Original Research Article

Effect of Shensong Yangxin on heart failure in preserved ejection fraction rat model

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Abstract

Purpose: To investigate the interventional effect of ShensongYangxin (SSYX) on heart failure (HF) using preserved ejection fraction (HFpEF) rat model.
Methods: HFpEF rat model was established using abdominal aorta coarctation method and randomly divided into a positive drug control group; SSYX at high, medium, and low dosage groups; and normal control group. After 8 weeks oral treatment of SSYX, echocardiography and cardiac catheterization were used to investigate the effects of SSYX on HFpEF rat cardiac functions, including mean heart rate (HR), left ventricle anterior and posterior wall thicknesses at end diastole (LVAWd + LVPWd), left ventricular internal diameter at end diastole (LVIDd), and left ventricle mass (LVM).
Results: SSYX markedly decreased heart weight and improved survival rate (p < 0.01) after 12 weeks of treatment. The expression of NT-proBNP decreased in a dose-dependent manner and was significantly lower in SSYX treatment groups (p < 0.01). Compared with normal control group, expression of CaMK II, PKA and RyR2 was significantly lower (p < 0.005), while expression level of SERCA2a significantly increased after 4 g/kg/day SSYX treatment (p < 0.01).
Conclusion: SSYX significantly attenuates HFpEF-induced cardiac dysfunction and increases survival rate, suggesting that SSYX may prevent HF via regulation of cytoplasmic Ca^{2+} handling. SSYX reduces plasma NT-proBNP levels, lending support for its therapeutic potential in HF management.

Keywords: Heart failure, Ejection fraction, ShensongYangxin

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is predominantly found in older adults with hypertension, diabetes mellitus, and atrial fibrillation [1,2]. HFpEF causes various cardiovascular complications related to diastolic left ventricular (LV) dysfunction [3]. HFpEF accounts for 50 % of HF cases, and its prevalence is rising at an alarming rate of 1 % per year [4].. In contrast to HFrEF, some studies on neurohumoral suppression in HFpEF failed to reach a positive outcome [6]. The diagnosis of HFpEF involves typical symptoms of HF such as the LV ejection fraction (LVEF) LVEF > 50, left ventricular end-diastolic volume index (LVEDVI) < 97 ml/m^2, left ventricular end-diastolic pressure (LVEDP) > 16 mmHg or pulmonary artery wedge

pressure (PCWP) > 12 mmHg, and Doppler mitral flow spectrum:peak diastolic velocity/early diastolic velocity (E/E') > 15. When the value of E/E' is between 8 and 15, other non-invasive evidence of LV diastolic dysfunctions such as peak E deceleration time or pulmonary venous flow spectrum analyses is necessary [7].

The alteration of cardiac structure has been associated with aberrant expression of calmodulin-dependent protein kinase II (CaMK II), sarcoplasmic reticulum cata-ATPase (SERCA2a), protein kinase A (PKA), phospholamban (PLB), and ryanodine receptor 2 (RyR2 receptor) [8]. In normal cardiomyocytes, phosphorylation of phosphoproteins during diastolic phase and dissociation from SERCA2a enhances the affinity between SERCA2a and Ca^{2+} [9]. When phosphorylated PLB binds SERCA2a, the affinity of SERCA2a for Ca^{2+} is decreased [10].

Brain natriuretic peptide (BNP) is a cardiovascular peptide hormone, mainly secreted by the ventricle, which can sensitively reflect LV functional changes [11]. When the cardiac volume load or pressure load increases, BNP is synthesized in the heart and secreted into the bloodstream, which increases the plasma levels of BNP[12].

The traditional Chinese medicine, *ShensongYangxin* capsules (SSYX), has been used effectively to treat cardiovascular disease. The bioactive components of SSYX include sodium *danshensu* (sodium 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate), chlorogenic acid, and gensenoside Rb1 [13]. However, the effects of SSYX on HFpEF remains unclear. The aim of this study was therefore to investigate the intervening effect of *SensongYangxi* in the HFpEF rat model to provide pharmacological evidence for its clinical application in HFpEF.

