Tropical Journal of Pharmaceutical Research August 2023; 22 (8): 1605-1611
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i8.11

Original Research Article

Dexmedetomidine pre-conditioning induces inhibition of ROS in myocardial ischemia-reperfusion injury in rats through AMPK pathway

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Sent for review: 12 April 2023 *Revised accepted: 26 July 2023*

Abstract

Purpose: *To elucidate the basis for the cardioprotective effect of dexmedetomidine pre-treatment on ROS-induced myocardial ischemia-reperfusion injury (IRI) in rats.*
Methods: *Sixty Sprague-Dawley (SD) rats were assigned to sham, model and dexmedetomidine intervention groups, each having 20 rats. Myocardial IRI was induced in the model and dexmedetomidine intervention groups using modified suture method. In sham group, chests of rats were opened, but without ligation, while dexmedetomidine intervention group was pre-treated with dexmedetomidine (5 μg/kg) before establishment of the IRI model. Protein expressions of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) was determined by Western blot assay. Mean fluorescence intensity of ROS was measured using flow cytometry.*
Results: *AMPK protein was significantly down-regulated in model rats, relative to sham rats, but significantly higher in dexmedetomidine intervention rats ($p < 0.05$). In model rats, mean ROS fluorescence intensity and degree of apoptosis of cardiomyocytes were higher than the corresponding values in sham rats ($p < 0.05$), but lower in dexmedetomidine intervention group.*
Conclusion: *Dexmedetomidine reduces oxidative stress in myocardial tissue and exerts a protective role by activating AMPK pathway and inhibiting mitochondrial generation of ROS. Therefore, this compound might have a potential clinical role in the management of IRI.*

Keywords: *Dexmedetomidine premedication, Adenylate-activated protein kinase, Reactive oxygen species, Myocardial ischemia-reperfusion injury, Myocardial protection*

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INTRODUCTION

At present, percutaneous coronary intervention (PCI) and coronary artery bypass grafting are major treatment strategies for myocardial infarction. These treatments help patients with myocardial infarction to achieve rapid myocardial ischemia-reperfusion and also prevent cardiomyocyte death and systolic dysfunction, thereby reducing symptoms of myocardial ischemia [1]. However, research has shown that myocardial ischemia-reperfusion therapy may aggravate myocardial injury, induce cardiomyocyte death, and worsen extent

of ischemia-reperfusion injury (IRI) [2]. Therefore, treatment of patients with reperfusion therapy with the aim of reducing or even preventing myocardial IRI is of great clinical significance for improving the clinical prognosis of coronary artery disease patients.

Dexmedetomidine, with the molecular formula of $C_{13}H_{16}N_2$, is an effective α2-adrenergic receptor agonist. Dexmedetomidine is used frequently in clinics as anesthetic drug. It is also widely used as adjuvant anesthesia for surgical patients and for sedation of Intensive Care Unit (ICU) patients. However, although dexmedetomidine exerts protective effect against IRI of heart, liver, brain, kidney and other organs, the specific mechanism of action has not been fully elucidated [3]. It has been reported that AMPK is highly sensitive to changes in cellular energy status. AMPK activation enhances the up-regulation of expression of protein uncoupler (UCP2), thereby suppressing reactive oxygen species (ROS) production [4]. This research was carried out to investigate cardioprotective effect of dexmedetomidine pre-treatment against ROS-induced myocardial ischemia-reperfusion injury in rats and ascertain mechanism of action.

EXPERIMENTAL

Materials

Sixty (60) Sprague Dawley (SD) rats were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co. Ltd. Some of the reagents used were fluorescence ROS detection kit and antibodies (Abcam Biotechnology Co. Ltd, UK). Superoxide dismutase (SOD) and malondialdehyde (MDA) assay kits were purchased from Nanjing Jiancheng Institute of Biological Function, China. Enzyme-linked immunosorbent assay kits for IL-6, TNF-α and IL-1β, and annexin VFITC/PI apoptosis kits were purchased from Shanghai Biyuntian Company.

