

Full Paper

Analysis of Cardioprotective Effects Using Purified *Salvia miltiorrhiza* Extract on Isolated Rat HeartsPiek Ngoh Chang^{1,†}, Jian Chun Mao^{2,†}, Shan Hong Huang³, Li Ning¹, Zhong Jing Wang¹, Todd On¹, Wei Duan³, and Yi Zhun Zhu^{1,4,*}*Departments of ¹Pharmacology and ³Biochemistry, Faculty of Medicine, National University of Singapore, 119260, Singapore**²Department of Internal Medicine, Long Hua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China**⁴School of Pharmacy and Institute of Biomedical Sciences, Fudan University, Shanghai 200032, China*

Received December 26, 2005; Accepted June 5, 2006

Abstract. The purpose of the current study is to evaluate the cardioprotective effects of purified *Salvia miltiorrhiza* extract (PSME) on myocardial ischemia/reperfusion injury in isolated rat hearts. Hearts were excised and perfused at constant flow (7–9 ml·min⁻¹) via the aorta. Non-recirculating perfusion with Krebs-Henseleit (KH) solution was maintained at 37°C and continuously gassed with 95% O₂ and 5% CO₂. KH solution with or without PSME (100 mg per liter solution) was used after 30-min zero-flow ischemia for the PSME and control group, respectively. Left ventricular (LV) developed pressure; its derivatives, diastolic pressure, and so on were continuously recorded via a pressure transducer attached to a polyvinylchloride balloon that was placed in the left ventricle through an incision in the left atrium. PSME treated hearts showed significant postischemic contractile function recovery (developed pressure recovered to 44.2 ± 4.9% versus 17.1 ± 5.7%, *P*<0.05; maximum contraction recovered to 57.2 ± 5.9% versus 15.1 ± 6.3%, *P*<0.001; maximum relaxation restored to 69.3 ± 7.3% versus 15.4 ± 6.3%, *P*<0.001 in the PSME and control group, respectively). Significant elevation in end-diastolic pressure, which indicated LV stiffening in PSME hearts might have resulted from the excess high dose of PSME used. Further study will be conducted on the potential therapeutic value with lower dose of PSME on prevention of ischemic heart disease.

Keywords: purified *Salvia miltiorrhiza* extract, cardioprotective effect, myocardial ischemia/reperfusion, isolated rat heart

Introduction

Salvia miltiorrhiza (SM, also known as DanShen) is one of the well-known Chinese herbal medicines, containing phenolic compounds and potent antioxidant moieties (1–3). These non-phenolic constituents were reported to be capable of effectively inhibiting reactions that resulted from inflammatory oxidants, peroxynitrite, hypochlorous acid, and hydroxyl radicals as well as iron-dependent lipid peroxidation (4).

Additionally, SM had demonstrated its effectiveness in preventing postoperative increase in endothelin-1 in order to decrease myocardial damage and attenuate postoperative vasoactive mediator imbalance (5) and reduction of infarct size and mortality rate in rats with acute myocardial infarction (MI) (1). Moreover, SM also encourages capillary growth in blood vessels that play an important role in reconstruction of myocardial function after MI (6).

Similarly, a purified SM extract (PSME, known as Angino[®]) has been discovered to contain significant antioxidant capacity which is essential for scavenging free radicals. PSME also shows its striking effects in inhibiting both pyrogallo red bleaching and DNA

*Corresponding author. phczhuzy@nus.edu.sg, zhuyz@shmu.edu.cn

†shared first authorship

Published online in J-STAGE: July 13, 2006

doi: 10.1254/jphs.FPJ05034X

damage (3). Rats treated with PSME had higher survival rates after acute MI and significant reduction of infarct size (3). The antioxidant effect and possible feature of angiogenesis of SM had been suggested as the main factor in protecting ischemic myocardium (1, 2).

