The Protective Effect of Salivae Miltiorrhizeae Liguspyragine Hydrochloride and Glucose Injection on Isoproterenol-Induced Acute Myocardial Infarction in Rats

ABSTRACT

Salivae miltiorrhiza liguspyragine hydrochloride and glucose injection (SGI) are widely used in the clinical treatment of ischemic cerebrovascular diseases, but researches on the prevention and treatment of acute myocardial infarction (AMI) and other cardiovascular diseases are rarely reported. So, the purpose of this study is to evaluate preventive effect of SGI on AMI in rats and to explore its possible mechanism. In this study, isoproterenol- (ISO) induced AMI model in rats was established. Based on that, we studied the effect of SGI on ECG and cardiac function. We then investigated the effect of SGI on heart infarction area and heart histomorphology in AMI rats. Moreover, to explore the possible mechanisms, we tested the activities of myocardial enzymes in blood. Our study found that, SGI can improve the ECG of AMI rats and promote cardiac function to normal. In addition, SGI can reduce the infarct size and inhibit myocardial injury. Moreover, SGI can reduce the content of serum CK, LDH, cTnl and BNP in AMI rats. Therefore, we confirmed that SGI possessed remarkably protective effects against ISO-induced AMI in rats.

KEYWORDS salivae miltiorrhiza, liguspyragine hydrochloride, acute myocardial infarction, protective effect, isoproterenol, cardiac function

INTRODUCTION

Cardiovascular disease (CVD) has become one of the most harmful human diseases with highest morbidity in the world, including coronary heart disease, angina, myocardial infarction (MI), hypertension, etc. About 17 million people die each year because of coronary heart disease around the world. The incidence of CVD is expected to continue to rise. It is expected that worldwide CVD-related deaths are projected to climb up to 23.3 million by 20301,2. So far, a lot of researches on the prevention and treatment of CVD have been done at home and abroad. Although we have a more profound understanding of the pathogenesis and risk factors of these diseases, many progresses have also been made in the diagnosis, but still limited to CVD reversal therapy. Therefore, the prevention and treatment of CVD is still a hot and difficult issue for medical and scientific researchers around the world.

The causes of acute myocardial infarction (AMI) mainly are coronary artery diseases and sudden blockage of arteries, which leads to a drastic reduction or interruption, serious and persistent myocardial ischaemia, and local myocardial necrosis. AMI is a serious hazard to human health and it is also a kind of disease that will produce a variety of serious consequences, including organ damage, other diseases and even death, if not timely treated3. At present, the treatment methods of MI mainly include general treatment, drug treatment and interventional therapy. Although having some advantages, these methods have deficiencies on clinical practice. Some commonly used drugs can bring down the mortality rate of MI significantly, if they are taken in the long-term, but that can cause a number of serious adverse reactions, including secondary re-infarction and heart failure, and lead...
patients to disability or death. For example, antiplatelet drugs, β-receptor blockers, ACEI, lipid-lowering drugs. Although interventional therapy can be faster and more directly to save dying myocardium, its widespread use is limited due to the operation conditions, high cost and some postoperative serious adverse reactions.

Salvia miltiorrhiza liguspyragine hydrochloride and glucose injection (SGI) is a chemical injection, which is originated from Salvia miltiorrhiza Bge and Ligusticum chuanxiong Hort., mainly contain salvia miltiorrhiziae and liguspyragine hydrochloride. It was approved by the CFDA in 2003 and listed in drug Standard of Ministry of Public Health of the Peoples in China. Because of its significant effect without obvious side effect, SGI is widely used in treatment of the occlusion of cerebrovascular disease and ischemic vascular diseases. However, its detailed therapeutic effects and mechanisms against AMI still lack complete research. Therefore, this study adopted isoproterenol (ISO) to establish AMI models in rats, and investigated the protective effects and possible mechanisms of SGI against ISO-induced AMI.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats 72, weight of 150–220 g, were provided by Guangdong Medical Laboratory Animal Center (certificate no.: SCXK (Guangdong) 2013-0002). The rats were maintained at 24°C, 65% humidity, and 12 h light/dark cycle with free access to standard laboratory rat chow and tap water in Laboratory Animal Management Center of Medical School of Jinan University (certificate no. SCXK (Guangdong) 2012-0117). Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of Jinan University according to the internationally accepted principles.

