EFFECTS OF GUANXINNING TABLETS ON THE AUTONOMIC NERVE REGULATION FUNCTION OF MI/ RI RATS BY ANTIOXIDATION AND INHIBITION OF INFLAMMATORY RESPONSE

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ABSTRACT

Objective: *To investigate the effects of Guanxinning tablets on the autonomic nervous system regulation of myocardial ischemia/reperfusion (MI/RI) rats through anti-oxidation and inhibition of inflammatory response.*

Methods: *One hundred (100) clean male Wistar rats were selected and randomly divided into a sham operation group, MI/RI group, and Guanxinning tablet low-dose group, middle-dose group, and high-dose group, using the random number table method. There were 20 rats in each group. Rats in the sham operation group and MI/RI group were injected with distilled water intraperitoneally, and those in the low, medium and high dose groups of Guanxinning were given different doses of Guanxinning tablets –60 mg/kg, 120 mg/kg, 180 mg/kg, respectively. Each group was continuously administered for 5 days. The MI/RI operation model was established, and the sham operation group was not treated. Suture lines in the MI/RI group and Guanxinning groups were tightened after administration. The changes in left ventricular function [left ventricular end-systolic pressure (LVESP) and left ventricular end-diastolic pressure (LVEDP)] of rats in each group, oxidative stress indicators [malonaldehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px)], inflammatory factors [interleukin-10 (IL-10), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α)], heart rate variability (HRV) (RR interval, total power, high frequency, low frequency, and low frequency/high frequency), and the pathological morphology of myocardial tissue in rats were observed.*

Results: *In comparison to the sham operation group, the levels of LVESP, SOD, GSH-Px, and IL-10 in the MI/RI group were significantly reduced, and the levels of LVEDP, MDA, IL-6, and TNF-α were significantly increased (P<0.05). In comparison to the MI/RI group, the levels of LVESP, SOD, GSH-Px, and IL-10 in the low, medium, and high-dose Guanxinning groups were significantly increased, and the levels of LVEDP, MDA, IL-6, and TNF-α were significantly decreased (P<0.05); With an increase in the dose of Guanxinning tablets, the levels of LVESP, SOD, GSH-Px, and IL-10 increased significantly, and the levels of LVEDP, MDA, IL-6, and TNF-α decreased significantly (P<0.05). In comparison to the sham operation group, the RR interval, total power, and high frequency in the MI/RI group were significantly reduced, and the low frequency and low-frequency/high-frequency ratios were significantly increased (P<0.05). In comparison to the MI/RI group, the RR interval, total power, and high frequency of the Guanxinning groups were significantly increased in the medium- and high-dose groups, and the low frequency and low-frequency/high-frequency ratios were significantly reduced (P<0.05). There was no significant difference in HRV indicators between the RI group and the Guanxinning low-dose group (P>0.05). In the sham operation group, myocardial fibres were arranged neatly, the nuclei were normal, and there was no inflammatory cell infiltration in the cardiac muscle stroma. In the MI/RI group, the myocardial fibres were distributed in a wavy shape, in a disordered arrangement, and some myocardial fibres and cell nuclei were broken or ruptured. The Guanxinning low-dose group exhibited slight improvements in comparison to the MI/RI group; in the medium-dose group, myocardial fibres were arranged neatly, and there was a small amount of inflammatory cell infiltration into the cardiac muscle stroma; the high-dose group was very similar to the sham operation group.*

Conclusion: *Guanxinning tablets can significantly improve the cardiac autonomic nervous regulation function of MI/RI rats through anti-oxidation and inhibition of inflammatory response.*

Keywords: *Guanxinning tablets, antioxidant, inhibition of inflammatory response, MI/RI, cardiac autonomic nerve regulation.*

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Introduction

Ischemic cardiomyopathy is a clinical syndrome caused by localized or diffuse fibrosis of the myocardium caused by long-term myocardial ischemia, resulting in impaired cardiac contraction or diastolic function. It commonly occurs in the elderly, and is indicative of conditions such as acute myocardial infarction[1, 2]. Myocardial ischemia/reperfusion injury (MI/RI) refers to the pathophysiological changes of myocardial cells and local blood vessels in the reperfusion area when the ischemic tissue resumes blood flow perfusion, thereby further promoting tissue damage[3]. It has been reported

that there sympathetic/parasympathetic dysfunction occurs in patients with ischemic cardiomyopathy, along with impaired autonomic regulation of the heart. The occurrence and development of MI/RI is closely related to oxidative stress and inflammatory responses. When the patient suffers from ischemic cardiomyopathy, the body's oxygen free radical production increases, and the levels of inflammatory factors increase significantly[4]. The main ingredients of Guanxinning tablets are salvia miltiorrhiza and Chuanxiong, which promote blood circulation, cease blood stasis, promote pulse and nourish the heart, and can reduce plasma viscosity and platelet aggregation rate[5].

