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Effects of dexmedetomidine on the degree of myocardial ischemia-reperfusion injury, oxidative stress and TLR4/NF-κB signaling pathway in rats

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Abstract: To investigate the effects of dexmedetomidine on the degree of myocardial ischemia-reperfusion injury (MIRI), oxidative stress and Toll-like receptor 4 (TLR4)/nuclear factor κB (NF-kappa B) signaling pathway in rats. Thirty-six adult healthy SD rats were randomly divided into experimental group, injury group and control group by random number table. Dexmedetomidine, 0.9% sodium chloride solution and pre-treatment with 0.9% sodium chloride solution for 1 hour were given respectively. CK-MB, cTn-I, NT-proBNP and LDH levels in the experimental group and the injury group were significantly higher than those in the control group; SOD activity in the experimental group and the injury group were significantly superior to that in the control group; GSH contents in the experimental group and the injury group were significantly less than that in the control group; The MDA contents of the experimental group and the injury group was significantly greater than that of the control group; the TLR4 and NF-κB protein expression levels of the experimental group and the injury group were significantly higher than that of the control group (all $P<0.05$). Dexmedetomidine can effectively reduce myocardial injury caused by myocardial ischemia-reperfusion (I/R), reduce the expression of TLR4 and NF-κB, negatively regulate its signaling pathway, alleviate oxidative stress response.

Keywords: Dexmedetomidine, I/R, injury, oxidative stress, TLR4, NF-κB signaling pathway.

INTRODUCTION

In recent years, the incidence of cardiovascular disease has been witnessing a trend of gradual increase. After patients receive treatment, I/R may damage cardiomyocytes and affect cardiac function, even leading to exacerbation of the disease, which further results in a somber prognosis and compromises the quality of life of patients. Although the mechanism of I/R-induced injury is still elusive, previous research has reported (Yi *et al*., 2020) that oxidative stress response may be involved in myocardial injury. The free radical scavenging capacity will be reduced after myocardial ischemia and a large amount of oxygen free radicals will destroy cytomembrane and mitochondrial function in a short time in wake of reperfusion. The inflammatory response (Zilan *et al*., 2018) may be another involving mechanism, as oxygen radicals induce over expression of specific adhesion factors on the surface of leukocytes to promote neutrophil adhesion, aggregation and infiltration, which leads to the formation of micro thrombi, thereby activating an inflammatory response that further gives rise to myocardial injury. Toll-like receptor4 (TLR4)/nuclear factor kappa-B (NF-κB) signaling pathway plays an important role in regulating inflammatory response (Yuying *et al*., 2020). Dexmedetomidine (Dex) is a sedative and analgesic that is generally used in clinical anesthesia. It has been reported that (Jingchun *et al*., 2019) Dex can regulate the expression of inflammatory factors in the body after surgeries to excessively produce antagonistic oxygen-free radicals for tissue and organ protection. However, Dex is less used in I/R. The study aimed to provide a theoretical basis for clinical prevention by investigating the effects of Dex on myocardial injury, oxidative stress, and the TLR4/NF-κB signaling pathway. The report is as follows.

MATERIALS AND METHODS

Laboratory animals

Aminals included in this study were thirty-six clean male adult healthy SD rats, weighted 250±20g, provided by our Laboratory Animal Center and this study was approved by our Animal Ethics Committee. The ethics approval number is 2018-11-17.

Reagents and instruments

Rat Creatine Kinase MB (CK-MB), cardiac troponin (cTn) -I, N terminal pro B type natriuretic peptide (NT-ProBNP), and lactate dehydrogenase (LDH) detection kits were purchased from Wuhan Cusabio Biotech Co., Ltd., Anti-TLR4 and Anti-NF-kB p65 antibodies were purchased from Abcam Shanghai Trading Co., Ltd., internal reference antibody and secondary antibody were purchased from Wuhan Boster Biological Technology Co., Ltd., superoxide dismutase (SOD) activity detection kits, glutathione (GSH) content detection kits and malondialdehyde (MDA) content detection kits were all

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purchased from Nanjing Jiancheng Bioengineering Institute, micro plate readers were purchased from American Berten Instrument Co., Ltd., both electrophoresis system and gel-imaging system were purchased from American Bio-rad, small animal ventilators were purchased from Shanghai Jiapeng Co., Ltd, and visible spectrophotometers were purchased from Shanghai Precision Instruments Co., Ltd.