Reagents

SGI (approval no.: H52010703, Guizhou Jingfeng Injection Co., Ltd., Guangzhou, China), panax notoginseng saponins (PSN, lot no.: 13122816, Guangxi Wuzhou Pharmaceutical Co., Ltd., Guangxi, China), tetrazolium chloride (TTC, lot no.: CBC7466, Sigma-Aldrich Co., Ltd., St. Louis, USA), ELISA kits for cTnI (lot no.: A21019886) and BNP (lot no.: F10019543) are both purchased from CUSABIO Biotech Co., Ltd., Wuhan, China. ISO hydrochloride (lot no.: E140513, Hunan Lunke Ruji Biology Technology Co., Ltd., China), 5% glucose injection (SGI) with different concentration, which is originated from Salvia miltiorrhiza Bge and Ligusticum chuanxiong Hort., mainly contain salvia miltiorrhiziae and liguspyragine hydrochloride. It was approved by the CFDA in 2003 and listed in drug Standard of Ministry of Public Health of the Peoples in China. Because of its significant effect without obvious side effect, SGI is widely used in treatment of the occlusion of cerebrovascular disease and ischemic vascular diseases. However, its detailed therapeutic effects and mechanisms against AMI still lack complete research. Therefore, this study adopted isoproterenol (ISO) to establish AMI models in rats, and investigated the protective effects and possible mechanisms of SGI against ISO-induced AMI.

Animal models

The rats were randomly divided into the following six groups: control (vehicle), model (vehicle), PSN (22.30 mg/kg), SGI (L) (low-dose, salvia miltiorrhiza 0.71 mg/kg and liguspyragine hydrochloride 3.57 mg/kg), SGI (M) (medium-dose, salvia miltiorrhiza 3.57 mg/kg and liguspyragine hydrochloride 17.85 mg/kg) and SGI (H) (high-dose, salvia miltiorrhiza 17.85 mg/kg and liguspyragine hydrochloride 89.25 mg/kg). Each group had 12 rats; all drugs were dissolved in the vehicle (i.e., 5% glucose injection) with different concentrations before administration. Rats were administered intravenously once daily for 7 consecutive days. On the 6th and 7th days, except control group, all groups received intraperitoneal injection with ISO (3 mg/kg), once daily for 2 consecutive days. Electrocardiograms (ECGs) were recorded after administration. If the typical ischemic ECG waveform (significant ST segment elevation) was observed, the AMI model was prepared5-6.

Cardiac function evaluation

Cardiac function was evaluated by Vevo 770 vivo micro-imaging system (Visual Sonics Inc. Toronto, Canada), left ventricular end systolic diameter (LVES), left ventricular end systolic volume (LVESV), left ventricular end diastolic volume (LVEDV), left ventricular fractional shortening (LVFS), heart rate (HR), ejection fraction (EF) and cardiac output (CO) were measured.

Determination of infarct size

After blood collection, rats were injected via abdominal aorta with 10 mL of 1% TTC in phosphate buffer (pH = 7.4), which was pre-incubated at 37°C, then the aorta was clamped for 15 min. Next, the hearts were cross-sectioned into five slices in 4°C phosphate buffer saline (PBS) and then incubated at 37°C in 1% TTC for 15 min. The infarcted myocardium was cut off and weighed by LAC-110.4 analytical balance (Sartorius Group, Germany). The infarct size could be obtained by

$$\frac{W_{\text{infarcted myocardium}}}{W_{\text{total myocardium}}} \times 100\%$$

H & E staining observation

After blood collection, the apex of the ischemic heart was separated, and it was washed in normal saline and dried with filter paper. Next, it was fixed in 10% formalin and sent to the pathology department of the First Affiliated Hospital of Jinan University (Guangzhou, China) for pathological section and hematoxylin and eosin (H & E) staining. At the end, the structure of myocardium was observed with Nikon ECLIPSE TS100 fluorescence inverted microscope (Nikon Co., Ltd., Japan).
**Assay of enzyme activity**

Blood was collected and left standing for 2 h. Then, it was centrifuged at 3,000 rpm at 4°C for 10 min by refrigerated centrifuge (Sigma Laborzentrifugen GmbH, Germany). The supernatant was collected and stored at −20°C for activity tests of CK, LDH, cTnI and BNP.

**Statistical analysis**

Software SPSS 17.0 was used for statistical analysis. All data were expressed as the mean ± standard error of the mean (SEM). Comparisons between groups were calculated by using one-way ANOVA and independent-samples t test, while comparisons between percentages were calculated by rank-sum test. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Electrocardiogram (ST segment)**

Compared with the control group, the ST segments of the model group elevated significantly within 5 min (P < 0.01), which demonstrated that the model of MI was successfully established. When compared with the model group, the ST segments of SGI and peripheral nervous system (PNS) groups declined significantly within 120 min (P < 0.01 or P < 0.05) (Figs. 1, 2).

