European Review for Medical and Pharmacological Sciences 2020; 24: 12400-12407

Dexmedetomidine promotes the recovery of renal function and reduces the inflammatory level in renal ischemia-reperfusion injury rats through PI3K/Akt/HIF-1α signaling pathway

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Abstract. – OBJECTIVE: To evaluate the protective effect of dexmedetomidine (Dex) against renal ischemia-reperfusion injury (RIRI) in rats through the phosphatidylinositol 3-hydroxy kinase (PI3K)/protein kinase B (Akt)/hypoxia-inducible factor-1α (HIF-1α) signaling pathway.

MATERIALS AND METHODS: (1) A Sprague-Dawley rat model of RIRI was established. Thirty rats were divided into Sham group, injury (RIRI) group, and Dex treatment (RIRI + Dex) group. Serum was collected to detect renal function-related indexes, and the levels of serum inflammatory factors were examined via enzyme-linked immunosorbent assay (ELISA). (2) The kidney tissues were separated, and the degree of tissue damage was determined using immunohistochemical staining. (3) Ribonucleic acids (RNAs) were extracted from tissues, and the mRNA levels of inflammatory factors were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). (4) The protein expressions of Akt, phosphorylated (p)-Akt, PI3K, p-PI3K, and HIF-1α were detected *via* Western blotting.

RESULTS: Compared with those in RIRI group, the levels of blood urea nitrogen and creatinine declined ($p<0.05$), the synthesized mRNAs of inflammatory factors in the kidney tissues were reduced ($p<0.05$), the secreted serum inflammatory factors was also reduced ($p<0.05$), and the phosphorylation levels of Akt and PI3K and the HIF-1α level rose ($p<0.05$) in RIRI + Dex group.

CONCLUSIONS: Dex promotes the recovery of renal function and reduces the inflammatory level in RIRI rats through the PI3K/Akt/HIF-1α signaling pathway.

Key Words:
Ischemia-reperfusion injury, Dexmedetomidine, PI3K/Akt/HIF-1α, Inflammation.

Introduction

Acute kidney injury (AKI) is a kind of destructive renal insufficiency syndrome caused by kidney surgery, sepsis or renal toxicity, which may trigger the onset of chronic kidney disease (CKD), lead to the accumulation of nitrogenous wastes such as blood urea nitrogen (BUN) and creatinine (Cr), and further cause renal failure, with higher morbidity and mortality rates[1,2]. However, the therapeutic effect is still unsatisfactory, bringing heavy social and economic burden. End-stage CKD is characterized by irreversible loss of renal function, and dialysis or kidney transplantation is needed for treatment[3-5]. Renal ischemia-reperfusion injury (RIRI) is a major cause of AKI, and it is reported that RIRI is manifested as the severe injury of renal tubular epithelial cells and vascular system, accompanied by strong inflammatory response[6]. Apoptosis also affects the occurrence of RIRI[7,8]. Therefore, the targeted therapy for oxidative stress, apoptosis, and inflammation is beneficial to the recovery of RIRI. Mesenchymal stem cells (MSCs) possess the inherent ability to produce the cellular phenotype of host organs and improve tissue repair and regeneration, benefitting the treatment of AKI[9,10].

Dexmedetomidine (Dex) is a kind of clinically common sedative. It has been detected that Dex reduces cerebral IRI and alters the components of the phosphatidylinositol 3-hydroxy kinase (PI3K)/protein kinase B (Akt) signaling pathway[11]. Hypoxia-inducible factor-1α (HIF-1α), a nuclear transcription factor, plays an important role in the activation of hypoxia state, which is regulated by the oxygen concentration and related to the activation

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of the PI3K/Akt signaling pathway[12,13]. It is reported that[14] Dex alleviates pulmonary IRI through the PI3K/Akt/HIF-1α signaling pathway. However, the protective effect of Dex against RIRI through the PI3K/Akt/HIF-1α signaling pathway has not been evaluated in studies. Therefore, this experiment aims to evaluate whether the PI3K/Akt/HIF-1α signaling pathway is involved in the protective effect of Dex against RIRI.

