Original Article

The activation of SIRT3 by dexmedetomidine mitigates limb ischemia-reperfusion–induced lung injury

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Background: The lung is one of the most sensitive organs, and is vulnerable to injury caused by limb ischemia-reperfusion (LIR). Dexmedetomidine, an anesthetic adjunct, has been shown to have therapeutic effects on lung injury secondary to LIR. This study aimed to investigate the role of dexmedetomidine in ameliorating LIR-induced lung injury in a mouse model of bilateral hind LIR.

Methods: In this study, 75 mice were randomly divided into 5 groups to prepare the LIR model. After the model was established, arterial blood was extracted for blood gas analysis. The pathological changes of lung tissue, lung wet/dry weight ratio, arterial blood gas analysis, detection of myeloperoxidase (MPO) activity, the content of reactive oxygen species (ROS), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in oxidative stress indexes, mitochondrial membrane potential (MMP), adenosine triphosphate (ATP) content and cytochrome c content were measured, and the relative protein expression levels of sirtuin-3 (SIRT3) and apoptosis factor Bcl-2 related X protein (Bax), B-cell Lymphoma 2 (Bcl-2), cleaved caspase 3, and nuclear factor erythroid 2-related factor 2 (Nrf2) and cytoplasmic heme oxygenase-1 (HO-1).

Results: Pretreatment with dexmedetomidine dramatically ameliorated LIR-induced lung injury, the wet/dry weight ratio, the arterial blood gas parameters, and enhanced SIRT3 expression. Moreover, dexmedetomidine significantly inhibits ROS and MDA level and restores antioxidant enzyme activities (SOD, GSH-Px). Of note, dexmedetomidine suppressed LIR-induced lung tissue apoptosis by modulating apoptosis-associated protein such as Bax, Bcl-2, and cleaved caspase 3. Moreover, dexmedetomidine inhibited the LIR-induced decreases in MMP, ATP levels, and the release of cytochrome c of LIR to maintain mitochondrial function. Latest study has shown that activating Nrf2 could promote SIRT3 expression to alleviate IR injury. Intriguingly, dexmedetomidine could facilitate nuclear Nrf2 and cytoplasmic HO-1 expression.

Conclusions: Our findings suggest that dexmedetomidine protects against LIR-induced lung injury by inhibiting the oxidative response, mitochondrial dysfunction and apoptosis. The mechanism appears to be at least partly mediated through the upregulation of SIRT3 expression.

Keywords: Dexmedetomidine; limb ischemia-reperfusion (LIR); lung injury; SIRT3

Submitted Jan 21, 2022. Accepted for publication Mar 14, 2022.
doi: 10.21037/atm-22-711
View this article at: https://dx.doi.org/10.21037/atm-22-711

Introduction

Limb ischemia-reperfusion (LIR) is a common disease in many clinical situations, such as atherosclerotic thrombosis, crush injury, and some surgical operations (1). It may be that ischemic hypoxia impairs the mitochondrial function of cells, resulting in cell swelling, necrosis, oxidative stress, and inflammatory reactions during reperfusion that further aggravate the tissue injury (2,3). The lung is a hypersensitive organ to damage during LIR, and its dysfunction is considered the main reason for morbidity and mortality (4).

Recent studies have shown that the dysfunction of mitochondrial morphology is an important hallmark of the development and progression of lung injury (5,6). Sirtuin-3 (SIRT3), a highly conserved nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase that is mainly expressed in mitochondria and it processes the most powerful deacetylase activity in all mitochondrial sirtuins (7). Emerging evidence suggests that SIRT3 regulates mitochondrial protein function of oxidative stress, fatty acid oxidation, and the antioxidant response system by deacetylation (8). In addition, SIRT3 could control the production of mitochondrial adenosine triphosphate (ATP) through its effect on the respiratory chain and also protects mitochondrial function by regulating the production of reactive oxygen species (ROS) through a variety of substrates, such as superoxide dismutase 2 (SOD2) (9) and transcription factor forkhead box O3a (FoxO3a) (10). However, little is known about the regulation of SIRT3 expression. Nuclear factor erythroid 2 related factor 2 (Nrf2) is a major key transcription factor and a major stress responder, its transcripts and activates genes with antioxidant and cytoprotective effects. *In vitro*, knockdown or overexpression of Nrf2 could regulate SIRT3 levels (11). Several studies indicated that SIRT3 attenuated tissue damage caused by cerebral (12), myocardial (13) and limb IR (14). Lee *et al.* (14) reported that the expression of SIRT3 was significantly increased in the aorta tissues of lower limb ischemic mice. Jiang *et al.* researched the effect of hydrogen sulfide on lung ischemia reperfusion damage and found that it may alleviate oxidative stress injury and mitochondrial dysfunction via SIRT3 (15). However, the role of SIRT3 in LIR-induced lung injury remains unclear.

There are many drugs to improve LIR related lung injury, such as fat emulsion, ulinastatin, taurine, angiotensin converting enzyme 2, etc., but they all have side effects to some extent. Dexmedetomidine is a highly selective α2 adrenergic receptor agonist with sedative, analgesic, anti-inflammatory, and antioxidant properties (16,17). Notably, recent research has shown that dexmedetomidine ameliorates hippocampus injury and cognitive dysfunction induced by hepatic ischemia/reperfusion by activating SIRT3-mediated mitophagy and inhibiting the activation of the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in young rats (18). Dexmedetomidine appears to protect against LIR-induced lung injury, but its underlying mechanisms are not yet clear. Thus, this study sought to explore the role of SIRT3 in LIR, and investigate the potential protection mechanisms of dexmedetomidine in LIR-induced lung injury. A protocol was prepared before the study without registration. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-711/rc).

