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EXPERIMENTAL STUDY

Dexmedetomidine protected COPD-induced lung injury by regulating miRNA-146a

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ABSTRACT

OBJECTIVE: To study the mechanism of protection provided by dexmedetomidine against COPD-induced lung injury.

METHODS: COPD rat model was determined by measuring lung function, and comparing HE staining between two different groups. We got the lung tissue and cells from the control and COPD groups. The cells were divided into three groups: control group, and blank and drug groups that were from the COPD rats. Cell apoptosis, relative gene expression and TNF-α and IL-1β from nutrient solution were measured.

RESULTS: The TV, PEF, EF50, FEV0.3 and FEV0.3/FVC in COPD group were significantly lower than in control group (1.26±0.17 vs 2.65±0.21; 17.61±0.35 vs 38.55±0.24; 1.20±0.14 vs 1.81±0.06; 2.52±0.28 vs 4.44±0.26; 63.39±0.22 vs 88.45±0.34, $p < 0.05$, respectively). Cell apoptosis was significantly different in blank and drug groups (21.65±0.86 vs 10.74±0.15; $p < 0.05$, respectively). The gene expressions of miRNA-146a, p53 and Bcl-2 were significantly downregulated compared with blank group.

CONCLUSION: Dexmedetomidine protected COPD-induced lung injury by inhibiting miRNA-146a expression to reduce cell apoptosis *(Tab. 1, Fig. 3, Ref. 25)*. Text in PDF *www.elis.sk.*

KEY WORDS: miRNA-146a expression, COPD, IL-1β, TNF-α.

Chronic obstructive pulmonary disease (COPD) is a kind of main damage located in airways and lung tissue, causing systemic disease progressively affecting other tissues and organs The symptoms are not reversible. Clinical research shows that the incidence of hospitalized patients with chronic pulmonary disease gradually increases (1). In 2040, COPD will become the world's most endangering disease (2, 3). COPD pathogenesis is not yet entirely clear, but the current study showed that the main causes of COPD include protease/anti-protease imbalance, oxidant/anti-oxidant imbalance stress (4–7), inflammatory/anti-inflammatory response imbalance (8), mechanical stretch injury (9), etc. Clinical treatment is often used for the purpose of mechanical ventilation after induction of anesthesia. Dexmedetomidine is a highly selective α2-adrenergic receptor agonist (10); it not only can effectively calm, but also has some anti-inflammatory effect (11). However, the mechanism of dexmedetomidine protection against COPD is unclear. The objective of the present study is to explain the latter mechanism.

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Material and method

Animals and cigarette smoke exposure

In our study, we used 6-week-old SD rats at body weight 110±20 g from the animal center, Nanfang Hospital of Southern Medical University. Before the experiment, the rats were accommodated for seven days. All procedures were in compliance with the institutional and national guidelines for the care and use of laboratory animals. The Study Protocol was approved by The Institutional Animal Care and Use Committee, Hebei Medical University. Rats were randomized into two groups: Control Group (n = 8) and Smoking Group (n = 16). The rats in smoking group were exposed to cigarette smoke in a smoking device manufactured by Shijiazhuang Jinyang Science and Technology Inc. (model: JY-01, Shijiazhuang, Hebei, China). Briefly, 20 cigarettes were burnt continuously and blown into a box together with oxygen, while the whole bodies of animals were exposed to the cigarette smoke in the box. Animals were exposed to cigarette smoke for one hour each time, twice a day, 5 days a week for 16 weeks. After 16 weeks, the rats were analyzed for relative index of COPD including tidal volume (TV), peak expiratory flow (PEF), 50 % exhaled tidal volume during expiratory flow rate (EF50), forced expiratory volume in 0.3 seconds (FEV0.3) and FEV0.3/ forced vital capacity (FVC).

Tissues and cell

The rats were sacrificed by femoral artery bleeding. Lung tissue was extracted and a proportion was examined after HE stain-

Tab. 1. Lung function compared between two groups.

