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Dexmedetomidine alleviates lung ischemia-reperfusion injury in rats by activating PI3K/Akt pathway

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Abstract. – OBJECTIVE: This research aims to investigate the role and mechanism of PI3K/Akt pathway in the pathological process of lung ischemia-reperfusion injury in dexmedetomidine-treated rats.

MATERIALS AND METHODS: Forty-five healthy male Sprague-Dawley rats were divided into three groups: sham operation group, lung ischemia-reperfusion group (IR group) and dexmedetomidine pretreatment group (Dex group). Rats in the sham operation group did not receive other procedures except for opening left chest. The left lung hilar of rats in the IR group was clamped with non-invasive vascular clamp after anesthesia to establish an ischemic model. After 1 hour, the vascular clamp was released and the rats were reperfused for 2 hours. As for rats in the Dex group, 3 μg/kg of dexmedetomidine (pumping time of 10 min) was pumped through the tail vein before releasing the left hilar clamp. After the experiment, blood samples and lung tissues were collected. Serum levels of interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), IL-10, and IL-1 in rats were examined. Activities of malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD) and catalase (CAT) in rat lung tissues were also detected. Besides, the expressions of hypoxia-inducible factor-Ia (HIF-Ia), p-Akt, Caspase-3, and Caspase-9 in lung tissues were detected by Western blot. The mRNA expression levels of HIF-1a, p-Akt, Caspase-3, and Caspase-9 in lung tissues were evaluated by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR).

RESULTS: Lung ischemia-reperfusion markedly increased the levels of IL-6, TNF-α, IL-10, and IL-1 in the IR group. In contrast, dexmedetomidine pretreatment decreased the expression levels of IL-6, TNF-α, IL-10, and IL-1in the Dex group. Also, the activities of MDA and MPO in lung tissues of rats in the IR group significantly increased after lung ischemia-reperfusion injury, whereas dexmedetomidine pretreatment reversed the elevated activities of MDA and MPO in the Dex group. Furthermore, dexmedetomidine pretreatment also improved the activities of SOD and CAT in rat lung tissues compared with rats with lung ischemia-reperfusion injury. In addition, dexmedetomidine pretreatment increased the expression levels of HIF-1α, p-Akt and HIF- in the Dex group when compared to those in the IR group. The mRNA expressions of HIF-1a, p-Akt, Caspase-3, and Caspase-9 in lung tissue of rats was significantly reduced after dexmedetomidine pretreatment.

CONCLUSIONS: Rat lung ischemia-reperfusion can induce severe lung injury. Dexmedetomidine treatment can attenuate lung ischemia-reperfusion injury by activating the PI3K/Akt signaling pathway at the transcriptional level.

Key Words:
Lung ischemia-reperfusion, Dexmedetomidine, PI3K/Akt, Lung injury.

Introduction

Lung ischemia-reperfusion injury (LIRI) is a common disease in clinical practice, such as in cardiopulmonary resuscitation, lung transplantation, single-lung ventilation, cardiopulmonary bypass and pulmonary embolism. LIRI is a form of acute aseptic lung injury with high morbidity and mortality[1]. The pathogenesis of LIRI is complex, involving many pathways and pathophysiological processes such as oxidative stress injury, calcium overload, endoplasmic reticulum stress injury, inflammatory injury, autophagy and apoptosis[2-4].

Dexmedetomidine (Dex) is a highly potent and highly selective adrenergic alpha 2 receptor agonist with sedative, anxiolytic, hypnotic, analgesic and sympathetic inhibitory effects. Dex is capable of reducing the dosage of conventional sedatives and analgesics. It has no incompatibility with other anesthetics, sedative and analgesic drugs, and has

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been widely used in clinical application[5,6]. Numerous studies[7-9] have shown that dexmedetomidine has a protective effect on tissues and organs including brain, heart, liver and kidney in ischemia-reperfusion animal models. Such a protective role of Dex is mainly through anti-inflammation, anti-oxidation and regulation of apoptosis, gradually highlighting its superiority and potential values in clinical application. It has been reported[10] that dexmedetomidine can prevent ischemia-reperfusion injury in a dose-dependent manner, the mechanism of which may be related to the inhibited inflammatory reaction and apoptosis of intestinal mucosal epithelial cells. In addition, studies[11-13] have shown that the degree of alveolar damage in LIRI model is significantly alleviated after the application of dexmedetomidine.