EXPERIMENTAL

Establishment of HFpEF animal model and treatments

Healthy Sprague-Dawley male rats weighing 180–240 g were purchased from Changzhou Experimental Animal Co. (China). This animal study was approved by the ethics committee of Dezhou People's Hospital (approval no. 20180521), and the guidelines for the use of experimental animals of the National Institutes of Health were followed [14]. The animals were kept under standard laboratory conditions (temperature: 22 °C; 12 h/12 h day-night cycle) and divided randomly into seven groups (each

group, n = 8): normal control, positive control (HFpEF rats), HFpEF rat + 4 g/kg SSYX, HFpEF rat + 0.8 g/kg SSYX, HFpEF rat + 0.2 g/kg SSYX, f) HFpEF rat + 0.5 g/kg metoprolol, and HFpEF rat + 0.5 g/kg metoprolol+ 0.8 g/kg SSYX. HFpEF rats were established as described previously [15].

Following standard methodology [16], the rats were fixed in a supine position after intraperitoneal anesthesia (20 % uratan injection of 5 mL/kg). The abdominal aorta was separated using a longitudinal incision on the median line of abdomen, and was ligated. In the sham operation group, the abdominal aorta and left renal artery were separated and threaded but not ligated. Penicillin (200,000 U/day for 3 days, intramuscular injection) was used to prevent infection, and the rats were fed with standard diet.

Reagents and preparation of SSYX

ShensongYangxin (SSYX), purchased from Shijiazhuang Yiling Pharmaceutical (Shijiazhuang, China), was resuspended in double-distilled water and was used to treat the rats. The SSYX was prepared daily. The herbal drug was authenticated and standardized based on marker compounds according to the Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2005).

Assessment of cardiac function

All groups of animals were anesthetized by intraperitoneal injection with 5 % sodium pentobarbital sodium (45 mg/kg).Thereafter the hair was removed and the rats were placed on a thermostatic heater in a supine position. The mean heart rate (HR), left ventricle anterior and posterior wall thicknesses at end diastole (LVAWd+LVPWd), left ventricular internal diameter at end diastole (LVIDd), left ventricle mass (LVM), left ventricular ejection fraction (EF %), and left ventricular short-axis contraction rate were measured using echocardiography (Visual Sonics, Toronto, Canada).The mean values of the measurements were calculated.

Western blot analysis

Heart tissues (30 µg) were homogenized in lysis buffer and protein concentration was assessed using the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA, USA). The protein samples were then resolved using 10 % SDS polyacrylamide gels and transferred to Immobilon-P transfer membranes and incubated in blocking solution (4 % non-fat milk). The

membranes were probed overnight with a 1:2,000 dilution of primary antibodies to: CaMK II (Abcam, Cambridge, MA, USA), PKA (Santa Cruz Biotechnology, Santa Cruz, CA, USA), RyR2 (Abcam), and SERCA2a (Abcam). Glyceraldehyde 3-phosphate dehydrogenase (Abcam) was used as an internal control. The blotted proteins were detected using a chemiluminescent system.

Statistical analysis

Data are presented as mean ± standard deviation (SD). The comparison between multiple groups was performed using one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$. SPSS software for Windows, version 17.0 (SPSS, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Effect of SSYX on survival rates and cardiac weight of HFpEF rats

To evaluate the effect of SSYX on HF, HFpEF rats were used to establish the HR model. The rat diastolic LV function was significantly increased, and the cardiovascular reserve function was impaired, indicating successful establishment of the HFpEF model. The survival rate after 12 weeks of treatment was analyzed, which showed a dose-dependent increase and the 12-week survival rate was significantly higher in the SSYX 4 g/kg/day group than in the model group (Figure 1A).