The purpose of the present study was to examine the cardioprotective effects of PSME by utilizing classic *ex vivo* preparations of isolated perfused Langendorff hearts, which had been widely used in studies of myocardial ischemia/reperfusion injury and evaluating the effect of drugs on the behavior and haemodynamic parameters changes of the heart. The major advantage of this preparation is to examine heart function in the absence of potential confounding factors such as systemic neurohormonal activation, pericardial constraint, and cardiovascular interaction and under constant conditions of temperature, gas composition, coronary flow, or pressure. Important parameters were used as indices for determining cardiac performance in isolated hearts including left ventricular developed pressure (LVDP), heart rate (HR), contractility ($+dp/dt_{max}$), and relaxation ($-dp/dt_{min}$) etc.

Materials and Methods

Animal model

Twelve adult male Wistar rats (weight 300–400 g) were supplied by the Laboratory Animal Center, National University of Singapore. The experiment was approved by the local animal ethics committee and performed based on international accepted guidelines for care and use of laboratory animals.

Purified *Salvia miltiorrhiza* extract (PSME)

PSME was supplied by CMM Corporation Pte Ltd. (batch No. IV-S-900-2, 30-4-2003; Singapore) which was originally developed by Shanghai Material Medica Bioengineering Institute). It comprises 4 Chinese medicinal bioactive ingredients: Salvianoic acid (*Danshensu*), Rosmarinic acid, and Salvianolic acid A and B as reported previously (3). Chemical structures of these 4 compounds are shown in Fig. 1.

Isolated perfused heart preparation

The rat was anesthetized with 7% Choral Hydrate (60 mg/kg) and heparinized (2 ml of 50 i.u.) via intraperitoneal injection. The heart was rapidly excised and arrested in ice cold, oxygenated Krebs-Henseleit (KH) solution (118.0 mM NaCl, 4.5 mM KCl, 1.4 mM KH_2PO_4 , 1.2 mM MgSO_4 , 25 mM NaHCO_3 , 1.4 mM CaCl_2 , and 11 mM glucose, pH 7) before the aorta was cannulated on the Langendorff apparatus. Non-recirculating mode of retrograde perfusion with KH

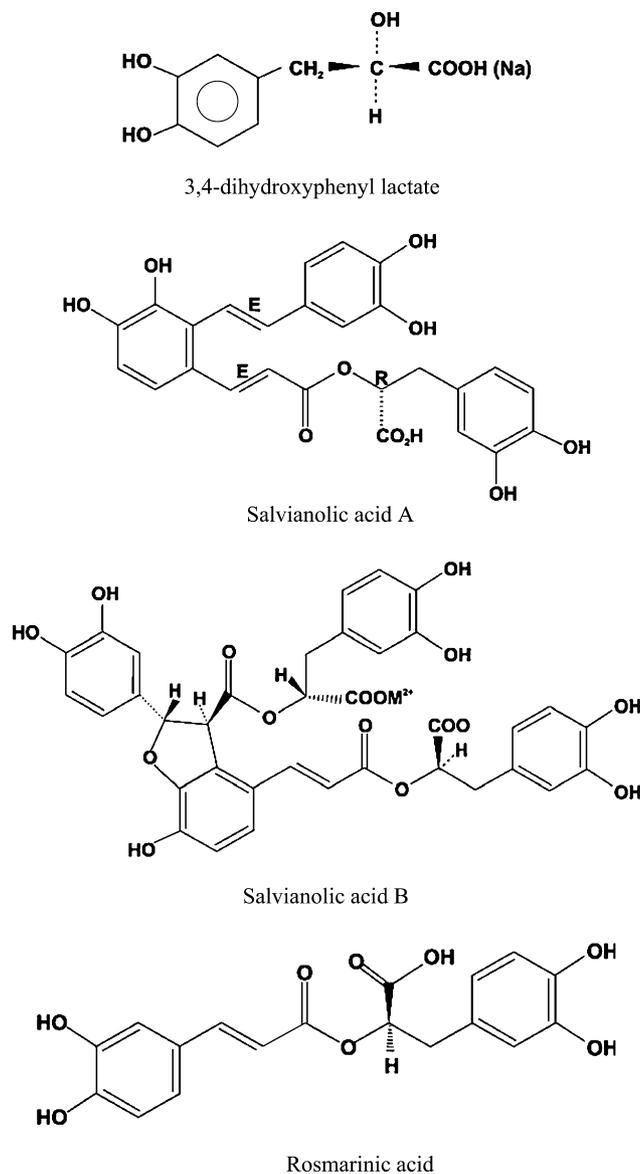


Fig. 1. Stereochemical structures of 4 active bioactive ingredients purified from *Salvia miltiorrhiza*.