Animal grouping and modeling

All rats were randomized into three groups by the random number table method, with 12 rats in each group. Rats in the experimental group were pretreated with Dex at 5 μg/kg by intravenous injection with micro-injection pumps, and rats in the injury group and control group were pretreated with 0.9% sodium chloride solution at an equal dose. Models were created in one hour, in which the control group was provided only with threading, without ligation. Rat I/R modeling (Enayati *et al*., 2018): rats were fixed on the laboratory table and anesthetized with Urethane at 1g/kg by intraperitoneal injection. Tracheotomy was conducted to connect the ventilator for mechanical ventilation, the 3-4th ribs were cut off along with the left sternal border and thoracotomy was operated to expose the heart. Ligation for the left anterior descending coronary artery was performed, the ligature was loosened 30 minutes later and reperfusion was provided for 120 minutes. ST-segment depression for 50% and above or soaring T segment in the electrocardiogram (ECG) was set as the criteria of successful modeling. Systemic blood was collected from rats after successful modeling and centrifuged at 3000r/min for 10 minutes. The supernatant was taken for storage.

Detection indexes

Detection of myocardial injury markers

Enzyme-linked immunosorbent assay (ELISA) was used to detect expression levels of myocardial injury markers, such as CK-MB, cTn-I, NT-proBNP and LDH in rat serum. The above procedures should be operated strictly by following the Instructions of ELISA kits.

Oxidative stress detection

The myocardial tissues of rats in each group were collected, crushed by ultrasound and then centrifuged at 1000r/min to obtain the supernatant. Visible spectrophotometry was used to detect SOD activity, GSH, and MDA contents for each group. The above procedures should be operated by following the kits Instructions strictly.

TLR4/NF-κB detection

The myocardial tissues of rats in each group were collected and the Western blot method was applied to detect relative expression of TLR4 and NF-κB protein (Pillai-Kastoori *et al*., 2020). The details were as follows (1) Protein extraction: Myocardial tissue was completely lysed with lysate and centrifuged for 16000×g to extract protein. The protein concentration was determined by the BCA method before boiling in the water bath at 100°C for 5 minutes; (2) Electrophoresis: 13% spacer gel was prepared and electrophoresis buffer was added, followed by the adding of sample and protein markers in the gel wells for electrophoresis at 80 V for 50 minutes. When the protein markers reached separation gel, electrophoresis was converted to 120 V for 2 hours. (3) Transfer: The separation gel was removed, a wet transfer system was assembled by the order of two slices of sponge, two filter papers, separation gel, PVDF membrane, two filter papers and two slices of sponge, and transfer buffered was added for wet transfer at low temperature and 100 V for 100 minutes; (4) Blocking: PVDF membrane was immersed in 5% skimmed milk for blocking, which was placed in an oscillator at room temperature for 2 hours. (5) Membrane washing: target band was cut off by referring to the protein marker, and the membrane was washed with TBST three times. (6) Antibody incubation: Primary antibody and secondary antibody were added successively for incubation and placed overnight in the oscillator at 4°C. (7) Color development: PCDF membrane was processed with ECL luminescence solution, exposed in a dark room for color development, and eventually analyzed with gel imager.

STATISTICAL ANALYSIS

The study data were analyzed with SPSS22.0 software. The measurement data were expressed with ($x \pm s$) and analyzed by an independent-sample t-test. Comparison among groups used one-way analysis of variance, while pair wise comparison applied LSD-t method. The statistical difference was indicated with $P<0.05$.

RESULTS

Comparison of myocardial injury indexes-serum CK MB, cTn-I, NT-proBNP and LDH among the three groups

The CK-MB, cTn-I, NT-proBNP and LDH levels of the experimental group and injury group were significantly higher than those of the control group ($P<0.05$), with lower results in the experimental group than the injury group ($P<0.05$). table 1.

Comparison of oxidative stress indexes-SOD, GSH and MDA among the three groups

The SOD activity of the experimental group and injury group was significantly superior to the control group ($P<0.05$), while the SOD activity of the experimental group was significantly inferior to the injury group ($P<0.05$). The injury group had the lowest level of GSH, followed by the experimental group and the then control group (all $P<0.05$). The highest level of MDA was observed in the injury group, followed by the experimental group, and then the control group (all

P<0.05). Table 2.

