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Original article

Cardioprotection of Sheng Mai Yin a classic formula on adriamycin induced myocardial injury in Wistar rats

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ABSTRACT

Background: Sheng Mai Yin (SMY), a well-known Chinese herbal medicine, is widely used to treat cardiac diseases characterized by the deficiency of Qi and Yin syndrome in China. SMY-based treatment has been derived from Traditional Chinese Medicine (TCM), officially recorded in the Chinese Pharmacopoeia.

Purpose: We aimed to clarify whether SMY attenuates myocardial injury induced by adriamycin in Wistar rats with chronic heart failure (CHF).

Methods: To quantify ginsenoside Rg1, ophiopogonin D, ophiopogonin D', schisandrin by HPLC. To establish CHF animal model, adriamycin was intraperitoneally injected in Wistar rats for 7 weeks at a dose of 2 mg/kg body weight. Overall, 180 rats were randomly assigned to six groups: control, CHF model, captopril (positive control), high dose (HSMY), medium dose (MSMY), and low dose (LSMY). Experimental rats were fed 0.625 mg/kg captopril and 90 mg/kg, 45 mg/kg, and 22.5 mg/kg SMY, respectively, over 7 weeks. The inflammatory cytokines TNF-α and IL-6 were measured using ELISA. Matrix metalloproteinases (MMPs) were identified using immunohistochemistry (IHC). Both IHC and RT-PCR were used for quantification of COL-IV expression levels in the heart tissues. Scanning electron microscopy (SEM) was used for the visualization of myocardium morphology.

Results: The concentration of ginsenoside Rg1, ophiopogonin D, ophiopogonin D' and schisandrin in SMY was found to be 25.63 ± 3.42 mg, 11.00 ± 1.17 mg, 7.02 ± 0.51 mg, and 25.31 ± 4.28 mg per gram of SMY, respectively. Compared with CHF model group, TNF-α levels were significantly lower ($p < .01$) in the four drug-administered groups. Moreover, except in the SYM low dose group, IL-6 levels in the other 3 drug-administered groups were also significantly reduced ($p < .01$). COL-IV expression was also significantly reduced on treatment with high SYM dose ($p < .05$). IHC results confirmed that SMY and captopril significantly reduced MMPs expression in the heart.

Conclusion: SMY could control or slow CHF progression by suppressing pathological changes in the myocardium in CHF models. This could be attributed at least partly to the downregulation of IL-6 and TNF-α and inhibition of overexpression of MMPs and COL-IV, which significantly relieved the cardiac-linked pathologies, decreased the risk of myocardial fibrosis, and inhibited cardiac remodeling. These findings suggested that SMY and captopril have similar efficacy for the treatment of adriamycin-induced myocardial injury. In addition, Chinese herbal preparation SMY may play a role in the treatment of cardiac diseases.

Introduction

Natural products possess an enormous structural and chemical diversity, which cannot be matched by synthetic libraries of small molecules and thus continue to inspire novel discoveries in chemistry, biology, and medicine. These natural chemicals have been evolutionarily optimized as drug-like molecules and remain the best known sources of drugs and drug-leads (Newman and Cragg, 2012). In the last

Abbreviations: CA, Captopril administration group; CHF, Chronic heart failure; CHFM, CHF model group; COL-IV, Collagen-IV; CON, Control group; ECM, Extracellular matrix; ELISA, Enzyme-linked immunosorbent assay; HF, Heart failure; HSMY, High dose Sheng Mai Yin administration group; IHC, Immunohistochemistry; IL-6, Interleukin-6; LSMY, Low dose Sheng Mai Yin administration group; MI, Myocardial infarction; MMP-2, Matrixmetalloproteinases-2; MMP-9, Matrixmetalloproteinases-9; MMPs, Matrix metalloproteinases; MSMY, Middle dose Sheng Mai Yin administration group; SEM, Scanning electron microscopy; SMY, Sheng Mai Yin; TCM, Traditional Chinese Medicine; TNF-α, Tumor necrosis factor-α

