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Original Research Article

Effect of Zhen-wu decoction on chronic heart failure in rats

Zhongyong Liu, Lin Li*, Shihua Luo and Jia Fang
Department of Cardiovascular, The Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Jiangxi Province, Nanchang, China

For correspondence:* ***Email:* *lilin494133@sina.com;* ***Tel:*** *+86 0791-86362753*

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Abstract

Purpose: *To investigate the effect of Zhen-wu decoction (ZWD) on oxidative stress and hemodynamics in chronic congestive heart failure (CHF) rats.*
Methods: *After Sprague Dawley (SD) rats were successfully prepared into CHF, they were randomly divided into normal control group, model (untreated CHF) group, captopril group, high-dose, middle-dose and low-dose of ZWD groups, and were treated with drugs for 4 weeks respectively. At the end of the experiment, hemodynamic function, whole heart weight index, blood creatinine kinase (CK), superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and nitric oxide synthase (NOS) were determined.*
Results: *Compared with normal control group, ZWD group showed decreased arterial systolic pressure (SBP, 89.16 ± 17.27 mmHg), diastolic pressure (DBP, 72.54 ± 22.36 mmHg), mean arterial pressure (MAP, 72.64 ± 11.87 mmHg), heart rate (HR, 368.25 ± 39.12 beats/min), left ventricular systolic peak (LVSP, 105.27 ± 15.23 mmHg), and left ventricular pressure change rate (dp/dt max) ($p < 0.05$), while left ventricular end diastolic pressure (LVEDP) (19.52 ± 1.89 mmHg), whole heart weight index (2.74 ± 0.16 mg/g), blood CK (0.98 ± 0.16 U/mL), MDA (17.28 ± 2.94 nmol/mL), NO (36.35 ± 3.27 umol/L), NOS (39.89 ± 3.56 U/mL) significantly increased ($p < 0.05$). High dose of ZWD significantly improved hemodynamic function, lowered MDA (8.85 ± 2.14 nmol/mL) and NO (24.25 ± 3.21 umol/L) levels ($p < 0.05$), and also decreased CK (0.58 ± 0.37 U/mL) and NOS (26.12 ± 3.87 U/mL) in CHF rats ($p < 0.05$).*
Conclusion: *ZWD improves adriamycin-induced chronic congestive heart failure in rats significantly, and therefore has potential to be developed for the management of chronic congestive heart failure.*

Keywords: *Zhen-wu decoction, Chronic heart failure, Hemodynamic function, Oxidative stress*

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INTRODUCTION

Chronic heart failure (CHF) is a common, complex clinical syndrome that arises from structural or functional cardiac disorder, including changes in electrophysiology, contraction, and energy metabolism [1]. Heart failure (HF) is becoming an increasing disease with an incidence approaching 1 % of the population over 65 years of age in developed countries [2]. In China, it was reported that the prevalence of HF in the adult population from ten provinces was 0.9 % [3]. The prognosis for CHF is poor and there are few therapeutic options, HF is even worse than many types of cancer [4]. Furthermore, there has been an increased hospitalization burden, and makes HF a global public health problem.

The most effective and commonly used drugs for treatment of HF are angiotensin-converting enzyme (ACE) inhibitors, β-adrenoceptor blockers, and digitalis [5-7]. The American Heart Association (AHA) and European Society of

Cardiology (ESC) have issued and updated the guidelines for diagnosis and management of CHF [2]. However, HF is still a leading cause of death worldwide [8], therefore it is necessary to seek novel effective drugs for HF. Traditional Chinese Medicine has gained popularity in the treatment of complex multifactor diseases by targeting multiple pathways to improve therapeutic efficacy and could reduce drug-related side effects and drug resistance. TCMs such as *Shengmai* [9], *Sini* decoction [10], *Shuanglong* formula [11], and *Huangqi* injection [12] have potential therapeutic effects in the treatment of cardiovascular diseases.

Zhen-wu decoction(ZWD) have the effects of promoting blood circulation and removing blood stasis, tonifying blood, arresting bleeding, and alleviating pain [13,14]. However, to the best of our knowledge, no studies on the therapeutic effect of *Zhen-wu* decoction on cardiovascular diseases have been reported.

In this work, the effect of ZWD on chronic congestive heart failure (CHF) was studied in adriamycin-induced CHF rats.

EXPERIMENTAL

Preparation of Zhen-wu decoction

Zhen-wu decoction was composed of Fuling (*Poria cocos (Schw.) Wolf)* 15 g, Baishao (*Paeonia lactiflora Pall.)* 15 g, Shengjiang (*Zingiber officinale Roscoe)* 15 g, Baizhu (*Atractylodes macrocephala Koidz.)* 10 g, Fupian (*Aconitum carmichaeli Debx*.) 7 g. These herbs were mixed, and decocted with 6000 mL of water twice, 45 min on each occasion. *After filtering and concentrating the* decoction, the *concentration of ZWD was* 480 mg/mL.

