

Original Article

Dexmedetomidine pretreatment reduces myocardial ischemia and reperfusion injury in rats by altering JAK/STAT and PI3K/Akt levels

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Abstract: Dexmedetomidine, as a highly selective α_2 -adrenoreceptor agonist, has been proved its protective effect in I/R injury, but the underlying mechanism remains unclear. Our study helps provide evidence for further investigation on myocardial I/R injury and possible interventional measures in clinical practice. In this study, Male Wistar rats were randomized into 3 groups (20 each) by random number table: Control group, Ischemia reperfusion (IR) group and Pretreatment (DEX) group. Serum markers of myocardial injury and Myocardial STAT3 and Akt expression of three groups were evaluated. Our results indicated that myocardial injury markers, CK-MB, cTnI and LDH in three groups were statistically different, with IR the highest followed by DEX, and control group the lowest. And a difference in STAT3, Akt by western blot and relevant mRNA expressions among the three groups was also noted, with that in DEX group the highest, and no marked difference between IR and control group. Therefore, dexmedetomidine pretreatment may provide protective effect for myocardial tissue by upregulating JAK/STAT and PI3K/Akt signaling pathways.

Keywords: Dexmedetomidine, myocardial I/R injury, JAK/STAT, PI3K/Akt levels

Introduction

Coronary heart disease is a major clinical event that draws worldwide attention, and is one of the leading contributors to disability and death. A major portion of such pathological process results from acute myocardial ischemia and reperfusion (I/R) injury [1]. Coronary artery bypass surgery, extracorporeal circulation and a number of other clinical treatment, may also induce I/R injury with a poor prognosis [2]. Current understanding on the mechanism of I/R injury involves increased production of active oxygen and calcium overload. A clinically effective measure to prevent myocardial reperfusion injury is still in need.

Dexmedetomidine is a highly selective α_2 -adrenoreceptor agonist for sedation in postanesthetic and critical care patients. It is also a commonly used supplement to anesthesia. A

growing number of researches have suggested the protective effect of dexmedetomidine in I/R injury [3, 4]; some primary researches have indicated its protective effect on rat myocardial cells in vitro [5], but the underlying mechanism remains unclear. The present study investigated the protective effect of dexmedetomidine pretreatment on myocardial cells of rat I/R injury model, and the possible underlying mechanism that is due to alterations on myocardial JAK/STAT and PI3K/Akt pathways. Our study helps provide evidence for further investigation on myocardial I/R injury and possible interventional measures in clinical practice.

Subjects and methods

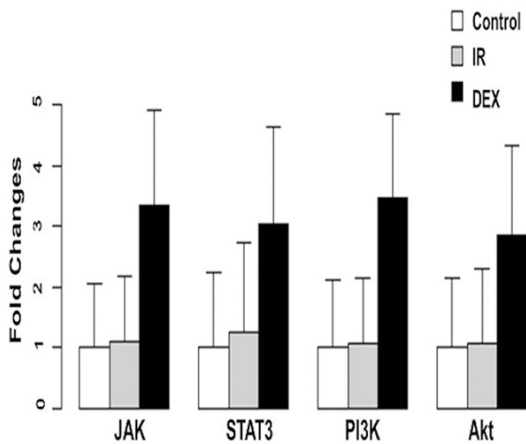
Animal model and grouping

A total of 60 SPF-grade Male Wistar rats (purchased from Vital River Laboratories, Beijing,

Table 1. Myocardial injury markers in between groups

Group	CK-MB (ng/ml)	cTnI (ng/ml)	LDH (IU/l)
IR	121.5±8.7	1.02±0.35	842.5±35.6
DEX	34.3±5.6	0.49±0.14	209.1±24.7
Control	18.2±2.5	0.18±0.07	68.3±6.5
<i>F</i>	8.421	9.873	10.124
<i>P</i>	0.013	0.004	0.001
<i>P</i> _{IR vs DEX}	0.005	0.018	0.034
<i>P</i> _{IR vs Control}	0.001	0.001	0.001
<i>P</i> _{DEX vs Control}	0.029	0.009	0.001

Note: *F* and *P* values were determined by ANOVA; *P*_{IR vs DEX}, *P*_{IR vs Control} and *P*_{DEX vs Control} were determined by SNK tests.

