MECHANISM OF GINSENOSIDE IN THE TREATMENT OF HEART FAILURE BY INTERVENING PPAR PATHWAY

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ABSTRACT

Objective: *To analyze the therapeutic effect and related mechanism of ginsenoside on heart failure.*

Methods: *A total of sixty male SD rats were randomly allocated to a control group, a model group and a drug group. The rats in the control group were not treated. The rats in the model group and the drug group were treated to establish a heart failure model. The rats in the model group were also given the total ginsenoside gavage treatment, and the heart function of the rats was analyzed using color Doppler ultrasound after treatment. The heart mass index of each group was calculated and serum levels of BNP, ATP and ADP were measured by ELISA. Western blot assay was used to determine levels of mitogen activated protease p38 (p38MAPK), phosphorylated mitogen activated protease p38 (P-P38MAPK), and peroxisome proliferator activated receptor (PRR) in rats of each group. Bcl-2 and Bax protein content was studied.*

Results: *LVDD and LVDS levels in the model group were significantly higher than those in the control group, and levels of LVEF, LVPWD and LVPWS in the model group were significantly lower than those in the control group. LVDD and LVDS levels in the drug group were significantly lower than those in the model group, and levels of LVEF, LVPWD and LVPWS in the drug group were significantly higher than those in the model group ($P<0.05$). The heart mass index of the model group was significantly higher than that of the control group, and the heart mass index of the drug group was significantly lower than that of the model group ($P<0.05$). Levels of BNP and ADP in the model group were significantly higher than those in the control group, and the ATP level in the model group was significantly lower than it was in the control group. Levels of BNP and ADP in the drug group were significantly lower than those in the model group, and the ATP level in the drug group was significantly higher than it was in the model group ($P<0.05$). Bax level in the model group was significantly higher than that in the control group, and Bcl-2 level was significantly lower than that in the control group. The Bax level of the rats in the drug group was significantly lower than that in the model group, and the level of Bcl-2 in the drug group was significantly higher than that in the model group ($P<0.05$). Levels of p38MAPK and P-P38MAPK in the model group were significantly higher than those in the control group. The PPAR- γ level in the model group was significantly lower than that in the control group. Levels of p38MAPK and P-P38MAPK in the drug group were significantly lower than those in the model group. The PPAR- γ level of the drug group was significantly higher than that of the model group ($P<0.05$).*

Conclusion: *Ginsenoside can delay the development of heart failure in rats with heart failure. The mechanism may be related to the regulation of PPAR pathway related proteins by ginsenoside.*

Keywords: *Ginsenoside, PPAR pathway, heart failure, treatment, mechanism.*

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Introduction

Chronic heart failure (CHF) is a pathological state in which various heart diseases progress to impaired heart function. Clinical symptoms such as dyspnea, fatigue, and fluid retention may occur; these symptoms indicate a persistent heart failure state, and are also the main cause of death in patients with cardiovascular diseases[1]. An acceleration in the pace of life combined with an aging population may be partly responsible for the recent trend of year-on-year increases in the incidence of CHF[2]. Current clinical goals in the treatment of heart failure include prevention and treatment of myocardial remodeling, improvement of patients' symptoms and quality of life, and reduction of the hospitalization and mortality rates of heart failure[3]. Ginseng is a plant of the araliaceae family, which is sweet in

nature and bitter in taste. Ginsenosides are the most important active ingredients of ginseng in terms of physiological activity: they can regulate the body's glucose and lipid metabolism, are anti-diabetic and anti-obesity, and are commonly used in the treatment of cardiovascular diseases[4].

Recent studies have found that ginseng total saponins have a certain efficacy in the treatment of heart failure, but the relevant mechanism has not yet been clarified[5]. In this experiment, 60 clean-grade male SD rats were selected as observation objects to analyze the therapeutic effect and related mechanisms of ginsenoside on heart failure.

Materials and methods

General information

Experimental animals

60 clean-grade male SD rats, weighing (210±10) g and aged (8±1) weeks old, were provided by the Animal Center of Heilongjiang University of Chinese Medicine, with experimental license number SYXK (black) 2016 -0035. The rats were bred in an incubator with natural light, (23±2) °C temperature, and 50%-60% humidity, and were given adequate food and drink. Operations performed upon these experimental animals were in compliance with the relevant standards of the 'Regulations on the Administration of Laboratory Animals'.