	TV (ml)	PEF (ml/s)	EF50 (ml/s)	FEV0.3 (ml)	FEV0.3/FVC (%)
Control	2.65±0.21	38.55±0.24	1.81±0.06	4.44±0.26	88.45±0.34
Model	1.26±0.17*	17.61±0.35*	1.20±0.14*	2.52±0.28*	63.39±0.22*

ing. After taking the alveolar epithelial cell from rats, the COPD group cells were divided into two groups (blank group and drug group). The cells of control and blank groups were cultured by DMEM (Gibico, U.S.A), while the cells of drug group were treated as those from the control group and added with 5 mM dexmedetomidine. The cells of all three groups were cultivated for 3 days.

Cell apoptosis analysis

Annexin V-FITC apoptosis detection kit (BD Biosciences; San Jose, CA, USA) was used to analyze cell apoptosis according to the manufacturer's protocols. In brief, cells were collected after the dissociation with EDTA-free trypsin, and then washed with cold phosphate-buffered saline (PBS). Then, cells were resuspended in the binding buffer with the addition of Annexin V-FITC and PI for an incubation period of 15 min in the darkness. Finally, flow cytometry analysis was performed immediately on the BD FACSCalibur (BD Biosciences).

Quantitative real-time PCR

TRIzol reagent (Invitrogen, Grand Island, NY, USA) was used to isolate total RNA from tissues or cultured cells, which was then transcribed into cDNA by the PrimeScript RT Reagent Kit (Takara, Dalian, China) with provided random primers, according to the manufacturers' protocol. Quantitative PCR was performed on ABI 7500 real-time PCR system (Applied Biosystems; Foster, CA, USA) by using the SYBR PrimeScript RT-PCR kit (Takara). The gene expression was quantified by calculating the ΔCT value, and results were normalized to the expression of GAPDH. The sequences of primers used here were as follows: miRNA146a:

F: 5'-CAGCTGCATTGGATTTACCA-3';
R: 5'-GCCTGAGACTCTGCCTTCTG-3'.
P53:
F: 5'-GACACGCTTCCCTGGATT-3';
R: 5'-CGACGCTAGGATCTGACTG-3'.
Bcl-2:
F: 5' -AGCGTCAACGGGAGATGTC-3';
R: 5'-GTGATGCAAGCTCCCACCAG-3'
GAPDH:
F: 5'-GCACCGTCAAGGCTGAGAAC-3';
R: 5'-TGGTGAAGACGCCAGTGGA-3'

Statistical analysis

All data were analyzed by SPSS 19.0 statistical software, and presented as mean±SD. One-way ANOVA was used for comparison of multiple groups, and Dunnett'sT-test was used for comparison of paired groups. It was considered statistically significant when $p < 0.05$.

Results

Comparison of rats' lung function in two groups

The TV, PEF, EF50, FEV0.3 and FEV0.3/FVC were significantly lower than in control group (Tab. 1).

H&E staining

There was more serous effusion and inflammatory cell infiltration in the bronchial and alveolar periphery (Fig. 1A). Alveo-

