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Effects of different doses of Savda Munziq on myocardial ischemia-reperfusion injury in rats with abnormal Savda syndrome

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ABSTRACT. To investigate the effects of different doses of abnormal Savda Munziq on myocardial ischemia-reperfusion injury (MI/RI) in rats with the abnormal Savda syndrome, 50 abnormal Savda animal models were randomly divided into a control group, a model group, a high-dose group, a middle-dose group, and a low-dose group, with each group containing 10 rats. The enzyme-linked immunosorbent assay was used to detect the serum myocardial enzyme and troponin levels, and hematoxylin and eosin (HE) staining was used to observe changes of the myocardial tissues in the different groups. Results showed that in the Munziq intervention groups, the serum creatine kinase and troponin levels were significantly lower than those in the model group, and the middle-dose group showed the lowest levels. The HE staining of myocardial tissue showed that the myocardial edema and muscle fiber proliferation levels were significantly higher in the

Munziq intervention groups than in the model group, and the middle-dose group showed the least cardiac tissue damage. Therefore, intervention with an intermediate Munziq dose could significantly reduce MI/RI in rats with abnormal Savda syndrome.

Key words: Munziq; Abnormal Savda syndrome; Rats; Ischemia-reperfusion injury

INTRODUCTION

Myocardial ischemia/reperfusion injury (MI/RI) occurs when the myocardial blood supply is interrupted for a short time, and restoration of the blood supply causes damage to the original ischemic myocardium that is more severe than the pre-restoration state (Schulze et al., 2007). The MI/RI mechanisms have not yet been completely elucidated, but a number of factors are known to initiate or aggravate the injury after reperfusion. Studies have shown that oxygen free radicals, calcium overload, energy metabolism disorders of myocardial fibers, vascular endothelial cells, nitric oxide, heat shock proteins, neutrophils, apoptosis, and complement participation (Lum and Roebuck, 2001; Mohl et al., 2008; Wagner et al., 2010) may all be involved in the pathogenesis of MI/RI (Hoffman et al., 2004; Ruiz-Ginés et al., 2000).

Uygur humoral medicine theory considers that when a variety of adverse factors (such as poor environment, diet, lifestyle, and psychological factors) affect the human body for a long time *in vitro* and *in vivo*, one or several Savda substances, phlegmatic substances, blood substances, and bile substances that are necessary to maintain the body's normal physiological functions will be burning. This would lead to the generation of pathological products with different features, namely abnormal Savda syndrome, abnormal phlegmatic status, abnormal blood status, and abnormal bile status. The formation of such pathological products can, on the one hand, lead to the imbalance of body fluids that were originally balanced, and on the other hand, lead to changes in the nutrients and properties contained in the body fluids, resulting in abnormal function of the transport and metabolism of nutrients contained in fluids. Consequently, the nutrients are not easily absorbed by the cells, tissues, and organs or the absorption of nutrients is reduced. The body might absorb the nutrients with abnormal properties and qualities, which may lead to property changes of cells, tissues, and organs, and inhibition of the normal physiological function of blood vessel wall tissues and cells. Simultaneously, because of the weakening of the forces caused by the abnormal body fluids (absorbing, holding, driving, and excreting forces) and the abnormal humoral physical changes (heavier weight, temperature reduction, or thickened viscous), the fluid flow rate slows down, precipitation can be easily produced in the vessel walls, the vessel wall can thicken, and its flexibility can become reduced, resulting in vascular stenosis. The vessel wall precipitates can also serve as a new source of stimulation to continue stimulating blood vessel walls. This would increase the inhibition of vascular wall tissues and cellular functions leading to atherosclerosis, hypertension, coronary heart disease, and other cardiovascular diseases (Upur, 2008). Most diseases treated with cardiac surgery present abnormal Savda symptoms. Therefore, in this study, an MI/RI injury model was established based on abnormal Savda substances to

better achieve better myocardial protection. We also explored the nature and pathogenesis of this mechanism to better understand the role of the traditional medicine Munziq in the prevention, treatment, and intervention of abnormal Savda qualitative heart disease.

Our previous studies showed that the traditional Uygur medicine abnormal Savda Munziq, which is heading toward a modernized formula, showed good resistance to oxidation (Kizaibek et al., 2012) and had protective effects against oxidative DNA damage (Yusup et al., 2011). Experiments showed that the abnormal Savda Munziq not only had protective effects on the lipid peroxidation damage caused by free radicals, but also improved mitochondrial sodium oxide dismutase (SOD) and Ca^{2+}-Mg^{2+}-ATPase activities, and maintained the structural integrity of the mitochondrial membrane (Yusup et al., 2004). It could also play a role at the subcellular level in the prevention and treatment of cancer, asthma, diabetes, hypertension, coronary heart disease, and other intractable diseases (Yusup et al., 2012).

