

## Protective Roles of Ginseng Saponin in Cardiac Ischemia and Reperfusion Injury

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**Abstract :** Ginsenosides, one of the most well-known traditional herbal medicines, are used frequently in Korea for the treatment of cardiovascular symptoms. The effects of ginseng saponin on ischemia-induced isolated rat heart were investigated through analyses of hemodynamic changes including perfusion pressure, aortic flow, coronary flow, and cardiac output. Isolated rat hearts were perfused and then subjected to 30 min of global ischemia followed by 60 min of reperfusion with modified Krebs's Henseleit solution. Myocardial contractile function was continuously recorded. Ginseng saponin administered before inducing ischemia significantly prevented decreases in perfusion pressure, aortic flow, coronary flow, and cardiac output. The ginseng saponin administered group significantly recovered all of the hemodynamic parameters, except heart rate, after ischemia-reperfusion (I/R) compared with ischemia control. The intracellular calcium ( $[Ca^{2+}]_i$ ) content in rat neonatal cardiomyocytes was quantitatively determined. Administration of ginseng saponin significantly prevented  $[Ca^{2+}]_i$  increase that had been induced by simulated I/R *in vitro* ( $p < 0.01$ ) in a dose-dependent manner, suggesting that the cardioprotection of ginseng saponin is mediated by the inhibition of  $[Ca^{2+}]_i$  increase. Overall, we found that the administration of ginseng saponin has cardioprotective effects on the isolated rat heart after I/R injury. These results indicate that ginseng saponin has distinct cardioprotective effects in an I/R-induced rat heart.

**Key words :** ginseng saponin, isolated heart, ischemia-reperfusion injury, hemodynamics, cardioprotection

### INTRODUCTION

The single largest cause of death and disability worldwide<sup>1,2)</sup> is ischemic heart disease (IHD). Acute myocardial infarction (AMI) is the clinical manifestation of a heart attack following coronary thrombosis. When an AMI is treated with early revascularization, this procedure leads to myocardial reperfusion. Ischemia-reperfusion (I/R) is associated with myocardial injury. When coronary flow is restored, the detrimental effects of reperfusion occur causing oxidative stress and intracellular calcium overload, which lead to mitochondrial alterations and cell death.<sup>3)</sup> The primary manifestations of myocardial I/R are myocyte death, arrhythmias, and contractile dysfunction.<sup>4,5,6)</sup> Patients surviving an AMI are susceptible to recurrent angina, reinfarction, arrhythmias, heart failure, and sudden cardiac death.<sup>7)</sup> During the last three decades, tremendous progress has been made in the treatment of myocardial infarction, and these advancements have

decreased mortality from IHD.<sup>8,9,10)</sup> While reperfusion-associated myocardial injury limits myocardial salvage, reperfusion therapies and interventions that are cardioprotective in animal models have not yet been successfully advanced to improve clinical outcomes.<sup>11)</sup> However, a number of mechanisms have been proposed that may mediate myocardial I/R injury. Intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) is a ubiquitous signal for regulating cellular function, including cell survival and death.<sup>12)</sup> While a small amount of  $[Ca^{2+}]_i$  is necessary for optimal physiological function of the heart, growing evidence suggests that an increased cytosolic free  $Ca^{2+}$  is one of the major contributors to myocardial I/R injury.<sup>13,14,15,16,17)</sup> Therefore,  $[Ca^{2+}]_i$  handling in post-ischemic myocardium is a prime target for the treatment of AMI.

*Panax ginseng* has been used as an herbal medicine in Asia for more than 2000 years and continues to occupy a prominent place among the tonic remedies in oriental medicine. It has become increasingly popular in the Western world for its alleged tonic effect and possible curative and restorative properties.<sup>18)</sup> There are a variety of reports

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explaining the pharmacological effects of *Panax* ginseng in certain chronic disease states.<sup>19,20,21)</sup>

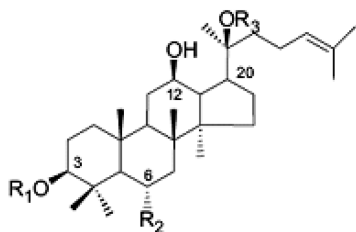
In humans, ginseng is a relatively safe adjuvant medication for hypertension.<sup>22)</sup> Ginseng improves the vascular endothelial dysfunction found in hypertensive patients possibly through increased synthesis of nitric oxide (NO).<sup>23)</sup> It also possesses some cardiac and vascular effects.<sup>24,25,26,27,28,29,30,31)</sup> Yet, the mechanisms of action of ginseng saponin (GS) remain largely unclear. Also, until now, there is no evidence to indicate that GS exhibits any cardioprotection on I/R injury *in vitro* and/or *ex vivo*.