In addition, compared with the SSYX 4 g/kg/day group, the survival rate was significantly increased in the 0.5 g/kg/day metoprolol + 0.8 g/kg/d SSYX group. Moreover, Figure 1B shows that the heart weights were significantly decreased in a dose-dependent manner with SSYX treatment. The heart weight in the 0.5 g/kg/day metoprolol + 0.8 g/kg/d SSYX group was found to be lower than that of the 4 g/kg/day SSYX or 0.5 g/kg/day metoprolol groups. These results suggest that SSYX may confer protective effect against HF, and the combination of metoprolol and SSYX exerts synergic protective effects against HF.

Effect of SSYX on cardiac function in HFpEF rats

To determine whether SSYX regulates cardiac functions in HF, the mean HR, LVAWd + LVPWd, LVIDd, LVM, EF %, and FS % were assessed using echocardiographic parameters. The data

for different groups of HFpEF rats are summarized in Table 1. Compared with the sham group, the HR, LVAWd + LVPWd, LVIDd, and LVM were significantly increased in the model group, which were accompanied by lower EF % and FS %. SSYX treatment induced a significant decrease in HR, LVAWd + LVPWd, LVIDd, and LVM, which were associated with increased EF% and FS%. Furthermore, treatment with 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX reversed the effects induced by HF. These results indicate that SSYX is able to regulate cardiac function during HF.
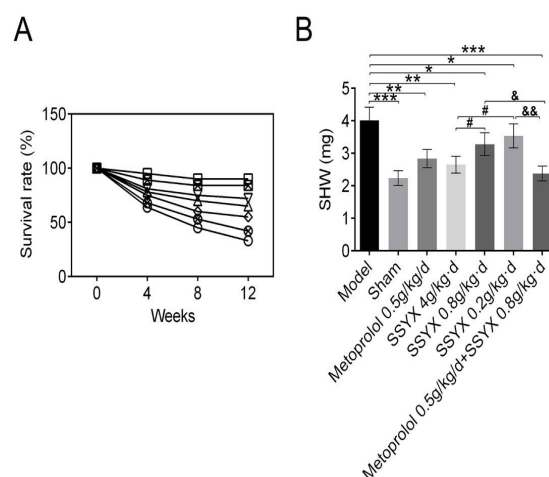

Figure 1: Cardioprotective effects of SSYX on survival rate and cardiac weight in HFpEF rats. (A) The survival rate in different treatment groups. ⊠: 0.5 g/kg/day metoprolol + 0.8 g/kg/day SSYX; ⊗: 0.2 g/kg/day SSYX; ◇: 0.8 g/kg/day SSYX; ▽: 4.0 g/kg/day SSYX; △: 0.5 g/kg/day metoprolol; □: sham; ○: model; (B) The cardiac weight in different treatment groups. Data are presented as mean ± SD, using Student's *t*-test; [*] compared with the model; [#] compared with 4 g/kg/d SSYX, and compared with 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX ([*]$p < 0.05$, [**]$p < 0.01$, [***]$p < 0.005$, [#]$p < 0.05$, [&]$p < 0.05$, and [&&]$p < 0.01$)

SSYX inhibits NT-proBNP expression in HFpEF rats

N-terminal brain natriuretic peptide (NT-proBNP) and BNP are peptide hormones released predominantly by ventricular myocytes. The specific NT-proBNP detection assay is therefore useful for the diagnosis of HF. As shown in Table 2, plasma NT-proBNP levels in the model group were significantly higher than that of the sham group. The expression of NT-proBNP decreased in a dose-dependent manner and was significantly lower in the 4 g/kg/d SSYX group than in the model group. Similarly, NT-proBNP levels decreased significantly in the 0.5 g/kg/d metoprolol + 0.8 g/kg/day SSYX groups.