solution was carried out at a constant flow ($7-9 \text{ ml} \cdot \text{min}^{-1}$) at 37°C and gassed with 95% O_2 and 5% CO_2 . The hearts were allowed to equilibrate for 30 min and then subjected to 30-min global and normothermic ischemia by halting solution perfusion and immersing the heart into a saline-filled organ bath. Twelve rats were randomly subjected to either reperfusion with PSME-containing KH solution (100 mg per liter) or KH solution only for 90 min immediately after ischemia.

Measurement of isovolumic cardiac performance

A water-filled polyvinylchloride balloon was attached to a pressure transducer and inserted through the mitral valve into the left ventricular (LV) through an incision in

the left atrium. The balloon was inflated until End-diastolic Pressure (EDP) achieved 20–30 mmHg and then held constant so that changes in EDP reflected changes in LV diastolic compliance (7). The pressure transducer was connected to a Powerlab (ADInstruments, Colorado Springs, CO, USA) data recording system. The following indices of cardiac performance were measured and averaged from 10 beats for each condition, and premature contractions were excluded from the analysis: EDP, LVDP, HR, the maximum and minimum values of the first derivative of left ventricular pressure ($+dp/dt_{max}$ and $-dp/dt_{min}$), ratio of $+dp/dt_{max}$ and $-dp/dt_{min}$, time to reach peak systolic pressure, and time to reach peak $+dp/dt_{max}$. Additionally, two hearts from each group were taken for ECG measurements by attaching spring clips and ground electrode to the atria and cannula, respectively. Baseline measurements: Cardiac performances were determined by examining both systolic and diastolic function by using isolated perfused heart preparation (Langendorff). Systolic function was assessed from systolic pressure, time to peak systolic, maximum contraction ($+dp/dt_{max}$), and time to reach peak maximum contraction. Diastolic function was assessed from the maximum relaxation ($-dp/dt_{min}$) and from time to reach 90% of relaxation (8).

Statistical analysis

Statistical software Minitab 14 was used. Paired Student's *t*-test was applied to compare the data in the pre- and postischemic condition. Unpaired Student's *t*-test was used for the function retaining rate after each

treatment. All values were expressed as the mean \pm S.E.M. Results were considered statistically significant if the *P*-value was less than 0.05.

Results

No significant differences were observed between the groups in terms of preischemic absolute values of contractile function. Acute myocardium injuries on isolated hearts were shown by significant reduction in LVDP, maximum contraction ($+dp/dt_{max}$) and maximum rate of pressure decay ($-dp/dt_{min}$) that resulted by zero-flow ischemia in both PSME and control groups. Comparison of postischemic variables between the PSME and control group showed significant differences in both systolic and diastolic functions (Table 1). LVDP was significantly higher in the PSME-treated group (17.03 ± 1.9 mmHg) when compared to the control (6.6 ± 2.2 mmHg) ($P < 0.05$). Similar observations were noted for $+dp/dt_{max}$ and $-dp/dt_{min}$: the PSME group (580 ± 60 mmHg/s) had a higher $+dp/dt_{max}$ versus the control (142 ± 59 mmHg/s) and lower $-dp/dt_{min}$ (-437 ± 46 mmHg/s) versus control (-106 ± 43 mmHg/s). The postischemic ratio of $+dp/dt_{max}$ to $-dp/dt_{min}$, which used to assess diastolic function relative to systolic function (8), was significantly lower in the PSME group compared to the control group ($P < 0.05$). After 30-min zero-flow ischemia, developed pressure recovered to $44.2 \pm 4.9\%$ versus $17.1 \pm 5.7\%$, $P < 0.05$; maximum contraction recovered to $57.2 \pm 5.9\%$ versus $15.1 \pm 6.3\%$, $P < 0.001$; maximum relaxation