However, there are few reports on the effect of Guanxinning tablets on the autonomic nervous regulation function of the heart in MI/RI rats. The purpose of this study is to analyse the effects of Guanxinning tablets on the autonomic nervous regulation of MI/RI rats through anti-oxidation and inhibition of inflammatory responses.

Materials and methods

Experimental animals

100 clean male Wistar rats were used (provided by Shanghai Ruitai Mos Biotechnology Co., Ltd., production license SCXK (Shanghai) 2016-0001), with weights of (200±40) g.

Primary instrumentation and reagents

Low-temperature high-speed centrifuge (German SIGMA, model: 3-18K); low-speed centrifuge (Changsha Xiangrui Centrifuge Co., Ltd., model: DT5-1B); flow cytometer [American Cell Signaling Technology (CST) company]; refrigerator (Qingdao Haier Group, model: BCD-470WDPG); cardiac-function tester (Tianjin Ruize Analytical Instrument Co., Ltd.); ELISA kit (Shanghai Thermo Fisher Scientific Technology Co., Ltd.); MDA detection kit (Mersak Biotechnology Co., Ltd.); nitric-oxide detection kit (Beijing Solibao Biotechnology Co., Ltd.); superoxide dismutase detection kit (Shanghai Jining Biotechnology Co., Ltd.); slicer (Jinhua Huasu Technology Co., Ltd.); ventilator (Jiangsu Sans Biotechnology Co., Ltd.); Guanxinning tablets [Yunnan Jinbuhuan (Group) Co., Ltd. Pharmaceutical Branch, Approval Number: SFDA approval number Z5180028, specification: 0.38 g]; and propofol injection (Sichuan Guorui Pharmaceutical Co., Ltd., approval number: SFDA approval number H20180079, specification: 10 ml: 0.1 g).

Rat model creation and grouping

All rats were raised in the laboratory at a temperature of (21±3) °C, humidity of (48±12) %, and were adaptively fed for 3 weeks. According to the random number table method, they were randomly divided into a sham operation group, an MI / RI group, and a low-dose group, a medium-dose group, and a high-dose Guanxinning group, with 20 rats in each group. Rats in the sham operation group and the MI/RI group were given distilled water by intraperitoneal injection. The rats in the Guanxinning group were given different doses of Guangxinning tablets – 60 mg/kg, 120 mg/kg, and 180 mg/kg. Each group was continuously administered for 5 days.

The rats fasted for 8 hours before the operation. The rats were anaesthetized with a propofol injection and placed on the experimental table. A fourth intercostal incision was made on the left side of the sternum. The chest was opened to expose the heart and the suture was used to pass through the left coronary artery [left coronary anterior descending branch, LAD, near the pulmonary artery (Pulmonary artery, PA) (coronary artery occlusion tube at 2.5–3.5 cm below)]. The sham operation group was not treated. Suture lines in the MI/RI group and the low-, medium-, and high-dose Guanxinning groups were tightened after administration. After 45 minutes, the suture was untied and observation continued for 60 minutes. The rats were killed after the experiment.

Observation indexes

Five millilitres of arterial blood was drawn from each rat, and the supernatant was stored in the refrigerator after centrifugation.

Changes in left ventricular function: the left ventricular end-systolic pressure (LVESP) and left ventricular diastolic pressure (LVEDP) of the rats were detected using a cardiac-function tester.

Oxidative stress indicators: Malondialdehyde (MDA) was measured using the thiobarbituric acid method, superoxide dismutase (SOD) was detected by the enzyme rate method, and changes in the activity of glutathione peroxidase (GSH-Px) were detected by the colourimetric method.

Inflammatory factors: changes in interleukin-10 (IL-10), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) levels were detected using enzyme-linked immunoadsorption analysis (ELISA).

Heart-rate variability (HRV) changes were monitored using an electrocardiograph.

The pathological changes of the myocardium in rats were observed by HE staining.

