Research Paper

Protective Effect and Mechanism of Dexmedetomidine Pretreatment Mediated AMPK Pathway on Hypoxic Reoxygenation Injury in Rat Cardiomyocytes

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To examine the effect and mechanism of dexmedetomidine pretreatment mediated adenylate adenosine monophosphate-activated protein kinase pathway on hypoxic reoxygenation injured rat cardiomyocytes. A total of sixty Sprague-Dawley rats were allocated into three groups, namely the sham surgery group, model group, and dexmedetomidine intervention group, utilizing a random number table. Each group consisted of twenty rats. The model group and the intervention group of dexmedetomidine were used to prepare a rat myocardial hypoxia reoxygenation injury model using an improved thread occlusion method. The sham surgery group only underwent thoracotomy without ligation, and the intervention group of dexmedetomidine was pretreated with dexmedetomidine before establishing the model. Western blot was used to detect adenosine monophosphate-activated protein kinase, uncoupling protein 2, and Kruppel-like factor 2 proteins; using flow cytometry to detect the average fluorescence intensity of reactive oxygen species; detection of cell apoptosis rate using terminal deoxynucleotidyl transferase dUTP nick end labeling method; using the enzyme-linked immunosorbent assay detection kit manual to detect serum interleukin-6 and tumor necrosis factor-alpha, interleukin-1 beta and the levels of various indicators such as superoxide dismutase and malondialdehyde. Adenosine monophosphate-activated protein kinase, Kruppel-like factor 2, and uncoupling protein 2 proteins in the myocardium of the model group rats were markedly lower than those of the sham operation group; adenosine monophosphate-activated protein kinase, Kruppel-like factor 2, and uncoupling protein 2 in the myocardium of rats in the dexmedetomidine intervention group were markedly higher than the model group. The superoxide dismutase level of the model group rats was markedly reduced than the sham operation group, with malondialdehyde, interleukin-6 and tumor necrosis factor-alpha, interleukin-1 beta was markedly higher than that of the sham surgery group; superoxide dismutase, malondialdehyde, interleukin-6 and tumor necrosis factor-alpha in the intervention group of dexmedetomidine were markedly higher than the model group, tumor necrosis factor-alpha, interleukin-1 beta was markedly reduced than that of the model group. Dexmedetomidine can activate adenosine monophosphate-activated protein kinase pathway, upregulation of uncoupling protein 2 expressions and the inhibition of mitochondrial reactive oxygen species production are observed, thereby restraining the oxidative irritability of myocardial tissue under hypoxia/reoxygenation conditions and playing a role in myocardial cytoprotection.

Key words: Dexmedetomidine, adenylate activated protein kinase, hypoxia reoxygenation injury, rats, myocardial cells

Ischemic heart disease is a prominent contributor to mortality within the realm of cardiovascular disease. Percutaneous Coronary Intervention (PCI) and coronary artery bypass grafting represent the primary therapeutic modalities employed in the management of ischemic heart disease, which can help people with ischemic heart disease achieve myocardial ischemia-reperfusion as soon

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as possible, prevent myocardial cell death and systolic dysfunction, and improve the symptoms of myocardial ischemia and hypoxia[1]. However, it has been found that myocardial ischemia-hypoxia-reperfusion therapy itself may aggravate myocardial injury, induce cardiomyocyte death, and lead to the expansion of ischemic infarction, i.e., myocardial ischemia-hypoxia-reperfusion injury[2]. Therefore, preventive treatment for patients with reperfusion therapy in order to reduce or even avoid myocardial ischemia-hypoxia-reperfusion injury has important clinical significance in order to enhance the clinical prognosis of individuals diagnosed with coronary heart disease. Dexmetomide is a commonly used anesthetic in clinic, which is widely used in adjuvant anesthesia in surgical patients and sedation in patients with Intensive Care Units (ICU). Research has demonstrated that dexmetomide exerts a protective function in the context of ischemia-reperfusion injury affecting vital organs such as the heart, liver, brain and kidney, but the specific mechanism has not been fully elucidated[3]. Recent research has demonstrated that adenylate Adenosine Monophosphate-activated Protein Kinase (AMPK), also known as Adenosine Monophosphate (AMP)-dependent protein kinase, plays a crucial role in monitoring cellular energy equilibrium. Serving as a pivotal kinase in orchestrating metabolic processes and maintaining energy balance, the activation of AMPK facilitates the enhancement of Uncoupling Protein 2 (UCP2) expression, consequently impeding Reactive Oxygen Species (ROS) generation and mitigating oxidative stress-induced damage[4]. The objective of this study is to investigate the protective effect and underlying mechanism of AMPK pathway mediated by dexmedetomidine preconditioning on cardiomyocytes of rats with hypoxia-reoxygenation injury, in order to offer guidance for the selection of clinical treatment.