Materials and Methods

Reagents

Normal saline was purchased from Guangzhou Minsheng Pharmaceutical Co., Ltd. (Guangzhou, China), the reagents used for evaluating oxidative stress and the Griess reagent from Sigma-Aldrich (St. Louis, MO, USA), radioimmunoprecipitation assay (RIPA) lysis buffer, Western blotting reagent, polyvinylidene difluoride (PVDF) membranes, Akt, phosphorylated (p)-Akt, PI3K, p-PI3K and HIF-1α antibodies and horseradish peroxidase (HRP)-coupled secondary antibodies from Abcam (Cambridge, MA, USA), and enzyme-linked immunosorbent assay (ELISA) kits from eBioscience (San Diego, CA, USA).

Establishment of Rat Model of RIRI

After 30 Sprague-Dawley rats weighing 220-280 g were anesthetized *via* intraperitoneal injection of 3% pentobarbital (50 mg/kg), the abdominal muscle was cut along the Hunter's line to open the abdominal cavity, and the left renal pedicle was dissociated to fully expose the left renal artery. The right renal pedicle vessels were dissociated, and the right kidney was removed. The left renal artery was clamped using the microvascular clamp to block the renal arterial blood flow for 45 min, during which the anesthetic was continuously infused. Then, the left renal blood perfusion was restored, and after the good renal blood supply and no active bleeding in abdominal cavity were confirmed, the abdominal cavity was sutured and closed. This study was approved by the Animal Ethics Committee of Dongzhimen Hospital Beijing University of Chinese Medicine.

Grouping

In Sham group (n=10), the rats only received surgical operation without ischemia treatment. In RIRI group (n=10), normal saline was injected (5 mL/mg) for intervention twice immediately and at 6 h after RIR. In Dex treatment (RIRI + Dex) group (n=10), Dex was injected (5 mL/mg) for intervention twice immediately and at 6 h after RIR. After 48 h, the blood was drawn, and the kidney tissues were taken, from which ribonucleic acids (RNAs) and proteins were extracted.

Evaluation of Oxidative Stress

Malondialdehyde (MDA) is the final product of lipid peroxidation. 1500 μL of acetic acid (20%), 1500 μL of TBA (0.8%) and 1500 μL of sodium lauryl sulfate (8.1%) were added into 200 μL of kidney homogenate, and the mixture was heated *via* boiling water bath for 60 min. After cooling, 4 mL of 1-butanol was added and mixed with the mixture for 1 min, followed by centrifugation at 3000 rpm for 15 min. Then, the absorbance of the upper layer was measured at a wavelength of 532 nm. After that, the antioxidant capacity of the kidney was determined using ferric ion reducing antioxidant power (FRAP) assay, and 50 μL of kidney homogenate was added into 1.5 mL of freshly-prepared FRAP reagent and incubated at 37°C for 5 min. Finally, the absorbance of the blue complex was measured at a wavelength of 593 nm.

Determination of Renal Function

At 24 h after reperfusion, the blood was collected, incubated at 37°C for 15 min, and centrifuged at 4000 rpm for 15 min to separate the serum. The serum was stored in a refrigerator at -20°C, and the concentrations of Cr and BUN were detected using the automatic analyzer.

Histological Examination

The degree of renal tissue damage was determined through hematoxylin-eosin (H&E) staining. Briefly, the kidney tissues were fixed with formaldehyde solution, dehydrated, embedded in paraffin, and serially sliced into 5 μm-thick sections, followed by H&E staining. Then, the degree of damage was detected based on the following scoring criteria: 0 point: no damage, 1 point: damage <10%, 2 points: damage =10-25%, 3 points: damage =26-45%, 4 points: damage =75%, and 5 points: damage >75%.

Detection of Serum Nitrite Level Using Griess Assay

The zinc sulfate powder (6 mg) and serum samples (400 mL) were mixed and vortexed for 1 min, followed by centrifugation at 12, 000 rpm and 4°C for 10 min. Then, 50 μL of supernatant was taken, added with 100 μL of Griess reagent and incubated at room temperature for 30 min.

Finally, the absorbance was measured at a wavelength of 450 nm[15].

Detection of Changes in mRNA Levels Using Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from kidney tissues, and reversely transcribed into cDNA. The mRNA level was quantified using SYBR Green PCR reagent (Promega, Madison, WI, USA). The reaction system volume was in total 25 μl, pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 3 min, with 35 cycles, and then extension at 72°C for 5 min. PCR products were stored at 4°C. Quantitative analysis was carried out using the ABI 7500 fluorescence PCR amplification instrument (Applied Biosystems; Foster City, CA, USA). With the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference, the relative mRNA expressions of interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) were calculated. The qRT-PCR primer sequences are shown in Table I.