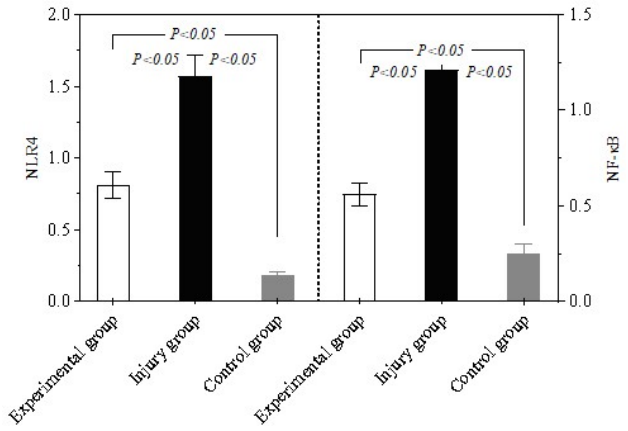

Fig. 1: Comparison of TLR4 and NF-κB expression levels among the three groups

Comparison of TLR4 and NF-κB expression levels among the three groups

The TLR4 expressions of the experimental group, injury group, and control group were 0.81±0.09, 1.57±0.15 and 0.18±0.03 respectively. The highest expression of TLR4 was obtained in the injury group, followed by the experimental group and then the control group (*P*<0.05). NF-κB expression of the experimental group was 0.56±0.06, that of the injury group was 1.21±0.11 and that of the control group was 0.25±0.05. The NF-κB expression of the experimental group and injury group was significantly higher than that of the control group (*P*<0.05), with higher outcomes in the injury group than the experimental group (*P*<0.05), as shown in fig. 1.

DISCUSSION

Cardiovascular disease will increase blood perfusion and oxygen supply to the heart, leading to myocardial ischemia and hypoxia, abnormal myocardial cell metabolism and impaired cardiac function, threatening the life and health of patients. Blood reperfusion is a method of treating myocardial ischemia to restore oxygen and blood supply to the heart. However, it has been reported that (Lianqin *et al*., 2018) after reperfusion, patients may experience more serious damage such as arrhythmia, enlarged myocardial infarction, cardiac decompensation, or even death, which poses a challenge to the clinical management of cardiovascular diseases. Currently, the primary treatments for I/R include administration of drugs for ischemic preconditioning, anti-oxygen free radical drugs, calcium overload inhibitors, VEC protective agent, antioxidants, and traditional Chinese medicines. Dex, as a new sedative and analgesic, was reported to be closely related to oxidative stress and inflammatory response (Tongwei *et al*., 2019).

CK-MB of this type mainly exists in the creatine kinase isoform of myocardial tissue, which catalyzes ATP to produce phosphokinase and ADP. CK-MB starts to elevate at 3-4 hours after acute myocardial infarction and peaks at 12-28 hours, which is highly indicative of myocardial injury (Wenyan *et al*., 2019). NT-proBNP, mainly discharged by ventricular myocytes, is the N terminal fragment without activity after the division of BNP prohormone, with its level being more stable in the body than BNP. But its elevation is mainly related to endotoxin and inflammatory factors, myocardial depressant factor, and circulating blood volume, which can reflect the myocardial dysfunction. It is one of the myocardial injury markers in clinical detection (Longzhu *et al*., 2020). cTn-I is a protein that can regulate calcium-mediated interaction between actin and myosin, mainly distributed in the myocardium. It starts to climb at 3-6

Table 1: Comparison of myocardial injury indexes-serum CK MB, cTn-I, NT-proBNP and LDH among the three groups

Group	n	CK-MB (U/L)	cTn-I(pg/ml)	NT-proBNP (pg/ml)	LDH(U/L)
Experimental group	12	1091.44±189.47^{ab}	61.41±5.47^{ab}	180.46±20.48^{ab}	2091.43±259.49^{ab}
Injury group	12	1594.56±258.29^{a}	74.56±6.38^{a}	274.55±28.28^{a}	2794.58±308.35^{a}
Control group	12	601.51±152.47	47.47±4.42	103.53±12.45	1421.53±182.47
F value		70.522	73.276	192.196	86.714
P value		<0.001	<0.001	<0.001	<0.001

Table 2: Comparison of oxidative stress indexes-SOD, GSH and MDA among the three groups