Methods

Animals

Experiments were performed using male healthy C57BL/6J mice weighing 25–30 g (Vital River Laboratories, Beijing, China). Mice were maintained in a room with a temperature of 22±1 ℃ and 45–55% humidity on a 12-h light/dark cycle, and had free access to water and chow. Experiments were performed under a project license (No. 2021002) granted by the Laboratory Animal Ethics Committee of The Fourth Hospital of Hebei Medical University, in compliance with the National Institutes of Health's guidelines for the care and use of animals. All experiments were performed in the Animal Experiment Center of the Fourth Hospital of Hebei Medical University.

Modeling

According to the pre-experimental results, 75 samples were examined. The 75 mice were randomly divided into the following 5 groups (15 mice per group) using the digital randomization method: (I) the sham-model group (the control group); (II) the ischemia-reperfusion group (the IR group); (III) the dexmedetomidine group (the Dex + IR group); (IV) the dexmedetomidine group + SIRT3 inhibitor group (the 3-TYP + Dex + IR group); and (V) the SIRT3 inhibitor group (3-TYP + IR group).

Referred to the literature (19) to prepare the LIR model. Specifically, after anesthetizing the mice with sodium barbiturate, hindlimb ischemia was caused by ligating

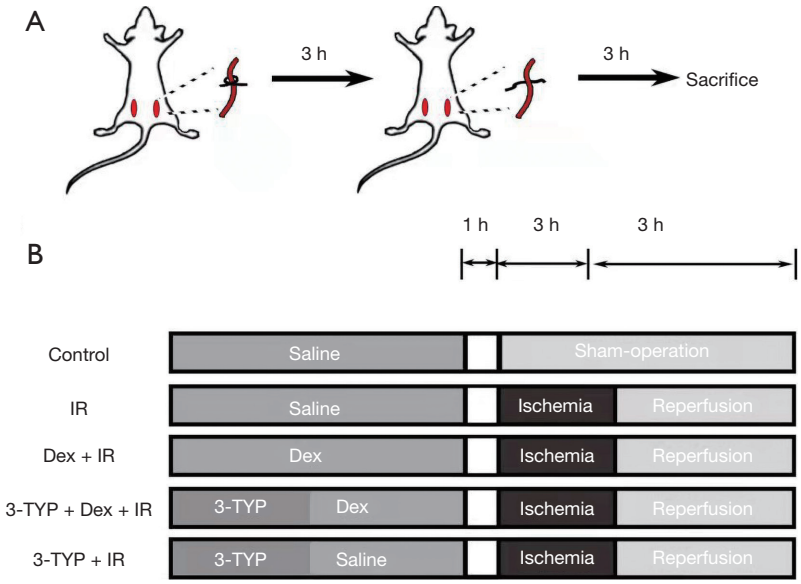

Figure 1 The diagram of animal experiments. (A) LIR was induced via hind limb ischemia. Mice were ligated with the tourniquet at the roots of bilateral hind limbs to cause ischemic. Three hours later, the tourniquet was removed. After 3-hour reperfusion, the mice were sacrificed, and the tissues were collected. (B) Mice in the control group only underwent post anesthesia thoracotomy and lung tissue separation; the other groups underwent surgery and clamping the blood vessels of the hind limbs. Saline was injected intraperitoneally into the control group and the IR group, and only the IR group was modeled after 1 hour. The Dex + IR group was injected with dexmedetomidine (50 μg·kg^{-1}) intraperitoneally, and the model was established 1 hour later. The 3-TYP + Dex + IR group received intraperitoneal injection of the SIRT3 selective inhibitor (3-TYP) (50 mg·kg^{-1}·d^{-1}) before dexmedetomidine (50 μg·kg^{-1}) intervention, and the model was established 1 hour later. The 3-TYP + IR group was injected with 3-TYP (50 mg·kg^{-1}·d^{-1}) intraperitoneally, and the model was established 1 hour later. IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine; LIR, limb ischemia-reperfusion; SIRT3, silent information regulator 3.

the roots of both the hindlimbs with a tourniquet, which was released after 3 h of ischemia. Both hindlimbs were then massaged to accelerate blood flow reperfusion, and after 3 h of reperfusion, the lung tissues were isolated and the peripheral blood was left for use, and the mice were sacrificed. Mice in the control group only underwent post anesthesia thoracotomy and lung tissue separation; the other groups underwent surgery and clamping the blood vessels of the hind limbs. The control group and IR group were injected with saline intraperitoneally, and 1 h later, only the IR group was modeled. The Dex + IR group was injected with dexmedetomidine (50 μg·kg^{-1}) intraperitoneally (20,21), and the model was established 1 hour later. The 3-TYP + Dex + IR group received intraperitoneal injection of 3-(1H-1,2,3-triazol-4-yl) pyridine (3-TYP) (50 mg·kg^{-1}·d^{-1}), a SIRT3 selective inhibitor, before dexmedetomidine (50 μg·kg^{-1}) intervention, and the model was established 1 hour later. The 3-TYP + IR group was injected with 3-TYP (50 mg·kg^{-1}·d^{-1}) intraperitoneally, and the model was established 1 hour later (see *Figure 1*).

Blood gas analysis

Arterial blood was extracted and arterial oxygen partial pressure (PaO_2) and arterial carbon dioxide partial pressure ($PaCO_2$) were recorded using a blood gas analyzer (ABL80, Denmark).

Lung tissue W/D weight ratio

The lung tissue was taken from each mouse, and the lung tissue sample was immediately weighed to obtain the wet weight. The lung tissue sample was then dried until the tissue weight did not change. Finally, the lung tissue sample

was reweighed to obtain the dry weight. The wet-to-dry (W/D) weight ratio of the lung tissue was calculated by dividing the wet weight by the dry weight.