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decade, de-replication has emerged as a hot research topic, leading to a huge publication boom since 2012. This blending of multiple disciplines in ways that provide important and novel conceptual and/or methodological advances has opened up vast research prospects (Gaudêncio and Pereira, 2015 Leung et al., 2011). This interdisciplinary research has also led to the identification of natural products like 6,6″-biapigenin, which is only the second inhibitor discovered so far for NEDD8-activating enzyme. Importantly, 6,6″-biapigenin was found to be enzymatically active in kinetics- and cell-based assays, with a potency in the micromolar range. Fong et al. (2007) showed that the extract of the rhizomes of *Alisma orientalis* (Sam) Juzep. has synergistic growth inhibitory effect with cancer drugs that are P-glycoprotein substrates including actinomycin D, puromycin, paclitaxel, vinblastine, and doxorubicin. Zhong et al. (2015) firstly reported that natural product-like compound 1 was the first natural product-like inhibitor and only the second inhibitor overall of TLR1-TLR2 heterodimerization, as potential agents for the treatment of inflammatory and autoimmune diseases. Amentoflavone was found to be JAK2 inhibitor by structure-based virtual screening of a natural product library, and its analogues might function as Type II inhibitor of JAK2 (Ma et al., 2014). Liu et al.(2014) indicated that natural product-like compound 1inhibited STAT3 DNA-binding activity in *vitro* and attenuated STATA3-directed transcription in *cellulo* with selectivity over STAT1 and with comparable potency to the well-known STAT3 inhibitor S3I-201, and also exhibited selective anti-proliferative activity against cancer cells over normal cells. Peptidyl-proline isomerases (PPIases) played a key role in cancer, neurodegeneration, and psychiatric disorders, however, macrocyclic natural products might create potent and selective inhibitors, such as FK506, rapamycin, and cyclosporin. Manivannan et al. (2017) illustrated that twelve novel silybin analogues had significantly greater efficacy than silybin, and derivative 15k as a novel tubulin inhibitor with significant activity against ovarian cancer cells. Nature has been extremely generous to the mankind in offering life-saving therapies, and the next great drug may be just around the corner: are we ready to seize the opportunity? (Shen, 2015).

Sheng Mai Yin (SMY) is a classical natural and effective formula, which is routinely used in China, and contains Radix Ginseng (Panax ginseng C.A. Mey., Araliaceae), Radix Ophiopogon (Ophiopogon japonicas (Thumb.) Ker-Gawl., Liliaceae) and Fructus Schisandrae (Schisandraechinensis (Turcz.) Bail., Magnoliaceae) (Chen et al., 2007; Wang et al., 2005; Committee of Pharmacopoeia of PR China, 2005). ``Sheng" and ``Mai" are the Chinese abbreviations for Panax ginseng and Ophiopogon japonicas, which have both been extensively used for centuries in China as effective drugs (Chen et al., 2007; Gillis, 1997). Medicinal plants have been used in patients with congestive heart failure as well as systolic hypertension (Rastogi et al., 2016). Specifically, Panax ginseng, Fructus Schisandrae, and Ophiopogon japonicas, which contain multiple bioactive components, have been shown to be effective against many diseases (Wang et al., 2005; Zhang et al., 2010). Given the excellent activity and safety of its components, SMY is widely used for the treatment of cardiac diseases, which are characterized by deficiency of Qi and Yin syndrome (Mo et al., 2015). Clinically, SMY has been shown to treat shock, coronary heart disease, angina, myocardial infarction (MI), viral myocarditis, pulmonary heart disease, heart failure etc. In addition, SMY can treat myocardial diseases, rheumatoid diseases, systemic lupus erythematosus, as well as epidemic hemorrhagic fever. These diverse curative effects of SMY explain why SMY in combination with other herbs has been used for the treatment of diabetes, nodular lupus erythematosus, mild brain dysfunction syndrome, optic atrophy, recurrent pneumothorax, iron deficiency anemia, severe infectious mononucleosis, and malignant tumor (with a maximum clinical dosage of 0.3 g/kg) (Zhang et al., 2010). Interestingly, SMY is also especially prescribed for coronary artery disease (Wang et al., 2002).

