Basic Investigations

Effects of Polysaccharides Extracted from Zhu Zi Shen (Rhizoma Panacis Majoris) on Oxidative Stress and Hemodynamics in Rats with Adriamycin-induced Chronic Heart Failure

CHEN Tao 陈涛, HU Yue-qin 胡月琴, DENG Li-rong 邓李蓉, GONG Zi-peng 巩仔鹂, and YU Xue-qin 余雪琴

Objective: To probe into the intervening action of polysaccharides of Zhu Zi Shen (Rhizoma Panacis Majoris) (PZZS) on oxidative stress and hemodynamics in rats with adriamycin-induced chronic congestive heart failure (CHF).

Methods: After SD rats were successfully modeled with adriamycin, they were randomly divided into a normal control group, a model group, a PZZS group, and a captopril group, and were administrated respectively. At the end of experiment, the hemodynamic function, whole heart weight index, and the blood CK, SOD, MDA, NO, NOS were detected; and the myocardial morphological examinations were carried out.

Results: Compared with the normal control group, the arterial systolic pressure (SBP), diastolic pressure (DBP), mean arterial pressure (MAP), heart rate (HR), left ventricular systolic peak (LVSP), and left ventricular pressure change rate (dp/dt max) significantly decreased, and left ventricular end diastolic pressure (LVEDP), whole heart weight index, the blood CK, MDA, NO, NOS significantly increased in the model group. PZZS significantly improved the hemodynamic function, lowered the MDA and NO levels, and decreased the CK and NOS activities in the CHF rats.

Conclusion: PZZS can improve the hemodynamic function, and alleviate the oxidative stress reaction in the CHF rat.

Keywords: Zhu Zi Shen (Rhizoma Panacis Majoris); chronic congestive heart failure (CHF); oxidative stress; hemodynamics

Zhu Zi Shen (Rhizoma Panacis Majoris), bitter and sweet in taste, and slight cold in nature, acts on the liver, lung and stomach channels. It has the effects of promoting blood circulation and removing blood stasis, enriching the blood and hemostasis, and subduing swelling and alleviating pain. Han, et al.¹ found that the total saponin of Zhu Zi Shen (Rhizoma Panacis Majoris) can improve the left ventricular function of the rabbit after ischemia-reperfusion. In addition, Zhuzishen–F, a monomer isolated and purified from the total saponin, has obvious inhibitory action on lipid peroxidation in the gerbil of reperfusion in a high oxygen environment after acute cerebral ischemia.² However, till now the study on PZZS for treatment of cardiovascular diseases has not been found yet. In the present experiment, the improving effect of PZZS on chronic congestive heart failure (CHF) was studied in the adriamycin-induced CHF rat.

Drugs and Reagents
Zhu Zi Shen (Rhizoma Panacis Majoris) was purchased from Yichang Municipal TCM Hospital; Adriamycin (lot No. 080501A) was produced by Zhejiang Haizheng Pharmaceutical Co. Ltd; Captopril (lot No. 090314) was made by Shantou Jinshi General Pharmaceutical Factory; Creatine kinase (CK), nitric oxide synthase (NOS), superoxide dismutase (SOD), nitric oxide (NO) and malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Biological Co. Ltd.

Preparation of Drugs
Rhizomes of Panax japonicus var. M grown in forest zone of Hubei Shen Nong Jia were dried over heat, weighted, ground into thick powder, and filled into a round bottom flask added with 10-fold volume distilled water for soaking for 2 h, which was heated for 8 h on an electric thermostat water bath. After filtration with neutral filter paper, the dregs of the decoction were removed; then the PZZS was extracted and purified according to the literature³,⁴ in an extraction rate of 4.8%, and 0.0458 g/mL PZZS solution was prepared.

