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Protective effect and mechanism of lithium chloride pretreatment on myocardial ischemia-reperfusion injury in rats

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ABSTRACT

Objective: To investigate the protective effect and mechanism of lithium chloride pretreatment on myocardial ischemia-reperfusion injury (I-RI) in rats. **Methods:** A total of 60 SD rats were randomly divided into control group, model group, lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group with 15 in each. The I-RI model was established in model group, the lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group by method of seaming along left anterior descending coronary artery myocardial, control group was only opened the chest without seaming, ST-elevation within 2 min was regarded as modeling success. Model group did not adopted any intervention, lithium chloride intervention group was treated with lithium chloride injection 15 mg/kg by jugular venipuncture preoperatively, L-arginine methyl ester + lithium chloride intervention group was treated with intraperitoneal injection of 30 $mg \cdot kg^{-1} \cdot d^{-1}$ L-arginine methyl ester 7 d before the test, and intravenous catheter of 15 mg/kg lithium chloride preoperatively. The hydroxybutyric acid dehydrogenase (HBDH), creatine kinase isoenzyme (CK-MB), superoxide dismutase (SOD), malondialdehyde (MDA) level and nitric oxide synthase (NOS) activites were tested. Each large area of myocardial ischemia tissue was extracted for determination of the MDA content, SOD activity in tissue and serum, and morphological changes of myocardial tissue. **Results:** SOD activity was highest in lithium chloride intervention group, followed by L-arginine methyl ester + lithium chloride intervention group, control group and model group ($P<0.05$); SOD activity was highest in L-arginine methyl ester + lithium chloride intervention group intervention group, followed by lithium chloride intervention group, control group and model group ($P<0.05$). MDA content of myocardial tissue was highest in model group, followed by L-arginine methyl ester + lithium chloride intervention group, the lithium chloride intervention group and control group ($P<0.05$); serum MDA content was highest in L-arginine methyl ester +lithium chloride intervention group, followed by model group, lithium chloride intervention group and control group ($P<0.05$). Compared with the control group, serum NOS was significantly higher in model group, lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group ($P<0.05$), there was no statistical difference of serum NOS activity between the three groups ($P>0.05$); HBDH and CK-MB of plasma were highest in model group, followed by L-arginine methyl ester + lithium chloride intervention group, lithium chloride intervention group and control group ($P<0.05$). A significantly lighter myocardial damage was observed microscopically in lithium chloride intervention group than that in L-arginine methyl ester + lithium chloride intervention group and model group. **Conclusions:** lithium chloride pretreatment can significantly reduce the myocardial I-RI, maintain structure and function of myocardial cells, improve the antioxidant ability of myocardial tissue, play an effective role on protecting myocardial I-RI.

1. Introduction

Ischemia-reperfusion injury (I-RI) refers to the pathological process of cell structure and metabolism impairment aggravating induced by reentry of the blood supply to the tissues after a period time of ischemia[1]. As the development of heart transplant surgery, the prevention and cure of myocardial I-RI has become the hot topic in clinical study[2]. According to WHO statistics[3], acute obstructive coronary artery disease is becoming the major cause of deaths worldwide, in the process of treatment, the existing operation and drug treatment of coronary artery

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recanalization can all cause I-RI[3]. With the deepening of clinical research on I-RI and the development of myocardial protection drugs, drug pretreatment has become the main method of myocardial protection. Lithium is widely existed in the body's bones, muscles and major organs, is one of the trace elements in the human body, is also the potential of the myocardial protective drugs. Studies have confirmed that[4], after a period of time, lithium chloride pretreatment can reduce nerve - mental symptoms of I-RI recovery phase in rats. Another study showed[5-7], lithium chloride has antiapoptotic effect on myocardial reperfusion, which can effectively reduce myocardial I-RI. To observe the protective effects and mechanism of lithium chloride on rat myocardial I-RI, the author selected SD rats for myocardial I-RI modeling after using lithium chloride pretreatment.

2. Materials and methods

2.1. Experimental animals

A total of 60 healthy adult SD rats were selected, weighting 250-300 g, male and female unlimited. They were purchased from Nanjing University Laboratory Animal Center, and provided with free food and water.

2.2. Instrument and reagent

Small animal breathing machine, electrocardiograph (Nanjing Yu's Biotechnology Company); MICROM HM325 tissue slicing machine (Zeiz Company, Germany); Spectrophotometric instrument (Olympus Company, Japan); BH-2 optical microscope (Olympus Company, Japan); MDA determination kit (MDA2003), SOD determination kit (GMS50002.1), anhydrous lithium chloride, L-arginine methyl ester, heparin injection, 2% pentobarbital sodium, bought from Nanjing Institute of Biological Engineering.

2.3. model establishMENT

All rats had intraperitoneal injection of 2% sodium pentobarbital for anesthesia, followed by endotracheal intubation assisted mechanical ventilation. Indwelling tube was placed in the jugular vein. Skin incision was performed along the left collarbone midline between 4th rib, and muscle blunt separation was carried out. Pericardium was cut after thoracotomy; the left anterior descending coronary artery was along the joint of left atrium and pulmonary arterial cone using 6-0 prolene string. String was cut down after 30 min of artery blocking, blood supply was restored, closed chest, and appearance of ST-elevation within 2 min was regarded as modeling success.