The phosphatidylinositol 3-kinase/serine-threonine protein kinase (PI3K/Akt) signaling pathway is a key pathway for cell signal transduction and is mainly involved in the regulation of cell proliferation and apoptosis[14]. Activated PI3K produces phosphatidylinositol triphosphate (PIP3), which acts as a second messenger to activate Akt. Subsequently, Akt downregulates the pro-apoptotic proteins including Bad, Bax and caspase, ultimately reducing the opening and lowering of mitochondrial permeability transition pores. The permeability of mitochondrial membrane inhibits apoptosis and enhances cell tolerance to hypoxia or nutrient deficiencies, and promotes cell survival and proliferation[15]. When the body tissues or organs are subjected to ischemia-reperfusion, the hypoxanthine in the body undergoes electronic conversion to jaundice, which can produce a large amount of oxygen free radicals. Ischemia or hypoxia leads to a decrease in the production of adenosine triphosphate (ATP) in the mitochondria, and ATP dysfunction eventually produces a large number of oxygen free radicals[16]. The activation of the PI3K/Akt signaling pathway may alleviate the ischemia-reperfusion injury of tissues or organs by reducing the generation of oxygen free radicals[17]. A recent study[18] suggested that the PI3K/Akt signaling pathway plays a key role in inflammation, ischemia-reperfusion and multi-organ protection.

In this work, a rat model of lung ischemia-reperfusion was established using non-invasive vascular clamping method to investigate the effect of dexmedetomidine on lung reperfusion injury. In addition, changes in the PI3K/Akt pathway were evaluated to explore the protective mechanism of dexmedetomidine in lung function after reperfusion injury in rats.

Materials and Methods

Animal Grouping

Forty-five male Sprague-Dawley rats weighing 220-270 g were randomly divided into three groups, including sham operation group, lung ischemia-reperfusion group (IR group) and dexmedetomidine pretreatment group (Dex group). Rats in the sham operation group did not receive other procedures except for opening the left chest. The left hilar of rats in the IRI group were clamped with a non-invasive vascular clamp to establish an ischemic model. After 1 hour, the vascular clamp was released and the rats were reperfused for 2 hours. As for rats in the Dex group, 3 μg/kg of dexmedetomidine (pumping time of 10 min) was pumped through the tail vein before releasing the left hilar clamp. This study was approved by the Animal Ethics Committee of Harbin Medical University Animal Center.

Rat Lung Ischemia-Reperfusion Model

Rats were anesthetized with 1% sodium pentobarbital (50 mg/kg). After anesthesia was sufficient, rats were intravenously injected with saline (10 mL/kg/h). Then the laryngoscope was intubated under the direct vision of the laryngoscope. The tracheal tube was connected to a small animal ventilator for mechanical ventilation. The specific parameters were maintained: the respiratory rate was 60 times/min, the tidal volume was 15 mL/kg, I: E = 1:2.5 and FiO2: 99%. Rat chest was opened by the fourth intercostal space and the subcutaneous tissue was blunt separated. The pleura was exposed and cut in the expiratory phase. The left thoracic cavity was exposed and the left hilar was carefully separated to expose the trachea. Then the portal vein was injected with 300 U/kg of unfractionated heparin via the tail vein. After 10 min, the left hilar was clamped with a non-invasive vascular clamp to establish an ischemic model. After 60 min, the non-invasive vascular clamp was released to restore the ventilation and blood supply of the left lung for 2 h.

Specimen Collection and Processing

At the end of the experiment, 2 mL of blood was collected from the internal iliac vein and stored at -80°C. The lung tissue was taken out, quickly washed with phosphate-buffered saline (PBS; Beyotime, Shanghai, China), and immediately placed in an 80°C refrigerator.

Detection of Serum Inflammatory Factors in Rats

Serum levels of interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), IL-10 and IL-1 in rats were measured according to the instructions of the enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA).

Examination of Malondialdehyde (MDA), Myeloperoxidase (MPO) Content and Superoxide Dismutase (SOD) and Catalase (CAT) Activities

After the lung tissue was homogenized, activities of MDA, MPO and SOD and CAT in the lung tissue were detected according to the instructions of the relative kits (Nanjing, Jiancheng Bioengineering Institute, Nanjing, China).