MATERIALS AND METHODS

Animals
SD male rats, sanitary degree, weighing (180±20) g, were purchased from Wuhan Tongji Medical College, certificate of quality No. SCXK (鄂)2004-0007, and each rat was raised in single cage, at environment temperature (20±2) °C, humidity 55%–65%, illumination 12 h, bred with forage for rodents, free access to water.
Grouping of Animals
Forty-eight rats were randomly divided into a normal control group (n=10) and a modeling group (n=38). After successful modeling, the survived rats were sub-divided randomly into a model group, a captopril group and a PZZS group.

Modeling and Administration
Each rat in the normal control group was administrated by intraperitoneal injection of 0.2 mL saline, once a week, 10 times in total. Other rats were administrated by intraperitoneal injection of adriamycin hydrochloride (2 mg/kg), once a week, for 6 times. Six weeks later, 2 rats were randomly selected from the survived rats with heart failure for detection of the cardiac function and histopathologic examination, and the unsuccessful modeling rats were continuously modeled for 4 weeks. The administration of drugs was given from the 7th week of the modeling. The rats in the normal control group and the model group were intragastrically administrated with 2 mL saline, once a day; the rats in the PZZS group were administrated intragastrically with PZZS, 97 mg/kg, once a day; The rat in the captopril group was administrated with captopril, 100 mg/kg, once each day. The administration lasted for 4 weeks.

Observation on General Behaviors, Physical Signs and Death of the Rats
During the experiment, the general conditions of the rats, including diet, respiration, stool, urine, hair color, were observed.

Detection of the Cardiac Function and Hemodynamics
Twenty-four h after the last intragastrical administration, the rats were anesthetized by intraperitoneal injection of 20% urethane solution in 6 mL/kg, and was fixed on a table in a spinal position. The right common carotid artery was separated with a self-prepared ventricular canula (cardiac catheter 1 mm in diameter) filled with 1.0% heparin inserted, which was connected with the Biopac multichannel physiologic sign collection and processing system via a pressure transducer; the SBP, DBP, MAP, HR were recorded. Then the cardiac catheter was slowly pushed, and at the same time the pressure oscilloscope was observed. When the wave form was changed and the pulse pressure obviously increased, indicating the cardiac catheter entering into the left ventricle, stop to advance. After stabilization for 3 min, the LVSP, LVEDP, +dp/dt max and –dp/dt max were recorded. For the hemodynamic indexes, 5 sections were respectively taken for calculation of the mean value. Then, 10 mL blood was taken from the abdominal aorta, 5 mL added with 200 uL EDTA-Na2 and 5 mL with no anti-coagulant, which were centrifuged at 3000 rpm for 15 min. The plasma and serum were kept at -20 °C for biochemical detection.

Determination of the Heart Weight Index
After hemodynamic detection and the blood sampling, the heart was rapidly separated with the blood stain washed with saline and the water absorbed with a filter paper. The whole heart was weighted, and the whole heart weight index was calculated. The whole heart weight index = whole heart weight (mg)/body weight (g).

Biochemical Determinations of the Blood
CK, MDA, SOD, NO and NOS in the plasma and serum were respectively determined according to the instruction of the kits.

Myocardial Histomorphologic Examination
Myocardial cellular form of the rat was observed with routine paraffin section and HE staining, and the myocardial cellular ultrastructure was investigated with trans-electric microscope.

Statistical Processing
The data were expressed as (X ± s), one -way ANOVA was used for comparison among multi-groups, and LSD method was used to conduct both side test for comparison between groups. If variance was irregular, Tamhane method would be used for both side test , and the results were analyzed with SPSS13.0 statistical software.