2.4. Animal grouping and pretreatment

A total of 60 SD rats were randomly divided into control group, model group, lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group with 15 in each. The I-RI model was set up in model group, the lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group by method of seaming along left anterior descending coronary artery myocardial. Rats in control group was only opened the chest without seaming, ST-elevation within 2 min was regarded as modeling success. Model group did not adopted any intervention, lithium chloride intervention group was treated with lithium chloride injection 15 mg/kg by jugular venipuncture preoperatively, L-arginine methyl ester + lithium chloride intervention group was treated with intraperitoneal injection of 30 $mg \cdot kg^{-1} \cdot d^{-1}$ L-arginine methyl ester 7 d before the test, and intravenous catheter of 15 mg/kg lithium chloride preoperatively. After 240 min of reperfusion, venous blood was extracted and centrifuged, the serum was saved in freeze. After sacrificing the rats, ischemic ventricular wall was sheared,for REDOX index and morphological observation.

2.5. Biochemical index determination

The hydroxybutyric acid dehydrogenase (HBDH), creatine kinase isoenzyme (CK-MB), superoxide dismutase (SOD), malondialdehyde (MDA) level and nitric oxide synthase (NOS) activites were detected.

2.6. Tissue morphology observation

0.3 cm×0.3 cm×0.3 cm size of ischemic myocardial tissue was processed under conventional fixation, embedding, sectioning and HE staining, to observe the histomorphology changes of the damaged myocardium.

2.7. Statistical analysis

Data were analyzed using SPSS19.0 statistical software, and expressed as mean±sd value. Comparison between groups was analyzed using single factor analysis of variance with q inspection, qualitative data was analyzed with χ^2 test, and $P<0.05$ was regarded as significant difference.

3. Results

3.1. Changes of SOD activity and MDA level of myocardium and serum

SOD activity of myocardium was highest in lithium chloride intervention group, followed by L-arginine methyl ester + lithium chloride intervention group, control group and model group ($P<0.05$); serum SOD activity was highest in L-arginine methyl ester + lithium chloride intervention group, followed by lithium chloride intervention group, control group and model group ($P<0.05$). MDA content of myocardial tissue was highest in model group, followed by L-arginine methyl ester + lithium chloride intervention group, the lithium chloride intervention group and control group ($P<0.05$); serum MDA content was highest in L-arginine methyl ester +lithium chloride intervention group, followed by model group, lithium chloride intervention group and control group ($P<0.05$), as shown in Table 1.

3.2. NOS, CK-MB and HBDH level

Compared with the control group, serum NOS was significantly higher in model group, lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group ($P<0.05$), there was no statistical difference of NOS activity between the three groups ($P>0.05$); HBDH and CK-MB of plasma were highest in model group, followed by L-arginine methyl ester + lithium chloride intervention group, lithium chloride intervention group and control group ($P<0.05$), as shown in Table 2.

3.3. Tissue morphology observation

Control group showed no pathological changes of myocardial tissue; myocardial tissue of model group, lithium chloride intervention group and L-arginine methyl ester + lithium chloride intervention group was microscopically showed with different degrees of pathological damage; Irregular cell nuclei, hyperchromatic cytoplasm, inflammatory cells infiltration and myocardial fibrosis were observed in model group. Some irregular cell nucleus and fibrous degeneration were observed in Lithium chloride intervention group, the hyperchromatic cytoplasm was not obvious, inflammatory cells infiltration degree was slighter than that of model group. The myocardial damage degree of L-arginine methyl ester + lithium chloride intervention group were between model group and lithium chloride intervention group, the results are shown in Figure 1.

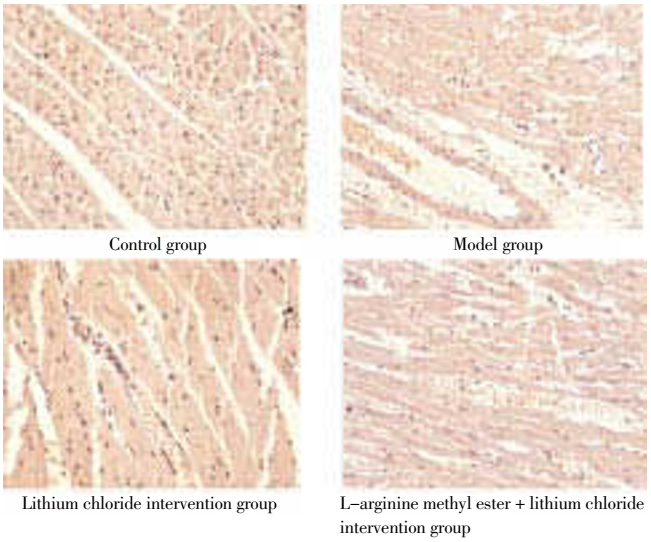

Figure 1. Tissue morphology observation (HE×200).