Experimental drug

Ginsenoside was provided by Baishan Changbaishan Pharmaceutical Co., Ltd.

Methods

A total of 60 SD rats were randomly divided into a control group, a model group and a medication group. The rats in the control group were not treated, while the rats in the model group and the medication group were treated to establish heart failure models. Isoproterenol was injected subcutaneously at multiple points daily for 14 days.

The dose on the first day was 20 mg/kg, the dose on the second day was 10 mg/kg, and the daily dose from day 3 to day 14 was 5 mg/kg. Measurement of left ventricular ejection fraction (LVEF) at ≤45% indicated that the heart failure rat model was successfully constructed. After successful modeling, rats were given 60 mg/kg ginsenoside by gavage, once per day, for 28 consecutive days. The rats in the model group were given the same amount of normal saline by gavage.

Observation indicators

Color Doppler ultrasound to detect cardiac function in rats

After treatment, the rats were anesthetized, laid supine and fixed. A probe was placed on the left edge of the sternum to detect the left ventricular end systolic diameter (LVDS), left ventricular end diastolic diameter (LVDD), left ventricular end diastolic posterior wall thickness (LVPWD), LVEF, and the left ventricular end systolic posterior wall thickness (LVP-WS) of each group of rats, taking the average of three consecutive tests.

Heart mass index

After treatment, the rats were anesthetized, the chest cavity was opened, the heart was taken out, the heart mass was weighed after washing, and the heart mass index of each group of rats was calculated.

Serum detection: After treatment, blood was collected from the abdominal aorta of the anesthetized rats. The blood was kept at room temperature for 20 minutes then centrifuged at 3000 r/min for 10 minutes. The serum was carefully separated and refrigerated at -70 °C for later use to avoid repeated freezing and thawing. Enzyme-linked immunosorbent assay was used to detect serum brain natriuretic peptide (BNP), adenosine triphosphate (ATP), and adenosine diphosphate (ADP) levels.

Western blot

Myocardial tissue from rats in each group was cut, ground and added to cell lysate. Protein was extracted from the tissue, protein concentration was detected by the BCA method, and a polyacrylamide gel electrophoresis test was carried out. Electrotransfer to a PDVF membrane was performed, blocking with skimmed milk for 1 hour, with the primary antibody overnight, and with the secondary antibody for 1 hour. Image analysis software was used to analyze the relative content of mitogen activated proteinase P38 (P38MAPK), phosphorylated mitogen activated proteinase P38 (p-P38MAPK), peroxisome proliferator activated receptor (PPAR-γ), apoptosis-related protein bcl-2, and bax protein.

Statistical methods

The data in this study were all statistically analyzed using the SPSS20.0 software package.

LVDD, LVDS, LVEF, LVPWD, LVPWS, BNP, ADP, ATP, P38MAPK, p-P38MAPK, PPAR-γ, Bcl-2, Bax and other measurement data all conformed to normal distribution, which was represented by ($\bar{x}\pm s$).

Comparison between multiple groups was performed by one-way analysis of variance, and pairwise comparison was performed by the SNK-q test. The statistical results were statistically significant at P<0.05.

Results

Comparison of heart function of rats in each group

Levels of LVDD and LVDS in the model group were significantly higher than those in the control group, and levels of LVEF, LVPWD and LVPWS were significantly lower in the model group than those in the control group. Levels of LVDD and LVDS in the medication group were significantly lower than those in the model group, and levels of LVEF, LVPWD and LVPWS in the medication group were significantly higher than those in the model group. The differences were statistically significant (P<0.05). See Table 1.

Group	LVDD (mm)	LVDS (mm)	LVEF (%)	LVPWD (mm)	LVPWS (mm)
Control	6.65±0.45	5.61±0.38	87.62±15.36	3.25±0.38	4.65±0.58
Model	9.78±0.56[a]	9.66±0.64[a]	36.52±5.38[a]	1.97±0.25[a]	1.23±0.28[a]
Medication	7.52±0.26[ab]	6.59±0.34[ab]	61.52±6.78[ab]	2.78±0.26[ab]	2.58±0.54[ab]
F	268.310	400.060	126.030	91.640	252.040
P	<0.001	<0.001	<0.001	<0.001	<0.001

Table 1: Comparison of heart function of rats in each group ($\bar{x}\pm s$).
Note: [a]indicates comparison with the control group, [a]P<0.05; [b]indicates comparison with the model group, [b]P<0.05.

