Sheng-Mai-San is Protective Against Post-Ischemic Myocardial Dysfunction in Rats Through Its Opening of the Mitochondrial KATP Channels

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The present study used isolated rat hearts to investigate whether (1) Sheng-Mei-San (SMS), a traditional Chinese formulation comprising *Radix Ginseng*, *Radix Ophiopogonis* and *Fructus Schisandrae*, is protective against post-ischemic myocardial dysfunction, and (2) whether the cardioprotective effect of SMS is related to scavenging of hydroxyl radicals and opening the mitochondrial KATP channels. The excised hearts of male Sprague-Dawley rats were perfused on a Langendorff apparatus with Krebs-Henseleit solution with a gas mixture of 95% O₂ and 5% CO₂. Left ventricular end-diastolic pressure (LVEDP, mmHg), left ventricular developed pressure (LVDP, mmHg), ±dP/dt (mmHg/s) and coronary flow (ml/min) were continuously monitored. All hearts were perfused for a total of 120 min consisting of a 30-min pre-ischemic period followed by a 30-min global ischemia and 60-min reperfusion. Lactate, lactate dehydrogenase (LDH) and 2,5-dihydroxybenzoic acid (2,5-DHBA) concentrations in the effluent were measured during reperfusion. Three days’ treatment with SMS (1.67 ml/kg per day) inhibited the rise in LVEDP and improved the post-ischemic LVDP and ±dP/dt significantly better than in the untreated control hearts during reperfusion. SMS increased the coronary flow at baseline, and during reperfusion. Pretreatment with 5-hydroxydecanoic acid (5-HD), a mitochondrial KATP channel blocker, abolished the inhibition of the rise in LVEDP, the increase in coronary flow and the improvement in LVDP and ±dP/dt induced by SMS. SMS significantly attenuated the concentrations of lactate, LDH and 2,5-DHBA during reperfusion, but the pretreatment with 5-HD restored them; 5-HD alone did not affect the concentrations. SMS improved the post-ischemic myocardial dysfunction through opening the mitochondrial KATP channels. (Circ J 2002; 66: 763–768)

Key Words: Free radicals; Ischemia-reperfusion; Isolated rat heart; Mitochondrial KATP channel; Sheng-Mai-San

Sheng-Mai-San (SMS), a traditional Chinese formulation comprising *Radix Ginseng*, *Radix Ophiopogonis* and *Fructus Schisandrae*, has long been used for the treatment of loss of essence-energy and excessive body fluid, and is especially prescribed for coronary artery disease in China.¹ We reported recently that a 3-day, but not 30 min before ischemia, treatment with SMS in rabbits significantly reduced the myocardial infarct size induced by 30 min of ischemia and then 48 h reperfusion;² however, it is unknown whether SMS is protective against post-ischemic myocardial dysfunction. Therefore, the aim of the present study was to clarify (1) whether SMS improves post-ischemic myocardial dysfunction, and (2) whether its cardioprotective effect is related to scavenging hydroxyl radicals and opening the mitochondrial KATP channels.

Methods

All rats received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethical Committee of Gifu University School of Medicine, Gifu, Japan.

Animal Selection

We used Male Sprague Dawley rats (Chubu-Kagaku-Shizai Co, Nagoya, Japan), each weighing 200–250 g and without any clinically evident infections.

Perfusion of Isolated Rat Hearts

The rats were anesthetized with pentobarbital (60 mg/kg, ip) and their hearts were excised into 4°C saline and perfused with oxygenated buffer within 30 s. The hearts were retrogradely perfused on a Langendorff apparatus with Krebs-Henseleit solution (5.5 mmol/L glucose, 1.2 mmol/L Ca²⁺, 4.7 mmol/L KCl, 25.0 mmol/L NaHCO₃) with a gas mixture of 95% O₂ and 5% CO₂. A water-filled polyethylene tube was inserted into the left ventricle through the apex and connected to a pressure transducer and adjusted to a left ventricular end-diastolic pressure (LVEDP) of 5 mmHg during the initial equilibration. This preload volume was held constant during the entire experiment to allow continuous recording of the ventricular pressure. Pacing wires were fixed to the pulmonary outflow tract, and the hearts...
were paced at 320 beats/min except during ischemia. Left ventricular developed pressure (LVDP, end-systolic-end-diastolic pressure, mmHg) and ±dP/dt (mmHg/s) were continuously monitored. Coronary flow (ml/min) was continuously measured with an electromagnetic flow probe that was placed around the tube connected to the aorta.