Western Blot Assay

The lung tissue was homogenized in radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China). After homogenization, the supernatant was centrifuged, and the total protein content was determined according to the bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL, USA). Protein samples were electrophoresed on polyacrylamide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk, the membranes were incubated with primary antibody of hypoxia-inducible factor-la (HIF-la), p-Akt, Caspase-3 and Caspase-9 (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. The membrane was incubated with the secondary antibody after rinsing with the Tris-buffered Saline and Tween 20 solution (TBST). Chemiluminescence was used to expose the protein bands on the membrane.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Analysis

Total RNA of the lung tissue was extracted according to the instructions. Complementary deoxyribose nucleic acid (cDNA) was synthesized by reverse transcription. The sequences of HIF-1a, p-Akt, Caspase-3 and Caspase-9 and glyceraldehyde 3-phosphate dehydrogenase (GADPH) (the internal reference) were obtained from GeneBank. Primers were designed using Primer 5.0 software, and the Ct values of the target gene and the reference gene were determined by qRT-PCR. The average value was calculated by the relative quantitative method, and the gene expressions in the treatment group relative to the blank group were calculated by the formula 2-ΔΔCt. Primer sequences used in this study were as follows: HIF-1a, F: 5'-CCTATGTAGTTGTGGAAGTTTATGC-3', R: 5'-ACTAGGCAATTTTGCTAAGAATG-3'; p-Akt, F: 5'-AGGAGGAGGAGGAGATGGA-3', R: 5'-GGTCGTGGGTCTGGAAAG-3'; Caspase-3, F: 5'- AGAACTGGACTGTGGCATTGAG-3', R: 5'-AAGCTTGTCGGCATACTGTTTC-3'. Caspase-9: F: 5'-CTGAGCCAGATGCTGTCCCATA-3', R: 5'-GACACCATCCAAGGTCTCGATGTA-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 17.0 statistical software (Chicago, IL, USA). Data were expressed as mean ± standard deviation. One-way analysis was used for comparison between groups, followed by Post-Hoc Test (Least Significant Difference). Paired t-test was used for comparison within the two groups. DUNNETT'S T3 analysis was used for comparing uneven variance. $p<0.05$ was considered statistically significant.

Results

Levels of Serum Inflammatory Factors

ELISA results showed that lung ischemia-reperfusion markedly increased the levels of IL-6, TNF-α, IL-10 and IL-1 in the IR group. In contrast, the expression levels of the above factors in the Dex group were lower compared with those in the IR group ($p<0.05$) (Figure 1A-1D).

MDA, MPO Content and SOD, CAT Activity in Lung Tissue

Activities of MDA and MPO in the lung tissue of the IR group significantly increased after LIRI, whereas dexmedetomidine pretreatment reversed the elevated activities of MDA and MPO in the Dex group (Figure 2A, 2B). Furthermore, LIRI decreased the activities of SOD and CAT in the IR group. However, dexmedetomidine pretreatment improved the activities of SOD and CAT in lung tissue of rats (Figure 2C, 2D).

Relative Protein Expression in Lung Tissues

The expression levels of HIF-1α and p-Akt in rat lung tissue of the IR group were markedly lower than those in the sham operation group. In