Animals

SD male rats, weighing (180 ± 20) g, was purchased from the Experimental Animal Center of Jiangxi Province (Certificate no. SYXK 2006-0001). Each rat was raised in single cage at a temperature of (20 ± 2) ^{o}C and relative humidity of 55 - 65 %. They were fed on rodent feed and had free access to water.

The rat experiment was approved by the Animal Care and Use Committee of Jiangxi University of Traditional Chinese Medicine (approval ref no. 20101005) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [15].

Preparation of chronic heart failure rats and treatment

Sixty SD rats were divided into normal control group, model (untreated CHF) group, captopril group, high-dose, middle-dose and low-dose of Zhen-wu decoction groups. There were 10 rats in each group. Normal control group was treated with intraperitoneal injection of 0.2 mL saline once a week. Other rats were administered intraperitoneal injection of adriamycin hydrochloride (2 mg/kg) once a week for 6 weeks. Six weeks later, 2 rats were randomly selected from the rats which survived heart failure while rats without CHF were further treated with adriamycin hydrochloride for 4 weeks. Treatments commenced from the 7th week after CHF was established. 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 ZWD group were administered intragastrically ZWD (1.2, 2.4 or 4.8 g/kg) daily. The rats in the captopril group were administered captopril (100 mg/kg) daily. The treatment lasted for 4 weeks.

Determination of cardiac function and hemodynamics

Twenty four hours after the last intragastrical administration, the rats were anesthetized by intraperitoneal injection of 20 % urethane solution in 6 mL/kg, and were fixed on a table in a spinal position. The right common carotid artery was separated with a ventricular canula (cardiac catheter l mm in diameter), which was connected with Biopac multichannel physiologic sign collection and processing system via a pressure transducer; the SBP, DBP, MAP, HR were recorded. The cardiac catheter was slowly pushed, and at the same time the pressure oscilloscope was observed. If the wave form changed and pulse pressure increased, it indicates that the cardiac catheter has entered into the left ventricle and cannot go further. After stabilization for 3 min, LVSP, LVEDP, + dp/dt_{max}, and - dp/dt_{max} were recorded. For the hemodynamic indices, 5 sections were taken for calculation of the mean value. Thereafter, 10 mL blood was taken from the abdominal aorta, 5 mL added with 200 uL $EDTANa_2$ and 5 mL with no anti-coagulant, which were centrifuged at 3000 rpm for 15 min. The plasma and serum were kept at -20 ^{o}C for other index detection.

Determination of heart weight index

After assessment of hemodynamic function and blood sampling, the heart was rapidly separated with the blood stain washed with saline and

water blotted with a filter paper. The whole heart was weighed, and whole heart weight index was calculated as the ratio of heart weight (mg) to body weight of the rat.

Biochemical profile

CK, MDA, SOD, NO and NOS in plasma and serum were determined according to the instructions that accompanied the kits (Shenzhen Xin Bo Sheng Biological Technology Co., Ltd., Shenzhen, China).

Data analysis

All the data were analyzed using Statistical Package SPSS 16.0 (SPSS Inc, Illinois, Chicago, USA) and are expressed as mean ± standard error of mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effects of ZWD on whole heart weight index of CHF rats

The heart weight index (HWI) of the model group was higher than that in the normal control group ($p < 0.05$), indicating that there were myocardial hypertrophy or stasis of blood in the model group. Compared with model group, HWI was not significantly changed in captopril and ZWD treatment groups (Table 1).

Table 1: Heart weight index of CHF rats (n = 10)

Group	Dose(g/kg)	HWI(mg/g)
Normal	-	2.23±0.24*
Model	-	2.74±0.16
Captopril	0.1	2.46±0.25
ZWD-L	1.2	2.66±0.37
ZWD-M	2.4	2.56±0.31
ZWD-H	4.8	2.52±0.28

*$P < 0.05$, **$p < 0.01$ compared with model group; ZWD-L: low dose of ZWD, ZWD-M: medium dose of ZWD, ZWD-H: high dose of ZWD

Effect of ZWD on hemodynamic parameters in CHF rats

Compared with the normal control group, SBP, DBP, MAP, HR, LVSP, dp/dt_{max} were significantly decreased ($p < 0.05$), and LVEDP was significantly increased in the model group ($p < 0.01$). ZWD significantly improved vasomotor and left ventricular functions of CHF rats ($p < 0.05$), while captopril did not (Table 2 and Table 3).