Detection of Serum Inflammatory Factors

First, the enzyme-linked immunosorbent assay (ELISA) kits were equilibrated at room temperature for 20 min. Then, the anticoagulant blood was centrifuged at 4000 rpm for 15 min, and the serum was collected to measure the levels of inflammatory factors *via* ELISA according to the instructions of the ELISA kit (eBioscience, San Diego, CA, USA).

Analysis of Related Protein Levels Using Western Blotting

Proteins were extracted from kidney tissues using radio immunoprecipitation assay (RIPA) lysis buffer and subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 60 μg/lane). Thereafter, they were transferred onto polyvinylidene difluoride (PVDF) membranes and sealed with Tris-buffered saline + 0.1% Tween-20 (TBST) containing 5% skim milk powder for 1 h. Subsequently, the proteins were incubated with Akt (1:3000), p-Akt (1:500), PI3K (1:100), p-PI3K (1:2000), HIF-1α (1:2000), and β-actin (1:2000) primary antibodies at 4°C overnight, and incubated again with horseradish peroxidase (HRP)-coupled secondary antibodies (1:3000) for 1 h. Finally, after washing with TBST, the color was developed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (SPSS, Chicago, IL, USA) software was used for statistical analysis. The numerical variables were expressed as mean ± standard deviation, and independent-samples *t*-test was performed for the comparison between two groups. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ suggested the statistically significant difference.

Results

Dex Improved Renal Function in RIRI Rats

The levels of renal function markers BUN and Cr were increased in RIRI group compared with those in Sham group ($p<0.05$), while they were significantly decreased in RIRI + Dex group compared with those in RIRI group ($p<0.05$). There were no significant differences in the levels of renal function markers BUN and Cr between Sham group and RIRI + Dex group (Figure 1).

Dex Improved Tissue Damage in RIRI Rats

The results of H&E staining showed that the histological structure of the kidney significantly changed and the tissue damage score was higher in RIRI group compared with that in Sham group ($p<0.05$). However, the tissue

Table I. qRT-PCR primer sequences.

Index	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
TNF-α	ATGAGCACTGAAAGCATGAT	CTCTTGATGGCAGAGAGGAG
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC

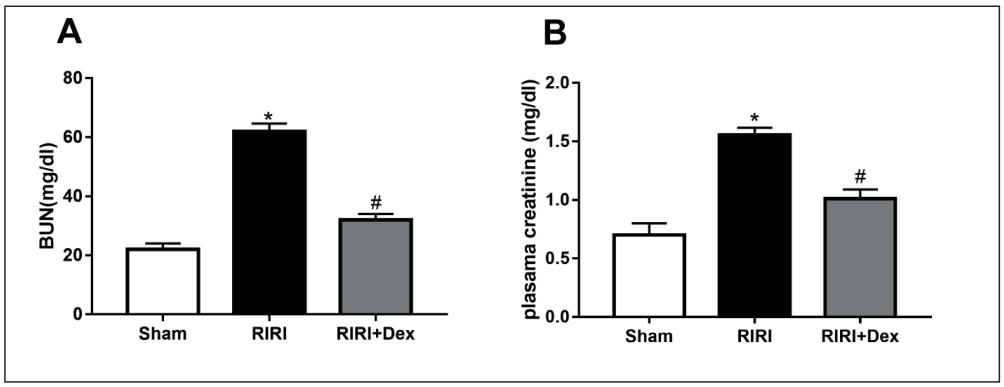

Figure 1. Effects of Dex on ischemia/reperfusion renal function markers: **A**, BUN, **B**, blood Cr. Note: The levels of BUN and Cr rise in RIRI group compared with those in Sham group (*p<0.05), and decline in RIRI + Dex group compared with those in RIRI group (#p<0.05).

damage score in RIRI + Dex group significantly declined compared with that in RIRI group (p<0.05) (Figure 2).