**Protocol for Measuring Cardiac Function**

All hearts were perfused for a total of 120 min consisting of a 30-min pre-ischemia period followed by a 30-min global ischemia and 60-min reperfusion. The heart was perfused with Krebs-Henseleit solution with and without 5-HD during the pre-ischemia period. The SMS group was treated with SMS for 3 days before the induction of ischemia.

![Fig 1. Experimental protocol. All hearts were perfused for a total of 120 min, consisting of a 30-min pre-ischemia period followed by a 30-min global ischemia and 60-min reperfusion. The heart was perfused with Krebs-Henseleit solution with and without 5-HD during the pre-ischemia period. The SMS group was treated with SMS for 3 days before the induction of ischemia.](image1)

![Fig 2. Time course of changes in left ventricular end-diastolic pressure (LVEDP) during baseline, ischemia and at 15, 30 and 60 min after reperfusion (Open circle). Control group, (open square) SMS group, (open triangle) SMS + 5-HD group, (closed triangle) 5-HD group. *p<0.05 vs control, #p<0.05 vs SMS + 5-HD group.](image2)

![Fig 3. Time course of changes in left ventricular developed pressure (LVDP) during baseline, ischemia and at 15, 30 and 60 min after reperfusion (Open circle). Control group, (open square) SMS group, (open triangle) SMS + 5-HD group, (closed triangle) 5-HD group. *p<0.05 vs control, #p<0.05 vs SMS + 5-HD group.](image3)

The dose of SMS used in the present study was the clinical maximal dose (20–100 ml, once a day) for the maximal effect of *Radix Ginseng*, which is a main element of SMS.3,4

**Measurement of Lactate and Lactate Dehydrogenase (LDH) in the Effluent**

The lactate and LDH concentrations in the effluent at 15, 30 and 60 min after reperfusion were measured in the control, SMS, SMS + 5-HD, and 5-HD groups. Lactate was measured spectrophotometrically by monitoring hydrogen peroxide formation resulting from the enzymatic reaction with lactate oxidase.5 LDH was assayed by an ultraviolet method using a LDH assay kit.

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Measurement of Hydroxyl Radicals

The amount of 2,5-DHBA (2,5-dihydroxybenzoic acid), an indicator of hydroxyl radicals, was measured using high-performance liquid chromatography coupled with electrochemical detection in the control group (n=8), SMS group (n=8), SMS + 5-HD group (n=7), and 5-HD group (n=7). This method is based on the chemical reaction of salicylic acid with hydroxyl radicals, yielding 2,3-DHBA, 2,5-DHBA and catechol as its derivatives in an approximate proportion of 49%, 40%, and 11%, respectively. It is reported that salicylic acid with a concentration of 1 mmol/L traps approximately 10% of the theoretically possible hydroxyl radicals formed in vivo. We used 2,5-DHBA rather than 2,3-DHBA because of its greater sensitivity for quantification of hydroxyl radicals production. The experimental procedure for the measurement of 2,5-DHBA was the same as just described except that the hearts were perfused with buffer containing 1 mmol/L salicylic acid. The pH of the buffer was readjusted after the addition of salicylic acid. Then the hearts were subjected to global ischemia for 30 min and reperfused for 30 min. The effluent

Fig 4. Time course of changes in (A) +dP/dt and (B) –dP/dt during baseline, ischemia, and at 15, 30 and 60 min after reperfusion (Open circle). Control group, (open square) SMS group, (open triangle) SMS + 5-HD group, (closed triangle) 5-HD group. *p<0.05 vs control, #p<0.05 vs SMS + 5-HD group.