The main aims of this study were to investigate whether the administration of GS facilitated the recovery of post ischemic heart function and to determine the optimized concentration of GS that exerted the cardioprotective effect *ex vivo*. Moreover, in order to explore the specific molecular mechanisms, we not only examined  $[Ca^{2+}]_i$  closely associated with I/R injury.<sup>32)</sup> Our results revealed that administering GS greatly attenuated I/R injury

## MATERIALS AND METHODS

### Drugs

Fig. 1 shows the chemical structure of GS. Ginseng saponin was kindly obtained from Korea Ginseng Corporation (Daejeon, Korea), and other chemicals that were of



Ginsenosides	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Rb <sub>1</sub>	-Glc <sub>2</sub> -Glc	-H	-Glc <sub>6</sub> -Glc
Rb <sub>2</sub>	-Glc <sub>2</sub> -Glc	-H	-Glu <sub>6</sub> -Ara(pyr)
Rc	-Glc <sub>2</sub> -Glc	-H	-Glc <sub>6</sub> -Ara(fur)
Rd	-Glc <sub>2</sub> -Glc	-H	-Glc
Re	-H	-O-Glc <sub>2</sub> -Rha	-Glc
Rf	-H	-O-Glc <sub>2</sub> -Glc	-H
Rg <sub>1</sub>	-H	-O-Glc	-Glc
Rg <sub>2</sub>	-H	-O-Glc <sub>2</sub> -Rha	-H
Rg <sub>3</sub>	-Glc <sub>2</sub> -Glc	-H	-H

**Fig. 1.** Structures of the nine representative ginsenosides. They differ at three side chains attached the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

analytical grade were purchased from Sigma (St. Louis, MO). GS was dissolved in modified Krebs-Henseleit (KH) buffer and the solution was centrifuged at 15,000 rpm for 10 min. The supernatant was transferred to another tube and filtered through a 0.2  $\mu$ m syringe filter. Modified KH buffer consisted of NaCl 120.0 mM, NaHCO<sub>3</sub> 25 mM, KCl 4.8 mM, KH<sub>2</sub>PO<sub>4</sub> 1.2 mM, CaCl<sub>2</sub> 1.25 mM, MgSO<sub>4</sub> 1.2 mM, and glucose 11.0 mM.

### Animals

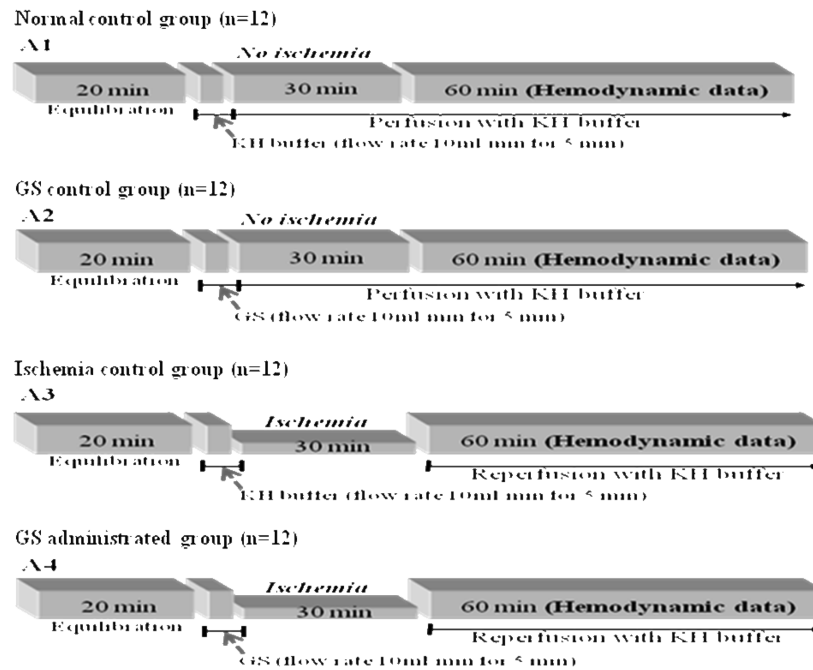
Male Sprague-Dawley rats weighing 250 to 300 g were supplied by Charles River (KFT). The rats were housed with free access to a standard rodent pellet chow (LSM, Bacutil, Poland) and tap water. Room temperature was 23 $\pm$ 1 $^{\circ}$ C, and the relative humidity was 50 $\pm$ 10%. All of the animals was kept in light (L)-dark (D) conditions L:D = 12:12. The rats were acclimatized to their environment for two weeks prior to experimentation. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the "Guideline for Institutional Animal Care and Use Committees (IACUC)" of Chonbuk National University (Jeonju, Korea).