Table 1. Preischemic and postischemic absolute values for control and PSME hearts

Parameter	Preischemic		Postischemic	
	Control	PSME	Control	PSME
LVDP (mmHg) [% of preischemic value]	38.5 ± 6.3	38.5 ± 6.2	6.6 ± 2.2 [17.1 \pm 5.7]	$17.0 \pm 1.9^{**}$ [44.2 \pm 4.9]
LVEDP (mmHg)	25.0 ± 4.3	20.53 ± 2.0	40.9 ± 7.6	37.4 ± 4.0
HR (BPM)	148 ± 22	136 ± 11	136 ± 38	118 ± 18
Maximum contraction ($+dp/dt_{max}$) (mmHg/s) [% of preischemic value]	940 ± 126	1014 ± 58	142 ± 59 [15.1 \pm 6.3]	$580 \pm 60^{***}$ [57.2 \pm 5.9]
Maximum relaxation ($-dp/dt_{min}$) (mmHg/s) [% of preischemic value]	-688 ± 73	-630 ± 62	-106 ± 43 [15.4 \pm 6.3]	$-437 \pm 46^{***}$ [69.3 \pm 7.3]
Time to reach peak (systolic pressure) (s)	0.086 ± 0.006	0.099 ± 0.09	0.071 ± 0.006	0.080 ± 0.006
Time to reach peak $+dp/dt_{max}$ (s)	0.042 ± 0.006	0.036 ± 0.002	0.028 ± 0.028	0.032 ± 0.002
Time to 90% relaxation (s)	0.065 ± 0.005	0.058 ± 0.010	0.013 ± 0.013	0.053 ± 0.005
Ratio of $+dp/dt_{max}$ and $-dp/dt_{min}$	-1.37 ± 0.08	-1.72 ± 0.16	-0.61 ± 0.27	$-1.33 \pm 0.04^*$

LVDP: left ventricular developed pressure, LVEDP: left ventricular end-diastolic pressure, HR: heart rate in unit beat per minute (BPM). Data are presented as the mean \pm S.E.M. $n = 12$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs control group (postischemia).

restored to $69.3 \pm 7.3\%$ versus $15.4 \pm 6.3\%$, $P < 0.001$ in the PSME and control group, respectively. Taken together, these results indicated that PSME showed cardioprotective effects against ischemic injuries compared to the control group.

However, EDP, a measurement of ventricular stiffness (7) was significantly increased in the PSME group after 30-min global ischemia ($P < 0.01$), while there were no changes in the control group. ECG measurements were carried out to determine whether PSME affected the electrical activity of cardiac muscles. Results of ECG suggested that hearts treated with PSME showed increased heart rate and it became much significant (tachycardia) after global ischemia, although they presented an improved ECG graph compared to the control group. An obvious postischemic ST increment was observed in control hearts. Further observations were noted, for example, a reduction in the amplitude of the QRS complex and shortened PR interval, which indicated that cardiac contraction and conduction time from atria to ventricle were altered. It might serve to compensate for the global ischemia.

Discussion

The main aim of the present study was to determine the myocardium protective effects of PSME. The majority of the statistically results were obtained by comparing data between the PSME and control group postischemically. The major finding of this study was the significant ischemic tolerance effects of PSME which caused a higher recovery in cardiac performance after 30-min zero-flow ischemia compared to the control group (Table 1). These results were further supported by ECG measurements showing an improved ECG graph in PSME-treated hearts, while there was an obvious increment in ST in control rats, which served as an indication of injury or transmural ischemia (9).

