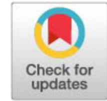
Pakistan J. Zool., vol. 54(4), pp 1757-1763, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20201103151131

Dexmedetomidine Alleviates Myocardial Ischemia-Reperfusion Injury through Mitochondrial and ER Oxidative Stress Pathways



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ABSTRACT

The objective of this study was to explore the mechanism by which dexmedetomidine (DEX) alleviates myocardial ischemia-reperfusion injury (MIRI) through mitochondrial and ER oxidative stress pathways. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd) of SD rats were measured by echocardiography, and the mRNA expression level of peroxisome proliferator-activated receptor gamma co-activator 1-a (PGC1 a), superoxide dismutase (SOD2) and citrate synthase (Cs) were detected by real-time PCR. Ultrastructural changes of myocardial mitochondria were observed by transmission electron microscope. The activity of Caspase-3 in heart tissue of SD rats was measured, and the expression of p-JNK, GRP78, Caspase 12 and CHOP in heart tissue of SD rats was determined by Western blot. We found that LVEDd and LVESd in MIRI group and MIRI + DEX group were significantly higher than those in sham operation group (P<0.05), and LVEF was significantly lower than that in sham operation group (P<0.05). Compared with MIRI group, the expression levels of PGC1-a, SOD2 and Cs genes in MIRI + DEX group were significantly lower(P<0.05), and mitochondrial structure was slightly damaged in MIRI + DEX group. Compared with the sham operation group, p-JNK, Caspase 12, CHOP and GRP78 in MIRI group and MIRI + DEX group increased significantly (P<0.05), Compared with MIRI group, the expression of p-JNK, Caspase-12 and CHOP protein in MIRI + DEX group decreased significantly (P<0.05), while the expression of GRP78 increased (P<0.05). It is concluded DEX can alleviate mitochondrial damage induced by ischemia reperfusion, inhibit excessive endoplasmic reticulum and improve myocardial function.

Article Information
Received 03 November 2020
Revised 18 November 2020
Accepted 04 December 2020
Available online 22 October 2021 (early access)
Published 22 April 2022

Authors' Contribution
ML, SD and YP conducted experiments. SD and HL documented and concluded the experiment. ML and SD completed the first draft.

Key words
Dexmedetomidine, Mitochondria, ER oxidative stress, Myocardial ischemia-reperfusion injury, peroxisome proliferator-activated receptor, γ co-activator 1, γ co-activator 1-a, Superoxide dismutase, Citrate synthase, Caspase

INTRODUCTION

For patients with ischemic heart disease, coronary reperfusion can effectively limit the infarct size. Unfortunately, reperfusion itself may lead to additional damage, called myocardial ischemia/reperfusion injury (MIRI) (Liu *et al*., 2016; Kumar *et al*., 2009). During reperfusion, multiple cytokines such as tumor necrosis factor (TNF-α) and interleukin 6(IL-6) are released, triggering an excessive regional inflammatory response and leading to further myocardial damage. The incidence of ischemic cardiomyopathy is on the rise. The disease can be caused by a variety of factors, causing decreased coronary blood flow, myocardial blood supply disruption, nutritional deficiencies and decreased metabolites, resulting in myocardial cell damage and even death. Many studies have shown that inhibiting excessive inflammation can reduce infarct size and improve cardiac dysfunction caused by MIRI damage (Liu *et al*., 2016). MIRI refers to a kind of serious myocardial function injury after restoring blood supply in the case of myocardial ischemia. It is a common pathophysiological process of clinical anesthesia, especially cardiac surgery. Dexmedetomidine (DEX) is a highly selective receptor agonist of epinephrine α2, which has analgesic, sedative and anti-anxiety effects and is widely used in clinical practice (Devasya and Sarpangala, 2015; Zhong *et al*., 2015). Studies have shown that DEX has a protective effect on myocardial ischemia reperfusion (Cai *et al*., 2014a). Endoplasmic reticulum oxidative stress can also protect vital organs by inhibiting oxidative stress, cell apoptosis and inflammatory response.

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0030-9923/2022/0004-1757 $ 9.00/0

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MATERIALS AND METHODS

General data

Thirty male SD rats weighing 230-250 g provided by the Animal Testing Center were randomly divided into sham operation group (A), MIRI group (B) and MIRI + DEX group (C), with 10 rats in each group.