**Figure 1.** Myocardial mRNA expressions of JAK, STAT3, PI3K and Akt in three groups.

China) with good health condition, aged 8 weeks and weighed 290-320 g (average 306±14 g), were fed adaptively for 2 weeks. Standard rat chow and water were available ad libitum. Animals were fasted 12 h before experiment (without water deprivation). All procedures in this study were approved by the ethics committee on laboratory animals.

Establishment of rat myocardial I/R model was in accordance with that published by Cai et al. [6]. Rats were anesthetized with 20% urethane (0.5 ml/100 g body weight, intraperitoneally), connected to biological functional system, intubated after dissection of trachea and connected to small animal ventilator (tidal volume, 1.0-1.5 ml/kg; respiration frequency, 60 bpm). Left parasternal thoracotomy was performed, followed by incision of the pericardium to expose the heart. Left anterior descending artery (LAD) was located, and a single suture was used to

penetrate the middle section of the LAD for backup purposes. A small water-inflated balloon was placed between the LAD and the ligation suture; LAD was then ligated to induce myocardial ischemia, which was proven by a marked ST or T elevation in precordial lead II on ECG. Water was completely drained from the balloon 30 mins later, and reperfusion of LAD was proven by a marked fall on ST segment in lead II. Sustain for 60 mins to complete myocardial I/R modeling.

Male Wistar rats were randomized into 3 groups (20 each) by random number table: 1) Control group, in which sham surgery was performed without ligation of the coronary artery to observe for 90 mins; 2) Ischemia reperfusion (IR) group, in which 30 µl saline was injected intraperitoneally, followed by ligation of LAD after 30 mins; reperfusion after 30 mins and sustained for 60 mins; 3) Pretreatment (DEX) group, in which Dexmedetomidine (100 µg/kg) was injected intraperitoneally, the dose of which followed that documented by Kocoglu H et al. [7]; ligation and reperfusion was the same as described in IR group.

Assay for serum markers of myocardial injury

At 60 min after myocardial reperfusion, 1 ml jugular vein blood was collected, let stand for 30 mins, and centrifuged for 10 mins (3000 rpm, radius 12 cm) to harvest the serum and preserved at -80°C. cTnI, CK-MB and LDH concentrations were determined by ELISA (Roche).

Myocardial STAT3 and Akt expression

mRNA expression of myocardial STAT3 and Akt was determined by qPCR. Total RNA was extracted and reversely transcribed to form cDNA by grinding of myocardial tissue. cDNA solution was used for qPCR reactions. STAT3 upstream primer: 5'-CGCCACTCTGGTGTTCATA-3', downstream primer: 5'-TTCGACGGTTGTGCTGATAG-3', product size 97 bp; Akt upstream primer: 5'-GGTTGCCACACGCTTACT-3', downstream primer: 5'-AAACACGCGCTCAGAGAC-3', product size 162 bp; β-actin upstream primer: 5'-CCCATCTATGAGGGTTACGC-3', downstream primer: 5'-TTTAATGTCACGCACGATTTC-3', product size 150 bp. A relative quantitative analysis was performed, Ct value (number of cycles to exceed threshold), was recorded, and target gene expression was determined using 2-ΔΔCt method.

