. *p<0.05, **p<0.01, ***p<0.001. NC: negative control, M: model group, WH-YM: nebulized dexmedetomidine, JM-YM: intravenous dexmedetomidine

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

DISCUSSION

VILI is a lung injury caused by hyperventilation or other causes during mechanical ventilation.[14] The typical pathological manifestations of VILI include increased lung permeability, pulmonary edema, collapsed lungs, and inflammatory response.[15] Changes in mechanical stretch result in VILI, accompanied by a series of biological factors involved in its pathogenesis. It is generally believed that oxidative stress and inflammatory responses are vital events for VILI.

The deformation of the lung occurs during inspiration and expiration. The inhaled air passes through the narrow airways of the trachea and bronchi and finally reaches the terminal bronchi. The inhaled air during inspiration overcomes airway flow resistance and viscoelastic recoil of the chest wall, and the latter is mainly formed by the surface tension at the air-liquid interface of alveoli. Mechanically, VILI induces the decline of lung compliance, which is attributed to the damage in cell connections and mitochondrial dysfunction in the lung epithelium.[16] Cell junctions are contact points between cells, which are classified into tight junctions, gap junctions, and anchoring junctions. The barrier formed by tight junctions effectively prevents lipid diffusion and intercellular substance exchange. Gap junctions allow small intercellular substances to pass through. VILI causes damage in the cell junctions of the lung epithelium. The unstable cell junctions between alveolar epithelium and pulmonary vascular endothelial cells eventually cause pulmonary edema by disturbing signaling transductions in the lung and increasing the alveolar permeability.[17] The opening of cell junctions during mechanical ventilation impairs cellular functions and even causes cell death. In addition to the biological injury, VILI also causes acute inflammatory response and oxidative stress. Previous data have validated the role of inhibiting the release of pro-inflammatory factors in alleviating VILI. Inhibition of oxidative stress also provides clinical benefits to VILI patients. We, therefore, created an oxidative stress-associated VILI model in rats and achieved gratifying results.

Mechanical ventilation is a crucial tool for patients with respiratory failure. However, excessive oxygen supplementation or hyperventilation may induce acute lung injury, which is especially unfavorable to critically ill patients. Hyperoxic lung injury can be attributed to oxidative stress and mitochondria-mediated cell death, and inhibition of oxidative stress is a promising way to improve the prognosis.[18] Abundant evidence has validated the role of oxidative stress in aggravating lung injuries, inducing VILI, or even causing death. Blocking the oxidative stress significantly protects VILI.

The Keap1/Nrf2/ARE signaling pathway is a classic signaling transduction against oxidative stress.[19] Under the stimuli, Nrf2 detaches from Keap1 and binds to ARE, thus regulating the downstream transcription of antioxidant proteins and enzymes.[20] In a lipopolysaccharide-induced ARDS mouse model, Nrf2 exerts a protective role against oxidative stress by regulating the polarization of macrophages.[21] In the present study, we also validated the involvement of the Keap1/Nrf2/ARE signaling pathway in in vivo VILI models.

DEX is a highly selective and potent α2-adrenergic receptor agonist, which is initially used for sedation in the intensive care unit and an adjuvant drug for general anesthesia. It is also used for the treatment of inflammatory lung diseases.[22,23] Through activating the α2-adrenergic receptor and inhibiting sympathetic nerve excitability, DEX significantly downregulates inflammatory factors by regulating the p38 signaling pathway.[24,25] In addition to the antioxidation property, DEX has been found to inhibit oxidative stress.[26] An intravenous administration of DEX protects against trauma-induced acute lung injury by suppressing inflammatory response and oxidative stress.[27] Moreover, the protective effect of DEX on lung tissues is linked with the inhibition of mitochondrial damage and cell apoptosis.[28]

In the present study, we created an in vivo VILI model of rats. Both the nebulization and intravenous infusion of DEX significantly protected VILI-induced pathological lesions in the lung and inhibited oxidative stress indexes and inflammatory factors, which were more pronounced in rats managed by nebulized DEX during the process of mechanical ventilation. In addition, the treatment of DEX significantly downregulated Keap1 and upregulated Nrf2 in rat lung tissues and serum samples, indicating the involvement of the Keap1-Nrf2-ARE signaling pathway in VILI, consistent with previous reports.[29-31]

Several limitations should be considered. First of all, we have validated that nebulization of DEX was superior to the intravenous route in the treatment of

VILI. However, we did not determine the optimal dosage and time point. Secondly, the long-term efficacy and safety of nebulized DEX on VILI required to be further explored. Thirdly, the clinical benefits of nebulized DEX to VILI patients needed clinical evidence. Future research should focus on exploring the optimal dosage of DEX and time point of medication, and its long-term influence on lung function and safety.