Establishment of ischemia-reperfusion rat model

The cerebral ischemia-reperfusion rat model was established by intraperitoneal injection of 5% chloral hydrate (10 mL/kg). After completion of anesthesia, the rats were fixed on the operating table. Rats were intubated in tail vein, and the MIRI+Dexmedetomidine (DEX) treatment group was pretreated with DEX at a loading dose of 5 g/kg, for continuous 1h. Sham operation group and MIRI group were given equal volume of normal saline for 1h. After pretreatment, the thoracic cavity of the rats was opened to expose the heart and coronary arteries, we ligated the anterior descending branch of the left coronary artery for 30min, removed the ligation site, put back the heart into the thoracic cavity, and finally sterilized and sutured the skin. ST segment elevation on electrocardiogram was a successful sign of MIRI. In the sham group only threading was performed without ligation.

Cardiac function test

Echocardiography was performed 2 weeks after operation. After intraperitoneal injection of 5% chloral hydrate, the rats were fixed in supine position to prepare chest skin. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd), and left ventricular end-systolic diameter (LVESd) of SD rats were detected by echocardiography.

Real-time PCR detection

After the cardiac function tests were completed and the rats died suddenly, five were removed from each group and the left ventricular apical myocardial tissue was separated. The peroxisome proliferation-activated receptor co-activator 1-α(PGC1α), superoxide dismutase (SOD2) and citrate synthase (Cs) genes were involved in mitochondria and played important roles. mRNA expression of these genes was detected by real-time PCR. Total RNA was extracted by Trizol method, and cDNA was amplified by real-time PCR after reverse transcription. The fluorescence intensity was quantitatively determined by ABI 7900 system. CT values are read and fusion curves were collected. CT value ≥40 means negative. CT values of target genes and internal reference genes were recorded, respectively. The 2-CT method was used to calculate the difference in gene expression, the internal parameters of the target gene of the sample to be tested were tested, and the CT value was obtained. The experiment was repeated three times. The reaction mixture for RTPCR comprised 5×PrimeScript Buffer, 4μL; 1×PrimeScript RT enzyme mix I, 1μL; Oligo dT primer (50μM), 1μL (25pmol); Random 6 mers (100μM), 1μL (50pmol); Total RNA, lμL; RNase Free dH2O, 12μL. The Light Cycler 480 SYBR Green I Master system was used for qPCR analysis. The thermal cycle for amplification: Denaturation at 95°C for10 min followed by 39 cycles each of denaturation at 94 °C for 30s, annealing/extension at 60°C for 1 min, fusion curve analysis at 95°C for 15s, 60°C for 1 min, 94°C for 15s, 60 °C for 15s. The primers are shown in Table I, and the reaction system composed5×PrimeEx Taq TM II (2×) 12.5μL, PCR Forward Primer (10μM) 1μL (0.4μM), PCR Reverse Primer(10μM) 1μL (0.4μM), DNA template 2μL, dH_2O (sterile purified water) 8.5μL, to make up total volume 25μL. CT values were calculated respectively for each treatment group and CT control group, and their relative values were plotted.

Transmission electron microscope observation

After the sudden death of the rat, the apical myocardial tissue was immediately cut off. The myocardial tissue was cut into a volume of about 1 cubic millimeter under low temperature, and then immobilized immediately in a 5% glutaraldehyde fixative. After immobilized, rinsed, dehydrated, impregnated, embedded and ultrathin sliced, 50-80nm was finally obtained. The ultrastructural changes of myocardial mitochondria were observed by transmission electron microscope.

Table I. Primers used for RT-PCR.

Gene	Upstream	Downstream
SOD2	5'- TGGACAAACCTGAGCCCTAA-3'	5'- GACCCAAAGTCACGCTTGATA-3'
Cs	5'- GCACGCCAGTGCTTCTTC -3'	5'- CATGCTGCTGTCTGAAGGTC -3'
PGC1α	5'- CCAAACCCACAGAGAACAGAA -3'	5'- TCTGGGGTCAGAGGAAGAGA -3'
GAPDH	5'- GCATGGCCTTCCGTGTTCCTACC -3	5'- GCCGCCTGCTTCACCACCTTCT -3'

Caspase-3 activity detection

Heart tissue (3-10mg) was added in 100 μL pyrolysis solution. The cracking liquid was ground into homogenate on the ice and transferred to a 1.5mL centrifuge tube for 5min. The supernatant was centrifuged at 4-20,000 r/min for 10-15 min, and then collected. According to the instructions of the caspase-3 activity detection kit, the activity of caspase-3 in heart tissues was detected by spectrophotometer.