Table 1: Effect of SSYX on echocardiographic parameters

Group	HR (bmp)	EF%	FS%	LVAWd+LV PWd (mm)	LVIDd (mm)	LVM (mg)
Control	335±21	50.14±5.06	21.69±2.74	4.32±0.41	55.69±5.68	1185.18±106.17
Sham	304±27**	68.70±7.24**	40.58±4.01**	3.21±0.31*	27.74±2.14**	820.63±125.64**
Metoprolol (0.5 g/kg/d)	327±22*	62.45±6.55*	30.54±3.01*	3.66±0.35*	38.12±3.55*	1039±130.15*
SSYX (4 g/kg/d)	310±20*	64.50±6.85*	32.55±3.21*	3.56±0.34*	33.66±4.21*	852±163.89*
SSYX (0.8 g/kg/d)	325±23*	60.12±6.38*#&	28.36±3.01*#&	3.71±0.36*&	39.26±3.02*&	1055±189.26*&&
SSYX (0.2 g/kg/d)	330±10	57.33±6.34*#&&	26.44±2.78*#&	4.02±0.40#&	43.55±4.31*#&	1100±169.29*#&
Metoprolol (0.5 g/kg/d) + SSYX (0.8 g/kg/d)	308±20**	64.25±6.15**	38.54±3.56**	3.35±0.33*	31.67±5.61**	830.45±100.25**

Compared with control group, #compared with 4 g/kg/d SSYX group, & compared with 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX group (*p < 0.05, **p < 0.01, #p < 0.05, &p < 0.05, and &&p < 0.01)

Table 2: Effect of SSYX on NT-proBNP plasma levels

Group	NT-proBNP /ng L^{-1}
Control	303.45±10.26
Sham	185.46±18.49**
Metoprolol (0.5 g/kg/d)	256.39±14.58*
SSYX (4 g/kg/d)	229.35±13.92*
SSYX (0.8 g/kg/d)	250.26±15.18*#&
SSYX (0.2 g/kg/d)	286.89±20.34#&
Metoprolol (0.5 g/kg/d + SSYX 0.8 g/kg/d)	196.42±12.46**

Compared with the control group, #compared with 4 g/kg/d SSYX, & compared with 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX group (*p < 0.05, **p < 0.01, #p < 0.05, &p < 0.05)

Effect of SSYX on Ca^{2+}/calmodulin-dependent protein expression in HFpEF rats

To determine whether CaMK II, PKA, RyR2, and SERCA2a are involved in the regulation of HF, the expression of these proteins was detected by western blotting. As shown in Figure 2, compared with the model group, the expression levels of CaMK II, PKA, and RyR2 were significantly decreased (Figure 2 A, B and Figure 3A). Furthermore, the expression level of SERCA2a was significantly increased after 4 g/kg/day SSYX treatment (Figure3 B). In accordance with the SSYX treatment, the 0.5 g/kg/day metoprolol + 0.8 g/kg/day SSYX treatment group also increased CaMK II, PKA, and RyR2 expression and enhanced the expressions of SERCA2a. These data provide further evidence that SSYX may be able to prevent HF.

DISCUSSION

Heart failure is a major public health problem in the world, and continues to increase in prevalence at an alarming rate [17]. This study first determined whether SSYX could prevent HF. The results showed that SSYX had a protective effect on HFpEF, and that the combination of metoprolol and SSYX exerted a synergic protective effect on HF. These findings are consistent with previous results which showed that SSYX effectively improved the arrhythmic in the heart [18].
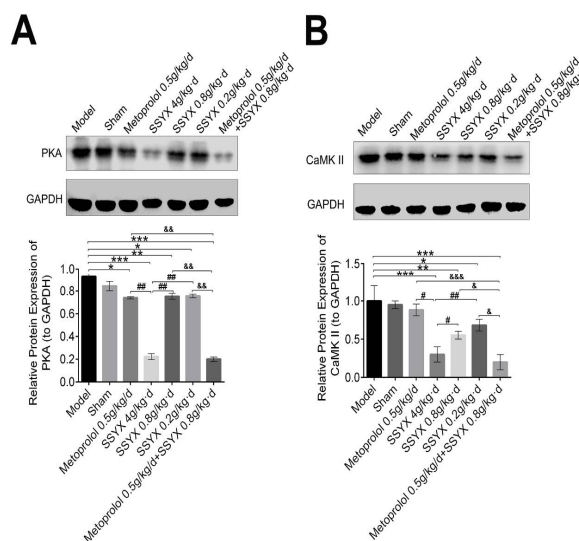