Comparison of heart mass index of rats in each group

The heart mass index of rats in the model group was significantly higher than that of those in the control group, and the heart mass index of rats in the medication group was significantly lower than that of those in of the model group. The difference was statistically significant (P<0.05). See Figure 1.

Comparison of serum indexes of patients in each group

BNP and ADP levels in the model group were significantly higher than those in the control group, and the ATP level was significantly lower in the model group than that in the control group. BNP and ADP levels in the medication group were significantly lower than in the model group, and the ATP level was significantly higher in the medication group than that in the model group. The differences were statistically significant (P<0.05). See Table 2.

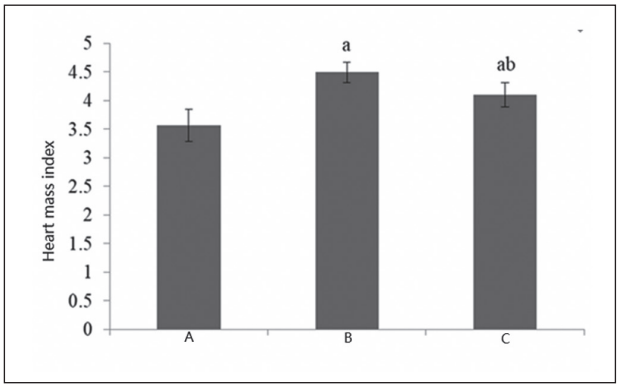

Figure 1: Comparison of heart mass index of rats in each group.
Note: [a]indicates comparison with the control group, [a]P<0.05; [b]indicates comparison with the model group, [b]P<0.05. A: Model group. B: Medication group. C: Control group.

Control	ATP (μg/L)	BNP (ng/L)	ADP (μg/L)
Model	762.58±185.24	63.52±11.52	234.61±50.64
Medication	387.95±154.23[a]	151.26±20.56[a]	596.34±125.64[a]
Control	582.62±256.34[ab]	105.62±15.82[ab]	352.64±80.46[ab]
F	17.010	143.400	82.250
P	<0.001	<0.001	<0.001

Table 2: Comparison of serum indexes of rats in each group ($\bar{x}\pm s$).
Note: [a]indicates comparison with the control group, [a]P<0.05; [b]indicates comparison with the model group, [b]P<0.05.

Comparison of myocardial apoptosis-related proteins in each group of rats

The Bax level of rats in the model group was significantly higher than that in the control group, and the Bcl-2 level in the model group was significantly lower than that in the control group. The Bax level of rats in the medication group was significantly lower than that in the model group, and the Bcl-2 level of rats in the medication group was significantly higher than that in the model group. The differences were statistically significant (P<0.05). See Figure 2.

Comparison of PPAR pathway related proteins in rats in each group

Levels of P38MAPK and p-P38MAPK in the model group were significantly higher than those in the control group, and levels of PPAR-γ were significantly lower in the model group than

those in the control group. Levels of P38MAPK and p-P38MAPK in the medication group were significantly lower than those in the model group, and levels of PPAR-γ were significantly higher than those in the model group. The differences were statistically significant (P<0.05). See Figure 3.

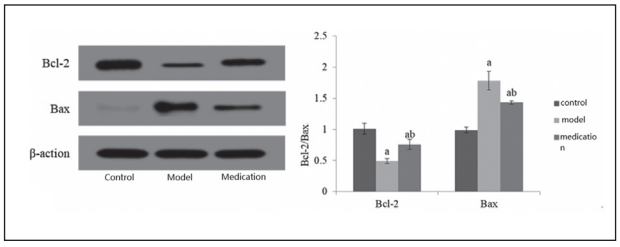

Figure 2: Comparison of myocardial apoptosis-related proteins in each group of rats.
Note: [a]indicates comparison with the control group, [a]P<0.05; [b]indicates comparison with the model group, [b]P<0.05.

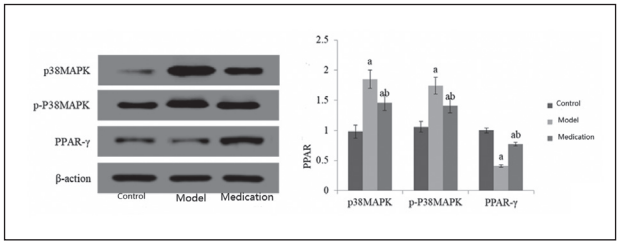

Figure 3: Comparison of PPAR pathway related proteins in each group of rats.
Note: [a]indicates comparison with the control group, [a]P<0.05; [b]indicates comparison with the model group, [b]P<0.05.

