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Original Research Article

Protective effect of dexmedetomidine pre-treatment against myocardial ischemia-reperfusion injury in rats

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Abstract

Purpose: *To determine the protective effect of dexmedetomidine pre-treatment against myocardial ischemia-reperfusion injury (MRI), and the involvement of ER stress and protein kinase R-like ER enzyme (PERK) signaling pathway in the process.*
Methods: *Sixty male Sprague-Dawley (SD) rats were assigned to three groups: sham, model (ischemia-reperfusion), and dexmedetomidine pre-treatment groups. Myocardial infarction area was measured using 2,3,5-triphenyl tetrazolium chloride (TTC) method. Changes in heart structure were examined using H & E, while myocardial cell apoptosis was determined with TUNEL assay. The protein expression levels of CHOP, glucose regulatory protein 78, ATF4, eukaryotic translation initiation factor 2-α (elf2-α), and PERK were assayed with Western blotting.*
Results: *RPP, myocardial infarction area, myocardial cell apoptosis rate, and the expression levels of CHOP, GRP78, ATF4, elf2-α, and PERK were significantly raised, relative to sham. The structure of myocardial cells in sham-operated rats was intact and orderly. In contrast, the arrangement of myocardial cells in the model group was disordered, and the myofibrils were broken or wavy. However, the arrangement of myocardial cells in the dexmedetomidine group was significantly improved, when compared with the model group.*
Conclusion: *Pretreatment with dexmedetomidine may exert a protective effect against MRI through suppression of ER stress and PERK signaling pathway. Further preclinical studies would be required to validate these findings.*

Keywords: *Dexmedetomidine, ER stress, PERK signaling pathway, Ischemia-reperfusion injury, Myocardial protection*

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INTRODUCTION

Ischemic heart disease has a high morbidity and high mortality, and its increasing incidence in the younger population constitutes a serious burden on patients' families and society [1]. Restoration of blood flow to ischemic areas has become an important method for treatment of ischemic heart disease. However, ischemia-reperfusion subsequently results in myocardial injury.

Myocardial ischemia-reperfusion injury is a multifactorial process that involves the activation of various cell signaling systems, changes in the expressions of effector genes, and increases in

the synthesis and release of protein molecules, all of which result in abnormal myocardial cell metabolism and function, and ultrastructural abnormalities [2]. In addition, apoptosis, an orderly and natural process of cell death regulated by genes, plays an important role during MRI [3]. Thus, strategies for reduction of MRI through inhibition of heart muscle cell death have gradually attracted the attention of clinicians. Dexmedetomidine, a new type of adrenal α2-receptor agonist, has little effect on hemodynamics and respiration. Some studies have shown that dexmedetomidine reduced spinal cord ischemia-reperfusion injury, and it significantly improved prognosis and reduced postoperative complications in mice. However, the exact mechanism through which dexmedetomidine exerts its protective effect against MRI has not been elucidated [4]. In the present investigation, dexmedetomidine pretreatment was used to ascertain the process involved in the protective effect of the drug against MRI in a rat model.

EXPERIMENTAL

Animals

Sixty (60) healthy male Sprague-Dawley (SD) rats purchased from Guangzhou Forbo Biotechnology Co. Ltd. (Production license SCXK; Guangdong = 2017-0011) were randomly selected. The rats were about 9 weeks old, with a mean body weight of 256 ± 24 g. The animals were maintained at a laboratory temperature of 25 ± 1 °C and humidity of 55 ± 5 % in an atmosphere with equal durations of night and daylight. The rats were raised adaptively for 7 days, with *ad libitum* access to standard feed and water.

Reagents

The main reagents used in this study, and their sources (in brackets) were: mouse anti-human CHOP monoclonal antibody (Shanghai Xuanling Biotechnology Co. Ltd.); mouse anti-human GRP78 monoclonal antibody (Beijing Sino Technology Co. Ltd.); mouse anti-human PERK monoclonal antibody (Wuhan Fein Biotechnology Co. Ltd), and dexmedetomidine (Jiangsu Hengrui Pharmaceutical Co. Ltd, approval number: National Medicine Zhunzi H20190248, specification: 200 g/2 mL).

Rat groups

Sixty SD rats were assigned to sham, model, and dexmedetomidine groups (n = 20 per group), with 20 rats in each group. Each rat was weighed and then anesthetized. They were fixed on the experimental table in supine position and connected to an ECG monitor. The sternum of each rat was cut to expose the heart. Sutures were passed through a coronary occlusion tube 2.5 - 3.5 cm below the left coronary artery near the pulmonary artery.

The sham operation group was left untreated, while the sutures in model and the dexmedetomidine-treated rats were tightened after drug administration. After 30 min, the sutures were loosened, and observation was continued for 120 min. All rats were sacrificed after the experiment. This research was approved by the Animal Ethical Committee Qingpu Branch of Zhongshan Hospital (approval no. 2022081), and conducted according to NIH guidelines for animal studies [5].