4. Discussion

Myocardial I-RI refers to the pathological phenomena of aggravating myocardial tissue damage degree induced by blood supply restore after blood supply of myocardial tissue ischemia for a period of time. Damage of schemic myocardium after restore blood flow can be aggravated on the ultrastructure, function and the electrophysiological

Table 1
Changes of SOD activity and MDA level in serum and myocardium.

Groups	SOD		MDA	
	Myocardium (U/mg protein)	Serum (U/mL)	Myocardium (U/mg protein)	Serum (U/mL)
Control group	72.10±10.34	188.27±26.92	0.86±0.17	4.15±1.34
Model group	67.25±5.36*	173.92±23.03*	2.05±0.61*	4.62±1.62*
Lithium chloride intervention group	85.51±15.92*#	205.37±21.25*#	1.31±0.23*#	3.68±0.64*#
L-arginine methyl ester + lithium chloride intervention group	78.89±8.44*#△	240.99±31.80*#△	1.70±0.35*#△	5.36±0.65*#△

Note: *$P<0.05$ compared with control group; #$P<0.05$ compared with model group; △$P<0.05$ compared with lithium chloride intervention group.

Table 2
NOS, CK-MB and HBDH level.

Groups	NOS (U/mL)	CK-MB (U/L)	HBDH (U/L)
Control group	55.96±8.85	695.31±143.59	111.34±52.71
Model group	62.16±12.88*	3 922.77±2109.88*	677.80±204.35*
Lithium chloride intervention group	63.64±8.03*	1 909.80±677.48*#	443.44±142.61*#
L-arginine methyl ester + lithium chloride intervention group	63.45±13.06*	2 228.32±614.84*#△	470.54±208.28*#△

Note: *$P<0.05$ compared with control group; #$P<0.05$ compared with model group; △$P<0.05$ compared with lithium chloride intervention group.

change, which resulted in the pathological basis of ischemia-reperfusion injury[8]. Clinical symptoms of myocardial I-RI are often characterized by abnormal changes of reperfusion myocardial enzyme, arrhythmia and myocardial ultrastructure[9-12]. Studies have shown that[13], ischemic heart disease perioperative cardiac events is the important cause of perioperative death. Therefore, to prevent or alleviate perioperative I-RI is of great significance in the process of treatment.

Mechanism of myocardial I - RI is not fully clear. Studies have shown that[14-16], myocardial energy metabolism disorder, calcium overload and free radical, neutrophils and apoptosis may participate in myocardial I-RI. Preconditioning refers to adding damage to tissues and organs in advance to stimulate adaptation and tolerance of the body tissues against harmful events[17]. Studies have shown that[18-20], repeatedly, transient myocardial ischemia-reperfusion can reduce myocardial cell damage due to long ischemia-reperfusion. According to the theory of the ischemic preconditioning protection mechanism, clinical used drugs as alternative ischemic stimulus in clinical application, in order to make the body produce medicine rational preadaptation, which is easy to control. Clinical use of inorganic lithium ion is more focused on the treatment of mental symptoms, recent studies have shown that[21], lithium salts can serve as an antioxidant to against ischemic anoxia. Other scholars found[22-24], lithium chloride pretreatment can reduce ischemia-reperfusion damage of neurons in rats, and can reduce the cerebral infarction volume. Therefore, the author carried on the study of lithium chloride pretreatment on myocardial I-RI.

In cardiac surgery, filling and began, with the oxygen molecules after myocardial ischemia can produce a large number of oxygen free radical, affecting lipid peroxidation of myocardial cell membrane, cell membrane and mitochondrial membrane, and increase membrane permeability, causing membrane dysfunction such as membrane receptors, ion channels dysfunction[16]. Studies have confirmed that[22,23], inhibition of oxygen free radicals, can reduce the I-RI. SOD is important metal antioxidant enzymes in the body by damaging free radicals and preventing the toxic effects of oxygen, so SOD activity can indirectly reflect the clearance ability of oxygen free radicals. MDA is the final production of lipid peroxides, its content changes can indirectly reflect the damage severity of tissue cells by free radicals, and can reflect the lipid peroxidation degree. Therefore, these two indicators can reflect oxygen free radical scavenging ability and damage severity of tissue cells attack by free radicals.

This study showed that compared with model group, lithium chloride intervention group had higher level of serum SOD and NOS activity ($P<0.05$), lower plasma HBDH, MDA and CK-MB content ($P<0.05$), suggesting the lithium chloride pretreatment can reduce the attacks of free radicals on cell membrane lipid peroxidation, directly decrease the mitochondrial membrane damage, protect myocardial I-RI. In this study, the degree of myocardial damage in the lithium chloride intervention group rat model group and L-arginine methyl ester + lithium chloride intervention group were significantly reduced, also confirmed that the lithium chloride pretreatment can reduce myocardial I-RI effectively.

This study showed that the drug preconditioning of lithium chloride can strengthen the antioxidant capacity of myocardial tissue, reduce myocardial I-RI injury, and play an effective role in protecting myocardial I-RI.

Conflict of interest statement

We declare that we have no conflict of interest.

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