Fig 5. Time course of changes in coronary flow during baseline, ischemia and at 15, 30 and 60 min after reperfusion (Open circle). Control group, (open square) SMS group, (open triangle) SMS + 5-HD group, (closed triangle) 5-HD group. *p<0.05 vs control, #p<0.05 vs SM + 5-HD group.

Fig 6. Lactate and LDH concentrations in the effluent at 15, 30 and 60 min of reperfusion after global ischemia. *p<0.05 vs control group, #p<0.05 vs SMS + 5-HD group, Bars = mean ± SEM.

Fig 7. Time course of changes in 2,5-DHBA during reperfusion following 30 min of global ischemia *p<0.05 vs control group, *p<0.05 vs before ischemia.
was collected at the end of the perfusion and at 1, 2, 3, 5, 10, 15 and 30 min in the reperfusion period and stored at
–80°C until assay.

Statistical Analysis
All values are presented as the mean ± SEM. The difference in hemodynamic variables over time between the
control and treatment groups was assessed by two-way repeated measures analysis of variance (ANOVA).
Student’s t test assessed the differences in lactate, LDH and 2,5-DHBA concentrations.

Chemicals and Herbal Materials
Five-hydroxydecanoic acid sodium salt (5-HD) was purchased from Sigma Chemical Co (St Louis, MO, USA)
and Shen-Mei-San was purchased from Pharmaceutical Factory of West China University of Medical Science (No.
980450, Chengdu, China).

Results
Effect of SMS on Post-Ischemic Recovery of LV Function
During the pre-ischemic period, all measured parameters, such as LVEDP, LVDP, ±dP/dt and coronary flow,
were comparable between the groups. The time course of changes in LVEDP, LVDP, ±dP/dt and coronary flow
in the control, SMS, SMS+5-HD and 5-HD groups are shown in Figs 2–5. Treatment with SMS inhibited the rise
in LVEDP and improved the post-ischemic LVDP and ±dP/dt significantly better than in the untreated control
hearts at 15, 30 and 60 min of reperfusion. Treatment with SMS increased the coronary flow at baseline, 15, 30
and 60 min after reperfusion. Pretreatment with 5-HD abolished the inhibition of the rise in LVEDP, the increase in
coronary flow and the improvement in LVDP and ±dP/dt induced by SMS.

Effect of SMS on Lactate and LDH Concentrations in the Effluent
The lactate and LDH concentrations in the effluent were both significantly attenuated in the SMS-treated group
compared with the control group at 15, 30 and 60 min of reperfusion, but were restored after pretreatment with 5-
HD (Fig 6). 5-HD alone did not affect the lactate and LDH concentrations.

Effect of SMS on 2,5-DHBA Concentrations in the Effluent
As shown in Fig 7, the 2,5-DHBA concentrations significantly increased during reperfusion in the control group.
The 3-day treatment with SMS significantly decreased the baseline concentrations of 2,5-DHBA and significantly
attenuated the rise of 2,5-DHBA concentrations during reperfusion. Pretreatment with 5-HD restored the baseline
level of 2,5-DHBA and blocked the attenuation of the increase in 2,5-DHBA level induced by SMS.

As shown in Table 1, the effects of SMS on post-ischemic left ventricular function of the heart perfused with
the buffer containing 1 mmol/L of salicylic acid were similar with those without the salicylic acid.