In this study, we established abnormal Savda substances myocardial ischemia and reperfusion models in rats and used abnormal Savda Munziq intervention to explore the mechanisms of MI/RI.

MATERIAL AND METHODS

Model establishment

The animal MI/RI models with abnormal Savda substance carriers were established according to previously described methods (Upur, 2008).

Study subjects

The experimental animals were provided by the Experimental Animal Center of the First Affiliated Hospital of Xinjiang Medical University. A total of 50 rats were included regardless of sex.

The 50 abnormal Savda animal models were randomly divided into a control group, a model group, a high-dose group, a middle-dose group, and a low-dose group, with each group containing 10 rats.

Experimental methods

One hour before the experiment on each day, 2.53 g/kg abnormal Savda Munziq was intragastrically administered to the low-dose group (2.5 g Munziq dissolved in 1 mL distilled water, at an aquatic dose of 8.58 g/kg), which was equivalent to the effective clinical dose. The middle dose was 5.069 g/kg (5.0 g Munziq dissolved in 1 mL distilled water at a raw dose of 17.16 g/kg), which was equivalent to double the effective clinical dose. The high dose was 10.12 g/kg (10 g abnormal Savda Munziq dissolved in 1 mL distilled water at a raw dose of 34.32 g/kg), which was equivalent to 5 times the effective clinical dose. For every 100 g, rats were given 1.5 mL Munziq solution. After 21 days of administration, the general biological characteristics of experimental animals were observed, such as body temperature, body weight, food intake, water intake, tongue, and the nature and shape of urine and stool.

To establish the ischemia-reperfusion model, the rats were intraperitoneally anes-

thetized with 30 g/L 30 mg/kg sodium pentobarbital, and then they were fixed on the small animal operating table in supine position and their limbs were subcutaneously punctured and connected to an electrocardiogram (ECG) machine. After 5 min, a normal ECG was recorded (100 mm/s paper speed, 20 mm/mV voltage) as the control. A tracheostomy was performed for ventilator-assisted breathing. The ventilator parameters were as follows: 50 beats/min respiratory rate, 20 mL/kg tidal volume, and 1:1 breathing ratio (Huang et al., 2007). The 3rd and 4th ribs were cut, the chest wall was opened with a wire retractor, a pair of lines was inserted through both sides of the chest wall muscle, and the small circle was left inside. The pericardium was carefully cut, the heart was exposed, and a cotton swab was lightly placed on the heart. The left coronary vein was taken as the marker, and a 520 non-invasive suture needle was inserted 3 to 4 mm of the lower edge of the left atrial appendage at a 115 mm approximate depth. The needle was pulled out obliquely in the top right of the pulmonary cone direction, and the stitch length was 3 to 4 mm. A fluted latex tube with a 115 mm inner diameter and a 0.15 cm length was set for the standby, and both ends of the line were pulled through the small circle, respectively. Ten minutes after steady breathing, the line was tightened and ligated with a latex tube. The latex tube was compressed with the left anterior descending coronary artery. The multi-lead ECG was recorded throughout the experiment. After 30 min of coronary artery ligation, the ligature was released to restore blood perfusion.

Detection indicators and methods

Five milliliters blood was obtained from the right atrium at the end of the perfusion. The blood was then injected into a test tube to be solidified, and centrifuged at 3000 rpm to separate the serum and test for aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB. AST was tested according to the kinetic method of the International Federation of Clinical Chemistry (IFCC). LDH was tested by using the IFCC recommended method. CK was tested with the N-acetylcysteine method, and CK-MB was tested with the immunosuppression method; all protocols were conducted in strict accordance with kit instructions.

Statistical methods

The sample content was based on the following data sample formula: $N = \frac{(Z_\alpha + Z_\beta)^2 \sigma^2}{\delta^2}(Q_1^{-1} - Q_2^{-1})$ ($\alpha = 0.05$, $\beta = 0.20$, $Q_1 = Q_2 = 0.5$). Based on previous studies, we determined that at least six samples were needed for each group sample size. All experimental data were entered into the computer, and the SPSS 11.5 statistical software was used for processing and analysis. Test results, such as water diet, urine output, and the amount of stool, are reported as means ± standard deviation. Univariate and multivariate analysis of variance were used, and body temperature and body weight were compared with variance analysis of repeated measurement data. The categorical data are reported as constituent ratios to describe their basic features. Myocardial function indicators were used for variance analysis of multiple mean comparisons. Differences among groups were compared with analysis of variance. Continuous data were compared using the χ^2 test. The test level was set to $\alpha = 0.05$.