Hematoxylin–Eosin (HE) staining

The removed lungs were embedded in paraffin and cut into 5-μm slices. The sections were then stained with hematoxylin (Solarbio, China) and eosin (Sangon, China). The stained sections were examined under a microscope at 200×/400× magnification (Olympus, Tokyo, Japan).

Detection of reactive oxygen species by dihydroethidine (DHE) staining

The lung tissues were cut into 10 μm sections on cryostat microtome (Leica, Nussloch, Germany) and then incubated with DHE (Beyotime, Shanghai, China) for 30 min without lighting, followed by three times washed by PBS. After staining, sections were examined under fluorescence microscope (Olympus, Tokyo, Japan).

Immunofluorescent staining

A series of 5 μm slices of lung tissue firstly dewaxed and rehydrated. After that, lung slices were permeabilized with PBS solution for three times and blocked in goat serum (Solarbio, Beijing, China) for 15 min at room temperature. Slices were then incubated with Nrf2 (1:50; ABclona, Wuhan, China) at 4 ℃ for overnight, and corresponding secondary antibody (Beyotime, Shanghai, China) for 1 h at room temperature in the dark. The cell nuclei were stained with 4, 6-diamino-2-phenylindole (DAPI) (Aladdin, Shanghai, China). The slices were finally mounted with antifluorescence quencher. The images were captured by fluorescence microscope at 400× magnification (Olympus, Tokyo, Japan).

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) assays

TUNEL assays were used to detect lung tissue apoptosis using an in-situ cell apoptosis detection kit (Roche, Switzerland). In brief, the dewaxed and rehydrated lung tissue slices were treated with TUNEL reagent, and then counterstained with DAPI. Photomicrographs were observed using a fluorescent microscope (Olympus, Japan).

Determination of biochemical indices

Cell lysate was added to part of the lung tissue, which was then centrifuged at 4 ℃ and 12,000 r/min for 15 min, after which the supernatant was left, and the concentrations of the proteins were analyzed. The level of malondialdehyde (MDA) (Jiancheng Bioengineering Institute, China), and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Jiancheng Bioengineering Institute, China) were then determined as per the directions of each kit.

Detection of myeloperoxidase (MPO) activity

The lung tissues were homogenized and centrifuged to obtain the supernatant for the following experimental detection. The activity of MPO was measured using MPO activity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) followed manufacturer's instructions.

Western blot analysis

Cell lysate and protease inhibitor phenylmethanesulfonyl fluoride (PMSF) were added to part of the left lung tissue homogenate, which was then lysed on ice for 2 h, and centrifuged at 4 ℃ and 12,000 r/min for 15 min. The supernatant was then taken and denatured at 95 ℃ for 10 min by Western blot, and transferred to polyvinylidene difluoride (PVDF) membranes after polyacrylamide gelelectrophoresis (PAGE) electrophoresis separation. Next, the primary antibody against the target was added, and it was then incubated in a shaking table for 2 h. The primary antibody was then discarded, and horseradish peroxidase labeled secondary antibody was added after tris-buffered saline with Tween (TBST) rinsing, and it was then incubated in a shaking table for 1 h, after which the secondary antibody was discarded. Finally, it was rinsed with TBST, exposed and developed using the chemiluminescence method, and analyzed with quantity 1 software. The ratio of the gray value of each target protein band to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) band reflects the expression of each target protein. The following primary antibodies were used: SIRT3 (A7307), Nrf2 (A0674), HO-1 (A19062), Bcl-1 (A0208) and Bax (A1968) (ABclona, Wuhan, China); cleaved-caspase 3 (#9654) and Cytochrome c (#11940) (Cell Signaling Technology; Danvers, MA, USA); COX IV (GTX49132);

GAPDH (60004-1-Ig) and Histone H3 (GTX122148) (Proteintech Group, Inc., Rosemont, IL, USA).

Mitochondrial function measurement

Detection of mitochondrial membrane potential (Δψm): Mitochondrial membrane potential (MMP) was measured using the 5, 5' 6, 6'-tetrachloro-1, 1' 3, 3'-tetraethylbenzi midazolylcarbocyanine iodide (JC-1) Assay Kit (Beyotime, Shanghai, China). Flow cytometry (ACEA Biosciences, San Diego, CA, USA) was used to detect the red/green fluorescence ratio. ATP measurement: Cellular ATP content was measured by ATP bioluminescence assay kit (Beyotime, Shanghai, China) and operated in accordance to the manufacturer's instructions.

Statistical analysis

SPSS 26.0 statistical software was used for the analysis. The measurement data with a normal distribution are expressed as the mean ± standard deviation, and the measurement data with a non-normal distribution are expressed as the median and interquartile interval [M (QL, QU)]. The Student's *t*-test or a 1-way analysis of variance was used for the comparisons. Differences were considered statistically significant at $P<0.05$.

Results

Dexmedetomidine alleviates LIR-induced lung injury

We investigated the effectiveness of dexmedetomidine on LIR. Compared with control group, the IR group showed significant lung injury changes: interstitial thickening, destruction of alveolar structures and massive inflammatory cell infiltration, while dexmedetomidine pretreatment obviously alleviated these changes (see *Figure 2A*). To further evaluate lung injury, the lung W/D ratio (see *Figure 2B*) and MPO activity (see *Figure 2C*) were significantly increased. And PaO_2 (see *Figure 2D*) in arterial blood gas decreased and $PaCO_2$ (see *Figure 2E*) increased in mice. Dexmedetomidine pretreatment dramatically reversed the levels of these lung injury indicators. As shown in *Figure 2E*, post-reperfusion, the protein expression levels of SIRT3 were markedly decreased, while an opposite trend was observed after dexmedetomidine pretreatment. In summary, we concluded that dexmedetomidine may alleviate LIR-induced lung injury by enhancing SIRT3 expression.