Discussion
The results of the present study demonstrated that (1) 3 days treatment with SMS significantly inhibited the rise in
LVEDP and improved the recovery of LVDP and ±dP/dt during reperfusion after 30 min of global ischemia, (2) the
effect of SMS was blocked by pretreatment with a mitochondrial KATP channel blocker, 5-HD, and (3) SMS
significantly attenuated the concentrations of lactate, LDH and 2,5-DHBA in the effluent during reperfusion.
Pretreatment with 5-HD restored the attenuated levels of

Table 1  Effects of Sheng-Mai-San on LV Function When Perfused With Buffer Containing Salicylic Acid

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Functional recovery of reperfusion</th>
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<tr>
<td></td>
<td></td>
<td>15 min</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>Control (n=8)</td>
<td>0.43±0.11</td>
<td>26.8±1.1</td>
</tr>
<tr>
<td>SMS (n=8)</td>
<td>0.38±0.3</td>
<td>14.8±2.0*</td>
</tr>
<tr>
<td>5-HD (n=7)</td>
<td>0.53±0.2</td>
<td>27.8±1.6</td>
</tr>
<tr>
<td>SMS+5-HD (n=7)</td>
<td>0.61±0.21</td>
<td>24.1±1.2</td>
</tr>
<tr>
<td>LV developed pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>74.6±1.4</td>
<td>37.4±1.1</td>
</tr>
<tr>
<td>SMS (n=8)</td>
<td>73.3±1.1</td>
<td>56.3±2.8*</td>
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<tr>
<td>5-HD (n=7)</td>
<td>71.4±1.8</td>
<td>34.4±3.6</td>
</tr>
<tr>
<td>SMS+5-HD (n=7)</td>
<td>68.8±1.6</td>
<td>37.6±2.8</td>
</tr>
</tbody>
</table>

Positive dp/dt (mmHg/s) |          |        |        |        |
| Control (n=8)       | 2.24±0.64| 1.08±0.14 | 1.24±0.19 | 1.19±0.13 |
| SMS (n=8)           | 2.14±0.48| 1.60±0.38* | 1.98±0.66* | 2.04±0.107* |
| 5-HD (n=7)          | 2.18±0.12| 1.00±0.12 | 1.33±0.19 | 1.26±0.17 |
| SMS+5-HD (n=7)      | 2.21±0.79| 1.17±0.14 | 1.36±0.12 | 1.14±0.95 |

Negative dp/dt (mmHg/s) |          |        |        |        |
| Control (n=8)       | 1.95±0.84| 1.01±0.16 | 1.14±0.12 | 1.00±0.13 |
| SMS (n=8)           | 1.89±0.92| 1.37±0.87* | 1.45±0.59* | 1.46±0.105* |
| 5-HD (n=7)          | 1.94±1.49| 0.95±0.79 | 1.09±0.14 | 1.11±0.13 |
| SMS+5-HD (n=7)      | 1.88±0.74| 0.90±0.17 | 1.08±0.06 | 0.96±0.04 |

Coronary flow (nl/min) |          |        |        |        |
| Control (n=8)       | 10.4±0.7 | 6.6±0.7 | 7.0±0.8 | 6.1±0.5 |
| SMS (n=8)           | 14.3±0.9*| 10.6±0.5* | 9.1±0.6* | 8.6±0.5* |
| 5-HD (n=7)          | 11.3±0.3 | 7.1±0.4 | 6.7±0.4 | 6.1±0.3 |
| SMS+5-HD (n=7)      | 11.1±0.3 | 6.8±0.6 | 6.0±0.7 | 5.5±0.5 |

SMS, Sheng-Mai-San; 5-HD, 5-hydroxydecanoic acid sodium salt; LV, left ventricular. *p<0.05 vs control group.
lactate, LDH and 2,5-DHBA.

Effect of Shen-Mai-San on LV Function

The 3-day treatment with SMS significantly improved post-ischemic myocardial dysfunction, but it is not clear from the present study which element of SMS improved the cardiac function. SMS decreased both the LDH and lactate concentrations in the effluent during reperfusion, suggesting that lactate formation during ischemia was attenuated by SMS, which in turn suggests that SMS protected the heart against ischemic cellular damage.