Western blot detection of pJNK, GRP78, CasPase12 and CHOP protein level

The heart tissue was crushed on ice, and split with 400 μL of RIPA for 20min, followed by absorption and centrifugal treatment of slurry at 4°C. The protein concentration was measured according to BCA protein quantitative kit and the standard curve were drawn, and the protein was denatured by boiling at 100 °C for 10min. Then the mixture was electrophoresed and transferred, and sealed with 5% skimmed milk powder at room temperature for 2h. After rinsing with TBST, I antibodies (P-JNK, JNK, and GRP78) were added and diluted at 1:1000, GAPDH and Caspase-12 were diluted at 1:500, and CHOP was diluted at 1:300), and kept at 4°C overnight. After incubation for 3 times, the protein was washed with TBST, incubated with II resistance for 1h at room temperature, and then washed with TBST. ECL developer was added for 3min for reaction before observing in the darkroom. Quantity One software was used to analyze the ratio of protein absorbance to internal reference absorbance. The ratio of p-JNK to JNK was calculated to reflect the level of p-JNK protein.

Data processing

Data were expressed as mean±standard deviation. One-way analysis of variance was used for comparison between groups. The difference was considered statistical significant when P<0.05.

RESULTS

Cardiac function

The changes of cardiac function in different treatment groups were recorded by echocardiography. The echocardiography showed that LVEDd and LVESd of MIRI group and MIRI+ DEX group were significantly higher than those of the sham operation group (P<0.05), LVEF was significantly lower than that of the sham operation group (P<0.05). LVEDd and LVESd in MIRI+ DEX group were significantly lower than those in MIRI group (P<0.05), and LVEF was significantly higher than that of MIRI group (P<0.05), as shown in Table II.

Table II. Effect of dexmedetomidine (DEX) on cardiac function of SD rats after myocardial ischemia-reperfusion injury (MIRI) determined by echocardiography.

Group	LVEDd(cm)	LVESd(cm)	LVEF(%)
Sham operation group	0.49±0.15	0.25±0.11	86.12±5.23
MIRI group	0.82±0.13	0.70±0.12	48.21±3.92
MIRI+DEX group	0.60±0.11	0.61±0.13	61.38±4.32
F	38.456	48.321	59.032
P	<0.05	<0.05	<0.05

LVEF, left ventricular ejection fraction; LVED, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter.

Mitochondrial function

Compared with the sham group, the expression levels of *PGC1-A, Sod2* and *Cs* genes in the MIRI group and MIRI+DEX group were significantly decreased (P<0.05). Compared with MIRI group, the expressions of *PGC1-A, Sod2* and *Cs* genes in MIRI+ DEX group were significantly decreased (P<0.05), as shown in Table III.

Table III. Effect of dexmedetomidine (DEX) on expression of genes related to mitochondrial function of SD rats after myocardial ischemia-reperfusion injury (MIRI) detected by real-time quantitative PCR.

Group	SOD2 mRNA	Cs mRNA	PGC1-a mRNA
Sham operation group	0.94±0.12	0.93±0.14	0.93±0.16
MIRI group	0.45±0.16	0.53±0.15	0.34±0.12
MIRI+DEX group	0.62±0.12	0.72±0.16	0.65±0.12
F	39.436	59.356	89.002
P	<0.05	<0.05	<0.05

SOD, superoxide dismutase; Cs, citrate synthase; PGC1, peroxisome proliferator-activated receptor γ activator 1.

Ultrastructural changes of myocardial mitochondria

In the sham group, the mitochondrial inner and outer membranes were intact without swelling, the mitochondrial cristae were clear and neatly arranged, and the matrix electron density was normal. In the MIRI group, the intima and intima were ruptured, integrity was lost, mitochondria swelled, and mitochondrial cristae disappeared. Compared with the MIRI group, the mitochondrial structure of MIRI+ DEX group was slightly damaged, and the damage degree was significantly reduced, as shown in Figure 1.

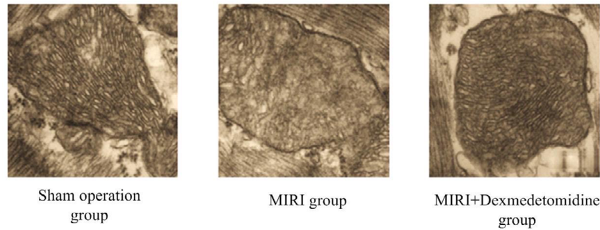

Fig. 1. Ultrastructure of myocardial mitochondria in different treatment groups by transmission electron microscopy.