Dexmedetomidine protects the lungs from LIR-induced oxidative stress

To investigate the protective mechanism of dexmedetomidine in LIR-induced lung injury, we detected some oxidative stress indexes. As a result, ROS content (see *Figure 3A*) and MDA level (see *Figure 3B*) in lung tissue were higher in the IR group relative to that in the control group, while dexmedetomidine pretreatment attenuated ROS accumulation and MDA level. In addition, SOD and GSH-Px activities were significantly decreased in IR group compared to the control group (see *Figure 3C*,*3D*). Dexmedetomidine treatment partially blocked these phenomena. Intracellular SOD and GSH-Px activities were restored to near-physiological levels by dexmedetomidine treatment.

LIR-induced apoptosis in the lungs is repressed by dexmedetomidine

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) staining was used to detect apoptotic cells in the lung tissues of each experimental group. The lung tissues of IR group showed stronger positive TUNEL-staining compared to the control group. Nevertheless, TUNEL-positive cells were remarkable reduced by treatment with dexmedetomidine (see *Figure 4A*). Meanwhile, western blotting showed that LIR significantly decreased the expression of anti-apoptotic B-cell Lymphoma 2 (Bcl-2) (see *Figure 4B*), and promoted the expression of pro-apoptotic B-cell Lymphoma 2 related X protein (Bax) (see *Figure 4C*). Of note, cleaved-caspase 3, mitochondrial apoptotic pathway mediators, expression levels were prominently increased (see *Figure 4D*). However, a profound reverse effect was observed in the Dex + IR group compared to the IR group, which suggests that dexmedetomidine plays an anti-apoptosis role in LIR-induced lung injury in mice.

Dexmedetomidine improves the mitochondrial function in the lungs after LIR

Based on the above research results, dexmedetomidine could inhibit mitochondrial apoptosis pathway. The next step is to examine whether the anti-apoptotic effect of dexmedetomidine was along with the improvement of mitochondrial function. The result showed that MMP (see *Figure 5A*) dissipated and ATP level lessened (see *Figure 5B*)

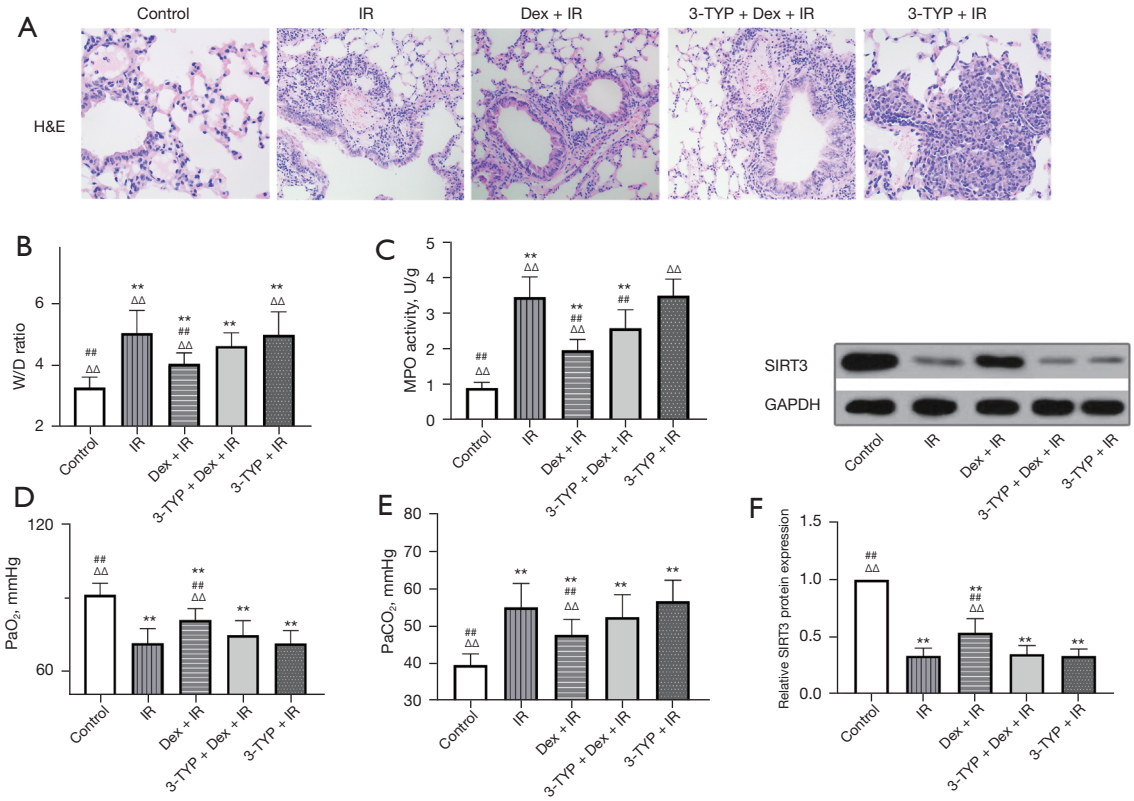

Figure 2 Dexmedetomidine alleviates LIR-induced lung injury. (A) H&E staining the morphological alterations in the lungs caused by LIR. Magnification: ×200. Scale =100 μm. (B) Pulmonary edema was assessed by W/D. (C) MPO activity in the lungs after LIR. (D) PaO_2 and (E) $PaCO_2$ were detected with arterial blood gas analysis. (F) Protein expression of SIRT3 in the lungs were determined by Western blot. The data were presented as the mean ± SD (n=15). **, P<0.05 versus Control; ##, P<0.05 versus IR; ΔΔ, P<0.05 versus 3-TYP + Dex + IR. H&E, Hematoxylin-Eosin; W, wet weight; D, dry lung weight; MPO, myeloperoxidase; PaO_2, the partial pressure of arterial O_2; CO_2, partial pressure of arterial; IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine; LIR, limb ischemia-reperfusion; SIRT3, silent information regulator 3.