Establishment of rat model of IRI

Rats were randomly divided into three groups namely: sham operation, model and dexmedetomidine intervention groups, with 20 rats in each group. Rats were anesthetized via intraperitoneal injection of 1 % pentobarbital sodium (30 mg/kg). They were fixed on an operation board, connected to electrocardiogram and monitored. Dexmedetomidine intervention rats were intravenously given dexmedetomidine (5 μg/kg) for 1 h, while rats in the model and sham operation groups were given equivalent volume of 0.9 % sodium chloride solution in place of dexmedetomidine. Myocardial IRI was induced in model and dexmedetomidine intervention groups, using the modified thread plug method [5]: ventilator for small animals was connected and tracheal intubation was performed. The left anterior descending branch of the coronary artery was ligated at the lower border of arterial protuberance to the left for 30 min. The ligation was marked by elevation of electrocardiogram in white left ventricle and ST segment and then reperfusion was done for 120 min. In sham operation group, thoracotomy with perforation but no ligature was performed on rats [6]. This research received approval from the Animal Ethical Authority of The Second Affiliated Hospital of Fujian Medical University, (approval no. SHFMU-2022034) and was conducted in line with the revised NIH guidelines for care of experimental animals [7].

Sampling and sample preparation

After establishment of model, blood (5 mL) was obtained from rat vein under 10 % pentobarbital sodium anesthesia. Thereafter, rats were sacrificed via rapid exsanguination [8] and the hearts were excised. The left ventricular myocardial tissues of rats were removed and rinsed repeatedly with normal saline. Half of the ventricular myocardial tissue was transferred to a -80 ^{o}C refrigerator for preservation, while the other half was paraffin-embedded and subjected to determination of apoptosis by staining with TUNEL kit in line with the manufacturer's instructions.

Determination of ROS fluorescence intensity [9]

Each heart sample was sheared in PBS buffer after ophthalmic scissors was used to cut the tissue. Then, the supernatant in EP tube was removed using a pipette, and after filtration into flow cytometry tube, cells were subjected to staining using DCFDA reagent (a cell-based ROS detection stain). Following incubation at 37 ^{o}C for 30 min, probe was added and ROS fluorescence intensity was analyzed flow-cytometrically.

Assay of protein expressions using western blot assay

Soluble proteins were extracted from heart tissues using RIPA buffer at 4 ^{o}C. Following centrifugation, protein content of supernatant was determined with BCA procedure. Proteins were subjected to SDS-PAGE and electro-transferred to PVDF films for 90 min. The films

were sealed using 5 % fat-free milk, prior to 12-h incubation at 4 °C with 1° immunoglobulins and thereafter with horseradish peroxidase-conjugated 2° immunoglobulin for 60 s at room temperature. After ECL, relative protein expressions were determined with BioRad image laboratory software [10].

Assay of serological indicators

Venous blood was centrifuged at 3000 rpm for 20 min and serum levels of interleukin 6 (IL-6), tumor necrosis factor (TNF), interleukin -α (IL-α), interleukin-1 β (IL-1 β), superoxide dismutase (SOD) and malondialdehyde (MDA) were determined using ELISA kits.

Statistical analysis

The SPSS software (version 20.0) was used for statistical analysis. Data are expressed as mean ± standard deviation (SD). Multiple groups were compared using analysis of variance (ANOVA). The LSD or Tamhane test was used for comparing the two groups. Statistical significance was assumed at $p < 0.05$.

RESULTS

Protein expression levels of AMPK and KLF2 in myocardium

As shown in Table 1, relative to sham operation rats, protein levels of AMPK and KLF2 were significantly down-regulated ($p < 0.05$) in model rats but were significantly up-regulated ($p < 0.05$) in dexmedetomidine intervention rats when compared to model rats.

Table 1: Protein expression levels of AMPK and KLF2 in myocardium of rats in each group (n= 20)

Group	AMPK	KLF2
Sham	0.68±0.20	0.83±0.35
Model	0.40±0.18[a]	0.59±0.17[a]
Dexmedetomidine intervention	0.62±0.24[b]	0.76±0.23[b]
F	10.03	4.47
P-value	0.00	0.02

[a]$p < 0.05$, vs. sham; [a]$p < 0.05$, vs. model

UCP2 protein level in myocardium

Expression level of UCP2 protein is presented in Table 2. The result shows that the level of UCP2 protein was significantly lower in model rats than in sham operation rats, but it was significantly up-regulated ($p < 0.05$) in dexmedetomidine-treated rats, relative to model rats.

Table 2: Protein expression level of UCP2 in rat myocardium

Group	UCP2 myocardial protein expression
Sham	0.74±0.23
Model	0.38±0.12[a]
Dexmedetomidine intervention	0.62±0.19[b]
F	19.50
P-value	0.00

[a]$p < 0.05$, vs. sham rats; [b]$p < 0.05$, vs. model rats

Mean fluorescence intensity of ROS and apoptosis of cardiomyocytes

As shown in Table 3, in model rats, the average fluorescence intensity of ROS in cardiomyocytes was significantly lower, while apoptosis rate was significantly higher than the sham group value ($p < 0.05$). However, ROS fluorescence intensity was significantly higher, while apoptosis rate was lower in the dexmedetomidine-treated rats when compared to model rats ($p < 0.05$).

