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ScienceDirect
Chinese Journal of Natural Medicines 2015, **13**(10): 0743−0750
Chinese Journal of Natural Medicines

·Research articles ·

Sheng-Mai-San attenuates contractile dysfunction and structural damage induced by chronic intermittent hypoxia in mice

MO Wei-Lan[1], CHAI Cheng-Zhi[1], KOU Jun-Ping[1], YAN Yong-Qing[1], YU Bo-Yang[1, 2*]

[1] *Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing 211198, China;*

[2] *Jiangsu Provincial Key Laboratory for TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing 211198 , China*

Available online 20 Oct., 2015

[ABSTRACT] Sheng-Mai-San (SMS), a well-known Chinese medicinal plant formula, is widely used for the treatment of cardiac diseases characterized by deficiency of Qi and Yin syndrome. A mouse chronic intermittent hypoxia (CIH) model was established to mimic the primary clinical features of deficiency of Qi and Yin syndrome. Mice experienced CIH for 28 days (nadir 7% to peak 8% oxygen, 20 min per day), resulting in left ventricle (LV) dysfunction and structure abnormalities. After administration of SMS (0.55, 1.1, and 5.5 $g \cdot kg^{-1} \cdot d^{-1}$) for four weeks, improved cardiac function was observed, as indicated by the increase in the ejection fraction from the LV on echocardiography. SMS also preserved the structural integrity of the LV against eccentric hypotrophy, tissue vacuolization, and mitochondrial injury as measured by histology, electron microscopy, and ultrasound assessments. Mechanistically, the antioxidant effects of SMS were demonstrated; SMS was able to suppress mitochondrial apoptosis as indicated by the reduction of several pro-apoptotic factors (Bax, cytochrome *c*, and cleaved caspase-3) and up-regulation of the anti-apoptosis factor Bcl-2. In conclusion, these results demonstrate that SMS treatment can protect the structure and function of the LV and that the protective effects of this formula are associated with the regulation of the mitochondrial apoptosis pathway.

[KEY WORDS] Sheng-Mai-San; Chronic intermittent hypoxia; Contractile dysfunction; Left ventricle; Mitochondrial apoptosis

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2015)10-0743-08

Introduction

Sheng-Mai-San (SMS), a classical Chinese herbal formula, is comprised of Panax ginseng, Schisandra chinensis, and Ophiopogon japonicas [1]. Many clinical reports have demonstrated the cardio-protective effects of SMS against heart attack and chronic lesions [2-4]. Since ancient times, this formula has been widely used for the treatment of cardiovascular diseases characterized by deficiency of Qi and Yin syndrome (DQYS) [5-7]. Epidemiological studies have shown that patients with DQYS often present with contractile dysfunction and ventricular remodeling in the left ventricle (LV), which are the risk factors for myocardial ischemia, stroke, and heart failure [8-9]. However, current reports addressing heart disease in patients with DQYS, and the mechanisms of SMS to treat this type of heart disease, are inadequate.

Our previous studies have demonstrated that the macroscopic signs in a mouse model under chronic intermittent hypoxia (CIH) mimic the primary clinical features of DQYS [10]. According to previous reports, CIH models could be applied to study different diseases including sleep apnea, atherosclerosis, pulmonary hypertension, and right ventricle abnormalities, as well as left ventricle inflammation [11-13]. However, few studies have revealed how the LV changes structurally and functionally under CIH [14].

It has been reported that, under the hypoxic conditions, oxidative stress is the primary factor that induces cardiac dysfunctions, such as heart dysfunction and ventricular remodeling [15-17]. Given the well-known studies of SMS on DQYS and its antioxidant activity [1, 18], we hypothesized

[Received on] 09-Oct.-2014

[Research funding] This work was supported by National Natural Science Foundation of China (No. 81303076), the Clinical Science and Technology Project of Department of Science and Technology of Jiangsu Province (No. BL2012060), and the Eleventh Five-Year Technology Support Project (No. 2008BAI51B03).

[*Corresponding author] Tel/Fax: 86-25-86185158, E-mail: boyangyu59@163.com

These authors have no conflict of interest to declare.

that SMS could protect LV structure and function in the CIH model.

Methods

Preparation and characterization of SMS decoction

The plant ingredients of SMS, Radix Ginseng (60 g), Radix Ophiopogonis (180 g) and Fructus Schisandrae (90 g), were purchased from Nanjing Traditional Chinese Medicine Out-patient Department (Nanjing, Jiangsu, China), and authenticated by Prof. WANG Chun-Gen of Nanjing University of Chinese Medicine. The voucher specimens were deposited in Jiangsu Provincial Key Laboratory for TCM Evaluation and Translational Research. The mixture of the dried plant materials was extracted three times (1 h per extraction at 100 °C) with 1 100 880, and 660 mL of water, respectively. The combined extracts were then concentrated to approximately 100 mL and stored at −20 °C. The sample was diluted to the required concentrations with double-distilled water at room temperature. HPLC fingerprint analysis of twelve batches of SMS extracts was performed, and a similarity analysis was employed to evaluate the SMS extracts from our previous study [19]. Forty-five compounds from the fingerprint were identified by HPLC-DAD-MS/MS [18]. The prepared sample was used to determine the cardioprotective effects in mice.