following LIR injury compared with control group, whereas dexmedetomidine prevented the reduction of MMP and ATP levels. The release of cytochrome c from mitochondria to cytoplasm is a major step to link mitochondrial damage with apoptosis. The data in *Figure 5C* implied that LIR-induced lung injury caused significant translocation of cytochrome c from mitochondria to cytoplasm compared with the control group. Unsurprisingly, dexmedetomidine remarkable reversed the translocation of cytochrome c. Collectively, these findings indicated that dexmedetomidine could improve weakened mitochondrial dysfunction during LIR-induced lung injury.

Dexmedetomidine activates the Nrf2/HO-1 pathway in the lungs

To further investigate the underlying protective mechanism of dexmedetomidine, the activation of Nrf2/HO-1 pathway was measured by immunofluorescence staining and western blotting from lung tissue. Immunofluorescence displayed raised nuclear accumulation of Nrf2 induced by dexmedetomidine (see *Figure 6A*). In IR group, the expression levels of Nrf2 and cytoplasmic heme oxygenase-1 (HO-1) were increased (see *Figure 6B,6C*). While dexmedetomidine pretreatment further enhanced the

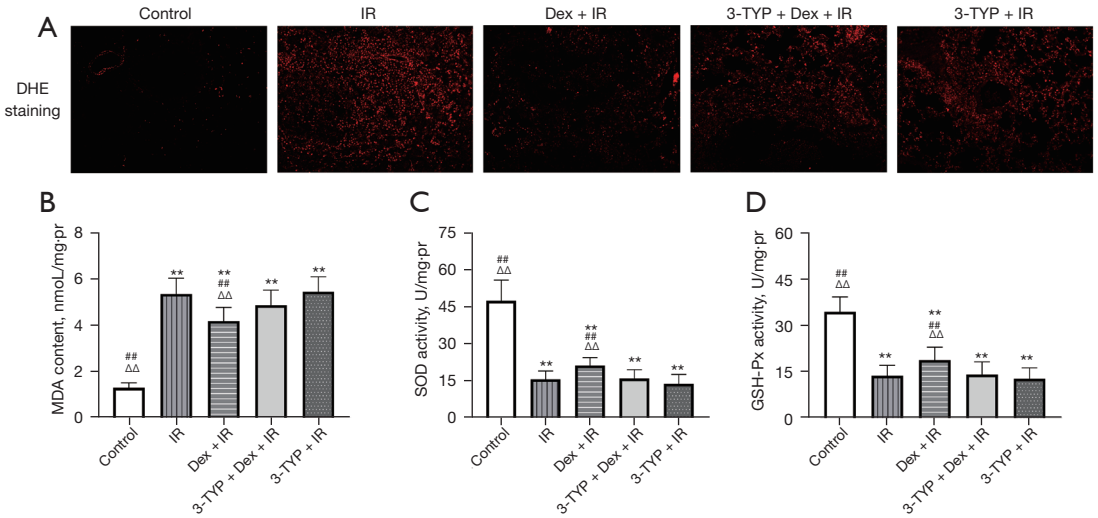

Figure 3 Dexmedetomidine prevents the lungs from LIR-induced oxidative stress. (A) ROS in the lungs after LIR was detected by dihydroethidium staining. Magnification: ×200. Scale bar =100 μm. (B) MDA level in the lungs after LIR. (C) SOD activity in the lungs after LIR. (D) GSH-Px activity in the lungs after LIR. The data were presented as the mean ± SD (n=15). **, P<0.05 versus Control; ##, P<0.05 versus IR; ΔΔ, P<0.05 versus 3-TYP + Dex + IR. DHE, dihydroethidine; IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; LIR, limb ischemia-reperfusion; ROS, reactive oxygen species.