The antioxidant and angiogenic effects of PSME could be the main factor in protecting ischemic myocardium (3). *Danshensu*, one of major component of PSME, is believed to be working as a preventive antioxidant, which manages to scavenge superoxide anions produced by the xanthine-xantine oxidase system and protect the myocardial mitochondrial membranes from lipid peroxidation. Tanshinone, another main substance of PSME, which functions as a chain-breaking antioxidant, scavenged the lipid free radicals synthesized from lipid peroxidation of the myocardial mitochondrial membranes (10).

Contradictorily, EDP, which indicated LV stiffening (7) increased significantly during zero-flow ischemia in PSME-treated hearts ($P < 0.05$) and its ratio of $+dp/dt_{max}$

to $-dp/dt_{min}$ decreased significantly ($P < 0.05$) when compared with the control group after ischemia. Since diastolic dysfunction become manifest as increased end-diastolic pressure and time to 90% relaxation (8), the elevated EDP and decreased ratio of $+dp/dt_{max}$ to $-dp/dt_{min}$ might indicate the impaired diastolic compliance in PSME-treated hearts. These explanations might further be supported by the observation of arrhythmia in the ECG of postischemic PSME hearts.

Impaired diastolic function in PSME heart could have resulted for several reasons. Firstly, the weakened diastolic function might be resulted by overdose of PSME used in buffer which poisoned the heart and caused it to malfunction. The changes in color of the PSME treated hearts (from reddish to dark brown, results not shown) might be an indicator of the excess amount of PSME used. However, the color changes could be also caused by the nature of the drug (PSME is brown), as no significant difference was observed in time to 90% relaxation, which served as another indicator of diastolic dysfunction (8). Hence, further experiments such as biochemical assay are required to further prove the results.

Other studies on cardioprotective effects of CHM (Chinese Herbal Medicine) had been carried out by using similar Langendorff heart preparations. Wang et al. (11) discovered *Sheng-Mai-San* (SMS), a traditional Chinese formulation containing *Radix Ginseng*, *Radix Ophiopogonis*, and *Fructus Schisandrae* is defensive against postischemic myocardial dysfunction by its ability to open of the mitochondrial K_{ATP} channels. In addition, *Radix Stephaniae tetrandrae* (RST) extract and its individual compounds had been reported to have significant cardioprotective and anti-arrhythmic effects without decreasing heart rate during ischemia (12).

There were no significant differences in time to reach peak systolic pressure, time to peak $+dp/dt_{max}$, and time to reach 90% relaxation in between the PSME and control group. The presence of noise-like waves (lacking of distinct peaks) in control hearts that were caused by severe impaired cardio-performance after 30-min global ischemia greatly affect the software's ability to process the data, and hence, caused inaccurate results. This might also be responsible for the insignificant differences, particularly in measuring the time duration between the PSME and control groups.

Heart rate measured in beats per minute was constant throughout the experiment for both the PSME and control group. This suggested that the hearts were able to restore their initial heart beats after severe injuries by lowering the beat intensities. Hence, PSME may provide better ischemic tolerance compared to a classical calcium channel blocker, verapamil, as they do not

decrease heart rate during ischemia (12).

The current experiment model might overlook several important aspects of clinical myocardial ischemia. Firstly, blood-free buffer perfusion alone may limit accurate measurement of left ventricular diastolic properties and exacerbate changes in diastolic functions, especially under conditions of elevated oxygen demand. The addition of erythrocyte inside the perfusate provides a better in vitro model for determining cardiac work that is physiologically superior to buffer perfusion (13). The suggestion was supported by Saupe et al. (7) who used coronary perfusate containing a normal hematocrit and oxygen content in order to mimic the significant differences in cardiac performance in vivo and ex vivo.

Secondly, it is important for physiological concentrations of the metabolic substrates normally consumed by the heart, namely, glucose, lactate, and free fatty acids, to be present in coronary perfusate because the myocardial response to ischemia is highly dependent on the concentrations of the metabolic substrates present during ischemia (14). Hence, significant differences in cardiac performance might exist in the current experiment due to the absence of erythrocyte, lactate, and free fatty.

In conclusion, the results showed that PSME exerts cardioprotective effects against ischemia-reperfusion injury. PSME at a lower dose might be used for prevention of ischemic heart disease.