RESULTS

General Behaviors, Physical Signs and Death Number of the Experimental rats
During the experimental period, the rats of the normal control group had lustrous hair, flexible eyes with spirit, quick activity, sensitivity to the environment changes. In the other 38 rats, after the third and forth sessions of interperitoneal injection of adriamycin, the symptoms and signs of CHF appeared successively, for example activity reduced, rolled up, ingestion reduced, loose furs, body weight reduced, etc. Afterwards, the symptoms gradually became more serious. After the 4th interperitoneal injection, 2 rats were taken for hemodynamic and histomorphologic examinations. After the 5th injection, ascites of different degrees, oliguria, loose stool, perioral cyanosis, pale ears, weak respiration, withered, disheveled and upright hair, and trichomadesis appeared; and in severe case, the rats had unstable walking, emaciation, cyanotic lips and ears, and started to die. Till the end of the 10th session of driumycin injection, 11 rats died, of which 4 was in the model group, 3 were in the PZZS group and 4 in the captopril group. Among the 33 rats investigated completely, 8 were in the normal group, 8 in the model group, 9 in the PZZS group, and 8 in the captopril group. In the survivaled rats of the medication groups, the symptoms improved obviously, especially in food- and water-intake with more activity, while the symptoms did not improve in rats of the model group.

Effects of PZZS on Whole Heart Weight Index in the Adriamycin-induced CHF Rats
The heart weight index (HWI) in the model group was higher than that in the normal control group, indicating that there were myocardial hypertrophy or stasis of blood
in the model group; and HWI was not significantly changed in both the medication groups (Table 1).

Table 1. Comparison of heart weight indexes in CHF rats of the groups ($\bar{x} \pm s, n=8$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg.d)</th>
<th>HWI (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>2.18±0.35</td>
</tr>
<tr>
<td>Model</td>
<td>-</td>
<td>2.55±0.12</td>
</tr>
<tr>
<td>Captopril</td>
<td>100</td>
<td>2.30±0.21</td>
</tr>
<tr>
<td>PZZS</td>
<td>97</td>
<td>2.39±0.39</td>
</tr>
</tbody>
</table>

Note: Compared with the normal control group, *$P<0.05$.

Effects of PZZS on Hemodynamic Parameters in the Adriamycin-induced CHF Rats

Compared with the control group, SBP, DBP, MAP, HR, LVSP, $dp/dt_{max}$ significantly decreased, LVEDP significantly increased in the model group. PZZS could significantly improve vasomotoricity and the left ventricular function of the rats with CHF; while captopril did not have significant effects on vasomotoricity and the left ventricular function of the rats with CHF (Table 2).

Table 2. Comparison of hemodynamic indexes of CHF rats in the groups ($\bar{x} \pm s, n=8$)

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR (beatss/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>117.25±16.21</td>
<td>100.87±19.10</td>
<td>113.10±15.14</td>
<td>428.72±33.35</td>
</tr>
<tr>
<td>Model</td>
<td>94.18±20.01</td>
<td>71.00±23.67</td>
<td>76.83±11.88</td>
<td>373.38±41.24</td>
</tr>
<tr>
<td>Captopril</td>
<td>107.95±28.73</td>
<td>88.07±30.41</td>
<td>102.71±29.49</td>
<td>406.8±58.41</td>
</tr>
<tr>
<td>PZZS</td>
<td>123.70±14.35</td>
<td>101.71±21.29</td>
<td>116.33±15.94</td>
<td>423.99±46.17</td>
</tr>
</tbody>
</table>

Notes: Compared with the control group, *$P<0.05$ and **$P<0.01$; Compared with the model group, $^*P<0.05$ and $^{**}P<0.01$.

Effects of PZZS on Blood CK, SOD, MDA, NO and NOS in the Adriamycin-induced CHF Rats

Compared with the normal control group, CK and NOS activities, MDA and NO levels significantly increased, SOD activity significantly decreased in the model group; compared with the model group, blood CK activity, MDA and NO levels significantly decreased in the captopril group, and MDA and NO levels, NOS activity significantly decreased in the PZZS group (Table 3).