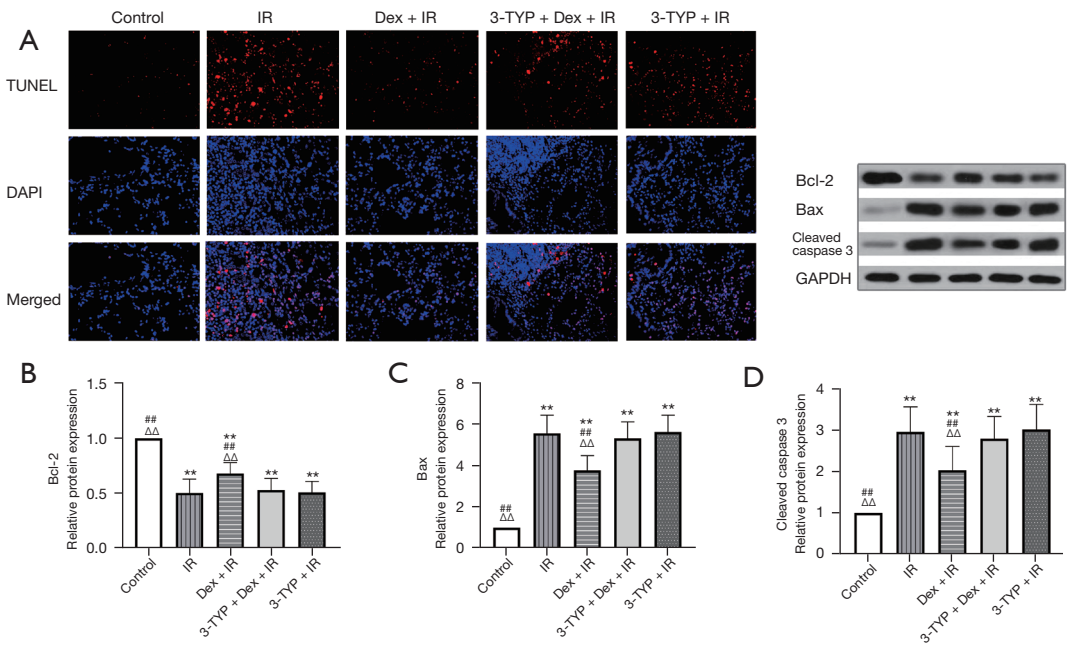

Figure 4 LIR-induced apoptosis in the lungs is repressed by dexmedetomidine. (A) TUNEL staining was performed to detect apoptosis in the lungs of mice. Magnification: ×400. Scale bar =50 μm. (B) The relative protein level of anti-apoptotic factor Bcl-2 was determined with Western blot. (C) The relative protein level of pro-apoptotic factor Bax was determined with Western blot. (D) The relative protein level of cleaved caspase 3 was determined with Western blot. The data were presented as the mean ± SD (n=15). **, P<0.05 versus Control; ##, P<0.05 versus IR; ΔΔ, P<0.05 versus 3-TYP + Dex + IR. TUNEL, transferase-mediated deoxyuridine triphosphate-biotin nick end labeling; DAPI, 4',6-Diamidino-2'-phenylindole; IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine Bcl-2, B-cell Lymphoma 2; Bax, Bcl-2 related X protein; LIR, limb ischemia-reperfusion.

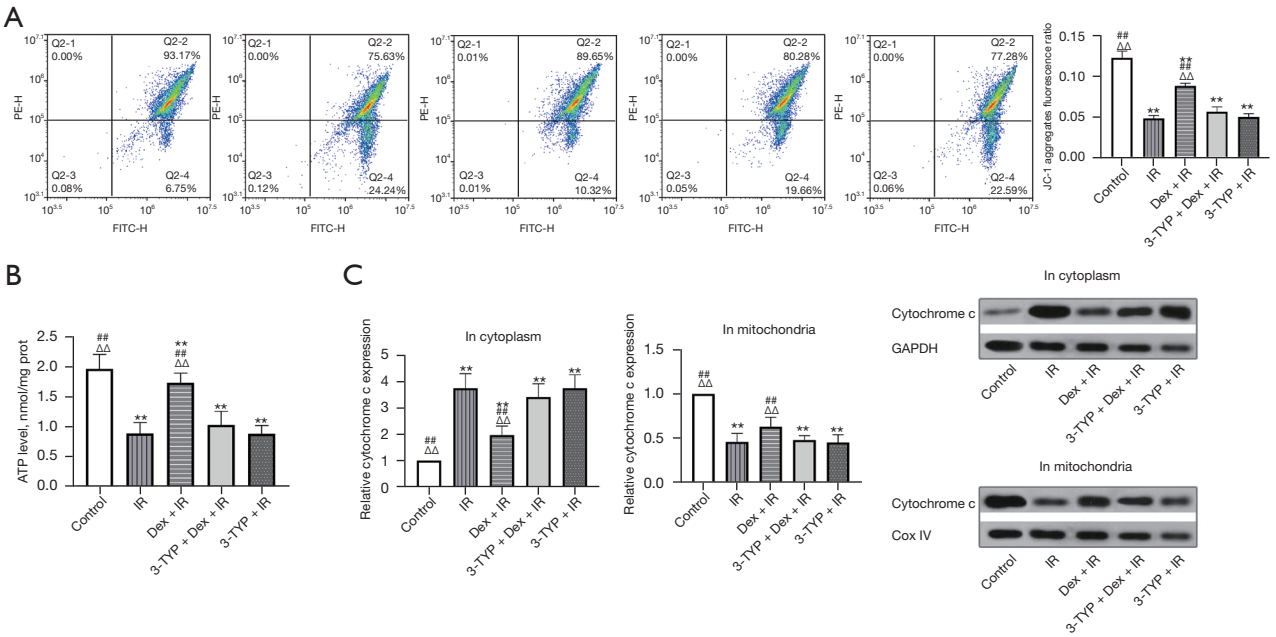

Figure 5 Dexmedetomidine improves the mitochondrial function in the lungs after LIR. (A) Changes in MMP were determined with JC-1 under flow cytometry. Representative images and fluorescence intensities ratio of JC-1 aggregates (red) to JC-1 monomer (green) were shown. (B) ATP level were examined in the lungs. (C) The relative protein level of cytochrome c in the cytoplasm and mitochondria was determined with Western blot. The data were presented as the mean ± SD (n=15). **, P<0.05 versus Control; ##, P<0.05 versus IR; ΔΔ, P<0.05 versus 3-TYP + Dex + IR. IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine; LIR, limb ischemia-reperfusion; MMP, mitochondrial membrane potential; ATP, adenosine-triphosphate.

expression of these proteins.

Discussion

In the present study, we found that: (I) SIRT3 was lowly expressed in the lung tissue of the LIR mouse model; and (II) dexmedetomidine alleviated LIR-induced lung injury, and its potential mechanism may be related to the reduction of oxidative stress, the promotion of mitochondrial function, and the inhibition of apoptosis.

Limb ischemia is a common clinical lesion, which is always treated by reperfusion to save the body. However, the recovery of blood circulation in ischemic limbs causes acute injury to the distal organs (22). Previous research has demonstrated that LIR may induce lung injury (23). In this experiment, after the reperfusion and ligation of the root of the hind limb, it was found that the blood gas index in the IR group deteriorated, the pathological section showed the destruction of the alveolar structure, and the lung injury score increased significantly, suggesting that the model of LIR lung injury had been successfully prepared.