### Experimental groups

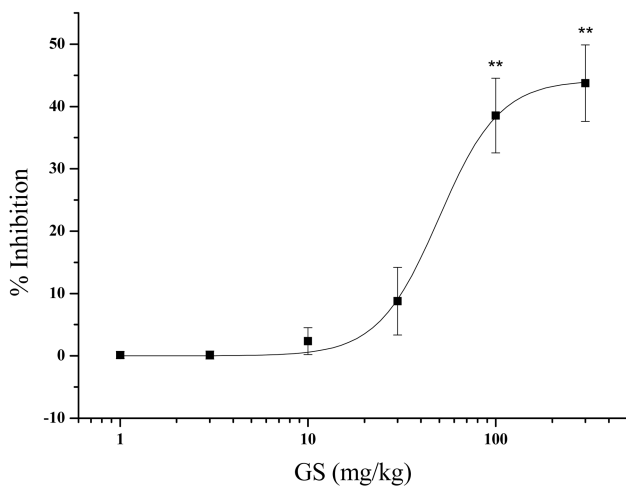
Forty eight animals were randomly divided into four groups (n=12, each group): A1, normal control group with modified KH solution with no ischemia: A2, Ginseng saponin administered group with no ischemia: A3, ischemia control group with no administration of GS: and A4, Ginseng saponin administered group with I/R injury (administration of GS before ischemia followed by reperfusion with modified KH buffer for 60 min). Ginseng saponin was added 5 min before ischemias were induced in the isolated hearts. All hearts were perfused for 20 min before the following perfusion protocol for equilibration (Fig. 2).

### Heart preparation and myocardial functions

Sprague-Dawley rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). Heparin (1000 U/kg) was injected through a femoral vein to prevent blood coagulation. In order to recover heart function, hearts were perfused in a retrograde fashion for 15 min according to the Langendorff method described previously.<sup>33)</sup> Briefly, the hearts were rapidly excised and placed in ice-cold (4 $^{\circ}$ C) modified KH buffer. Aorta and left atrium cannulations were performed rapidly, and the hearts were perfused in the Langendorff mode at a pres-



**Fig. 2.** Experimental protocol. All experimental groups began with a 20 min perfusion period to allow for stabilization of the isolated hearts. Then, the hearts were divided into the normal control group (A1; n=12), the GS control group (A2; n=12), the ischemia control group (A3; n=12) and the GS administered group (A4; n=12), which received administration of GS before ischemia induction.



**Fig. 3.** Inhibitory effect of GS on I/R-induced  $[Ca^{2+}]_i$  increase in rat neonatal cardiomyocytes. Responses evoked by I/R were quantified according to GS dose (1-100 mg/kg) to detect the maximal anti-calcium effect. Data are expressed as percentage inhibition change compared with the control response as 100% elicited by I/R. Each bar presents the mean  $\pm$  SEM from 25-30 cells per group under I/R-induced intracellular calcium increase. \*\*Significantly different from the control group under I/R induction ( $p < 0.01$ ) compared to the GS treatment group (100 and 300 mg/kg) based on Student's *t*-test.