On the cellular level, SMY suppresses mitochondrial apoptosis as indicated by reduction in several pro-apoptotic factors (Bax, cytochrome c, and cleaved caspase-3) and up-regulation of the anti-apoptosis factor Bcl-2 (Mo et al., 2015). The improvement in cardiac contractile function afforded by SMY treatment is likely mediated by an increase in Ca^{2+} release from SR through L-type Ca^{2+} current-activated RyRs (Zhang et al., 2008). On the other hand, SMY improves the post-ischemic myocardial dysfunction by opening the mitochondrial KTAP channels (Wang et al., 2002)., improving the heart structure and reducing Cx43 expression after MI. SMY also inhibits myocardial fibrosis in rats with diabetic cardiomyopathy, and significantly delays the formation of diabetic cardiomyopathy through multiple signaling pathways (Ni et al., 2011). In view of these considerations, the aim of our study was to investigate the basis of the protective function of SMY on myocardial injury and on the regulation of IL-6 and TNF-α levels. Our study also explores the regulatory role of SMY on MMPs and COL-IV to achieve myocardial remodeling as well as the protective function of SMY against the pathological changes of the myocardium.

Table 1
Primers (F, forward; R, reverse) used for relative mRNA quantifications by real-time PCR.

Target	Primer sequence(5′−3′)	Length (pb)
COL-IV	F: GAGGGTGCTGGACAAGC	5300.50
	R: TAAATGGACTGGCTCGGAATTC	6774.48
GAPDH	F: TGTGAGGGAGATGCTCAGTG	6253.13
	R: GGCATTGCTCTCAATGACAA	6101.04

Materials and methods

Preparation of SMY

SMY was purchased from Chiatai Qingchunbao Pharmaceutical Co. (Zhejiang, China) (Batch no.- 1410014). This SMY preparation was a 1320 g mixture of three common Chinese herbal medicines: Ginseng radix, Ophiopogonis Radix and Schisandrae Chinensis Fructus mixed in a ratio of 1:2:1.

Reagents and drugs

Adriamycin was purchased from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China). Captopril was purchased from North China Phar. Co. (Hebei, China). Rat IL-6, TNF-α enzyme-linked immunosorbent assay (ELISA) assay kits were obtained from RayBiotech. Inc. (GA, USA). Histostain-Plus kits was purchased from ZSBIO. Inc. (Beijing, China). Antibodies for type IV collagen, matrixmetalloproteinases-2 and matrixmetalloproteinases-9 were purchased from Boster Co. (Beijing, China). All other agents used in this study were of commercially available grade and purity.

Animals and CHF model

Adult Wistar rats weighing 160–200 g, with an equal proportion of males and females, were provided by the Animal Breeding Center of Lanzhou Military Region General Hospital. These animals were housed under controlled conditions at a temperature of 25 ± 2 °C, humidity of 40 ± 5% and on a 12 h light-dark cycle. The rats had free access to solid rodent chow and tap water. Animals were allowed a 1 week acclimatization period prior to entry into any experimental protocol. The entire laboratory procedure was carried out under the permission and surveillance of local ethics committee. The experimental procedures were approved by Lanzhou Institute of Husbandry and Pharmaceutical Sciences, CAAS.

Adriamycin was used to establish the animal model of CHF. It was injected intraperitoneally for 7 weeks at a dose of 2 mg/kg body weight (Li et al., 2006; Cheng , 2011). The rats were randomly assigned into six groups. Group 1 (CON) was normal controls comprising of 30 healthy