SIRT3, a deacetylase, is shown to play a vital role in IR injury, such as cerebral, intestinal and renal IR injury (24,25). Our research first proved that SIRT3 may participate in the pathogenesis of lung injury after LIR, which provided a novel target and mechanism for this pathogenesis. The histological results of this study are consistent with those of previous research that confirmed that LIR-induced lung injury in mice (26). Some evidence suggests that inflammation may participate in mediating LIR injury (27). We found different indications of inflammation in the lung tissue of the LIR group, which was characterized by an obvious increase in vascular dilation and hyperemia ($PaO_2/PaCO_2$). Additionally, pulmonary edema was detected, and a significant increase in the wet/dry weight ratio was observed. These findings showed that LIR may induce lung inflammation. However, these effects were reversed by dexmedetomidine, which also attenuated the histopathological damage. A recent study reported that dexmedetomidine increases the expression of SIRT3 in hepatic ischemia-reperfusion (26), which was consistent with our results. Our data showed that dexmedetomidine

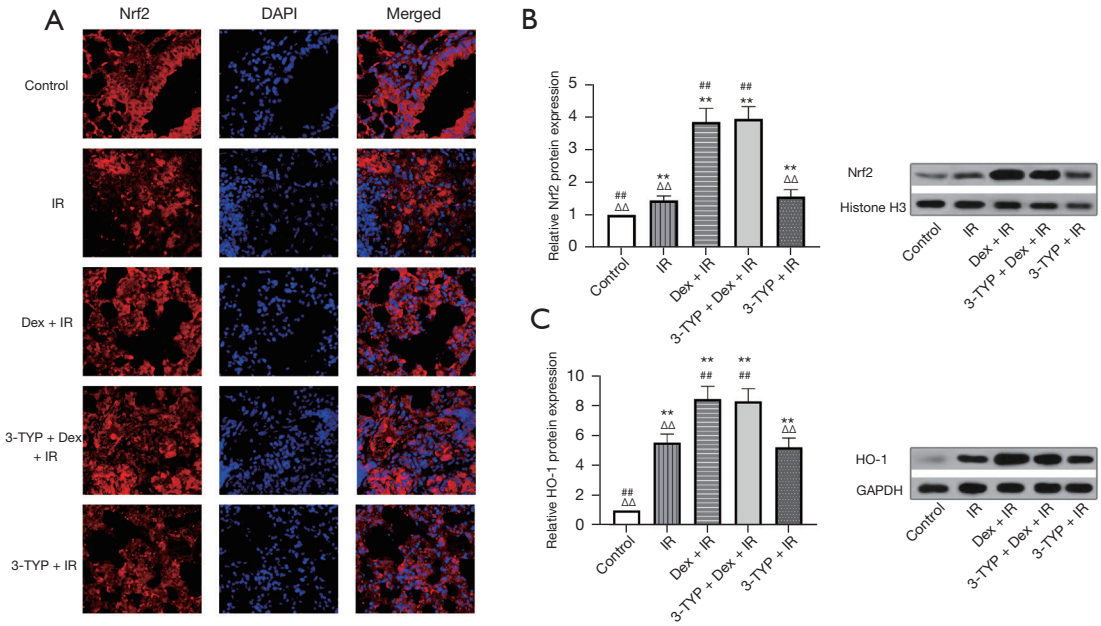

Figure 6 Dexmedetomidine activates the Nrf2/HO-1 pathway in the lungs. (A) Immunofluorescence staining of Nrf2 in the lungs. Magnification: ×400. Scale bar =50 μm. (B,C) Representative images and quantification of Nrf2 expression in the nucleus and HO-1 expression in the lung tissues. The data were presented as the mean ± SD (n=15). **, P<0.05 versus Control; ##, P<0.05 versus IR; ΔΔ, P<0.05 versus 3-TYP + Dex + IR. Nrf2, nuclear factor erythroid 2-related factor 2; DAPI, 4',6-Diamidino-2'-phenylindole; IR, ischemia-reperfusion; Dex, dexmedetomidine; 3-TYP, 3-(1H-1,2,3-triazol-4-yl) pyridine; HO-1, cytoplasmic heme oxygenase-1.

significantly increases SIRT3 expression. Taken together, these results indicate that dexmedetomidine prevents lung injury after LIR, and partly does so via the upregulation of SIRT3.

LIR-induced lung injury is a complex pathophysiological process, in which oxidative stress plays a key role. During ischemia, endothelial cells, neutrophils and macrophages will produce a large amount of ROS (28). In addition, the increased ROS could lead to protein and DNA damage through lipid peroxidation, which is considered to be a crucial cause of oxidative damage to cell membrane, leading to cell death (29,30). The increasing levels of MDA in lung tissue after ischemia-reperfusion will exacerbate inflammatory response and pulmonary dysfunction, leading to pulmonary edema and gas exchange impedance (31). SOD and GSH-Px are key antioxidant enzymes in cells to eliminate reactive free radicals. The loss of antioxidant enzymes significantly led to the accumulation of free radicals, which further aggravated lung injury (32). In the current study, dexmedetomidine treatment obviously decreased the levels of ROS and MDA and enhanced the activities of SOD and GSH-Px in lung tissue. The antioxidant capacity of dexmedetomidine have been reported in some studies (33). A recent study reported that dexmedetomidine protects against oxidative injury through the regulation of oxidative stress via SIRT3 after intestinal ischemia reperfusion (34). In addition, several researches have reported that SIRT3 could regulate ROS generation mainly by changing the acetylation of SOD2, thereby controlling redox equilibrium (35,36). Therefore, we inferred that a component of dexmedetomidine's protective effect may stem from its effect on the SIRT3 mediating oxidative stress, but the specific mechanism needs further study.