sure of 100 cm H<sub>2</sub>O with modified KH buffer with 95% O<sub>2</sub>/5% CO<sub>2</sub> at pH 7.4 and at a constant temperature of 37°C. In the non-working heart model of the Langendorff apparatus, perfusion fluid (perfusate) entered the heart via the aorta retrograde from the aortic reservoir located 100 cm above the heart. The aortic reservoir, which was the thermostatically maintained oxygenator, carried out a perfusate to the aorta at a 100 cm H<sub>2</sub>O hydrostatic pressure maintained with the use of a constant head device. This system maintained the function of the heart, but did not maintain perfusate circulation to the ventricle. Such a system is used to recover and maintain heart function for non-working periods after isolation. In the working heart model, the left atrium cannula and aortic cannula were open and perfusate entered the heart via the left atrium from an atrial bubble trap located 20 cm above the heart. The left ventricle ejected perfusate via the aorta into an elasticity chamber (aortic pressure chamber) against a 20 cm H<sub>2</sub>O hydrostatic pressure to the aortic bubble trap. Perfusion pressure was recorded by a pressure transducer connected to the aortic cannula. Aortic flow was measured by the volume ejected from the aorta to the cannula located 100 cm above the heart. Coronary flow also was measured by the timed collection of perfusate from the pulmonary trunk (ml/min). Cardiac output was calculated

by summing the aortic and coronary flows. A 20 min equilibration period was used prior to administration of KH buffer or GS, which were administered into the aortic line for 5 min (flow rate 10 ml/min). Global ischemia was then induced by clamping both the aortic and atrial lines for 30 min. Reperfusion was conducted for 60 min during the post-ischemic period. The resulting hemodynamic data among the A1, A2, A3 and A4 groups were compared by analyzing perfusion pressure, aortic flow, coronary flow, and cardiac output to observe the cardioprotection of GS. This system makes it possible to compare the recovery of heart function before and after ischemia. Aortic and coronary perfusates were not recirculated in the present study. The entire apparatus was thermostatically maintained by a water jacket and a coil heat chamber. Heart rate was obtained by an ECG monitoring system (S & W Medico Teknik A/S, Denmark) with three electrodes attached to the epicardium. Systolic and diastolic perfusion pressures were measured throughout the working heart model perfusion periods in the aortic outflow line with a hemodynamic monitoring system (S & W Medico Teknik A/S, Denmark).

#### Preparation of cardiomyocytes

Cardiomyocytes were isolated from 1- to 2-day-neonatal Sprague-Dawley rats as described previously.<sup>34)</sup> Briefly, the hearts were isolated and minced with scissors into 1-2 mm fragments and then enzymatically digested in HEPES containing 0.1% collagenase. The liberated cells from each digestion were collected in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), centrifuged, and then re-suspended in DMEM with 10% FBS. The cell suspension was filtered through a 100  $\mu$ m-diameter nylon mesh, and the cells were plated onto a 10 cm culture dish for 2 h cultivation (37°C in humid air with 5% CO<sub>2</sub>) for purification. After that, cells were plated onto six-well plates (2×10<sup>6</sup> cells/well) and cultured in DMEM supplemented with 10% FBS and penicillin (100 U/ml)/streptomycin (100  $\mu$ g/ml) at 37°C in humid air with 5% CO<sub>2</sub>. Within two days of isolation, a confluent monolayer of spontaneously beating cardiomyocytes had formed, and the cells were used as described below.

#### Simulated ischemia and reperfusion *in vitro*

To mimic the ischemic injury *in vitro*, I/R was induced based on the previously described method.<sup>34)</sup> Briefly, after a three-day culture, cardiomyocytes were exposed to ischemia by replacing the medium with modified Esumi

ischemic buffer. This buffer has a high potassium content and a low pH and hence mimics the conditions of cardiomyocytes that have been exposed to low levels of oxygen. Esumi ischemic buffer contains 137 mM NaCl, 12 mM KCl, 0.49 mM MgCl<sub>2</sub>, 0.9 mM CaCl<sub>2</sub>·2H<sub>2</sub>O, 4 mM HEPES, and 20 mM sodium lactate (pH 6.2). The cells were incubated in the hypoxic/ischemic chamber (Kendro, Germany) at 37°C for 4 h in a humidified atmosphere of 5% CO<sub>2</sub> and 95% nitrogen. After 4 h of ischemia, the cardiomyocytes were returned to DMEM and then incubated in an atmosphere of 21% O<sub>2</sub> and 5% CO<sub>2</sub> for 16 h. In control normoxia experiments, the cells were incubated with DMEM in an atmosphere of 21% O<sub>2</sub> and 5% CO<sub>2</sub> for 20 h. Ginseng saponin at a range of doses from 1 to 300 mg/kg was added 5 min before the cardiomyocytes were exposed to I/R injury. The cardiomyocytes that had KH buffer added 5 min before I/R injury served as the 100% control.