Dex Affected Oxidative Stress Level in RIRI Rats

To determine the oxidative stress level in RIRI rats after Dex treatment, the content of MDA and FRAP in serum and kidney tissues were detected. The results revealed that the level of MDA was markedly increased and FRAP significantly enhanced in serum and kidney tissues in RIRI group compared with those in Sham group (p<0.05). Besides, the level of MDA markedly declined and FRAP was significantly enhanced in serum and kidney tissues in RIRI + Dex group compared with those in RIRI group (p<0.05) (Figure 3).

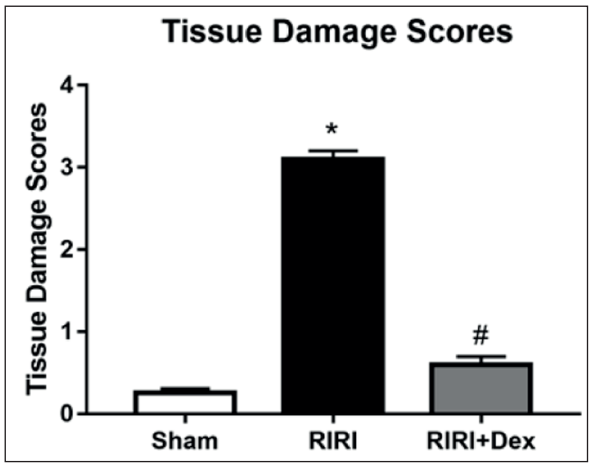

Figure 2. Dex improves tissue damage in RIRI rats. Tissue damage score. Note: RIRI group has a higher tissue damage score and severer damage than Sham group (*p<0.05). RIRI + Dex group has a significantly lower tissue damage score than RIRI group (#p<0.05).

Dex Reduced Serum Nitrite Level in RIRI Rats

To determine whether Dex causes changes in the serum nitrite level in RIRI rats, the serum nitrite level was measured. It was found that RIRI group had an evidently higher serum nitrite level than Sham group (p<0.05), while RIRI + Dex group had a significanttly lower serum nitrite level than RIRI group (p<0.05) (Figure 4).

Dex Lowered Inflammatory Level in RIRI Rats

To determine whether Dex causes changes in the inflammatory level in RIRI rats, the levels of inflammatory factors in tissues and serum were measured. It was found that RIRI group had remarkably higher levels of inflammatory factors in tissues and serum than Sham group (p<0.05), while RIRI + Dex group had lower levels of inflammatory factors in tissues and serum than RIRI group (p<0.05; Figure 5).

Dex Increased Phosphorylation of PI3K and Akt and HIF-α Level in RIRI Rats

To determine whether Dex leads to changes in the PI3K/Akt/HIF-1α pathway, the protein levels of PI3K, p-PI3K, Akt, p-Akt, and HIF-1α were measured. The results manifested that the levels of