Evaluation of parameters/indices

Cardiac function index

Heart rate (HR), LVEDP, LVDP, RPP and dp/dt_{max}, and left ventricular peak descending velocity ($-dp/dt_{max}$) of rats were compared amongst the groups.

Morphological features of rat heart

The rats were sacrificed. Then, coronary thrombosis areas of rats in each group were measured using TTC procedure. Heart tissue was fixed in 4 % formaldehyde solution. Paraffin sections of the heart were routinely prepared, dewaxed with xylene, dehydrated with alcohol gradient, stained with H & E, dehydrated, cleared, and mounted.

Structural changes in the rat heart were examined under a biological microscope (Beijing Century Kexin Scientific Instrument Co. Ltd., model: XSP-100SM).

Apoptosis

Apoptosis of cardiomyocytes in each group was determined using TUNEL assay kit.

Expressions of related proteins

The protein expression levels of cardiac tissue C/EBP, CHOP, glucose regulatory protein 78 (GRP78), transcriptional activation expression of activator of transcription 4 (ATF4), eukaryotic translation initiation factor 2α (eIF2α), and protein kinase R-like endoplasmic reticulum enzyme (PERK) in each group, were measured using Western blotting.

Statistics

Statistics was done with SPSS 20.0. Comparison of measurement results was done with *t*-test, while ANOVA was used for comparing the same index at different time points. Differences amongst groups at each time point were determined using independent samples *t*-test. Time-based differences between groups were determined with LSD *t*-test. Differences were assumed statistically significant at $p < 0.05$.

RESULTS

Levels of cardiac function indices

Before ischemia, HR, LVEDP, LVDP, RPP, and ± DP/dt_{max} were comparable amongst all groups. However, after reperfusion, relative to the sham group, the levels of HR, LVEDP, LVDP, and ± DP/dt_{max} in the model group were significantly reduced, but RPP level was significantly raised. Levels of HR, LVEDP, LVDP, ± DP/dt_{max} of rats in dexmedetomidine group were significantly increased when compared with the model rats. These data are presented in Table 1.

Changes in myocardial infarction area

Myocardial infarction area was significantly higher in model rats than in sham operation rats, but myocardial infarction area was significantly lower in dexmedetomidine-treated rats than in the model group ($p < 0.05$). These data are presented in Table 2.

Changes in cardiac structure of rats

The structure of heart muscle cells in sham-operated rats was complete and orderly, but myocardial cells in model rats were disordered, with broken or wavy myofibrils. In contrast, the cell arrangements in dexmedetomidine group were significantly improved, relative to model.

Cardiomyocyte apoptosis of rats

Table 3 depicts that there was a significantly increased apoptosis rate in cardiomyocytes in model rats, relative to sham operation rats, but myocardial apoptosis rate in dexmedetomidine-treated rats was significantly reduced, relative to that in model rats.

Changes in expression levels of CHOP, GRP78, ATF4, eIF2α, and PERK

In model group, the concentrations of CHOP, GRP78, ATF4, eIF2α, and PERK were significantly raised, relative to the corresponding expressions in sham-operated rats. However, the levels of CHOP, GRP78, ATF4, eIF2α, and PERK in the dexmedetomidine-exposed rats were significantly downregulated, relative to those in model rats, as depicted in Table 4.

Table 1: Comparison of levels of cardiac function indices amongst the groups

Index	Group	Before ischemia	At the end of reperfusion
HR (time/min)	Sham	285.48±1.01	302.91±1.71
	Model	284.79±2.88	103.73±1.11*
	Dexmedetomidine	285.58±2.87	218.16±1.42*#
LVEDP (mmHg)	Sham	3.28±1.30	13.09±1.79
	Model	4.45±1.38	25.85±1.29*
	Dexmedetomidine	4.37±0.98	16.73±2.85*#
LVDP (mmHg)	Sham	84.44±2.70	72.30±2.59
	Model	86.29±0.96	35.43±3.31*
	Dexmedetomidine	84.16±2.08	45.68±3.69*#
RPP	Sham	24469.36±688.52	21535.97±875.13
	Model	24407.46±941.02	3538.15±364.24*
	Dexmedetomidine	24404.83±965.71	9704.85±812.14*#
dp/dt_{max} (mmHg/s)	Sham	2448.75±211.87	2010.90±162.23
	Model	2317.32±388.24	758.99±62.17*
	Dexmedetomidine	2238.23±230.04	1087.87±106.92*#
$-dp/dt_{max}$ (mmHg/s)	Sham	1308.33±48.02	1006.74±52.94
	Model	1431.08±179.54	486.99±67.48*
	Dexmedetomidine	13512.06±47.65	778.48±97.78*#

*$P < 0.05$, vs sham operation; #$p < 0.05$, vs model rats

Table 2: Myocardial infarction area of rats in each group

Group	Myocardial infarction area (% of myocardial area)
Sham	3.12±1.02
Model	69.02±2.80*
Dexmedetomidine	15.58±2.76*#
F	4427.28
P-value	<0.001