#### Cytosolic Ca<sup>2+</sup> level in cardiomyocyte

Cytosolic Ca<sup>2+</sup> levels at the end of reperfusion were measured using the fluorescent Ca<sup>2+</sup> indicator Fluo-3/AM as described.<sup>35)</sup> Cardiomyocytes were incubated with 5  $\mu$ M Fluo-3/AM at 37°C for 30 min in the dark. The cells were then washed three times with PBS to remove the extracellular Fluo-3/AM dye, and then cytosolic Ca<sup>2+</sup> levels were determined with excitation at 488 nm and emission at 526 nm. Fura-2AM was dissolved in dimethyl sulfoxide plus pluronic acid, since dimethyl sulfoxide at this concentration does not affect [Ca<sup>2+</sup>]<sub>i</sub> levels nor cell length.<sup>36)</sup> The data were expressed as the relative fluorescence intensity. Inhibitory effects of GS were expressed as a percentage of the response to a maximal value of [Ca<sup>2+</sup>]<sub>i</sub> induced by the cardiomyocytes undergoing both no treatment of GS and I/R induction (as 100%). All imaging data were collected and analyzed using Universal Imaging software (West Chester, PA).<sup>37)</sup> In all cases, each experiment was repeated four or five times.

#### Statistical analysis

The results are presented as the mean±SEM. Statistical significance was compared between the treatment and control groups by Student's *t*-test. The results were considered statistically significant at a value of *p*<0.05.

## RESULTS AND DISCUSSION

#### GS inhibited I/R-induced [Ca<sup>2+</sup>]<sub>i</sub> level elevation

Ischemia-reperfusion injury led to a significant eleva-

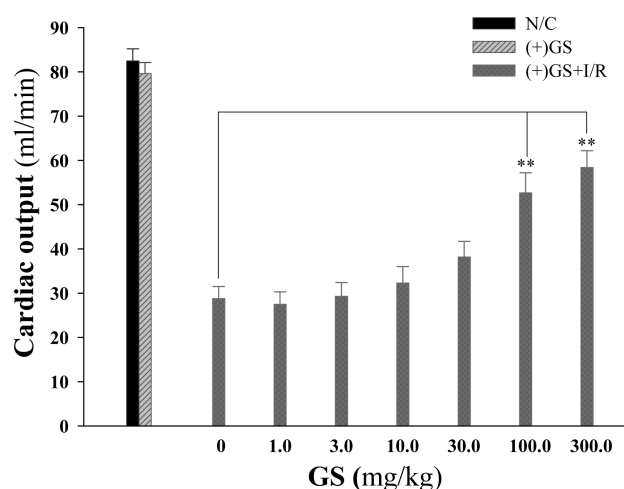
tion in  $[Ca^{2+}]_i$ .<sup>34)</sup> The effect of GS on  $[Ca^{2+}]_i$  elevation induced by simulated I/R *in vitro* was assessed to explore the underlying mechanism of the anti-ischemic effects of GS. Administration of GS in the range of 1 to 30 mg/kg did not attenuate I/R-induced  $[Ca^{2+}]_i$  elevation, but concentrations of GS >30 mg/kg significantly inhibited I/R-induced elevation of  $[Ca^{2+}]_i$  (Fig. 3;  $IC_{50}=49.76\pm 3.07$  mg/kg;  $n=25-30$ ). Namely, I/R-induced  $[Ca^{2+}]_i$  increases in cardiomyocytes were significantly reversed by the administration of 100 and 300 mg/kg GS (38.54±5.99% inhibition with 100 mg/kg GS and 43.73±6.15% inhibition with 300 mg/kg GS vs. 100% in the I/R control (\*\* $p<0.01$ , Fig. 3). Nevertheless, the administration of GS with no ischemia did not change  $[Ca^{2+}]_i$  (data not shown). This result suggests that GS attenuates I/R injury-induced  $[Ca^{2+}]_i$  elevation originating from external  $Ca^{2+}$  and, as a result, may represent the cellular mechanism for its cardioprotective role.