MATERIALS AND METHODS

Materials and reagents:

60 Sprague-Dawley (SD) rats were from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. ROS fluorescence detection kit (Abcam Biotechnology Co., Ltd.); Erythropoietin (EPO) injection (Shenyang Sansheng Pharmaceutical Co., Ltd.); AMPK, Kruppel-Like Factor 2 (KLF2), UCP2, Beta (β)-actin antibody (Abcam Biotechnology Co., Ltd.); anti-rabbit second antibody, gel electrophoresis preparation kit (Shanghai Biyuntian Co., Ltd.). Superoxide Dismutase (SOD) and Malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Institute of Biological function; Interleukin (IL)-6, Tumor Necrosis Factor-Alpha (TNF-α), IL-1β Enzyme-Linked Immunosorbent Assay (ELISA) Kit and Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) apoptosis kit (Shanghai Biyuntian Co., Ltd.).

Animal model:

A total of 60 SD rats were allocated into three groups; sham operation group, model group and dexmedetomidine intervention group. The rats were anesthetized using intraperitoneal injection of 1 % pentobarbital sodium at a dosage of 30 mg/kg, then fixed to the operation board, connected to the Electrocardiogram (ECG) and monitored. In the dexmetomidine intervention group, dexmetomidine 5 μg/kg loading dose was given intravenously, and then 5 μg/(kg·h) was infused continuously for 1 h. 0.9 % sodium chloride solution of the same volume was given intravenously in the model group and the sham operation group. The rat model of myocardial hypoxia-reoxygenation injury was established by modified thread occlusion method in model group and dexmedetomidine intervention group. Small animal ventilator was connected and endotracheal intubation was performed. The muscle tissue was bluntly separated layer by layer on the left side of the sternum, the cardiac capsule was cut and the heart was exposed. The ligation of the left anterior descending coronary artery (30 min) occurred at the inferior boundary of the left atrial appendage. The successful ligation was marked by the whitening of the left ventricle and the elevation of ST segment in ECG, and then 120 min was perfused. The sham operation group only opened the chest without ligation.

Sampling and sample preparation:

After the establishment of the model, venous blood 5 ml was taken from rats under anesthesia, and then the hearts of rats were removed by rapid bloodletting, and the left ventricular myocardial tissue of rats was removed and washed repeatedly with normal saline, half of which was transferred to -80° refrigerator for detection; the other half of the tissue was embedded in paraffin and

stained according to the instructions of Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) kit to detect the rate of apoptosis.

ROS fluorescence intensity analysis:

The heart was put into the Eppendorf (EP) tube, the tissue was treated by shearing method, the 1 ml Phosphate-Buffered Saline (PBS) buffer was added, the tissue was cut up by ophthalmology, and the supernatant from the EP tube was filtered by pipette. The cell suspension was passed through a flow tube and subsequently stained with Dichlorodihydrofluorescein Diacetate (DCFDA), incubated with 30 min at 37°, and then added with a probe. ROS fluorescence intensity was analyzed by flow cytometry.