Figure 2: Effect of SSYX on CaMK II and PKA expression in HFpEF rats. (A, B) Western blot data show suppression of PKA and CaMK II after treatment with SSYX and metoprolol. Statistical analysis was performed according to the Material and Methods. Data are presented as the mean ± SD, using Student's *t*-test, *Compared with the model group, # compared with the 4 g/kg/d SSYX group, and compared with the 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX group (*p < 0.05, **p < 0.01, ***p < 0.005, #p < 0.05, &p < 0.05, and &&p < 0.01)
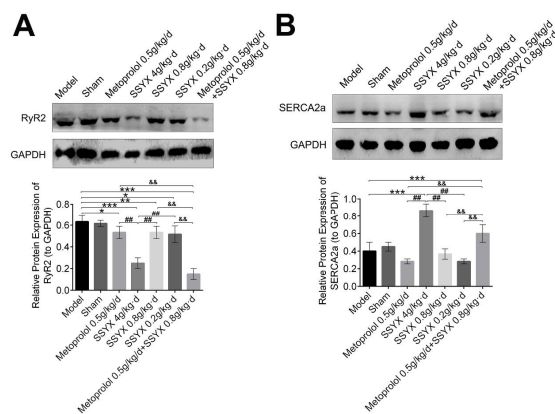
Figure 3: Effect of SSYX on RyR2 and SERCA2a expression in HFpEF rats. (A) Western blots show suppression of RyR2 levels after treatment with SSYX and metoprolol. (B) Western blots show increased SERCA2a expression after SSYX and metoprolol treatment. Statistical analysis was performed according to the Material and Methods. Data are reported as the mean ± SD, using Student's *t*-test, * Compared with the model group, # compared with the 4 g/kg/d SSYX group, and compared with the 0.5 g/kg/d metoprolol + 0.8 g/kg/d SSYX group (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, #$p < 0.05$, &$p < 0.05$, and &&$p < 0.01$)

To evaluate the effects of SSYX on cardiac function, the mean HR, LVAWd + LVPWd, LVIDd, and LVM were assessed using echocardiography. Subsequently, the heart weight in different treatment groups was also investigated. Treatment with SSYX reversed the effects induced by HF and decreased the heart weight, which was increased by HFpEF. Furthermore, the expression of NT-proBNP was decreased with SSYX treatment. The results showed that SSYX might be able to regulate cardiac function during HF in human patients. CaMK II is involved in some key aspects of acute cellular Ca^{2+} handling related to cardiac function [19]. CaMKII activity changes in the myocardium during HF, enhances the activity of RyR2, and promotes the release of Ca^{2+} from the sarcoplasmic reticulum [9,20].

These results therefore determined whether the effect of Ca^{2+}-mediated cellular responses could assist the diagnosis of cardiac hypertrophy and HF. In the present study, when SSYX was used to treat different groups of HFpEF rats, the expression levels of CaMKII, PKA, and RyR2 were significantly decreased and SERCA2a was significantly increased. These results are consistent with previous studies showing that the Ca^{2+}-ATPase mRNA levels for SERCA2a were decreased in LV failure and that SSYX was able to prevent HF by regulating cytoplasmic Ca^{2+} levels[21].

CONCLUSION

These findings show that SSYX significantly attenuates HFpEF-induced cardiac dysfunction and thus increases survival rate in rats. SSYX prevents HF by regulating cytoplasmic Ca^{2+} handling and inhibiting the expression of NT-proBNP. Therefore, these results provide a possible new therapeutic strategy for the treatment of HFpEF.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We 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 the authors. Weiwei Zhang and Qian Sun designed all the experiments and revised the paper. HaoChen performed the experiments, Qiang Zhang as corresponding author wrote the paper and approved the final version of the manuscript.

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