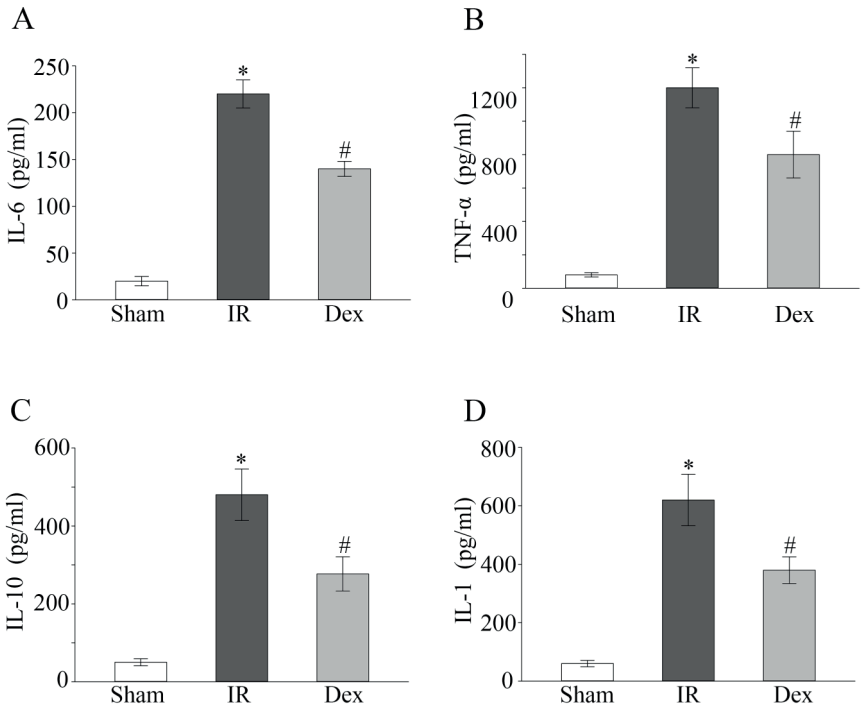

Figure 1. Levels of inflammatory factors in rats of different groups. ***A***, Comparison of serum level of IL-6 in three groups of rats. ***B***, Comparison of serum level of TNF-α in three groups of rats. ***C***, Comparison of serum level of IL-10 in three groups of rats. ***D***, Comparison of serum level of IL-1 in three groups of rats. *: The difference was statistically significant compared with the sham operation group (p<0.05); #: the difference was statistically significant compared with the IR group (p<0.05).

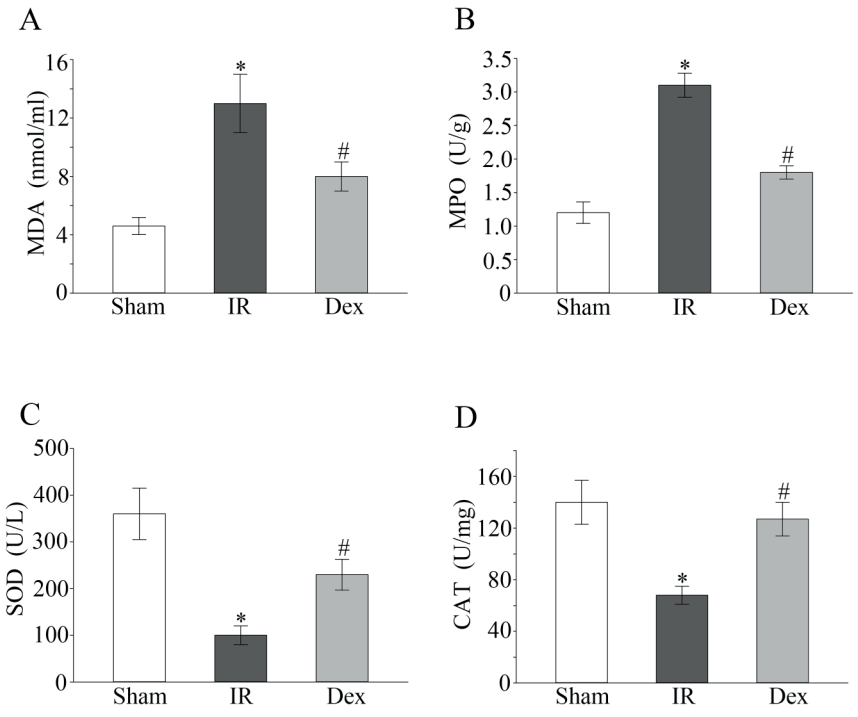

Figure 2. Activities of MDA, MPO, SOD and CAT in lung tissue of three groups of rats. ***A,*** Comparison of MDA content in lung tissue of rats in three groups of rats. ***B,*** Comparison of MPO content in lung tissue of rats in three groups of rats. ***C,*** Comparison of SOD activity in lung tissue of three groups of rats. ***D,*** Comparison of CAT activity in lung tissue of three groups of rats. *: The difference was statistically significant compared with the sham operation group (p<0.05); #: the difference was statistically significant compared with the IR group (p<0.05).