Western blot:

After adding protein lysate to homogenate at 4° to make 10 % homogenate, the supernatant was acquired through the process of centrifugation, and the concentration of protein was assessed using the Bicinchoninic Acid (BCA) assay method, gel was prepared, 90 min electrophoresis was done, gel was cut and transferred, and milk was sealed. After cleaning, it was incubated with primary and secondary antibodies; developed, and the data was subjected to analysis using the Bio-Rad image laboratory software.

Serological index:

20 min was separated by 3000 r/min in venous blood, and the supernatant was reserved for examination. IL-6, TNF-α, IL-1β, SOD and MDA were detected strictly according to the instructions of ELISA detection kit.

Statistical method:

Statistical analysis and processing of data were carried out using the Statistical Package for the Social Sciences (SPSS) 22.0 statistical software, and the measurement information was expressed by (x±s), and the comparison was made by t-test.

RESULTS AND DISCUSSION

AMPK and KLF2 in myocardium in model group were reduced than the sham operation group, whereas AMPK and KLF2 in myocardium in dexmedetomidine intervention group were markedly higher than the model group as shown in Table 1.

UCP2 protein in the model group exhibited decrease compared to the sham operation group, whereas the dexmedetomidine intervention group demonstrated increase in UCP2 protein than the model group as shown in Table 2. The average fluorescence intensity of myocardial ROS and the rate of cardiomyocyte apoptosis in the model group were markedly higher in comparison with the sham operation group, whereas the dexmedetomidine intervention group were markedly reduced than the model group as shown in Table 3.

The level of SOD in the model group was lower than the sham operation group, and the level of MDA in the model group was higher than the sham operation group. The level of SOD in the dexmedetomidine intervention group was higher than the model group, and MDA in the model group was lower than the model group as shown in Table 4.

IL-6, TNF- α and IL-1β in the model group were markedly higher than the sham operation group, whereas the levels of IL-6, TNF-α and IL-1β in the dexmedetomidine intervention group were lower than the model group as shown in Table 5.

TABLE 1: COMPARISON OF AMPK AND KLF2 IN MYOCARDIUM OF RATS IN DIFFERENT GROUPS

Group	n	AMPK	KLF2
Sham operation	20	0.68±0.20	0.83±0.35
Model	20	0.40±0.18[a]	0.59±0.17[a]
Dexmedetomidine intervention	20	0.62±0.24[b]	0.76±0.23[b]
F		10.031	4.474
p		0.000	0.016

Note: Compared to the sham operation group, [a]p<0.05 and compared to the model group, [b]p<0.05

TABLE 2: COMPARISON OF UCP2 PROTEIN EXPRESSION IN MYOCARDIUM OF RATS IN DIFFERENT GROUPS

Group	n	UCP2 cardiac protein expression
Sham operation	20	0.74±0.23
Model	20	0.38±0.12[a]
Dexmedetomidine intervention	20	0.62±0.19[b]
F		19.497
p		0.000

Note: Compared to the sham operation group, [a]p<0.05 and compared to the model group, [b]p<0.05

TABLE 3: COMPARISON OF ROS AVERAGE FLUORESCENCE INTENSITY AND CARDIOMYOCYTE APOPTOSIS RATE AMONG DIFFERENT GROUPS OF RATS

Group	n	Average fluorescence intensity expression of ROS	Cardiomyocyte apoptosis rate (%)
Sham operation	20	11.57±1.13	3.97±0.83
Model	20	15.81±1.23[a]	27.51±2.13[a]
Dexmedetomidine intervention	20	13.64±1.15[b]	18.34±1.25[b]
F		65.587	1244.37
p		0.000	0.000

Note: Compared to the sham operation group, [a]p<0.05 and compared to the model group, [b]p<0.05

TABLE 4: COMPARISON OF OXIDATIVE STRESS INDEXES IN RATS OF EACH GROUP

Group	n	SOD (kU/g)	MDA (μmol/g)
Sham operation	20	88.38±14.32	4.14±1.13
Model	20	51.52±12.49[a]	8.23±2.69[a]
Dexmedetomidine intervention	20	64.28±14.39[b]	6.34±1.47[b]
F		37.033	23.553
p		0.000	0.000