#### Determination of the maximal effective dose of GS

The maximum effective dose of GS was assessed by measuring cardiac output, the direct parameter of heart function, as increasing the doses of GS from 1, 3, 10, 30, 100 to 300 mg/kg. As shown in Fig. 4, there was no difference between the normal control (A1) and the 100 mg/kg GS control (A2) [79.6±2.5 ml/min (100%) vs. 82.5±2.7 ml/min (103.6%, compared with the 100% of the normal control)]. This result suggests that 100 mg/kg GS itself does not influence cardiac output in these conditions. Again using the doses of 1, 3, 10, 30, 100 and 300 mg/kg GS, the maximum recovery effect for cardiac output after 30 min of ischemia was obtained with the 100 and 300 mg/kg GS. The recovery effect on cardiac output after I/R injury was increased with doses of 100 and 300 μM (52.7±4.5 ml/min and 58.4±3.8 ml/min, respectively). However, 300 mg/kg GS is a very high concentration, and the recovery effects for cardiac output after I/R injury using 100 or 300 mg/kg GS were not significantly different (\*\* $p>0.05$ , Fig. 4). Therefore, the dose of 100 mg/kg was determined to be the appropriate dose of GS to optimize the anti-ischemic effect on ischemia-induced isolated rat hearts.

#### Effect of GS on heart rate

Since it is well-known that heart rate does not significantly change under ischemic conditions,<sup>38,39)</sup> the heart rates of I/R-induced isolated rat hearts were assessed. As shown in Table 1, the heart rates between pre-ischemic and post-ischemic conditions were not significantly different. Also, the heart rates of each group were not signif-



**Fig. 4.** Determination of maximum dose of that results in maximum anti-ischemic effect. Cardiac output was measured throughout the working heart model perfusion periods in the aortic flow plus coronary flow with a hemodynamic monitoring system in both groups to detect the maximal anti-ischemia effect according to dose of GS (0-300 mg/kg). Black histograms represent the mean±SEM from twelve rats per control group without any treatment under no ischemia conditions (A1). Striped histograms represent the mean±SEM from twelve rats per treatment group of GS under no ischemia conditions (A2). Gray histograms represent the mean±SEM from twelve rats per treatment group of GS under post ischemia conditions according to dose of GS (1-300 mg/kg). \*\* $p<0.01$  vs. ischemia control group based on Student's *t*-test.

icantly different ( $p>0.05$ , Table 1). These results indicate that heart rate does not change in I/R-induced isolated rat hearts regardless of administration of GS.

#### Effects of GS on perfusion pressure

Anti-ischemic effects of the GS were continuously observed for 60 min during the post-ischemic period in each group. The perfusion pressures of the normal control (A1) during the post-ischemic periods were 89.7±3.12, 90.6±3.07, 90.5±3.13, 89.7±2.98, 90.3±3.14 and 89.3±3.03 ml/min at the times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the GS control (A2) were 90.3±2.95, 89.7±2.87, 89.1±3.02, 89.5±3.13, 90.5±3.2 and 89.4±3.28 ml/min at the corresponding post-ischemic times, respectively (Fig. 5). Similarly, the perfusion pressures in the ischemia control (A3) were 56.7±5.56, 55.6±4.32, 56.8±3.71, 55.7±3.81, 55.8±3.97 and 54.6±4.03 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS administered group (A4) were 62.8±5.78, 66.7±4.2,

**Table 1.** Heart rate in ischemia-induced isolated rat hearts

	Pre-ischemia (min)		Post-ischemia (min)					
	10	20	10	20	30	40	50	60
A1	289.7±28.5	282.7±31.3	278.5±27.2	279.7±33.7	283.7±25.4	278.3±28.7	286.9±23.5	281.8±29.4
A2	294.3±28.8	289.6±23.8	276.9±26.3	286.1±24.6	290.3±30.1	289.3±24.7	277.1±28.4	281.4±29.8
A3	279.6±23.9	276.4±27.4	286.9±31.5	291.2±35.3	289.4±29.7	290.0±28.6	288.6±29.1	286.1±28.7
A4	283.2±26.8	288.1±29.9	295.3±31.6	290.5±30.6	286.4±28.8	293.1±29.3	285.6±26.3	288.4±26.7