Acknowledgments

The study was supported by a research grant of National Medical Research Council of Singapore (R-184-000-066-213 and R-184-000-109-213) and CMM Corporation Pte. Ltd. (N-184-000-005-001). The authors wish to thank Prof. Benny Tan for valuable discussion.

References

- Ji X, Tan BK, Zhu YC, Linz W, Zhu YZ. Comparison of cardioprotective effects using ramipril and DanShen for the treatment of acute myocardial infarction in rats. *Life Sci.* 2003;73:1413–1426.
- Ji X, Tan BK, Huang SH, Whiteman M, Zhu YC, Linz W, et al. Effects of *Salvia miltiorrhiza* in ischemic myocardium in experimental rats. In: Tan B, Bay BH, Zhu YZ, editors. *Perspective: Novel compounds from natural products in new millenniums*. Singapore: World Scientific Press; 2004. p. 152–162.
- Sun J, Huang SH, Tan B, Whiteman M, Wu YJ, Ng YK, et al. Effects of purified *Salvia miltiorrhiza* on ischemic rat myocardium after acute myocardial infarction. *Life Sci.* 2005;76:2849–2860.
- Zhu YZ, Huang SH, Tan BK, Sun J, Whiteman M, Zhu YC. Antioxidants in Chinese herbal medicines: a biochemical perspective. *Nat Prod Rep.* 2004;21:478–489.
- Xia Z, Gu J, Ansley DM, Xia F, Yu J. Antioxidant therapy with *Salvia miltiorrhiza* decreases plasma endothelin-1 and thromboxane B2 after cardiopulmonary bypass in patients with congenital heart disease. *J Thorac Cardiovasc Surg.* 2003;126:1404–1410.
- Sun J, Tan B, Huang SH, Whiteman M, Zhu YZ. Effects of natural products on ischemic heart diseases and cardiovascular system. *Acta Pharmacol Sin.* 2002;23:1142–1151.
- Saupe KW, Lim CC, Ingwall JS, Apstein CS, Eberli FR. Comparison of hearts with 2 types of pressure-overload left ventricular hypertrophy. *Hypertension.* 2000;35:1167–1172.
- Knollmann BC, Blatt SA, Horton K, de Freitas F, Miller T, Bell M, et al. Inotropic stimulation induces cardiac dysfunction in transgenic mice expressing a troponin T (I79N) mutation linked to familial hypertrophic cardiomyopathy. *J Biol Chem.* 2001;276:10039–10048.
- Ortega-Carnicer J. Giant R wave, convex ST-segment elevation, and negative T wave during exercise treadmill test. *J Electrocardiol.* 2004;37:231–236.
- Sun J, Huang SH, Zhu YC, Wang MJ, Tan B, Whiteman M, et al. Antioxidant effects of the extract of the *Herba Leonuri*, a traditional Chinese medicinal herb, on ischemic rat myocardium after acute myocardial infarction. *Life Sci.* 2005;76:3043–3056.
- Wang N, Minatoguchi S, Arai M, Uno Y, Nishida Y, Hashimoto K, et al. Sheng-Mai-San is protective against post-ischemic myocardial dysfunction in rats through its opening of the mitochondrial K_{ATP} channels. *Circ J.* 2002;66:763–768.
- Yu XC, Wu S, Wang GY, Shan J, Wong TM, Chen CF, et al. Cardiac effects of the extract and active components of *radix stephaniae tetrandrae*. II. Myocardial infarct, arrhythmias, coronary arterial flow and heart rate in the isolated perfused rat heart. *Life Sci.* 2001;68:2863–2872.
- Brooks WW, Apstein CS. Effect of treppe on isovolumic function in the isolated blood-perfused mouse heart. *J Mol Cell Cardiol.* 1996;28:1817–1822.
- Taegtmeyer H, Goodwin GW, Doenst T, Frazier OH. Substrate metabolism as a determinant for postischemic functional recovery of the heart. *Am J Cardiol.* 1997;80:3A–10A.