Statistical methods

The SPSS 23.0 software package was used for statistical data analysis. Measurement data were compared using single-factor and multiple-sample means. The statistical results were deemed to be statistically significant with P<0.05, and statistically insignificant with P>0.05.

Results

Comparison of left cardiac function of rats in each group

In comparison to the sham operation group, the LVESP level in the MI/RI group was significantly reduced, and the LVEDP level was significantly increased (P<0.05). In comparison to the MI/RI group, the LVESP levels of Guanxinning low-dose, medium-dose and high-dose groups were significantly increased, and the level of LVEDP decreased significantly (P<0.05). With the increase in the dose of Guanxinning tablets, the level of LVESP gradually increased, but the level of LVEDP increased (P<0.05). Please check Table 1 for further details.

Groups	n	LVESP	LVEDP
Sham operation group	20	132.98±11.07	2.42±0.61
MI/RI group	20	83.77±8.91*	12.23±0.96*
Guanxinning low-dose group	20	102.87±10.26*#	8.71±1.02*#
Guanxinning medium-dose group	20	116.25±9.65*#	6.97±0.49*#
Guanxinning high-dose group	20	128.34±12.03*#	4.29±0.58*#

Table 1: Comparison of left cardiac function of rats in each group ($\bar{x}\pm s$).

*Note: *indicates P<0.05 compared with the sham operation group, #indicates P<0.05 compared with the MI/RI group.*

Comparison of oxidative stress indexes of rats in each group

In comparison to the sham operation group, the MDA level in the MI/RI group was significantly increased, and the SOD and GSH-Px levels were significantly decreased (P<0.05).

In comparison to the MI/RI group, the MDA level in the low-dose, medium-dose, and high-dose Guanxinning groups was significantly reduced, and the SOD and GSH-Px levels were significantly increased (P<0.05).

With the increase of the dose of Guanxinning tablets, the MDA level significantly reduced, and the SOD and GSH-Px levels significantly increased (P<0.05), as shown in Table 2.

Groups	n	MDA (mmol/L)	SOD (U/L)	GSH-Px (U/L)
Sham operation group	20	3.27±1.32	69.87±9.05	71.51±1.13
MI/RI group	20	9.72±1.03*	41.54±8.67*	50.51±8.59*
Guanxinning low-dose group	20	8.16±3.01*#	53.37±5.38*#	54.12±5.39*#
Guanxinning medium-dose group	20	6.69±2.54*#	58.96±5.43*#	59.56±6.19*#
Guanxinning high-dose group	20	5.11±1.84*#	64.26±8.67*#	63.79±8.39*#

Table 2: Comparison of the oxidative stress indexes of rats in each group ($\bar{x}\pm s$).

*Note: *indicates P<0.05 compared with the sham operation group, #indicates P<0.05 compared with the MI/RI group.*

Comparison of the inflammatory factors of rats in each group

Compared with the sham operation group, the levels of IL-10 in the MI/RI group were significantly reduced, and the levels of IL-6 and TNF-α were significantly increased (P<0.05). In comparison to the MI/RI group, the levels of IL-10 in the low-, medium-, and high-dose Guanxinning groups were significantly increased, and the levels of IL-6 and TNF-α were significantly reduced (P<0.05), as shown in Table 3.

Groups	n	IL-10 (pg/mL)	IL-6 (ng/L)	TNF-α (pg/mL)
Sham operation group	20	92.49±18.74	131.65±40.06	134.37±13.85
MI/RI group	20	52.84±11.03*	344.91±64.04*	286.22±15.14*
Guanxinning low-dose group	20	63.24±16.27*#	296.53±35.97*#	247.87±14.29*#
Guanxinning medium-dose group	20	73.22±14.49*#	211.52±29.13*#	200.88±14.43*#
Guanxinning high-dose group	20	82.88±12.38*#	175.76±30.61*#	165.29±13.65*#

Table 3: Comparison of inflammatory factors of rats in each group ($\bar{x}\pm s$).

*Note: *indicates P<0.05 compared with the sham operation group, #indicates P<0.05 compared with the MI/RI group.*

Effect of guanxinning tablets on HRV in MI/ RI rats

Compared with the sham operation group, the RR interval, total power, and high frequency of the MI/RI group were significantly reduced, and the low frequency and low-frequency/high-frequency ratios were significantly increased (P<0.05).

Compared with the MI/RI group, the RR interval, total power, and high frequency of the Guanxinning medium- and high-dose groups were significantly increased, and the low frequency and low-frequency/high-frequency ratios were significantly reduced (P<0.05). There was no significant

difference in HRV indicators between the RI group and the low-dose Guanxinning group (P>0.05), as can be seen in Table 4.