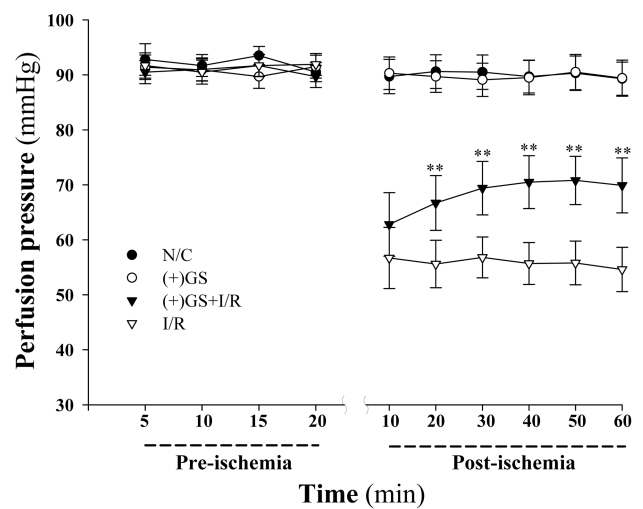
(heartbeats per min.)

A1: Normal control group with KH solution,  
 A2: GS administered group with no ischemia,  
 A3: Ischemia control group with no administration of GS  
 A4: 100 mg/kg GS administered group with I/R injury

69.4±4.86, 70.5±4.8, 70.8±4.38 and 69.9±4.99 ml/min at the corresponding post-ischemic times, respectively. On average, perfusion pressure on ischemia control (A3) was substantially decreased to an average of 55.86±4.12 ml/min under post-ischemic conditions for 60 min compared to an average 90.02±3.11 ml/min for the normal control (A1) (Fig. 5). However, such decreases were inhibited to an average of 68.35±4.88 ml/min by the administration of 100 mg/kg GS compared to that of the ischemia control (55.86±4.12 ml/min) (\*\**p*<0.01). Especially, the differences in perfusion pressure between the GS control (A2) and the normal control (A1) were not significant during the 0 to 20 min pre-ischemic period or in the 0 to 60 min post-ischemic period (Fig. 5).

### Effect of GS on aortic flow

The volumes (ml/min) of aortic flow in the normal control group (A1) were 58.7±3.0, 58.9±3.0, 60.4±3.0, 73.4±2.9, 61.8±2.8 and 61.5±2.54 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS control group (A2) were 57.7±2.9, 58.0±3.0, 59.3±2.6, 59.5±2.4, 59.1±2.8 and 58.5±2.9 ml/min at the corresponding post-ischemic times, respectively (Fig. 6). Similarly, the volumes of aortic flow in the ischemia control (A3) were 20.2±3.2, 20.5±3.2, 19.7±3.8, 18.9±4.3, 18.1±4.3 and 18.5±4.6 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS administrated group (A4) were 32.8±4.4, 32.7±4.1, 35.6±4.5, 39.4±4.8, 39.8±4.8 and 40.5±4.7 ml/min at the corresponding post-ischemic times, respectively. On average, aortic flow on was substantially decreased to an average of 19.3±4.2 ml/min in ischemia control for 60 min compared to an average 60.3±2.9 ml/min for the normal control (A1). However, such decreases were inhibited to an average of 36.8±4.5 ml/min by the administration of 100 mg/kg GS compared to that of the