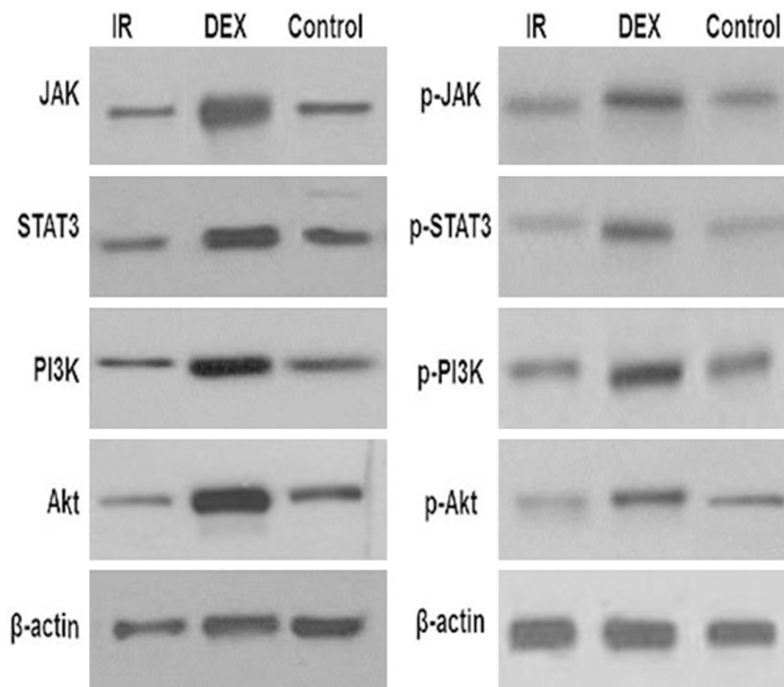


Figure 2. Western Blot analysis of JAK/STAT and PI3K/Akt protein and their phosphorylation products in three groups.

Protein expression and phosphorylation of myocardial JAK/STAT and PI3K/Akt

Myocardial JAK/STAT and PI3K/Akt protein expression and phosphorylation was determined by Western blot. Myocardial tissue was cut into 20 mg-sized dices and placed in total protein extraction solution (prepared at the dose of 200 μ l protein lysis buffer per 10 mg tissue). Homogenate was grinded ice for complete cell lysis, and then centrifuged for 10 mins (4°C, 12 000 rpm) to preserve supernatant, which subsequently underwent quantitative protein analysis, polyacrylamide gel electrophoresis, transfer, blocking, incubated with antibody and left overnight at 4°C, repeated washing for 3 times in TBST (10 mins per washing). Goat anti-rabbit IgG-HRP second antibody was diluted by blocking agent and incubated for 2-3 h, which was then washed for 3 times (5 mins per washing) in TBST. Chemical luminescence was performed, and the antibodies were developed and fixed.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0. Quantitative data were presented as $\pm$ s.

Statistical significance was determined by performing ANOVA between the three groups and SNK test between two groups. A value of $\alpha=0.05$ was used as significant level, and a P value of <0.05 was considered statistically significant.

Results

Myocardial injury markers

A significant difference was observed in CK-MB, cTnl and LDH between the three groups ($P<0.05$), with a result of IR > DEX > Control (all P values were <0.05) (**Table 1**).

Protein and mRNA level of JAK/STAT and PI3K/Akt in myocardial tissue

A significant difference was observed in STAT3 and Akt protein, their phosphorylation product, and mRNA expression between groups (all P values were <0.05); DEX group recorded the highest results ($P<0.05$), whereas no significant difference was observed between IR and control group ($P>0.05$) (**Figure 1**).

In Western Blot analysis, JAK/STAT and PI3K/Akt protein and their phosphorylation products in DEX group were also found elevated compared to IR and control group (**Figure 2**). The grey value analysis of Western blot were well consistent (**Table 2**).

Discussion

It has been proven clinically that coronary artery thrombolysis, percutaneous coronary angioplasty, coronary artery bypass graft, and other coronary recanalization procedures may induce myocardial reperfusion injury. Thus, in-depth investigation on the specific molecular signal transduction pathway in myocardial I/R injury, as well as approaches to alleviate myocardial reperfusion injury, are current research hotspots [8]. Commonly used animal model to investigate myocardial I/R injury is achieved by ligating and recanalizing the left anterior

Table 2. Grey value analysis of the results of western blot

Group	JAK	STAT3	PI3K	Akt	p-JAK	p-STAT3	p-PI3K	p-Akt
IR	0.28±0.06	0.36±0.08	0.45±0.09	0.22±0.07	0.24±0.05	0.30±0.05	0.37±0.07	0.19±0.02
DEX	1.51±0.18	1.82±0.35	1.48±0.37	1.34±0.42	1.43±0.13	1.67±0.19	1.48±0.24	1.11±0.08
Control	0.25±0.03	0.23±0.06	0.33±0.05	0.18±0.04	0.21±0.04	0.21±0.07	0.30±0.03	0.15±0.03
<i>F</i>	8.034	7.984	7.812	8.045	8.102	8.001	7.876	8.139
<i>P</i>	0.019	0.021	0.025	0.018	0.017	0.020	0.023	0.014
<i>P</i> _{IR vs DEX}	0.016	0.026	0.024	0.015	0.015	0.019	0.021	0.012
<i>P</i> _{IR vs Control}	0.079	0.074	0.079	0.080	0.076	0.077	0.073	0.068
<i>P</i> _{DEX vs Control}	0.019	0.021	0.029	0.013	0.021	0.022	0.025	0.018