Note: Compared to the sham operation group, [a]p<0.05 and compared to the model group, [b]p<0.05

TABLE 5: COMPARISON OF SERUM INFLAMMATORY INDEXES IN RATS OF EACH GROUP

Group	n	IL-6 (μg/l)	TNF-α (ng/l)	IL-1β (pg/l)
Sham operation	20	97.86±11.28	50.71±8.22	9.34±1.76
Model	20	180.28±18.04[a]	170.33±17.11[a]	30.28±4.84[a]
Dexmedetomidine intervention	20	150.37±16.09[b]	121.41±13.14[b]	16.36±2.13[b]
F		146.788	407.156	219.423
P		0.000	0.000	0.000

Note: Compared to the sham operation group, [a]p<0.05 and compared to the model group, [b]p<0.05

Ischemic heart disease is a kind of cardiovascular disease caused by acute and persistent myocardial ischemia and hypoxia on the basis of sharp decrease or even interruption of vascular blood supply caused by coronary artery disease. It is related to many factors such as overwork, emotional agitation, cold stimulation, overeating and so on[5]. The condition of patients with ischemic heart disease changes rapidly, and chest pain can be relieved in mild cases, but in severe cases, it is feasible to encounter complexity when dealing with severe complications such as cardiogenic shock, which affects the life, health and safety of patients[6]. At present, PCI and coronary artery bypass grafting are the main methods for the treatment of myocardial infarction, which can help patients with myocardial infarction to achieve myocardial ischemia-reperfusion as soon as possible. However, although the myocardial blood supply of some patients recovered after reperfusion treatment, the infarct area did not decrease, but further enlarged, suggesting that myocardial ischemia-hypoxia-reperfusion injury occurred, and resulting in an increase in mortality[7]. Therefore, taking scientific and effective treatment measures to prevent myocardial hypoxia-reoxygenation injury is instrumental in enhancing the prognosis of individuals afflicted with myocardial infarction. It has been found that after the occurrence of hypoxic-ischemic myocardial injury, the body initiates a series of sequence reactions, including apoptosis, inflammation, disturbance of energy metabolism, oxidative stress and so on[8,9].

Mitochondria serve as the central regulatory hub for cellular oxidative stress and energy metabolism, earning them the moniker "the cellular powerhouses". In the process of oxidative phosphorylation of mitochondria with oxygen, a small number of electrons leaked from the respiratory chain are easy to combine with oxygen to form ROS[10]. In myocardial infarction, myocardial ischemia and hypoxia lead to the decrease of Adenosine Triphosphate (ATP) production in mitochondria, which leads to the initiation of apoptosis cascade including ion imbalance, resulting in the production of ROS and the aggravation of oxidative stress after ischemia-reperfusion. When the production of ROS exceeds the scavenging capacity of cell antioxidant system, the balance of oxidation and antioxidation is broken, cells undergo oxidative stress, and mitochondrial function disorder and Deoxyribonucleic Acid (DNA) damage are caused. Activation of apoptotic protease to promote cardiomyocyte apoptosis[11]. As an endogenous neuroprotective factor, endogenous protective protein plays a protective role in myocardium after acute hypoxic-ischemic injury by means of antioxidant stress, inhibition of apoptotic body formation and clearance of ROS[12]. Numerous types of endogenous protective proteins, such as uncoupling protein, heat shock protein, and neuroglobin, are expressed within myocardial tissue[13]. UCP2 is classified as a mitochondrial inner membrane protein that actively engages in the facilitation of proton transport within the mitochondrial inner compartment, reduces the transmembrane proton gradient through uncoupling, and has the function of dissociating oxidative phosphorylation coupling of respiratory chain. UCP2 has the functions of regulating ATP production, mitochondrial function, regulating thermogenesis, apoptosis and oxidative stress response by this intervention demonstrates a notable protective impact on the myocardium[14]. It is found that UCP2 can increase the proton permeability of mitochondrial inner membrane, reduce $\Delta\mu H^+$, and reduce the production of ROS through the "proton pump" mechanism, so as to play the role of antioxidant stress, weaken the oxidative stress and reduce the death of cardiomyocytes[15]. Previous research has indicated that AMPK, an upstream protein of UCP2, serves as the primary regulator of cellular energy metabolism. The elevation of the AMP/ATP ratio in hypoxic-ischemic myocardium results in the activation of AMPK, thereby enhancing the efficacy of cellular oxidative phosphorylation. This, in turn, triggers the activation of UCP2 and augments the expression of UCP2[16]. The findings of this study indicate decrease in the UCP2 protein in the model group than the sham operation group. Furthermore, the dexmetomidine intervention group exhibited increase in the UCP2 protein compared to the model group. The average fluorescence intensity of myocardial ROS and the rate of cardiomyocyte apoptosis in the model group were markedly higher than the sham operation group, whereas the average fluorescence intensity of myocardial ROS and the rate of cardiomyocyte apoptosis in the dexmedetomidine intervention group were markedly reduced than the model group. The SOD