Groups	n	RR interval (ms)	Total power (ms2/HZ)	Low frequency (HZ)	High frequency (HZ)	Low frequency/ high frequency (HZ)
Sham operation group	20	150.08± 40.27	59.44± 20.57	21.72± 6.41	67.98± 5.14	0.46±0.23
MI/RI group	20	108.29± 12.67*	12.01± 3.17*	62.94± 8.58*	21.99± 4.78*	1.53±0.62*
Guanxinning low-dose group	20	125.47± 20.78*	21.64± 10.27*	54.11± 10.26*	37.04± 5.22*	1.28±0.74*
Guanxinning medium-dose group	20	132.53± 21.46#	35.41± 15.49*#	42.28± 8.91*#	46.55± 7.56*#	1.05±0.81*#
Guanxinning high-dose group	20	141.29± 30.42#	175.76± 30.61*#	165.29± 13.65*#	175.76± 30.61*#	165.29±13.65*#

Table 4: Effect of Guanxinning tablets on HRV in MI/RI rats ($\bar{x}\pm s$).

*Note: *indicates P<0.05 compared with the sham operation group, #indicates P<0.05 compared with the MI/RI group.*

Comparison of changes in myocardial tissue of the rats in each group

In the sham operation group, myocardial fibres were arranged neatly, the nuclei were normal, and there was no inflammatory cell infiltration into the cardiac muscle stroma; in the MI/RI group, the myocardial fibres were distributed in a wavy shape, and the arrangement was disordered, and some myocardial fibres and cell nuclei were broken or ruptured. The Guanxinning low-dose group was slightly improved in comparison to the MI/RI group; in Guanxinning medium-dose group, myocardial fibres were arranged neatly, and there was a small amount of inflammatory cell infiltration into the cardiac muscle stroma; the Guanxinning high-dose group was very similar to the same as the sham operation group. Please check figure 1(A-E) for more details.

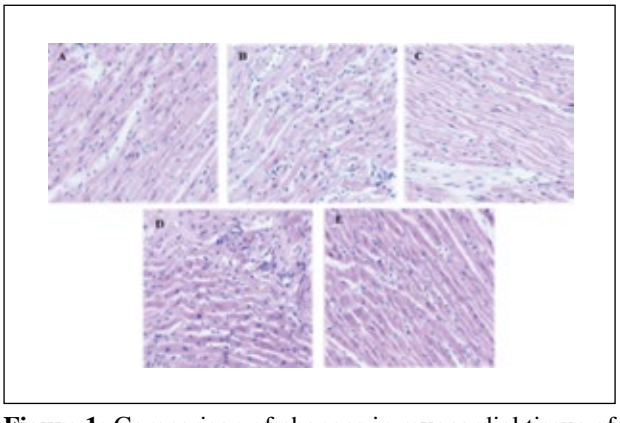

Figure 1: Comparison of changes in myocardial tissue of the rats in each group.

A: sham operation group; B: MI/RI group; C: Guanxinning low-dose group; D: Guanxinning medium-dose group; E: Guanxinning high-dose group.

Discussion

Ischemic cardiomyopathy mostly takes the form of coronary artery obstruction, stenosis caused by coronary artery disease, or that caused by diffused disease, which leads to severe myocardial dysfunction. This myocardial dysfunction can manifest as an enlarged heart, heart failure, or arrhythmia[6]. With an increase in age, mortality rates generally increase. This is especially the case regarding cardiovascular disease, which is increasing year on year, and is currently the biggest killer of humans.

In most cases, reperfusion after ischemia can restore the function of tissue and organs, repair damaged structures, and improve the condition of patients; however, sometimes reperfusion after ischemia not only fails to restore the function of tissues and organs, but also aggravates tissue and organ dysfunction and structural damage[7]. After the blood flow is restored on the basis of ischemia, tissue damage is actually exacerbated, and irreversible damage could occur. This phenomenon is called ischemia-reperfusion injury[8]. With the application of coronary arterial blood transfusion reconstruction technology, myocardial ischemia-reperfusion injury has received more and more attention. Studies have found that Guanxinning tablets can prevent myocardial ischemia and reperfusion injury by resisting free-radical damage, inhibiting myocardial enzyme release, and reducing myocardial cell apoptosis. Danshen contains tanshinone, which can inhibit the production of active oxygen species, directly scavenge free radicals, improve vascular endothelial function, and has the effect of promoting blood circulation, mitigating stasis, regulating qi, and stopping pain[9]. Chuanxiong has the effects of dilating coronary arteries, inhibiting fibroblast proliferation, resisting myocardial ischemia-reperfusion injury, inhibiting platelet activation, and improving vascular endothelial function. It can also relieve smooth muscle spasms and reduce heart load[10]. The purpose of this study is to observe the effects of Guanxinning tablets on the autonomic nervous system regulation of MI/RI rats through anti-oxidation and inhibition of inflammatory responses.