**Myocardial infarct size**

TTC staining of myocardium revealed that MI injury was strongly related to the development and progression of irreversible ischemic injury of myocardium (Fig. 3). The proportion of infarct size in the model group was remarkably greater than that in the control group (P < 0.01), and the infarct size was decreased significantly with treatments of SGI (H), SGI (M), SGI (L) and PNS (P < 0.01 or P < 0.05) (Fig. 4).

**Heart morphology observation**

The H & E staining of myocardial tissue showed that the myocardial fibres of rats in the control group were in an orderly arrangement, and there were no atrophy,
hypertrophy, necrotic foci, inflammatory cell infiltration or any other pathological changes. However, the myocardial damages were evident in the model group. The myocardial fibres were in a disorderly arrangement. Myocardial interstitial edema was found, and the tissue space was significantly widened. Neutrophil infiltration was also present. Many myocardial fibres were swollen, lysed or ruptured, and the cross striation was vague or even disappeared. The myocardial cells underwent vacuolar degeneration. The myocardium damages in SGI and PNS groups were milder than those in the model groups. Local mild swelling was found, mild interstitial edema was present and the tissue space was slightly widened. A few inflammatory cells were infiltrated, and vacuolar degeneration could be observed sporadically (Fig. 5).

**Cardiac function evaluation**

Compared with the model group (the same below), SGI groups displayed a remarkable decrease on LVESD and LVESV ($P < 0.01$ or $P < 0.05$). Besides, they showed a significant increase in LVFS and LVEF ($P < 0.01$ or $P < 0.05$). In addition, SGI improves LVESD, LVESV, LVFS and LVEF in a dose-dependent manner. It is interesting that SGI (M) can effectively improve LVEDV and CO. However, SGI groups showed no differences with the model group on HR ($P > 0.05$) (Table 1).

**Activity of serum enzymes**

As shown in Table 2, SGI improved CK, LDH, cTnI and BNP in a dose-dependent manner. Compared with the model group (the same below), all SGI groups displayed a remarkable decrease on activity of serum CK ($P < 0.01$ or $P < 0.05$). SGI (H) and SGI (M) showed a significant decrease on activity of serum cTnI and BNP ($P < 0.01$ or $P < 0.05$). Besides, SGI (H) can lower the activity of serum LDH ($P < 0.01$).

**DISCUSSION**

Methods to establish MI model mainly are medicine method, ligation, minimally invasive and interventional methods etc. Among them, the most frequently used method is coronary artery ligation, but the actual operation is difficult and will cause great injury to experimental animals. Drug-induced MI is a kind of method that has the advantages of simple operation, little injury and higher success rate. ISO (a catecholamine drug) and pituitary urotensin are commonly used to establish AMI model. Both of them induce AMI by promoting coronary artery contraction, enhancing myocardial contractility and increasing oxygen consumption. The ECG results of
this study suggest that rats AMI model can be prepared successfully by injecting intraperitoneally with ISO (3 mg/kg) for 2 consecutive days.

TTC staining is widely used in the observation of ischaemia infarction of mammalian tissues, because of its good sensitivity and convenience to observe. In this research, the TTC staining results showed that SGI can effectively reduce myocardial infarct size and improve the pathological state of myocardial ischemic necrosis.

H & E staining is one of the most widely used technical methods of histology, embryology and pathology researches. And it is important for reflecting the pathological changes intuitively. In our research, H & E staining results indicated that SGI could improve the pathological conditions of AMI rats.

AMI is a common and clinical disease in clinic. Detection of biochemical markers in blood is one of the important means for reflecting the degree of myocardial damages. Under normal physiological conditions, CK and LDH are specific enzymes in myocardial cytoplasm, cTnI is the important regulatory protein of striated muscle, and synthesis and secretion of BNP are mainly in ventricular myocytes. However, CK, LDH, cTnI and BNP can penetrate the cell membrane into the blood circulation when myocardial cells are injury, which will cause the content of these serum enzymes increased significantly. Our study showed that SGI intervention can significantly reduce the levels of LDH, CK, cTnI and BNP activity in serum, which suggested that SGI can improve the myocardial injury caused by ISO in rats.

Cardiac function detection is commonly used in clinical diagnosis of CVDs, and has an important significance in the pathophysiology evaluation of cardiovascular system. LVESD, LVESV, LVEDV and LSR can reflect the left ventricular size and left ventricular systolic/diastolic function, while LVEF and CO can reflect the left ventricular pump function. Our research showed that SGI could improve the above-mentioned cardiac function indexes, and SGI (H) group showed better curative effect, for cardiac function indexes could be restored to near normal level.

In summary, SGI could reverse the myocardial injury in rats after MI by improving cardiac function and pathological state of myocardium, reducing MI area and adjustment of levels of LDH, CK, cTnI and BNP activity in serum. Therefore, SGI had outstanding protective action on AMI rats caused by ISO.

REFERENCES