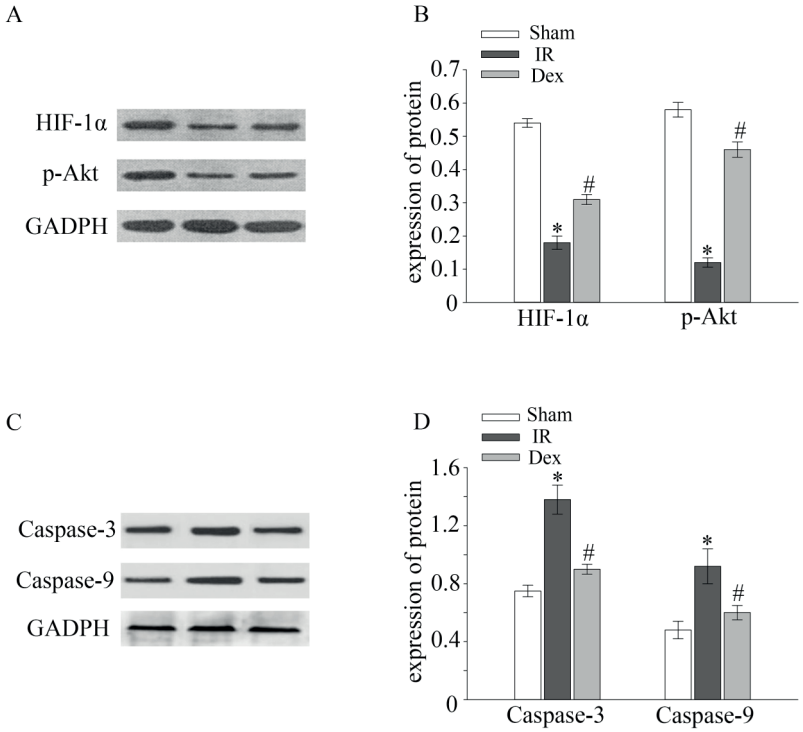

Figure 3. Western blot analysis of PI3K/Akt-related protein expression in lung tissue of three groups of rats. ***A,*** Expressions of HIF-1α and p-Akt in lung tissue of three groups of rats. ***B,*** Comparison of expression levels of HIF-1α and p-Akt in lung tissue of rats. ***C,*** Expressions of caspase-3 and caspase-9 in lung tissue of three groups of rats. ***D,*** Comparison of expression levels of caspase-3 and caspase-9 in lung tissue of three groups of rats. *: The difference was statistically significant compared with the sham operation group (p<0.05); #: the difference was statistically significant compared with the IR group (p<0.05).

addition, dexmedetomidine pretreatment markedly enhanced the expressions of HIF-1α and p-Akt in the Dex group (p<0.05) (Figure 3A, 3B). Besides, the expressions of Caspase-3 and Caspase-9 in the rat lung tissue of the IR group was remarkably higher than those in the sham operation group (p<0.05). However, dexmedetomidine pretreatment reversed this elevation (p<0.05) (Figure 3C, 3D).

Expression of HIF-1a, p-Akt, Caspase-3 and Caspase-9 mRNA in Lung Tissue

The mRNA levels of HIF-1αand p-Akt in rat lung tissue of the IR group markedly decreased compared to those in the sham operation group. However, dexmedetomidine pretreatment increased the expressions of HIF-1α, p-Akt and HIF- in the Dex group when compared to those in the IR group (Figure 4A, 4B). Besides, the levels of Caspase-3 and Caspase-9 in rat lung tissues of the IR group significantly increased after LIRI, but were conversely reduced after dexmedetomidine pretreatment (Figure 4C, 4D).

Discussion

Ischemia-Reperfusion (I/R) injury is a condition of tissues suffering from ischemia and hypoxia induced by various causes. After blood flow reperfusion, cell metabolism disorder and structural damage are further aggravated and some organ functions undergo further deterioration, which is a common surgical injury[19]. LIRI is a pathological phenomenon in which tissue damage is aggravated and even irreversible damage occurs after blood flow restores in the ischemic tissues and organs. The essence of LIRI is that the reversible injury of the ischemic period is further aggravated or converted into irreversible damage after restoring blood flow[20]. At present, the main pathogenesis of LIRI may be explained by free radicals, intracellular calcium overload, leukocyte activation and complement activation, but it has not been fully elucidated yet. In recent years, various theories have emerged to explain its underlying mechanism, including autoimmune theory, apoptosis theory, etc.[21-24].