Discussion

CHF is the terminal stage of multiple-cause heart disease, which is a heart disease with high incidence and mortality rates. Statistical analyses in previous studies demonstrate that the probability of death within 5 years among patients hospitalized with CHF is as high as 50%, which not only seriously endangers the life and health of patients but also represents a heavy economic burden for their families and wider society to bear[6-8]. CHF has become a focus of medical research worldwide in recent years due to its high morbidity and mortality rates. In Chinese medicine, CHF belongs to the category of heart palpitations, asthma, edema and chest numbness. Pathogenesis is based on root deficiency and branch excess: root deficiency presents with heart qi and heart yang deficiency, and branch excess with blood stasis, water stagnation and internal resistance of phlegm and stasis. Heart-qi and heart-yang deficiency is an important pathogenic factor of CHF[9]. According to 'Shen Nong's Materia Medica', ginseng can nourish the five internal organs, and also has the functions of calming the spirit, calming the soul, removing evil spirits, improving eyesight, and making people happy and wise[10].

Saponins are important active components in ginseng because they are steroid compounds which play a certain role in the treatment of cardiovascular diseases such as myocardial ischemia and myocardial hypertrophy[11]. Zhou Qin et al.[12] found that ginsenoside Rh2 can increase the level of serum VEGF and the number of peripheral blood endothelial progenitor cells after myocardial ischemia-reperfusion in rats fed on high-fat diets, thereby reducing myocardial ischemia-reperfusion injury. Zang Anyuan et al.[13] found that ginsenoside Rb1 exerts a protective effect on myocardial ischemia by regulating the expression of related proteins in the PI3K-Akt-eNOS signal transduction pathway. In this experiment, heart mass index along with the LVDD, LVDS, BNP, and ADP levels of rats in the model group were significantly higher than those of rats in the control group, and levels of LVEF, LVPWD, LVPWS, and ATP were significantly lower in rats in the model group than those of rats in the control group. Levels of LVDS, BNP, and ADP in rats in the medicated group were significantly lower than those of the model group, and levels of LVEF, LVPWD, LVPWS, and ATP were significantly higher in rats in the medicated group than those of the model group.

These results suggest that saponins can inhibit the heart function of rats with heart failure, inhibit the process of heart remodeling, and delay the development of heart failure: Dong Yanhong et al.[14] achieved similar results and drew similar conclusions. Ventricular remodeling refers to thechanges in gene expression, molecules, cells, and myocardial interstitium caused by heart injury. Apoptosis, excessive extracellular matrix proliferation, and myocardial hypertrophy are all pathological features. In this experiment, the Bax level of rats in the model group was significantly higher than that of rats in the control group, and the Bcl-2 level of rats in the model group was significantly lower than that of rats in the control group. The Bax level of rats in the medication group was significantly lower than that of rats in the model group, and the Bcl-2 level of rats in the medication group was significantly higher than that of rats in the model group. This suggests that saponin can delay the process of ventricular remodeling by inhibiting the apoptosis of cardiac cells. PPARs are a type of nuclear transcription factor activated by ligands, belonging to the type II nuclear

receptor superfamily. They are mainly expressed in tissues found in the intestine, heart, kidney and breast, and are involved in the body's glucose and lipid metabolism, atherosclerosis, tumor cell differentiation and in other biological processes[15-17]. Previous studies have shown that the PPAR signaling pathway is involved in the regulation of myocardial energy metabolism conversion. Wu Y Y et al.[18] showed that saponin and TZDs, as PPAR-γ activators, have similar biological effects, but whether they can activate the PPAR pathway is still problematic. In this experiment, levels of P38MAPK and p-P38MAPK in the model group were significantly higher than those in the control group, and the level of PPAR-γ was significantly lower than that in the control group. Levels of P38MAPK and p-P38MAPK in the medication group were significantly lower than those in the model group, and the level of PPAR-γ was significantly higher than that in the model group.

In summary, ginsenoside can improve the heart function of rats with heart failure and delay the development of heart failure. The mechanism may be related to the regulation of ginsenoside intervening in PPAR pathway related proteins.

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