Taken together, nebulized DEX effectively alleviated VILI-induced lung injury, oxidative stress, and inflammatory response in in vivo models by regulating the Keap1-Nrf2-ARE signaling pathway. Nebulized DEX provides a better efficacy on VILI-induced oxidative stress than that of intravenous administration.

STATEMENT OF ETHICS

The experiment has been approved by the Animal Ethics Committee of the Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences(2022-B31)

FUNDING

Wannan Medical College Young and Middle-aged Research Fund (EYR202301, High-level Talent Introduction Fund).The Key Project Research Fund of the Second Affiliated Hospital of Wannan Medical College (YK2023Z04).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Wannan Medical College Young and Middle-aged Research Fund (EYR202301, High-level Talent Introduction Fund). The Key Project Research Fund of the Second Affiliated Hospital of Wannan Medical College (YK2023Z04).

REFERENCES

1. Fan E, Villar J, Slutsky AS. Novel approaches to minimize ventilator-induced lung injury. BMC Med. 2013;11:85.
2. Li W, Zhao X, Yu TT, Hao W, Wang GG. Knockout of PKC θ gene attenuates oleic acid-induced acute lung injury via reduction of inflammation and oxidative stress. Iran J Basic Med Sci. 2021; 24(7):986-91.
3. Gu J, Chen J, Xia P, Tao G, Zhao H, Ma D. Dexmedetomidine attenuates remote lung injury induced by renal ischemia-reperfusion in mice. Acta Anaesthesiol Scand. 2011;55(10):1272-8.
4. Shi QQ, Wang H, Fang H. Dose response and mechanism of protective function of selective alpha-2 agonist dexmedetomidine on acute lung injury in rats. Saudi Med J. 2012;3(4):375--81.
5. Si Y, Bao H, Han L, Shi H, Zhang Y, Xu L, et al. Dexmedetomidine protects against renal ischemia and reperfusion injury by inhibiting the JAK/STAT signaling activation. J Transl Med. 2013;11(2):141
6. Afonso J, Reis F. Dexmedetomidine : current role in anesthesia and intensive care. Rev Bras Anestesiol. 2012;62(1):118-33.
7. Hu H, Shi D, Hu C, Yuan X, Zhang J, Sun H. Dexmedetomidine mitigatesCLP-stimulated acute lung injury via restraining the RAGEpathway. Am J Transl Res. 2017;9(12):5245-58.
8. Zhu CH, Yu J, Wang BQ, Nie Y, Wang L, Shan SQ. Dexmedetomidine reducesventilator-induced lung injury via ERK1/2 pathway activation. Mol Med Rep. 2020;22(6):5378-84.
9. Xu B, Gao H, Li D, Hu C, Yang J. Nebulized dexmedetomidine improves pulmonary shunt and lung mechanics during one-lung ventilation: a randomized clinical controlled trial. Peer J. 2020;8:e9247.
10. Tu W, Wang H, Li S, Liu Q, Sha H. The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases. Aging and disease. 2019;10(3):637.
11. Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M. Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol Cell Biol. 2014;34(5):832-46.
12. Lu MC, Ji JA, Jiang ZY, You QD. The Keap1–Nrf2–ARE pathway as a potential preventive and therapeutic target: an update. Med Res Rev. 2016;36(5):924-63.
13. Ahn CB, Je JY, Kim YS, Park SJ, Kim BI. Induction of Nrf2-mediated phase II detoxifying/antioxidant enzymes in vitro by chitosan-caffeic acid against hydrogen peroxide-induced hepatotoxicity through JNK/ERK pathway. Mol Cell Biochem. 2017;424(12):79-86.
14. Tonetti T, Vasques F, Rapetti F, Maiolo G, Collino F, Romitti F, et al. Driving pressure and mechanical power:

new targets for VILI prevention. Ann Transl Med. 2017;5(14):286.
15. Fan E, Villar J, Slutsky AS. Novel approaches to minimize ventilator-induced lung injury. BMC Med. 2013;11:85.
16. Feng Z, Wang JW, Wang Y, Dong WW, Xu ZF. Propofol Protects Lung Endothelial Barrier Function by Suppression of High-Mobility Group Box 1 (HMGB1) Release and Mitochondrial Oxidative Damage Catalyzed by HMGB1. Med Sci Monit. 2019;25:3199-11.
17. Jiang L, Zhang Y, Lu D, Huang T, Yan K, Yang W. Mechanosensitive Piezo1 channel activation promotes ventilator-induced lung injury via disruption of endothelial junctions in ARDS rats. Biochem Biophys Res Commun. 2021;556:79-86.
18. Yang Z, Yin Q, Olatunji OJ, et al. Activation of cholinergic anti-inflammatory pathway involved in therapeutic actions of α-mangostin on lipopolysaccharide-induced acute lung injury in rats. Int J Immunopathol Pharmacol. 2020;34:2058738420954941.
19. Hassanein EHM, Sayed AM, Hussein OE, Mahmoud AM. Coumarins as modulators of the Keap1/Nrf2/ARE signaling pathway. Oxid Med Cell Longev. 2020;22:1675957.
20. Liu S, Pi J, Zhang Q. Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. Redox Biol. 2022:102389.
21. Abreu CC, Cardozo L, Mafra D. Could physical exercises modulate Nrf2-Keapl pathway in chronic kidney disease. Med Hypotheses. 2015;152(84):44-6.
22. Wang J, Yi X, Jiang L, Dong H, Feng W, Wang S, et al. Protective effects of dexmedetomidine on lung in rats with one-lung ventilation. Exp Ther Med. 2019;17(1):187-92.
23. Lee SH, Kim N, Lee CY, Ban MG, Oh YJ. Effects of dexmedetomidine on oxygenation and lung mechanics in patients with moderate chronic obstructive pulmonary disease undergoing lung cancer surgery: a randomised double-blinded trial. Eur J Anaesthesiol. 2016;33(4):275.
24. Zhu CH, Yu J, Wang BQ, Nie Y, Wang L, Shan SQ. Dexmedetomidine reduces ventilator-induced lung injury via ERK1/2 pathway activation[J]. Mol Med Rep. 2020;22(6):5378-84.
25. Huang CY, Deng JS, Huang WC, Jiang WP, Huang GJ. Attenuation of lipopolysaccharide-induced acute lung injury by hispolon in mice, through regulating the TLR4/PI3K/Akt/mTOR and Keap1/Nrf2/HO-1 pathways, and suppressing oxidative stress-mediated ER stress-induced apoptosis and autophagy. Nutrients. 2020;12(6):1742.
26. Zeng L, Zhao C, Cui C. Dexmedetomidine improves DM-induced oxidative stress injury to protect liver function through Nrf2 pathway. Am J Transl Res. 2023;15(3):1715-29.
27. Li J, Chen Q, He X, Alam A, Ning J, Yi B, et al. Dexmedetomidine attenuates lung apoptosis induced by renal ischemia–reperfusion injury through α 2 AR/PI3K/Akt pathway. J Transl Med. 2018;16:1-11.
28. Wang Y, Mao X, Chen H, et al. Dexmedetomidine alleviates LPS-induced apoptosis and inflammation in macrophages by eliminating damaged mitochondria via PINK1 mediated mitophagy. Int Immunopharmacol. 2019;73:471-81.
29. Wang Y, Mao X, Chen H, Feng J, Yan M, Wang Y, et al. Activation of the KEAP1-NRF2-ARE signaling pathway reduces oxidative stress in Hep2 cells[J]. Molecular Medicine Reports, 2018, 18(3): 2541-2550.
30. Zhang ZT, Xie K, Luo RJ, Zhang DY, He ZW, Li KF, et al. Dexmedetomidine alleviates acute lung injury by promoting Tregs differentiation via activation of AMPK/SIRT1 pathway. Inflammopharmacology. 2023;31(1):423-38.
31. Yu X, Chi X, Wu S, Jin Y, Yao H, Wang Y, et al. Dexmedetomidine pretreatment attenuates kidney injury and oxidative stress during orthotopic autologous liver transplantation in rats. Oxid Med Cell Longev. 2016,4675817.