*$P < 0.05$, vs sham operation rats; #$p < 0.05$, vs model rats

Table 3: Apoptosis of cardiomyocytes of rats in each group

Group	The apoptosis rate of myocardial cells (%)
Sham	0
Model	59.18±0.58*
Dexmedetomidine	12.31±1.46*#
t	133.425
P-value	<0.001

*$P < 0.05$, vs sham operation; #$p < 0.05$, vs model

DISCUSSION

Timely restoration of normal circulation of blood to the ischemic area is the main goal of treatment for ischemic myocardium. However, reperfusion of ischemic tissue may cause more serious damage to myocardial tissue due to myocardial ischemia-reperfusion injury. There are no effective drugs and methods for the treatment of reperfusion injury [6]. With deepening of clinical research, investigations have revealed that MRI is a long-term and complex pathological process which may involve biological changes such as oxidative stress, apoptosis, and mitochondrial dysfunction. Endoplasmic reticulum stress-mediated pro-survival and pro-apoptotic mechanisms play important roles in the occurrence and development of myocardial ischemia-reperfusion injury [7].

Dexmedetomidine is a highly selective α2 adrenergic receptor agonist which inhibits sympathetic nerve excitability and enhances vagus nerve excitability. It is often used to maintain hemodynamic stability during general anesthesia, and for reducing cardiac adverse reactions in perioperative period [8]. In recent years, studies have found that dexmedetomidine maintains circulatory stability and provides cardio-protection during cardiac surgery, thereby preventing ischemia-reperfusion-induced myocardial injury, probably through activation of α2 adrenergic receptors, anti-inflammatory or mitochondrial apoptotic pathways [9]. However, there are no extant studies on the effect of dexmedetomidine on endoplasmic reticulum stress and myocardial ischemia-reperfusion injury. This research investigated the protective influence of dexmedetomidine on myocardia of rats with ischemia-reperfusion, and the involvement of ER stress pathway in it. The endoplasmic reticulum is a multifunctional organelle that participates in various cellular processes such as regulation of synthesis, transport, and folding of proteins, as well as synthesis of lipids and sterols, thereby maintaining a dynamic balance in cellular functions [10].

Studies have found that ischemia, hypoxia, oxidative stress, and other stimuli cause disorders in protein folding in the endoplasmic reticulum, resulting in misfolded proteins or abnormal accumulation of folded proteins, thereby triggering endoplasmic reticulum stress [11]. When endoplasmic reticulum stress occurs, cells reduce the load on the endoplasmic reticulum by reducing their protein synthesis and promoting the correct pathway of protein processing [12].

Some researchers have found that myocardial ischemia-reperfusion injury induces excessive endoplasmic reticulum stress. The CHOP is a specific transcription factor for endoplasmic reticulum stress and a downstream signaling molecule of GRP78. Under normal circumstances, its expression is very low. However, when myocardial ischemia-reperfusion injury occurs, CHOP levels are significantly raised, thereby aggravating the disease [13]. The first discovered marker protein for ER stress is GRP78.

Table 4: Expression levels of CHOP, GRP78, ATF4, eIF2α and PERK in myocardial tissue of rats in each group

Group	CHOP	GRP78	ATF4	eIF2α	PERK
Sham	0.32±0.07	0.26±0.05	0.24±0.06	0.27±0.05	0.41±0.09
Model	1.86±0.17*	1.67±0.18*	1.55±0.16*	1.64±0.14*	1.37±0.12*
Dexmedetomidine	0.89±0.13*#	0.73±0.09*#	0.67±0.09*#	0.68±0.08*#	0.86±0.10*#
F	32.654	28.642	26.843	27.648	19.467
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

*$P < 0.05$, vs sham operation group; #$p < 0.05$, vs model

During ER stress, GRP78 dissociates from transmembrane proteins and enhances the transfer of proteins from the ER to the cytoplasm, thereby ameliorating ER damage [13]. Protein kinase R-like ER enzyme (PERK), a type I integral membrane protein in ER, belongs to a subfamily of factor 2α kinases in the eukaryotic translation initiation family. Studies have shown that PERK regulates the activities of eIF2α and ATF4, reduces the entry of newly synthesized proteins into the endoplasmic reticulum, and decreases pressure on the endoplasmic reticulum arising from new protein folding, thereby exerting a protective effect against ischemia-reperfusion injury on the myocardium [14].

CONCLUSION

Myocardial ischemia-reperfusion injury significantly reduces cardiac function in rats, increases the size of myocardial infarction, and promotes myocardial cell apoptosis. However, dexmedetomidine significantly improves myocardial function in rats with ischemia-reperfusion injury, inhibits myocardial cell apoptosis, and down-regulates the expressions of CHOP, GRP78, ATF4, eIF2α, and PERK. Dexmedetomidine preconditioning, therefore, protects the myocardium of rats from ischemia-reperfusion injury via inhibition of endoplasmic reticulum stress and the PERK signaling pathway. Further preclinical studies would be required to validate these findings.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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