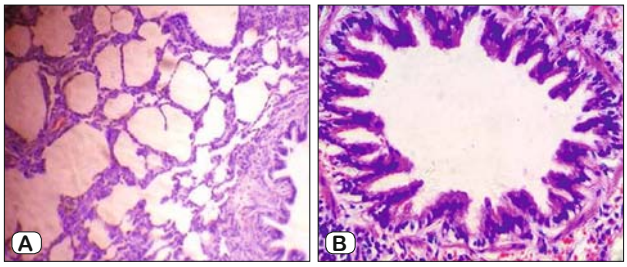
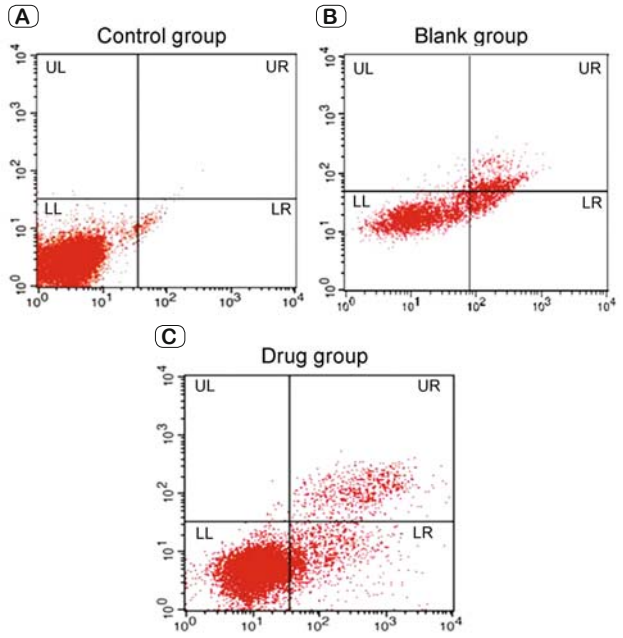


Fig. 1. H&E stain. A) The lung tissue from COPD model rat. B) The lung tissue from normal control rat.



	Control group	Blank group	Drug group
UL	0.42±0.25	2.65±0.86**	1.74±0.15*,**
UR	1.13±0.41	20.52±0.54**	19.44±0.31**
LR	5.65±1.21	30.19±1.61**	11.15±0.51*,**
LL	94.41±0.25	48.35±0.86**	69.26±0.15*,**

Fig. 2. A) Cell apoptosis in control group. B) Cell apoptosis in blank group. C) Cell apoptosis in drug group.

lar tissue and bronchial epithelial tissue had good integrity, and there was a small amount of inflammatory cell infiltration in the lung (Fig. 1B).

Cell apoptosis in three groups

The apoptosis rate in blank and drug groups was significantly higher compared with that in control group (30.19±1.61 and 11.15±0.51 vs 5.65±1.21; $p < 0.05$, respectively), and there were significant differences in blank and drug groups (30.19±1.61 vs 11.15±0.51; $p < 0.05$, respectively) (Fig. 2).