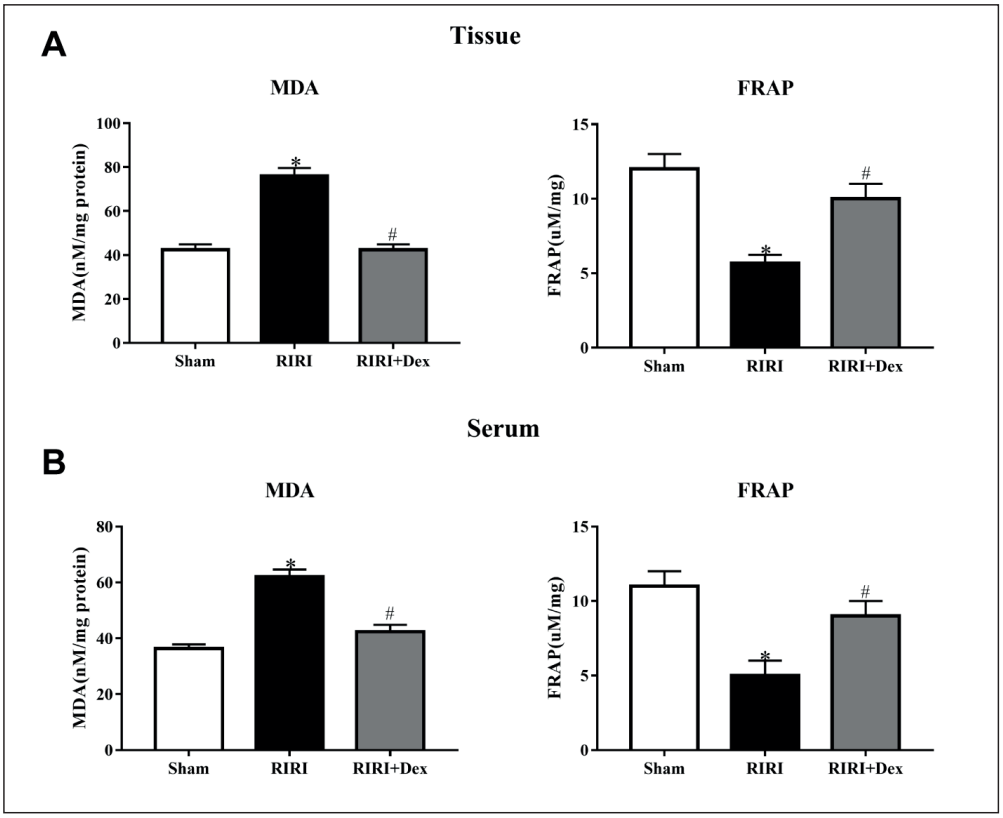

Figure 3. Dex affects the oxidative stress level in RIRI rats. **A**, MDA content and FRAP in the kidney tissues in Sham group, RIRI group, and RIRI + Dex group. **B**, MDA content and FRAP in serum in Sham group, RIRI group, and RIRI + Dex group. Note: The level of MDA is increased and FRAP declines in serum and kidney tissues in RIRI group compared with those in Sham group, showing statistically significant differences (*p<0.05). The level of MDA declines and FRAP is increased in serum and kidney tissues in RIRI + Dex group compared with those in RIRI group ($^{\#}p$<0.05)

p-PI3K, p-Akt and HIF-1α declined in RIRI group compared with those in Sham group (p<0.05), while they rose in RIRI + Dex group compared with those in RIRI group (p<0.05; Figure 6).

Discussion

It is widely recognized that organ ischemic injury and the resulting necrosis may activate the rapid immune response, further inducing the inflammatory response and release of free radicals and ultimately leading to severe organ damage[16]. RIRI is characterized by the pathophysiological conditions due to temporary restriction of blood to the kidney, and subsequent restoration of blood flow and tissue reoxygenation, which contributes to the development of AKI, and leads to the decline in renal function[17]. In this study, it was observed that Dex inhibited the production of BUN and Cr in RIRI rats, suppressed the production of MDA in serum and kidney tissues, and enhanced FRAP. The reactive oxygen species (ROS) produced during ischemia-reperfusion are involved

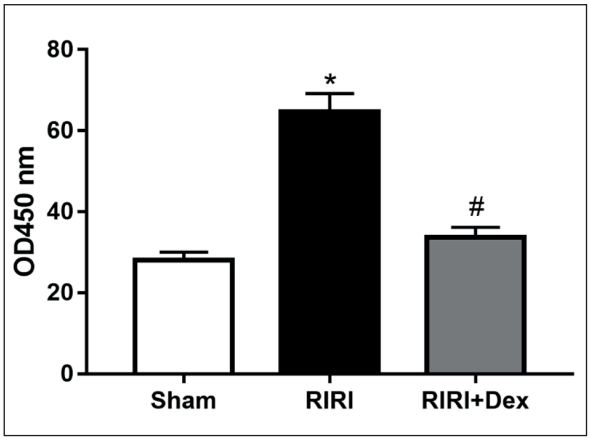

Figure 4. Dex influences the serum nitrite level in RIRI rats. Note: RIRI group has a higher serum nitrite level than Sham group, showing a statistically significant difference (*p<0.05), while RIRI + Dex group has a lower serum nitrite level than RIRI group ($^{\#}p$<0.05).