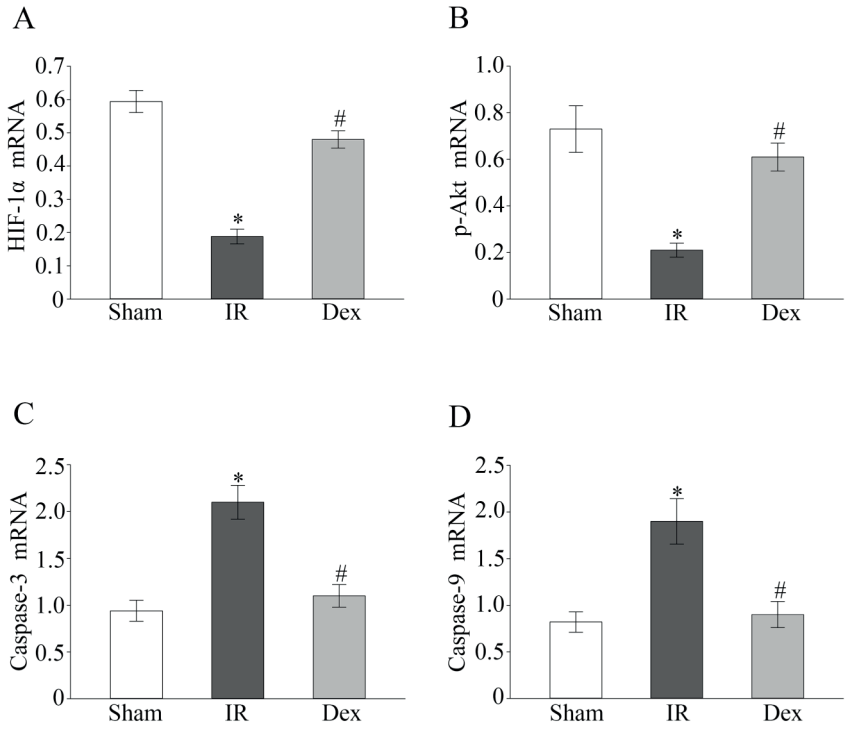

Figure 4. QRT-PCR detection of lung tissue-associated mRNA expressions in three groups of rats. ***A,*** Comparison of mRNA expression of HIF-1α in lung tissues of three groups of rats. ***B,*** Comparison of mRNA expression of p-Akt in lung tissue of rats in three groups of rats. ***C,*** Comparison of mRNA expression of caspase-3 in lung tissue of three groups of rats. ***D,*** Comparison of mRNA expression of caspase-3 in lung tissue of three groups of rats. *: The difference was statistically significant compared with the sham operation group (p<0.05); #: the difference was statistically significant compared with the IR group (p<0.05).

Dexmedetomidine is a second-generation, highly selective a2 adrenergic receptor agonist with an a2/a1 receptor selectivity ratio of 1620:1, which is 8 times that of clonidine. As a widely applied drug, dexmedetomidine can be used as preoperative sedatives, general anesthesia adjuvants, regional anesthesia adjuvants, postoperative sedation, analgesia, an so on[25]. In addition to these characteristics, dexmedetomidine has recently been found to inhibit inflammatory reactions in the brain, heart and kidney, exhibiting a protective effect on their function after ischemia and reperfusion[26,27]. In this study, dexmedetomidine significantly reduced the levels of IL-6, TNF-α, IL-10, and IL-1 in the LIRI model in rats. The activities of MDA and MPO in lung tissue was significantly down-regulated, while the activities of SOD and CAT were enhanced, indicating that dexmedetomidine can exert an anti-inflammatory effect by inhibiting the expressions of inflammatory factors in lung tissue, and can inhibit the process of oxidative stress damage. It is suggested that dexmedetomidine exerts its protective effect on the function of organs suffering from ischemia-reperfusion.

PI3K is an important member of the phospholipase kinase family. It is a heterodimer composed of a p110 catalytic subunit and a p85 regulatory subunit. It has lipid kinase and protein kinase activity and is an important signal transduction molecule in cells[28]. As an important downstream molecule of PI3K, Akt plays a key part in the regulation of cell proliferation, growth and survival, and is at the intersection of multiple signaling pathways[29,30]. In the PI3K/Akt signal transduction pathway, PI3K binds to and activates Akt by phosphorylating Akt at Ser473 and Thr30831. The caspase family is one of the important downstream effector molecules of the PI3K/Akt signaling pathway. It is mainly in the form of inactive zymogen in living cells and plays an essential role in the process of apoptosis. Apoptosis activates the irreversible hydrolysis substrate, caspase-9, serving as a promoter and caspase-3 serving as an effector. The PI3K/Akt signaling pathway regulates the cycle of apoptosis by inhibiting caspase

family activity, thereby affecting apoptosis[32-34]. In our study, Western blot assay showed that the protein levels of HIF-1α and p-Akt in the Dex group significantly increased compared to those in the IR group. However, Caspase-3 and Caspase-9 levels significantly decreased compared to those in the IR group, suggesting that Akt was activated and phosphorylated. Therefore, we hypothesized that the PI3K/Akt signaling pathway was inhibited after LIRI, leading to ischemia-reperfusion injury in rats. By comparison, dexmedetomidine pretreatment could protect the lung function of rats in the IR group by activating the PI3K/Akt signaling pathway.

Conclusions

The rat lung ischemia-reperfusion could result in severe lung injury. We showed that dexmedetomidine treatment can alleviate this injury by activating the PI3K/Akt signaling pathway at the transcriptional level.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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