Table 3. Comparison of Blood CK, SOD, MDA, NO and NOS of the rat in the groups ($\bar{x} \pm s, n=8$)

<table>
<thead>
<tr>
<th>Group</th>
<th>CK (U/mL)</th>
<th>SOD (U/mgprot)</th>
<th>MDA (nmol/mL)</th>
<th>NO (umol/L)</th>
<th>NOS (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.46±0.17</td>
<td>89.10±8.44</td>
<td>5.51±0.96</td>
<td>23.44±1.52</td>
<td>25.32±3.57</td>
</tr>
<tr>
<td>Model</td>
<td>0.91±0.17</td>
<td>76.46±2.97</td>
<td>15.21±2.80</td>
<td>32.76±3.64</td>
<td>33.70±4.51</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.47±0.11</td>
<td>82.77±8.08</td>
<td>7.31±1.89</td>
<td>25.71±6.30</td>
<td>31.34±2.24</td>
</tr>
<tr>
<td>PZZS</td>
<td>0.59±0.44</td>
<td>60.81±8.60</td>
<td>9.55±2.52</td>
<td>25.04±3.45</td>
<td>26.33±4.37</td>
</tr>
</tbody>
</table>

Notes: Compared with the normal group, *$P<0.05$; Compared with the model group, $^*P<0.05$ and $^{**}P<0.01$.

Myocardial Histomorphologic Comparison

Gross inspection showed that the volume of the heart was normal with red color, smooth surface and without any spot in the normal control group; the volume of the heart enlarged significantly in the model group, but it was reduced in the medication groups as compared with the model group.

Comparison of Ultrastructures of the Cardiac Muscle Cells

It was found with transmission electron microscope that the mitochondria were intact and neat in the normal control group; the mitochondria were rarefaction and significantly reduced, and the mitochondria of a part of cardiac muscle cells broken with mitochondrial crista cracked and indistinct structures, and for another part of mitochondria, the volume reduced in the model group; a part of mitochondrial structures was injured with both loose arrangement and dense arrangement in the PZZS group; the mitochondrial arrangement was more loose, but dense arrangement could be seen in the captopril group (Figure 1–4).
Comparison of the Cardiac Muscle Cell Forms
Observation of myocardial tissue paraffin sections with HE staining under microscope showed that the myocardial form was normal and the arrangement was in good order with clear plasma trip in rats of the normal control group; in the model group, the nuclei of a part of cardiac muscle cells were significantly larger than those in the normal control group, with myocardial fibers thickened and the cytoplasm stained darkly; different hypertrophic degree’s cardiac muscle cell groups were seen with cellular irregular arrangement, breaking, resolving, inflammatory infiltration in the PZZS group and the captopril group, which were milder than those in the model group (Figure 5–12).
DISCUSSION

Hemodynamic parameters are the important indexes reflecting cardiac functions. Polysaccharides extracted from Zhu Zi Shen (Rhizoma Panacis Majoris) can strengthen diastolic and contractile functions of the artery and left ventricle, and significantly improve the left ventricular function with no change of heart rate, showing better effects than that of captopril.

Oxidative stress is one of the key factors for heart failure. In heart failure, a large number of active oxygen families and nitric oxide are produced. A great number of
free radicals induce lipid peroxidation, injuring the cellular membrane and inducing inflammation and cell apoptosis. In physiologic state, the organism has an anti-oxidation system, for example SOD can clear away superoxide anions in time, and reduce lipid peroxidation. In physiologic state, NO has the function of dilating blood vessels, but a great quantity of NO can not only induce production of free radicals, but also mediate serious neurotoxicity and cytotoxicity, promoting injury of tissues. Synthesis of NO needs participation of NOS. It was found in the experiment that polysaccharides extracted from Zhu Zi Shen (Rhizoma Panacis Majoris) could significantly decrease the blood MDA and NO levels, and CK and NOS activities, but it did not effect on blood SOD activity. Therefore, it is considered that the improvement of oxidative stress state by Zhu Zi Shen (Rhizoma Panacis Majoris) in CHF rats is not carried out by SOD.

In brief, the present study indicates that polysaccharides extracted from Zhu Zi Shen (Rhizoma Panacis Majoris) can improve chronic congestive heart failure in the CHF model rats, which is possibly related with alleviation of oxidative stress and improvement of the left ventricular function.

REFERENCES


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