Oxidative stress (OS) is the negative effect of free radicals. It refers to the imbalance between the oxidative and antioxidant systems in the body, which leads to inflammatory infiltration of neutrophils and increased secretion of proteases, resulting in a large number of oxidative intermediates. MDA is one of the most important products of membrane-lipid

peroxidation, and its level can indirectly reflect the state of the cells in the body attacked by free radicals, making it an important index for evaluating the severity of oxidative stress injuries of myocardial cells[11]. SOD is a new type of enzyme preparation and is an important antioxidant enzyme in the human body. It is the first substance to scavenge free radicals, to the best of our knowledge, and is beneficial in reducing damage caused by oxidative stress; therefore, it can be used as an effective index for judging the antioxidant capacity of myocardial tissue[12]. GSH-Px is a peroxolytic enzyme that is prevalent throughout the body, and can protect the integrity of cell-membrane structure and function[13]. The results of this study show that the MDA level in the MI/RI group was significantly higher than that in the sham operation group and in the low-, medium-, and high-dose Guanxinning groups; further, the levels of SOD and GSH-Px were significantly lower in the sham operation group and in the low-, medium-, and high-dose Guanxinning groups ($P<0.05$), showing that Guanxinning tablets can increase the body's antioxidant enzyme levels, reduce the body's lipid peroxidation, reduce myocardial damage, and delay the protection of heart function in MI/RI rats.

Some studies suggest that, in MI/RI, the occurrence and development of cardiovascular disease are closely related to the increase in inflammatory factor levels. IL-10 is a multifunctional cytokine secreted by T-lymphocytes and plays an important role in suppressing the inflammatory response. TNF-α is a polypeptide cytokine with multiple biological roles, produced by monocytes and macrophages. It can induce granulocytes to produce IL-6 and is closely related to the body's immune response and inflammatory response, making it an important inflammatory factor[14]. According to reports, during MI/RI, a large number of pro-inflammatory factors are produced by ischemic cells in the body, such as TNF-α, IL-6, etc. In the study results, the level of IL-10 in the MI/RI group was significantly lower than that in the sham operation group and in the low-, medium-, and high-dose Guanxinning groups; the difference was statistically significant ($P<0.05$). Guanxinning tablets can significantly reduce the levels of IL-6 and TNF-α, while increasing the levels of IL-10.

HRV refers to the ability to respond to cardiac autonomic nerve activity and quantitatively evaluate the tension and balance of cardiac sympathetic and vagus nerves, and subsequently judge its important indexes for the condition of cardiovascular disease. It can be judged by the degree of change in heart rate[15]. In this group of studies, the RR interval, total power, and high frequency of the MI/RI group were significantly lower than those of the sham operation group and the low-, medium-, and high-dose Guanxinning groups, and the low-frequency and low-frequency/high-frequency ratios were significantly higher than those of the sham operation group and the low-, medium-, and high-dose Guanxinning groups. The LVESP level in the MI/RI group was significantly lower than that in the sham operation group and the low-, medium-, and high-dose Guanxinning groups, and the LVEDP level was significantly higher than that in the sham operation group and low-, medium-, and high-dose Guanxinning groups. Further, the LVESP level gradually increased with the increase in the dosage of Guanxinning tablets, while the LVEDP gradually decreased, showing that when MI/RI occurs, the sympathetic nerves are over-stimulated and HRV is significantly reduced. Guanxinning tablets can increase the HRV of MI/RI rats by inhibiting the activity of the sympathetic nerves, and then correct the autonomic nervous regulatory dysfunction of the heart, improving left ventricular function in rats.

In conclusion, Guanxinning tablets can correct the disturbances in cardiac self-regulation functions of MI/RI rats by reducing the inflammatory response and inhibiting the oxidative response, consequently improving left heart function, which could potentially have significant clinical value.

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