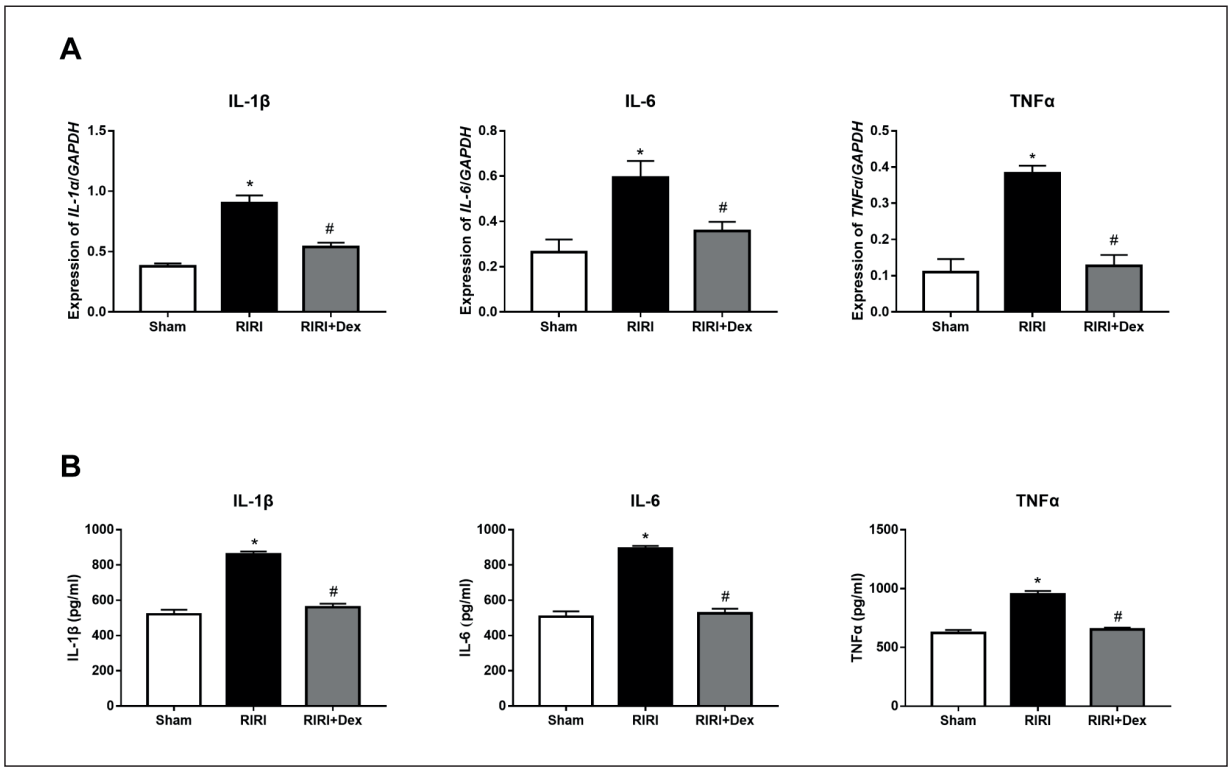

Figure 5. Levels of inflammatory factors in RIRI rats after Dex treatment. ***A***, Levels of inflammatory factors in tissues. ***B***, Levels of inflammatory factors in serum. Note: RIRI group has higher levels of IL-1β, IL-6 and TNF-α in tissues and serum than Sham group, displaying statistically significant differences (*p<0.05), while RIRI + Dex group has lower levels of IL-1β, IL-6 and TNF-α in tissues and serum than RIRI group ($^{\#}p$<0.05).