Accumulated ROS in mitochondria are the main source of intracellular oxidative stress. The imbalance between ROS production and antioxidant defense makes mitochondria particularly vulnerable to damage and leads to the initiation of apoptosis that involved in the pathogenesis of IR injury (37,38). Bcl-2 and Bax are members of the Bcl-2 family, which are responsible for regulating mitochondria or internal apoptotic pathway (39). Bax is a pro-apoptotic protein of cell apoptosis, which mainly exists in cytoplasm of normal cell. Bcl-2 is the anti-apoptotic protein that prevents

apoptosis by preventing the loss of outer mitochondrial membrane integrity (40). Bcl-2/Bax low ratio is considered to be a critical factor directly related to apoptosis, and lead to the activation of caspase 3 and apoptosis (41). Moreover, it was reported that Bcl-2 protein could form holes in the outer membrane of mitochondrial, and the activation of Bax would be translocated to mitochondria and other membrane sites, thereby resulting in mitochondrial dysfunction (42,43). These will result in increased permeability of the mitochondrial membrane, mitochondrial membrane potential (MMP) reduction, mitochondrial ATP synthesis stop, and finally cytochrome c released from mitochondria to cytoplasm (44). The release of cytochrome c is a key step in the process of apoptosis (45). Cytochrome c will trigger the activation of caspase 3, eventually inducing to cell apoptosis (46). It was reported that the IR-induced down-regulation of SIRT3 was accompanied by the increased of apoptotic-related molecular changes (47), the overexpression of SIRT3 could increase the ratio of Bcl-2/Bax to protect cells from apoptosis (48). The role of SIRT3 in regulating mitochondrial function and anti-apoptotic effect has been extensively demonstrated (49). Similar to our results, our data showed that dexmedetomidine pretreatment could promote ATP synthesis, inhibit the dissipation of MMP, prevent the release of cytochrome c, thus inhibiting caspase 3 and restoring the imbalance of Bax and Bcl-2 expression profiles to prevent cell apoptosis. Chen *et al.* have proved that dexmedetomidine protects against acute stress-induced kidney injury in rats by reducing oxidative stress and apoptosis via caspase-3 dependent pathway (50). In conclusion, our results suggested that dexmedetomidine may improve mitochondrial function and down regulate lung cell apoptosis, so as to reduce lung injury after LIR.

To clarify the mechanism of dexmedetomidine triggered SIRT3 activation, then we studied whether it could activate Nrf2/HO-1 pathway. Recent work has reported that Nrf2 could bind to SIRT3 promoter to regulate SIRT3 expression (51). Moreover, Nrf2 signal pathway also plays a vital role in IR injury (52,53). Under normal physiological conditions, Nrf2 and kelch-like ECH-associated protein 1 (Keap1) are bound together. Once activated, Nrf2 breaks away from Keap1-Nrf2 binding and transfers to the nucleus, where it activates genes driven by antioxidant response elements such as SOD and HO-1 (54). HO-1 is considered to be a cytoprotective protein activated by Nrf2. It can prevent apoptosis and oxidative stress under a variety of pathological conditions. A large number of studies have shown that the overexpression of Nrf2 and HO-1 could reduce apoptosis and increase antioxidant capacity after IR injury (55,56). Feng *et al.* have found that activation of Nrf2/SIRT3 signaling pathway can alleviate oxidative damage of PC12 cells induced by oxygen glucose deprivation and reoxygenation (57). In the study, we found that in the condition of LIR, the expression levels of Nrf2 and HO-1 were slight increased indicating that LIR induced oxidative stress. While dexmedetomidine deeply promoted Nrf2 nuclear translocation and enhanced the expression of Nrf2 and HO-1, suggesting the protection mechanism of dexmedetomidine at least partly by further activating Nrf2/HO-1 pathway. Our study has proved that dexmedetomidine could promote the expression of SIRT3. Based on above findings, it can be reasonably assumed that Nrf2/HO-1/SIRT3 axis is involved in the protective effect of dexmedetomidine in lung injury after LIR. However, our study only detected the change of Nrf2, HO-1 and SIRT3 and did not reveal any specific relationship among them. This is a limitation of our research. In the future, we could conduct further studies to reveal the relationship among Nrf2, HO-1 and SIRT3 and to demonstrate the exact protection mechanism of dexmedetomidine in LIR-induced lung injury.

In conclusion, the current research shows that dexmedetomidine could largely attenuate LIR-induced lung injury, probably by activating Nrf2/HO-1/SIRT3 axis, which in turn reduces inflammatory, alleviated oxidative stress, promotes mitochondrial function, and inhibits apoptosis. Dexmedetomidine appears to be a new beneficial therapy for LIR-induced lung injury, but it is a prelude to a new study that needs a large number of experiments to verify.

Acknowledgments

Funding: This funding was provided by the Science and Technology Bureau of Baoding (No. 2141ZF092) and the Key Science and Technology Research Plan of Hebei Provincial Department of Health (No. 20221176).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-711/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-711/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-711/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. 2021002) granted by the Laboratory Animal Ethics Committee of The Fourth Hospital of Hebei Medical University, in compliance with the National Institutes of Health's guidelines for the care and use of animals.

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Cite this article as: Wang L, Ding Y, Bai Y, Shi J, Li J, Wang X. The activation of SIRT3 by dexmedetomidine mitigates limb ischemia-reperfusion–induced lung injury. Ann Transl Med 2022;10(6):319. doi: 10.21037/atm-22-711