Animals

ICR male mice (8 weeks old) were provided by the Experimental Animal Center of Yangzhou University, Yangzhou, China. All animals were housed in a temperature (23 ± 1 °C), humidity (30%–40%) and light controlled (in 12 h light/dark cycle) room with food and water *ad libitum*. All animal welfare and experimental procedures were complied with National Institutes of Health Guide for the Care and Use of Laboraroty Animals, and the protocols used were approved by the Animal Ethics Committee of the School of Chinese Materia Medica, China Pharmaceutical University, Nanjing, China.

Animal CIH model and treatment

The mice were randomly divided into five groups (10 mice per group) and treated with either distilled water (for the control and model groups) or oral doses of SMS (0.55, 1.1, and 5.5 $g \cdot kg^{-1} \cdot d^{-1}$). All groups, except for the control group, were exposed to CIH in a hypoxic chamber (nadir 7% to peak 8% oxygen) for 20 min per day. After 28 consecutive days, the animals were anesthetized with chloral hydrate (4% chloral hydrate, i.p.) for echocardiography. Blood and samples of the left ventricle were collected and immediately stored at −70 °C until analysis.

Echocardiography

Echocardiography was performed under anesthetized conditions (4% chloral hydrate, i.p.) using the Vevo2100 imaging system (VisualSonics Inc., Toronto, ON, Canada) with a 30-MHz probe. Stable images of the parasternal long axis view were collected in M-Model. The following structures for both end-diastole and end-systole were acquired: interventricular septum (IVS), left ventricle interior diameter (LVID), and left ventricle posterior wall (LVPW).

Blood analysis

Blood samples from the five groups were collected, treated with EDTA (1.5 $mg \cdot mL^{-1}$) and sent to the Hospital of Southeast University (Nanjing, China) for routine tests. Serum samples were obtained by centrifugation of the blood samples at 3 500 $r \cdot min^{-1}$ for 15 min at 4 °C. The malondialdehyde and heme-oxygenase-1 levels were determined using respective kits (Nanjing Jian Cheng Biotech Co., Ltd., Nanjing, China) to evaluate oxidative stress, following the manufacturer's instructions.

Histopathology

The left ventricle was fixed in 10% buffered formalin overnight and sectioned (5 μm) after embedding with paraffin. The slices were stained with hematoxylin-eosin for structural evaluation under a light microscope (DFC450 C, Leica Microsystems Ltd., Buffalo Grove, United States).

Electron microscopy

The left ventricle samples were immersed in a 4% paraformaldehyde solution containing 2.5% glutaraldehyde for 24 h. After fixing, the ultrathin sample was prepared as previously described [20], and the ultrastructure was detected using transmission electron microscopy (JEM-1001, JEOL Ltd., Tokyo, Japan).

Western blotting

The total protein from the LV samples was extracted, and the protein concentration was measured as previously described [21]. The protein samples (Control, Model, SMS 5.5 $g \cdot kg^{-1}$ [22-25]) were separated on SDS-polyacrylamide gels (15% SDS gel for Bax, Bcl-2, cytochrome c, and cleaved caspase-3; 10% SDS gel for GAPDH) and subjected to Western blotting analysis. The blots were incubated with primary antibody at 4 centigrade for twelve hours [Bax: 1 : 4 000 dilution, Bcl-2: 1 : 4 000 dilution (Abcam, Cambridge, UK); cytochrome c: 1 : 1 000 dilution, caspase-3: 1 : 1 000 dilution (Cell Signaling Technology Inc., Boston, CA, USA); GAPDH: 1 : 5 000 dilution, Biogot Technology, Nanjing, China]. The secondary antibodies used were peroxidase-conjugated goat anti-mouse IgG or anti-rabbit IgG (1 : 10 000 dilution, Biogot Technology, Nanjing, China), depending on the primary antibody. The band densities were quantified by Quantity one (Bio-rad Laboratories, Hercules, Canada).

Statistical analysis

The results are presented as the mean ± SEM. Differences between the groups were analyzed by one-way ANOVA followed by either Dunnett's posthoc test or student t test using GraphPad Prism 5.0 software (GraphPad Software, Inc., California, United States). A P value less than 0.05 was considered statistically significant.