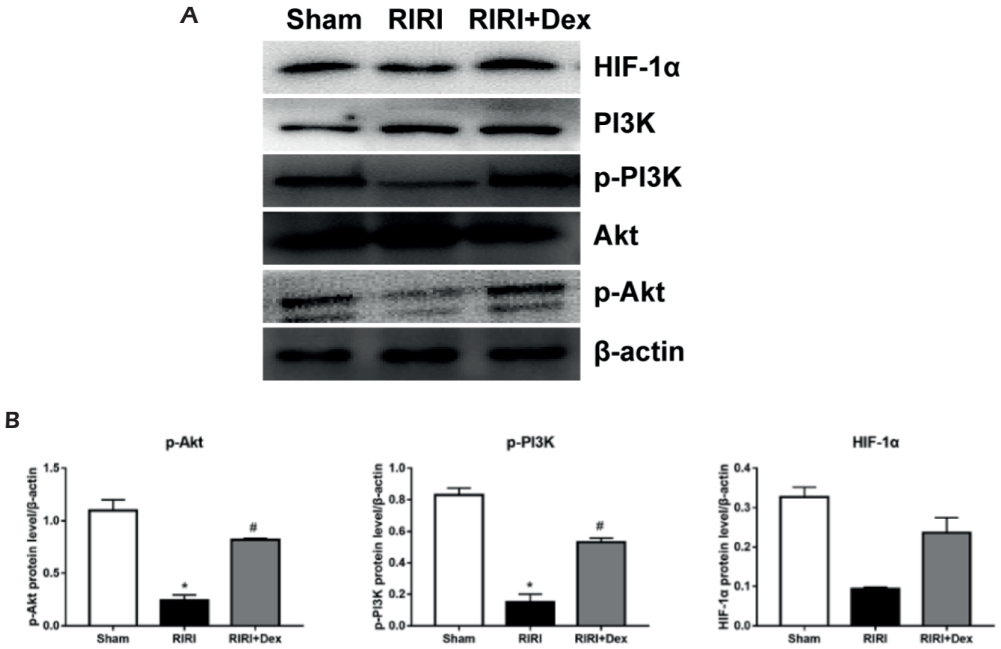

Figure 6. Level of PI3K/Akt/HIF-α pathway in RIRI rats after Dex treatment. Note: The levels of p-PI3K, p-Akt and HIF-1α decline in RIRI group compared with those in Sham group (*p<0.05), while they rise in RIRI + Dex group compared with those in RIRI group ($^{\#}p$<0.05).

in the ischemia-reperfusion process by activating the cellular stress signaling pathway[17-19]. The control and treatment of various diseases including RIRI with antioxidants have attracted considerable attention[20]. Recently, some studies have demonstrated that antioxidants can reduce RIRI-related damage and inflammatory complications. Besides, HIF is rapidly induced under hypoxic conditions and up-regulated in response to hypoxia in a cell-specific manner. HIF-1α is the most critical HIF for hypoxia response[21,22]. The PI3K/Akt/HIF-1α signaling pathway is involved in the recovery of IRI[14].

Dex is often clinically used as sedative, and studies have shown that Dex directly prevents IRI in the lungs and heart[23,24]. The cardioprotective effect of Dex has been observed in several clinical studies and animal experiments[25,26]. Therefore, Dex is considered as a novel and promising strategy for heart protection during the perioperative period. However, there have been no studies on Dex involved in the recovery of RIRI. Therefore, this study aims to discover the protective effect of Dex against RIRI through the PI3K/Akt/HIF-1α pathway. MDA is a biomarker for the oxidative stress of free radicals *in vivo*, and its increased level indicates the aggravated cell membrane damage. The production of free radicals may cause an elevation in the MDA level. In addition, the elevated level of serum MDA may be the cause of many diseases associated with oxidative stress, such as diabetes mellitus[27]. As an important molecule that plays a key role in the body physiology, nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) can induce DNA damage and structural degradation of many lipids and proteins[28]. Ischemia-reperfusion seems to cause cell damage through up-regulating Inos[29]. The results of this study showed that the MDA level was increased and FRAP declined in RIRI rats, while Dex could inhibit the MDA level and promote FRAP in RIRI rats.

Different factors are associated with the progression of acute renal failure and renal tubular injury, which can result in elevated levels of BUN and Cr, as well as tissue damage. Therefore, the damage to kidney tissues will cause changes in serum biochemical factors[30,31]. In this study, the levels of BUN and Cr in RIRI group were significantly increased compared with those in Sham group, while Dex could lower the levels of BUN and Cr after RIRI, and reduce the MDA level in serum and tissues. It is proposed that sensitive markers of glomerular filtration rate (GFR) may be better indexes for renal function[32]. In current studies, the collection of enough urine samples is one of the limitations in the evaluation of GFR. Due to the difference in species, the clinical application of Dex remains to be further proved in clinical practice, so there are still some deficiencies in this study. In the future, in-depth research is needed, so as to realize the application of Dex in the clinical treatment of RIRI, and reduce the degree of renal damage in RIRI patients.

Conclusions

This study demonstrates that Dex is involved in the recovery of RIRI, reduces the levels of MDA, BUN and serum Cr, and enhances the FRAP, and its mechanism is to reduce tissue damage in RIRI rats through the PI3K/Akt/HIF-1α signaling pathway, which lay a theoretical foundation and a scientific basis for the treatment of RIRI with Dex. Therefore, Dex is expected to be a potential therapeutic drug for postoperative recovery of RIRI.

Conflict of Interests

The authors declare that they have no conflict of interests.

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