Table 3: Mean ROS fluorescence intensity and cardiomyocyte apoptosis rate

Group	Mean ROS fluorescence intensity	Cardiomyocyte apoptosis rate (%)
Sham	0.74±0.23	3.97±0.83
Model	0.38±0.12[a]	27.51±2.13[a]
Dexmedetomidine intervention	0.62±0.19[b]	18.34±1.25[b]
F	65.59	1244.37
P-value	0.00	0.00

[a]$P < 0.05$, vs. sham rats; [b]$p < 0.05$, vs. model rats

Oxidative stress index levels

Table 4 shows that activity of SOD in model rats was significantly reduced ($p < 0.05$) when compared to activity in sham-operated rats, but MDA level was significantly higher than that in the sham-operation group ($p < 0.05$). However, SOD activity in dexmedetomidine intervention group was significantly higher ($p < 0.05$) than that in model group, while MDA level was significantly lower ($p < 0.05$) in the dexmedetomidine group when compared to model rats.

Serum levels of inflammatory indices in rats

Table 5 shows significantly higher levels ($p < 0.05$) of serum IL-6, IL-1β and TNF-α in model rats than in sham operation rats. However, these indices were found to decrease

significantly ($p < 0.05$) in dexmedetomidine intervention rats compared to model rats.

Table 4: Oxidative stress index levels in rats in each group

Group	SOD (kU/g)	MDA (μmol/g)
Sham	88.38±14.32	4.14±1.13
Model	51.52±12.49[a]	8.23±2.69[a]
Dexmedetomidine intervention	64.28±14.39[b]	6.34±1.47[b]
f	37.03	23.55
P-value	0.00	0.00

[a]$P < 0.05$, vs. sham rats; [b]$p < 0.05$, vs. model rats

Table 5: Levels of inflammation-linked indices in the rat groups

Group	IL-6 (μg/L)	TNF-α (ng/L)	IL-1 β (pg/L)
Sham	97.86	50.71	9.34±1.76
Model	180.28[a]	170.33[a]	30.28±4.84[a]
Dexmedetomidine intervention	150.37[b]	121.41[b]	16.36±2.13[b]
F	146.79	407.16	219.42
P-value	0.00	0.00	0.00

[a]$p < 0.05$, vs. sham rats; [b]$p < 0.05$, vs. model rats

DISCUSSION

Myocardial infarction is a cardiovascular disease caused by acute and sustained myocardial ischemia, hypoxia and necrosis due to intense reduction or interruption of blood supply caused by lesions in coronary artery of the heart. It is attributed to many factors such as overwork, emotional excitement, cold stimulation and over-eating [11]. Condition of patients with acute myocardial infarction fluctuates rapidly. In mild cases, chest pain may be self-limiting. However, serious complications such as cardiogenic shock easily occur in severe cases, thereby affecting lives, health and safety of patients [12].

At present, PCI and coronary artery bypass grafting are main strategies used for treatment of myocardial infarction. These treatments help patients with myocardial infarction to achieve myocardial ischemia-reperfusion as soon as possible. However, although some patients recover blood supply to myocardium after reperfusion treatment, infarct size does not shrink but expands further, suggesting the occurrence of myocardial IRI, a situation that results in increased mortality [13]. Therefore, scientific and effective treatment measures for preventing the occurrence of myocardial ischemia-reperfusion injury positively improve prognosis of patients with myocardial infarction. Studies have found that after hypoxic-ischemic myocardial injury, the body initiates a sequence of biological events, including apoptosis, inflammation, impairment of energy metabolism and oxidation-related changes [14,15].

Mitochondria, also known as the "power workshop of cells", are regulatory centers for generation of cellular energy and free radicals. During the process of oxidative phosphorylation in mitochondria, small amounts of electrons escape from the electron transport chain. These electrons rapidly combine with O_2, resulting eventually in formation of ROS [16]. In myocardial infarction, myocardial ischemia and hypoxia lead to reduction of ATP production in mitochondria, thereby initiating apoptotic cascade, ion imbalance and production of large amounts of ROS, all of which aggravate oxidative stress after ischemia-reperfusion. When generation of ROS exceeds scavenging capacity of cellular antioxidant system, balance between oxidative threat and antioxidant potential is broken. Therefore, cells undergo oxidative stress, resulting in dysfunctional mitochondria, DNA lesions, induction of apoptosis and enhancement of apoptotic changes in cardiomyocytes [17]. When myocardium is subjected to acute hypoxic-ischemic injury, endogenous protective proteins exert myocardial protection through scavenging of ROS (antioxidant effect), thereby inhibiting formation of apoptotic bodies [18].