Table IV. Effect of dexmedetomidine on caspase-3 activity of SD rats after MIRI.

Group	Caspase-3 activity
Sham operation group	30.94±5.62
MIRI group	100.45±9.16
MIRI+DEX group	80.62±8.12
F	89.439
P	<0.05

Caspase-3 activity

Caspase-3 activity was significantly higher in the MIRI group and the MIRI+ DEX group than in the sham group (P<0.05). Caspase-3 activity in MIRI+ DEX group was significantly lower than that in MIRI group (P<0.05), as shown in Table IV.

Protein expression of p-JNK, caspase-12, chop and GRP78

Compared with the sham group, p-JNK, Caspase12, CHOP and GRP78 were significantly increased in the MIRI group and MIRI+ DEX group (P<0.05). Compared with the MIRI group, the water expression levels of p-JNK, Caspase-12 and CHOP in the MIRI+ DEX group were significantly decreased (P<0.05), the expression level of GRP78 was increased (P<0.05), as shown in Figure 2 and Table V.

Table V. Effect of dexmedetomidine on gray value of protein expression of SD rats after MIRI.

Group	p-JNK	Caspase-12	CHOP	GPP78
Sham operation group	0.18±0.02	0.19±0.04	0.19±0.06	0.2±0.03
MIRI group	0.65±0.46	0.61±0.31	0.64±0.22	0.43±0.11
MIRI + DEX group	0.41±0.32	0.40±0.12	0.41±0.16	0.66±0.15
F	59.476	79.358	84.032	59.349
P	<0.05	<0.05	<0.05	<0.05

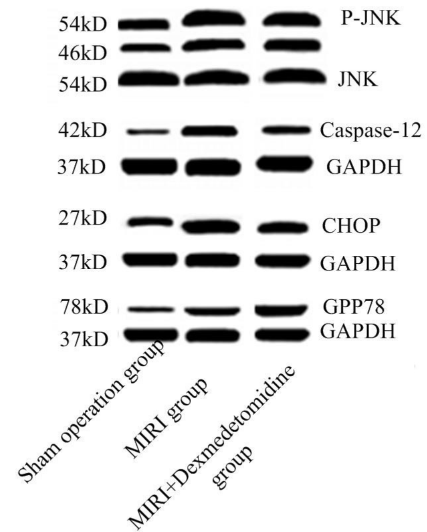

Fig. 2. Effect of dexmedetomidine on protein expression by various genes of SD rats after MIRI as detected byWestern bloting.

DISCUSSION

DEX is a highly selective α2 adrenergic receptor agonist that is widely used as a sedative in clinical anesthesia, intensive care unit (ICU) management, and pain management (Niu *et al*., 2013; Friesen *et al*., 2013; Obayah *et al*., 2010). Previous studies (Ji *et al*., 2013) have shown that DEX can improve cardiac prognosis in non-cardiac surgery. In addition, DEX pretreatment has been reported to significantly reduce the incidence and infarction area of reperfusition-induced ventricular arrhythmias in animal myocardial I/R models (Yoshitomi *et al*., 2012). In addition, more studies have shown that DEX plays an anti-inflammatory role in aseptic and infectious inflammatory models by reducing serum levels of inflammatory cytokines, including IL-6 and TNF-α (Huang *et al*., 2014; Tan *et al*., 2015).

Ischemia-reperfusion injury refers to the serious structural and functional damage of the heart during myocardial ischemia-reperfusion (Turer and Hill, 2010; Hausenloy and Yellon, 2013) caused by various reasons. After ischemia reperfusion, myocardial cell apoptosis and infarction area are increased, which aggravates cardiac function damage. This study also found that myocardial function was impaired after ischemia-reperfusion, which was manifested as reduced left ventricular ejection fraction, enlarged left ventricular end-diastolic diazmeter, and enlarged left ventricular end-systolic diameter. A large number of studies have shown that inhaled anesthetics such as sevoflurane, enflurane and isoflurane can affect the