in the model group was markedly reduced than the sham operation group, whereas MDA, IL-6, TNF-α and IL-1β in the model group were increase than the sham operation group. The SOD in the dexmedetomidine intervention group was increase than the model group, and MDA, IL-6, TNF-α and IL-1β in the model group was markedly reduced than the model group. There is a suggestion that UCP2 as an endogenous protective factor increases during myocardial hypoxia-reoxygenation injury, thus reducing the production of ROS and protecting cardiomyocytes from oxidative stress.

AMPK is classified as a serine/threonine protein kinase and plays a crucial role in the regulation of cellular energy homeostasis and inflammation. Its expression is observed in a diverse range of metabolic organs and tissues. It can be activated by various stimuli by sensing changes in the state of cell energy metabolism, thus affecting multiple links of cell material metabolism to coordinate body metabolism and energy balance[17]. It has been found that when there is an imbalance between metabolism and energy, activation of AMPK can regulate the expression of downstream malonyl-Coenzyme A (CoA) and lipid synthesis genes through phosphorylation, regulate the biosynthesis of fatty acids, thus inhibit inflammation and oxidative stress, and restore the energy balance of the body[18]. In addition, AMPK and UCP2 proteins have been proved to have a functional correlation mechanism. AMPK can promote the activity of mitochondrial enzymes, increase the efficiency of oxidative phosphorylation, increase the expression of UCP2, reduce oxidative stress and promote the balance of energy metabolism[19]. The findings indicate that the AMPK and KLF2 were notably diminished in the model group compared to the sham operation group. Conversely, the dexmedetomidine group exhibited significantly elevated levels of AMPK and KLF2 expression than the model group. There is a suggestion that the levels of AMPK and KLF2 in myocardial tissue of rats with hypoxia-reoxygenation injury are significantly decreased. Dexmetomidine can up-regulate the level of UCP2 by activating AMPK pathway, thus reduce the production of ROS and protect cardiomyocytes from oxidative stress. Dexmetomidine is a highly selective and specific α2-adrenergic receptor agonist. It has been reported that dexmetomidine can increase the production of endothelial nitric oxide, promote angiogenesis and protect cardiomyocytes from ischemia and hypoxia by promoting the AMPK protein and up-regulating KLF2[20]; in addition, dexmedetomidine can induce the phosphorylation of AMPK, increase the activity of mitochondrial enzyme, increase the expression level of UCP2, activate mitochondrial uncoupling, reduce the production of mitochondrial ROS, reduce oxidative stress induced by myocardial ischemia-reperfusion injury and provide a protective effect on the myocardium[21].

In conclusion, dexmetomidine has the ability to enhance UCP2 protein and suppress the generation of mitochondrial ROS through the activation of the AMPK pathway, so as to reduce the oxidative stress of myocardial tissue under hypoxia/reoxygenation and play a protective role in cardiomyocytes.

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Conflict of interests:

The authors declared no conflict of interests.

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