Several kinds of endogenous protective proteins are expressed in myocardial tissue including uncoupling protein, heat shock protein and neuroglobin [19]. An inner mitochondrial membrane protein, UCP2, which participates in H^+ transport in inner membrane of mitochondrion, reduces transmembrane proton gradient through uncoupling, thereby uncoupling phosphorylation (ATP formation) from oxidation in the respiratory chain. It has been reported that UCP2 regulates ATP production and mitochondrial function and it exerts a protective influence on myocardium [20]. In addition, UCP2 makes the mitochondrial membrane more permeable to protons, lowers thylakoid membrane $\Delta\mu H^+$ and decreases ROS levels, resulting in anti-oxidative stress, weakening of cellular oxidative stress and reduction of death of cardiomyocytes [21].

Another study found that AMPK, upstream protein of UCP2, is the master switch of cellular energy metabolism. Increases in myocardial AMP/ATP proportion due to hypoxia/ischemia lead to priming of AMPK, enhancement of efficiency of cellular oxidative phosphorylation,

activation of UCP2 and up-regulation of expression of UCP2 [22].

In the present research, UCP2 protein level was lower in model rats than in sham rats but was significantly ($p < 0.05$) up-regulated in dexmedetomidine intervention rats relative to model rats. Average fluorescence intensity of ROS in model rat cardiomyocytes was lower, while apoptosis rate was higher, relative to sham operation rats. However, ROS fluorescence intensity and apoptosis rate were significantly higher and lower, respectively, in dexmedetomidine intervention rats than in model rats. Moreover, model rat SOD activity was significantly lower ($p < 0.05$) than what was observed in sham rats, and levels of IL-1 β, MDA, IL-6, and TNF- α were significantly increased in model rats compared to sham-operation rats. However, SOD activity in dexmedetomidine intervention group was higher than that in model rats, while levels of MDA, IL-6, TNF- α and IL-1 β were significantly reduced compared to model rats ($p < 0.05$). These data suggest that expression of UCP2, an endogenous protective factor, was increased during myocardial ischemia-reperfusion injury, thereby reducing ROS production and protecting cardiomyocytes from oxidation-induced injury.

Adenylate-activated protein kinase (AMPK), which is crucial for cellular energy balance, is activated by various stimuli through sensing of changes in cellular energy metabolism, thereby affecting multiple links involved in metabolism and energy balance [23]. Studies have found that when there is an energy imbalance, AMPK activation regulates lipogenesis through modification of gene expression. These events regulate the biosynthesis of fatty acids and inhibit inflammation and oxidative insult, thereby restoring cellular ATP balance [24]. Moreover, UCP2 and AMPK are functionally associated: AMPK enhances mitochondrial enzyme activities and it is involved in up-regulation of UCP2 expression [25]. In the present investigation, there were significant reductions in protein levels of AMPK and KLF2 in model rats, relative to the corresponding values in sham operation rats, but protein expressions were up-regulated in dexmedetomidine intervention group when compared with model group. Thus, significant decreases in levels of AMPK and KLF2 were observed in myocardial tissue of rats with myocardial ischemia-reperfusion injury. Dexmedetomidine up-regulated level of UCP2 by activating AMPK pathway thereby reducing ROS concentration and protecting cardiomyocytes from oxidation-associated damage.

Dexmedetomidine is a highly selective and specific α_2 adrenergic receptor agonist. Research has shown that dexmedetomidine increases the production of NO in the endothelium, enhances angiogenesis and shields cardiomyocytes from lesions due to ischemia and hypoxia by enhancing AMPK protein expression and up-regulating the expression of transcription factor KLF2 [26]. In addition, dexmedetomidine induces AMPK phosphorylation, raises activities of enzymes in the mitochondrion, enhances expression of UCP2, decreases production of ROS caused by myocardial IRI, and protects myocardial tissues from damage [27].

CONCLUSION

Dexmedetomidine reduces myocardial tissue oxidative stress and plays a myocardial protective role by activating AMPK pathway, upregulating UCP2 expression and suppressing ROS generation in the mitochondria.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

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

We 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 the authors. All authors read and approved the manuscript for publication. Jinwei Liang conceived and designed the study. Wanping Hong and Xue'e Su collected and analyzed the data, while Shanhu Wu wrote the manuscript.

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