Note: *F* and *P* values were determined by ANOVA; *P*_{IR vs DEX}, *P*_{IR vs Control} and *P*_{DEX vs Control} were determined by SNK tests.

descending (LAD) coronary artery. Rodents have a very similar anatomy and regulation mechanism of the cardiovascular system, therefore we used the I/R injury model in rats by ligating and recanalizing LAD in the present study. The LAD of rat is more slender in nature, therefore ligation should not exceed 30 mins to prevent permanent occlusion and subsequent failure of modeling. All animal models were successfully established in the present study, indicating that such method is appropriate when investigating in vivo I/R injury.

The specific molecular signal transduction process of myocardial ischemia perfusion injury remains unclear. A vast number of basic researches have suggested the involvement of JAK/STAT, PI3K, Akt, ERK, NF-κB, and other signaling pathways, in which JAK/STAT and PI3K/Akt received the most attention [9]. Myocardial ischemia activates a survival activated enhancing route that is distinct from I/R salvage kinase pathway to produce a potent myocardial protection effect [10]. PI3K/Akt signaling pathway also serves as a crucial survival pathway. It is proven that PIP3 level is markedly elevated following PI3K activation; as secondary messenger, PIP3 activates downstream target molecule Akt that is subsequently phosphorylated, and such phosphorylated product lowers proapoptotic molecules such as Bad, Bax and caspase to improve mitochondrial function, decrease mitochondrial membrane permeability, inhibit intracellular calcium overload, down-regulate apoptosis factors released by mitochondria, and eventually inhibit cell apoptosis [11]. Researches have also discovered that JAK/STAT and PI3K/Akt have potential mutual effects.

Dexmedetomidine is a highly selective α₂-adrenergic receptor with sedative, analgesic, antisympathetic properties, and inhibits catecholamine release in serum. It is widely used clinically for sedative and analgesic purposes [12]. Recent studies have proven its organ-protective effect in I/R injury, for instance, that dexmedetomidine downregulates Bax and upregulates Bcl-2 expression by highly selective activation of α₂-adrenergic receptor to reduce neuron deaths and provide neuroprotective effects under incomplete ischemia/reperfusion injury in rats. Other researches have proven that dexmedetomidine lowers serum catecholamine concentration in cerebral ischemia in rats, with fair neuroprotective effects. However, the extent of such effects in myocardial ischemia and underlying mechanism regarding specific molecular signaling transduction pathways still needs further investigations. Thus, our present study intended to discuss whether dexmedetomidine exerts its protective effect in myocardial I/R injury by altering JAK/STAT and PI3K/Akt signaling pathways.

Our results indicated that myocardial injury markers, CK-MB, cTnl and LDH in three groups were statistically different, with IR the highest followed by DEX, and control group the lowest, suggesting that dexmedetomidine effectively reduced damage caused by myocardial I/R injury. A difference in STAT3, Akt and relevant mRNA expressions among the three groups was also noted, with that in DEX group the highest, and no marked difference between IR and control group. This suggests that pretreatment with dexmedetomidine markedly increases JAK/STAT and PI3K/Akt molecular level. Further research is still required to elucidate whether

dexmedetomidine triggers JAK/STAT and PI3K/Akt activation via G protein-coupled receptors, and whether the effect is achieved with highly expressed peripheral $\alpha 2B$ subtype.

Conclusions

The present study discovered that dexmedetomidine pretreatment reduced myocardial ischemia/reperfusion injury in rats and altered JAK/STAT and PI3K/Akt pathway levels, suggesting that dexmedetomidine pretreatment may provide protective effect for myocardial tissue by upregulating these signaling pathways.

Acknowledgements

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Disclosure of conflict of interest

None.

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