Group	n	SOD(U/mgprot)	GSH (nmol/mL)	MDA (nmol/mL)
Experimental group	12	64.37±8.49^{ab}	108.46±29.51^{ab}	40.57±5.28^{ab}
Injury group	12	81.47±10.46^{a}	69.43±19.44^{a}	54.54±7.45^{a}
Control group	12	48.59±6.29	194.61±38.34	26.41±3.53
F value		44.039	54.324	74.308
P value		<0.001	<0.001	<0.001

Note: A as compared with control group, *P*<0.05; b As compared with injury group, *P*<0.05.

hours after the onset of acute myocardial infarction and reaches a peak at 14-20 hours. cTn-I is one of the diagnostic markers of high specificity of myocardial injury (Zejiang *et al*., 2019). LDH is a type of NAD-dependent kinase with multiple isoforms, extensively existing in kidneys, myocardium, and skeletal muscles. It begins to increase at 6-9 hours after the onset of myocardial infarction and reaches a peak at 36-60 hours. It shows great potential as an ancillary diagnostic criterion for acute myocardial infarction at a later stage (Lingzhi *et al*., 2018). Study results showed that CK-MB, cTn-I, NT-proBNP, and LDH levels of the injury group after I/R were significantly increased, while those of experimental group administered Dex were significantly lowered, indicating that I/R caused severe injury to myocardial tissue and that Dex can protect myocardial tissue from myocardial injury.

Oxygen radicals and unsaturated fatty acids on cell membranes can peroxidize membrane lipids, further causing cardiomyocyte damage, producing lipid peroxides that disrupt the integrity of cell membranes, mitochondrial membranes, and lysosomal membranes and increase membrane fluidity and permeability (Wei and Jinqiao, 2019). SOD can be converted to oxygen and hydrogen peroxide by catalyzing superoxide through disproportionated reaction. It is an important antioxidant in animals, plants and microorganisms that plays a role in anti-aging, inhibition of cardiovascular diseases, immune system disorders, chronic diseases and anti-fatigue. Excessive oxide in the body may result in the activation and release of SOD which can serve as an oxidative stress response index (Jia *et al*., 2018). GSH is an active tripeptide substance distributed in the organs and tissues of human body. As an important reducing agent in cells, GSH protects important cellular components from damage by reactive oxygen substances and heavy metals, and binds to form glutathione disulfide, which is reduced to GSH by glutathione reductase to regulate and maintain redox homeostasis and participate in the regulation of proliferation, differentiation and apoptosis in a variety of cellular and other life activities. The presence of large amounts of oxygen free radicals in the body may lead to a significant decrease in GSH levels (Lan *et al*., 2020). MDA is a major metabolite of oxygen free radicals in the body and its content directly reflects the per oxidation rate and intensity of histocytes, which shows its potential to be an important marker of oxidative stress injury (Xue and Yazhe, 2018). The study result demonstrated that SOD activity in myocardial cells after I/R was enhanced, GSH content was reduced and MDA content was elevated, while the activity of experimental group given Dex was significantly decreased, GSH content was significantly increased, and MDA content was obviously dropped, suggesting that I/R may produce large amount of oxygen free radicals to enhance the oxidative stress response for myocardial cells. However, Dex can relieve such oxidative stress to protect the myocardial cells from injury.

During the I/R process, ischemic myocardial cells may release large amount of damage associated molecular patterns (DAMPs) to involve in immune response and TLR4 and NF-κB signals are activated by a cascade of reaction to promote release of downstream inflammatory factors, further inducing inflammatory response and myocardial tissue injury (Guo *et al*., 2016; Yafen and Jun, 2018). The study result demonstrated that after I/R in the injury group, TLR4 and NF-κB levels were obviously elevated, while those of the experimental group treated with Dex were obviously reduced, indicating that Dex was the negative regulator in TLR4/NF-κB pathway, which is conducive to relieving inflammatory response induced by I/R and reducing myocardial tissue injury.

CONCLUSION

Dex can effectively reduce I/R induced myocardial injury and expressions of TLR4 and NF-κB, negatively regulate the signaling pathway, relieve oxidative stress response, contribute to the recovery of myocardial function and protect myocardium. We should focus on the study of rational dose of Dex in the future, as low-dose cannot achieve efficacy, but high-dose may induce adverse reactions.

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