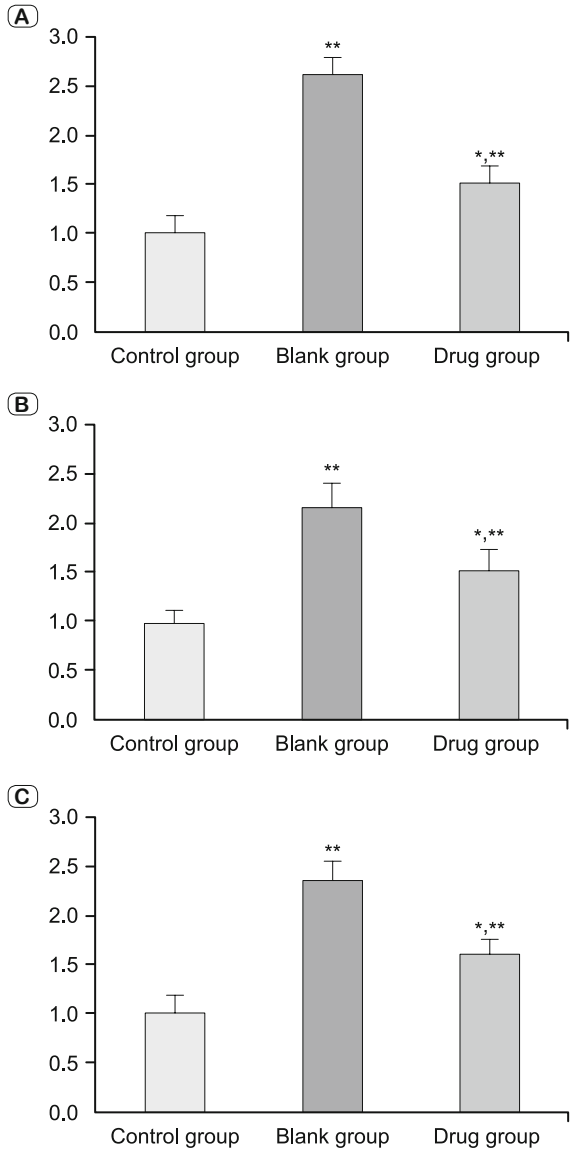


Fig. 3. Gene expression in three groups. A) Gene expression of miRNA-146a in three groups. B) Gene expression of p53 in three groups. C) Gene expression of Bcl-2 in three groups. *p < 0.05, compared with blank group, **p < 0.05, compared with control group, UL: damaged cells, UR: necrotic cells, LR: apoptotic cells, LL: viable cells*: p < 0.05, compared with blank group **: p < 0.05, compared with control group*: p < 0.05, compared with control group.

Gene expression in the three groups

The miRNA 146a, p53 and Bcl-2 gene expressions in the blank and drug groups were significantly higher compared with those in control group, while the gene expression of miRNA 146-a was significantly lower compared with that in the blank group (Fig. 3).

Discussion

So far, the mechanism of COPD remains unclear. A previous study found three causes of induced COPD, namely oxidative stress and oxidative stress imbalance, inflammation and inflammation disorders and protease and antiprotease imbalance (13). In our present study, we observed that TV, PEF, EF50, FEV0.3 and FEV0.3/FVC, which are relevant to lung function, were significantly lower compared with those in control group; in the COPD group, there was a large number of inflammatory cells such as neutrophils, monocytes, and macrophages in small bronchi and small bronchial mucosa. The changes were consistent with the pathological changes in COPD rats (14).

Dexmedetomidine is a new type of receptor agonist highly selective for α2-adrenergic receptor (α2-AR) located in the brain and spinal cord. Dexmedetomindine is used in order to inhibit sympathetic nerve activity, and further sedation, analgesia, antianxiety and other effects (15). Some previous studies reported that dexmedetomidine could inhibit the inflammatory reaction by blocking the cascade reaction (16–18). In those studies, dexmedetomidine was shown to improve inflammation by depressing the expression of IL-1β, and TNF-α which promote cell apoptosis (19, 20). We supposed that dexmedetomidine might have direct effects on decreasing the cell apoptosis.

The previous studies did not research the mechanism of dexmedetomidine's anti-inflammatory effect. The miRNA-146a was induced in response to lipopolysaccharide (LPS) and pro-inflammatory mediators (21), and stimulated P53 and Bcl-2 expression (22–25). In this study, the drug group cells were treated by dexmedetomidine for 3 days. When compared with the blank group, the apoptosis rate was significantly lower and the gene expression of miRNA-146a, P53 and Bcl-2 was also significantly downregulated.

In conclusion, dexmedetomidine protected COPD-induced lung injury by inhibiting miRNA-146a expression, and subsequently controlling P53 and Bcl-2 expression and reducing cell apoptosis.

References

1. Zhong N, Wang C, Yao W et al. Prevalence of chronic obstricitive pulmonary disease in China: a large population-based survey. Am J Resp Crit Care Med 2007; 176 (8): 753–760.

2. Jemal A, Ward E, Hao Y et al. Trends in the leading causes of death in the Unitied States, 1970–2002. JAMA 2005; 294 (10): 1255–1259.

3. Huertas A, Palalange P. COPD: amultifactorial systemic disease. Ther Adv Respir Dis 2011; 5 (3): 217–224.

4. Min T, Bodas M, Mazur S et al. Critical role of proteostasis-imbalance in pathogenesis of COPD and severe emphysema. J Mol Med 2011; 89 (6): 577–593.

5. MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005; 2 (1): 50–60.

6. NacNee W. Oxiants and COPD. Curr Drug Targets Inflamm Allergy 2005; 4 (6): 627–640.

7. Sadeghnejad A, Meyers DA, Bottai M et al. IL-13 promoter polymorphism 112C/T modulates the adverse effect of tobacco smoking on lung function. Am J Repir Crit Care Med 2007; 176 (8): 748–752.