Possible Mechanisms of Cardioprotection by SMS

Treatment with SMS significantly attenuated the rise in 2,5-DHBA concentrations in the effluent during reperfusion compared with the control group, suggesting that SMS inhibited the production of hydroxyl radicals in the heart during reperfusion. It has been reported that Panax ginsen, one of the elements of SMS, has an anti-oxidant effect via activation of the Cu, Zn-superoxide dismutase gene;8 post-ischemic myocardial dysfunction is mediated in part through the generation of reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radicals;9 and of these, hydroxyl radical has the most important role.10,11 Therefore, it is highly likely that the improvement of cardiac function by SMS is the result of blockade of hydroxyl radical production during reperfusion. Furthermore, the attenuated concentration of 2,5-DHBA during reperfusion by SMS was restored by treatment with 5-HD, a mitochondrial KATP channel blocker, suggesting that the opening of mitochondrial KATP channels reduces the production of hydroxyl radicals during reperfusion. This is a somewhat different results from the study by Pain et al who reported that the opening of mitochondrial KATP channels produces free radicals and activates MAP kinase, thus protecting the heart against ischemia–reperfusion injury.12 On the other hand, it has been reported that a KATP channel opener, nicorandil, scavenges hydroxyl radicals and inhibits superoxide anion production in neutrophils in vitro.13 Therefore, it is controversial whether the opening of KATP channel produces or scavenges hydroxyl radicals. One possible explanation is that the small amount of free radicals that mediates the ischemic preconditioning effect may be related to the opening of KATP channels, but the large amount of free radicals that is associated with ischemia–reperfusion injury may be scavenged by the opening of KATP channels, as shown in the present study.

Secondly, the cardioprotective effect by SMS may be mediated by opening the mitochondrial KATP channels of cardiac myocytes, because the improvement in myocardial dysfunction by SMS after global ischemia was completely abolished by pretreatment with a mitochondrial KATP channel inhibitor, 5-HD. Recently, it was reported that activation of protein kinase C opens the mitochondrial KATP channels;14 and that opening is currently believed to be critically important in protecting the myocardium against ischemic injury.15 Furthermore, nicorandil, which opens both sarcolemmal and mitochondrial KATP channels, has been reported to protect the heart against ischemic injury.16,17

Thirdly, in the present study, treatment with SMS increased the coronary flow at baseline before global ischemia and at 15, 30 and 60 min of reperfusion as compared with the control saline-treated group. This suggests that SMS by itself has a vasodilating action and blocks coronary artery vasoconstriction and thrombus formation in intramyocardial small vessels, thus inhibiting the so-called ‘no-reflow’ phenomenon during reperfusion. The improvement in myocardial dysfunction after global ischemia may also be related to this coronary flow-increasing effect of SMS. The increase in coronary flow by SMS before ischemia and during reperfusion was completely blocked by the pretreatment with 5-HD, suggesting that the increase in coronary flow by SMS was caused by opening the mitochondrial KATP channel. However, as it is widely accepted that all types of KATP channel blockers affect not only the mitochondrial KATP channels but also the sarcolemmal KATP channels to some extent, it is likely that the dilatation of the coronary artery by SMS was caused by the opening of the sarcolemmal KATP channels. In addition, it is well known that coronary artery dilatation is mediated through the production of nitric oxide,18 and hydroxyl radicals are reported to impair nitric oxide-mediated coronary vasodilatation in rats by reducing nitric oxide release.19 Therefore, the increase in coronary flow by SMS might also be caused by a reduction of hydroxyl radical production during reperfusion.

Clinical Implications

SMS clearly protected the myocardium against post-ischemic myocardial dysfunction in rats when given daily for 3 days before ischemia. Basically, SMS is chronically administered to patients with coronary artery disease in China and if given to patients with angina pectoris, will protect against post-ischemic myocardial dysfunction. Furthermore, it can be expected that SMS would improve the myocardial dysfunction during reperfusion after percutaneous transluminal coronary angioplasty for myocardial infarction. Consequently, SMS may be beneficial for patients with coronary artery disease and further clinical trials are warranted.

References

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