Effect of ZWD on blood CK, SOD, MDA, NO and NOS in CHF rats

Compared with the normal control group, CK and NOS activities, MDA and NO levels were significantly increased ($p < 0.01$),

Table 2: Hemodynamic index of CHF rats (n = 10)

Group	Dose (g/kg)	SBP (mmHg)	SBP (mmHg)	MAP (mmHg)	HR (beat/min)
Normal	—	121.34±14.25*	102.36±17.35*	108.34±12.46*	435.27±32.41*
Model	—	89.16±17.27	72.54±22.36	72.64±11.87	368.25±39.12
Captopril	0.1	112.23±25.64	90.24±31.25	99.37±27.25	412.37±53.32
ZWD-L	1.2	94.38±21.17	79.36±28.46	79.32±17.38	383.43±35.26
ZWD-M	2.4	108.38±18.27*	95.33±19.65	96.38±15.29	392.35±32.67
ZWD-H	4.8	125.48±12.52**	100.39±19.38**	107.38±13.83**	426.33±40.67*

*$P < 0.05$, **$p < 0.01$ compared with model group; ZWD-L: low dose of ZWD, ZWD-M: medium dose of ZWD, ZWD-H: high dose of ZWD*

Table 3: Hemodynamic index of CHF rats (n = 10)

Group	Dose (g/kg)	LVSP (mmHg)	LVEDP (mmHg)	$+dp/dt_{max}$	$-dp/dt_{max}$
Normal	—	146.34±13.65*	7.32±1.35**	3621.35±426.37*	3832.45±425.65*
Model	—	105.27±15.23	19.52±1.89	2236.24±658.91	2436.71±672.36
Captopril	0.1	112.24±33.65	17.38±2.43	3412.56±835.62	3322.14±721.33
ZWD-L	1.2	114.33±18.25	17.37±2.12	2673.26±563.21	2638.53±654.83
ZWD-M	2.4	120.38±16.54*	15.39±2.86	3126.37±497.85*	3267.12±547.35*
ZWD-H	4.8	141.27±14.27*	10.26±2.43*	3587.26±526.37*	3789.47±524.45*

*$P < 0.05$, **$p < 0.01$ compared with model group; ZWD-L: low dose of ZWD, ZWD-M: medium dose of ZWD, ZWD-H: high dose of ZWD*

Table 4: Blood CK, SOD, MDA, NO and NOS of the rats (n=10)

Group	Dose(g/kg)	CK(U/mL)	SOD(U/mgprot)	MDA(nmol/mL)	NO(umol/L)	NOS (U/mL)
Normal	-	0.42±0.14**	92.31±7.35**	6.14±1.27**	21.26±1.78**	24.41±3.23**
Model	-	0.98±0.16	70.33±3.56	17.28±2.94	36.35±3.27	39.89±3.56
Captopril	0.1	0.45±0.12**	81.26±8.25	8.11±1.58**	23.44±3.58**	35.64±2.98
ZWD-L	1.2	0.78±0.24	71.84±7.26	12.36±2.64	31.42±4.22	37.38±4.15
ZWD-M	2.4	0.63±0.27	74.28±7.32	10.24±2.37	28.38±3.86*	34.43±3.78
ZWD-H	4.8	0.58±0.37	75.36±6.37	8.85±2.14**	24.25±3.21**	26.12±3.87**

*$P < 0.05$, **$p < 0.01$ compared with model group; ZWD-L: low dose of ZWD, ZWD-M: medium dose of ZWD, ZWD-H: high dose of ZWD*

and SOD activity was significantly decreased in the model group ($p < 0.01$). Compared with the model group, blood CK activity, MDA and NO levels were significantly decreased in the captopril group ($p < 0.01$), and MDA and NO levels, NOS activity significantly decreased in the ZWD group ($p < 0.01$, Table 4).

DISCUSSION

Hemodynamic parameters are important indices reflecting cardiac functions. Zhen-wu decoction strengthened the diastolic and contractile functions of the artery and left ventricle, and significantly improved the left ventricular function with no change of heart rate, showing better effects than that of captopril.

Oxidative stress is one key factor for heart failure [16]. In heart failure, a large quantity of reactive oxygen species and nitric oxide are produced. A lot of free radicals induce lipid peroxidation, injuring the cellular membrane and inducing inflammation and cell apoptosis. In the 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. However, a large quantity of NO did not only induce the production of free radicals, but also mediated serious neurotoxicity and cytotoxicity, promoting injury of tissues. Synthesis of NO requires the participation of NOS [17]. It was observed that ZWD significantly decreased blood MDA and NO levels, as well as CK and NOS activities, without affecting blood SOD activity. Therefore, it is seems that the improvement in oxidative stress state by ZWD in CHF rats does not involve SOD.

CONCLUSION

The findings of this study indicate that ZWD improves chronic congestive heart failure in CHF model rats, and this is possibly related to the alleviation of oxidative stress and improvement in left ventricular function. Thus, ZWD has the potential to be developed as an alternative medicine for the treatment of chronic heart failure in future.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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