RESULTS

Comparison of myocardial enzymes and troponin levels in different Munziq intervention groups

As shown in Table 1, the serum CK and troponin levels in the model group and the Munziq low-dose, middle-dose, and high-dose groups were significantly higher than that of the control group ($P < 0.05$), indicating that the modeling was successful.

Table 1. Comparison of serum creatine kinase and troponin levels in different groups.

Group	Cases	CK-MB (U/L)	Troponin T (ng/mL)
Control	10	70.2 ± 3.2	17.7 ± 0.8
Model	10	100.9 ± 2.7*	28.6 ± 0.8*
Low dose	10	92.3 ± 1.8*	23.7 ± 0.8*
Middle dose	10	80.6 ± 2.9*#	21.2 ± 1.2*#
High dose	10	89.8 ± 2.5*	22.3 ± 0.9*

Compared to the controls, *$P < 0.05$; compared to the low-dose group and high-dose group, #$P < 0.05$.

The serum CK and troponin levels in the Munziq low-dose, middle-dose, and high-dose groups were significantly lower compared with that of the model group ($P < 0.05$, $P < 0.01$).

Myocardial hematoxylin and eosin staining and structural changes in different groups

As shown in Figure 1, there was no significant edema and degeneration in myocardial cells of the normal control group, the abnormal Savda syndromes model group, or any of the abnormal Savda Munziq dose groups. Proliferation of myocardial fibers was not obvious in the normal control group or middle-dose group, and the myocardial interstitial blood vessels showed minimal dilation. The cardiac muscle fibers showed mild proliferation in the model, low-dose, and high-dose groups. The myocardial interstitial vascular reactivity was moderate in the model and the low-dose groups, but the reactivity was mild in the middle-dose group.

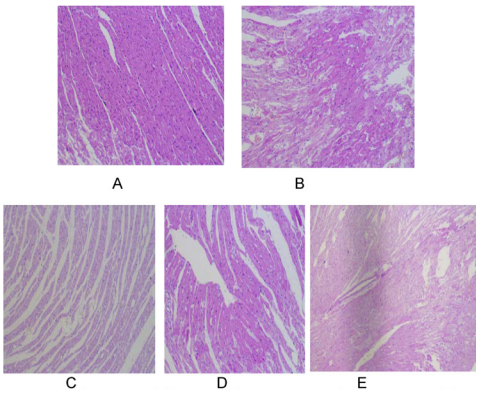

Figure 1. Hematoxylin and eosin staining myocardial structural changes. **A.** Control group; **B.** model group; **C.** low-dose-group; **D.** middle-dose group; **E.** high-dose group.

DISCUSSION

In the present study, we found that different Munziq doses had significant impacts on the MI/RI outcome of abnormal Savda substance rats. The Munziq intervention could significantly reduce MI/RI, and the best dose was the middle dose.

Abnormal Savda syndrome is a complex disease, and is the main contributor to diseases such as cancer, diabetes, hypertension, and coronary heart disease. A variety of factors can cause abnormal Savda syndrome with diet, environment, and psychological factors playing the most important roles. The long-term consumption of dry and cold properties of food and drugs, long-term residence in a dry and cold environment, exposure to cold air and serious environmental pollutants, and excessive anxiety, anger, and panic can all weaken the function of dominant organs such as the brain, liver, and heart, and affect the generation of the humoral system and the adjustment process. When the main intake ability of body organizations becomes weakened, heat is easily distributed, and the heat loss can make the body, tissues, and organs dry and cold. This results in an increase in human body Savda fluids and a change in their functions, eventually leading to abnormal Savda syndrome. In this study, on the basis of abnormal Savda animal models, we established MI/RI animal models with abnormal Savda substances. We found that different doses of Munziq intervention in the models could significantly reduce ischemia-reperfusion injury.

In this experiment, we focused on observations of cardiac markers and changes in serum CK and troponin. We found that CK-MB and troponin levels in the model group were significantly higher than those in the control group were. The use of different doses of Munziq intervention showed that regardless of dose, any Munziq intervention could significantly reduce MI/RI, with the most obvious effects observed in the middle-dose group. Histological and morphological observations showed no obvious proliferation of myocardial fibers in the normal control and middle-dose groups, and the myocardial interstitial blood vessels showed minimal dilation. The cardiac muscle fibers showed mild proliferation in the model group, low-dose group, and high-dose group, which was consistent with the observed changes in the myocardial enzymes.

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