ROS, protease pathway and KATP pathway. The effects of openness, inflammatory factor release and apoptosis can reduce the ischemia reperfusion injury of tissue cells (Agarwal *et al*., 2014; Mosbahi *et al*., 2014; Van Allen *et al*., 2012). DEX is a new intravenous anesthetic widely used in clinic. The results showed that DEX pretreatment could up-regulate the expression of Bcl-2 protein, down-regulate the expression of Bax protein, and reduced the release of inflammatory factors in MIRI rats, thus playing an anti-apoptotic role. UCH acts as TNF-α, IL-6 to reduce the damage of myocardial cells after ischemia reperfusion injury (Cai *et al*., 2014). This study aims to investigate the effects of DEX pretreatment on myocardial mitochondrial structure and function in rats with ischemia-reperfusion injury. Mitochondria are the most important organelles for ATP synthesis and the energy source of cell survival. PGC-1AlpA is a member of the peroxisome proliferator-activated receptor γ activator 1 (PGC1) family. Studies have shown that PGC1-α can increase mitochondrial respiration, enhance mitochondrial oxidation ability, reduce the toxicity of ROS, and promote the metabolic balance of tissues and cells (Park *et al*., 2017). CS is an acid cycle involving a regulatory enzyme of tricarboxylic acid (Zhang *et al*., 2016). SOD2 is mainly responsible for scavenging superoxide anion free radicals and fighting against cell damage caused by free radicals (Peterman *et al*., 2015). The above three gene levels are closely related to mitochondrial function. In this study, the expression level and mitochondrial ultrastructure of these genes were significantly decreased after ischemia-reperfusion injury. Structural damage indicates impaired mitochondrial function, while DEX pretreatment may reduce mitochondria by increasing the expression of PGC1-α, Cs and SOD2 genes (such as high expression of PGC1-α) , thereby promoting mitochondrial synthesis and enhancing mitochondrial oxidation, reducing structural and functional damage, and thus improving myocardial function.

In the process of organ ischemia/reperfusion, there are many intracellular signal transduction pathways, among which endoplasmic reticulum (ER) stress and JNK signal transduction pathways play important roles in organ I/R. ATP depletion, ischemia and hypoxia, oxidative stress and glucose/nutrient deficiency can lead to er stress (ERS). Moderate ER stress can protect the body, but sustained excessive ER stress can lead to apoptosis (Yousefi *et al*., 2014). The 4 proteins including JNK, CSPAS-12, CHOP and GRP78 detected in the experiment were all ER stress-related proteins. GRP78 is a calcium-binding molecular chaperone located in the ER. When cells are stimulated by the external environment and stressed by the ER, a large number of misfolded or unfolded proteins accumulate in cells. At this time, GRP78 is also expressed in large quantities to bind misfolded and unfolded proteins in ER and maintain the inner ring, thus maintaining stability. Therefore, the rapid up-regulation of GRP78 is considered to be the most sensitive marker of ER stress (Xia *et al*., 2014). As one of important MAPKs signaling pathways, JNK signaling pathway is related to apoptosis induced by stress and can be activated by various extracellular stresses to induce apoptosis. Studies have shown that JNK is over activated in the process of organ I/R injury, and inhibition of JNK activation before ischemia or reperfusion can significantly reduce apoptosis and reduce organ I/R injury (Sekulic-Jablanovic *et al*., 2017; Zhang *et al*., 2016; Peterman *et al*., 2015). Caspase12 is the main apoptotic signaling molecule of ER stress, which widely exists in various tissues of mice. Imbalance of calcium ions in the ER or excessive accumulation of ER protein will lead to the expression of caspase-12 and eventually result in apoptosis (Poone *et al*., 2015). CHOP is a specific ER stress transduction factor, which is an important signal molecule promoting apoptosis. Under normal conditions, its expression level is very low, but will be significantly increased under ER stress. In addition, it is considered as a marker of er stress (Du *et al*., 2013). Apoptosis is activated by mitochondrial pathway, releasing mitochondrial apoptotic protein and apoptotic induction factor (AIF), activating caspase cascade reaction and inducing apoptosis, which is characterized by increasing the activity of caspase-3 apoptotic protease. The experimental results showed that the expression levels of JNK, Caspase12, CHOP and GRP78 proteins in the MIRI and MIRI+ DEX groups showed an upward trend compared with the sham operation group. Compared with the MIRI group, the expression levels of JNK, Caspase-12, CHOP and caspase-3 apoptotic protease activity in the MIRI+ DEX group were lower, suggesting that DEX may inhibit the ER overload, so that stress response may reduce the levels of JNK, CasPase12 and CHOP.

To sum up, DEX can reduce the mitochondrial injury caused by ischemia reperfusion, inhibit the ER excess, and improve the myocardial function.

Statement of conflict of interest

The authors have declared no conflict of interest.

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