8. Shuh M, Bohorquez H, Loss GE, Jr. Tumor Necrosis Factor-α: Life and Death of Hepatocytes During Liver Ischemia/Reperfusion injury. Ochsner J 2013; 13 (1): 119–130.

9. Pinhu L, Whitehead T, Evans T et al. Ventilator associated lung ingury. Lancet 2003; 361: 332–340.

10. Ozaki M, Takeda J, Tanaka K et al. Safety and efficacy of dexmedetomidine for long-term sedation in critically ill patients. J Anesth 2014; 28 (1): 38–50.

11. Pandharipande PP, Sanders RD, Girard TD et al. Effect of dexmedetomidine versus lorazepam on outcome in patients with sepsis: an a priori-designed analysis of the MENDS randomized controlled trial. Crit Care 2010; 14 (2): R38.

12. Taniguchi T, Kurita A, Kobayashi K et al. Dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats. J Anesth 2008; 22 (3): 140–147.

13. Foschino Barbaro MP, Carpaqnano GE, Spanevello A et al. Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease. Int J Immunopathol Pharmacol 2007; 20 (4): 753–763.

14. Huertas A, Palalange P. COPD: amultifactorial systemic disease. Ther Adv Resp Dis 2011; 5 (3): 217–224.

15. Carollo DS, Nossaman BD, Ramadhyani U. Dexmedetomidine-a review of clinical applications. Curr Opin Anaesthesiol 2008; 21 (4): 457–461.

16. Memis D, Hekimoglu S, Vatan L et al. Effects of midazolam and dexmedetomidine on inflammatory responses and gastric intramucosal pH to sepsis, in critically ill patients. Br J Anaesth2007; 98 (4): 550–552.

17. Hofer S, Steppan J, Wagner T et al. Central sympatholylies prolong survival in experimental sepsis. Crit Care 2009; 13 (1): R11.

18. Koca U, Olgnner CG, Ergur BU et al. The effects of dexmedetomidine oH secondary acute lung and kidney injuries in the rat model of intraabdominal sepsis. Sci World J 2013; 292687.

19. Li X, Du W, Ma FX et al. High Concentrations of TNF-α Induce Cell Death during Interactions between Human Umbilical Cord Mesenchymal Stem Cells and Peripheral Blood Mononuclear Cells. PLoS One 2015; 10 (5): e0128647.

20. Hildebrand D, Bode KA, Rieß D. Granzyme A produces bioactive-IL-1- through a nonapoptotic inflammasome-independent pathway. Cell Rep. 2014; 9 (3): 910–917.

21. Taganov KD, Boldin MP, Chang KJ et al. NF-kB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA 2006; 103 (33): 12481–12486.

22. Ghose J, Bhattacharyya NP. Transcriptional regulation of microRNA-100, -146a, and -150 genes by p53 and NFκB p65/RelA in mouse striatal STHdh (Q7)/ Hdh (Q7) cells and human cervical carcinoma HeLa cells. RNA Biol 2015; 12 (4): 457–477.

23. Sandhu R, Rein J, D'Arcy M et al. Overexpression of miR-146a in basal-like breast cancer cells confers enhanced tumorigenic potential in association with altered p53 status. Carcinogenesis 2014; 35 (11): 2567–2575.

24. Ghose J, Sinha M, Das E et al. Regulation of miR-146a by RelA/NFkB and p53 in STHdh (Q111)/Hdh (Q111) cells, a cell model of Huntington's disease. PLoS One 2011; 6 (8): e23837.

25. Zhang F, Wang J, Chu J et al. MicroRNA-146a Induced by Hypoxia Promotes Chondrocyte Autophagy through Bcl-2. Cell Physiol Biochem 2015; 37 (4): 1442–1453.

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