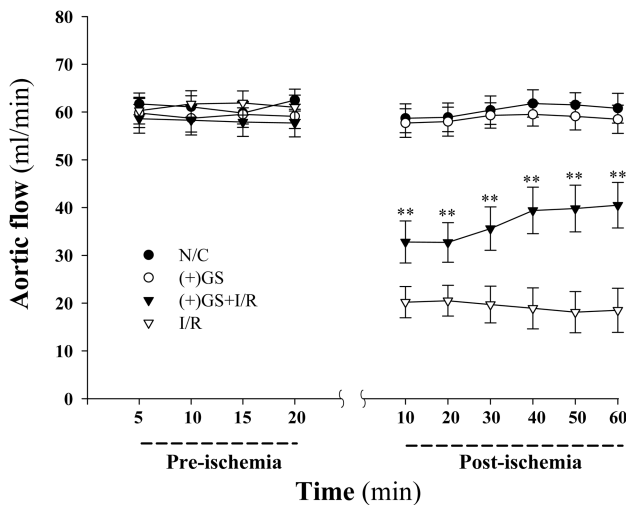


**Fig. 5.** Recovery effect of the GS on perfusion pressure of ischemia-reperfusion (I/R) induced isolated rat heart. Perfusion pressure was measured throughout the working heart model perfusion periods in the aortic outflow line with a hemodynamic monitoring system in the control and treatment groups of GS to detect an anti-ischemia effect. Each symbol represents the mean±SEM from twelve rats per group with (●) denoting the normal control group without any treatment and ischemia (A1), (○) the treatment group of GS under normal conditions without ischemia (A2), (▼) the treatment group of GS under ischemic conditions (A3), and (▽) the I/R group without any treatment (A4). \*\**p*<0.01 vs. ischemia control group based on Student's *t*-test.

ischemia control group for 60 min (\*\**p*< 0.01, Fig. 6). Especially, the differences between the GS control (A2) and the normal control (A1) in aortic flow were not significant during the 0 to 20 min pre-ischemic period or in the 0 to 60 min post-ischemic periods (Fig. 6).

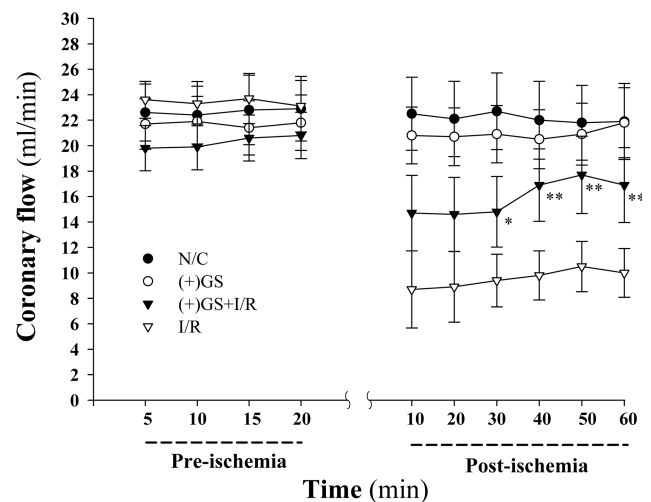
### Effect of GS on coronary flow

The volumes (ml/min) of coronary flow in the normal



**Fig. 6.** Recovery effect of the GS on aortic flow of ischemia-reperfusion (I/R) induced isolated rat heart. Aortic flow was measured by timed collection of perfusate from the aortic cannula in each group to detect an anti-ischemia effect. Each symbol represents the mean $\pm$ SEM from twelve rats per group with (●) denoting the normal control group without any treatment and ischemia (A1), (○) the treatment group of GS under normal conditions without ischemia (A2), (▼) the treatment group of GS under ischemic conditions (A3), and (▽) the I/R group without any treatment. \*\* $p$ <0.01 vs. ischemia control group based on Student's  $t$ -test.

control (A1) were 22.5 $\pm$ 2.8, 22.1 $\pm$ 2.9, 22.7 $\pm$ 3.0, 22.0 $\pm$ 3.0, 21.8 $\pm$ 2.9 and 21.9 $\pm$ 2.9 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS control (A2) were 20.8 $\pm$ 2.2, 20.7 $\pm$ 2.2, 20.9 $\pm$ 2.2, 20.5 $\pm$ 2.3, 20.9 $\pm$ 2.4 and 21.8 $\pm$ 2.7 ml/min at the corresponding post-ischemic times, respectively (Fig. 7). Similarly, the volumes of coronary flow in ischemia control (A3) were 8.7 $\pm$ 3.3, 8.9 $\pm$ 2.7, 9.4 $\pm$ 2.0, 9.8 $\pm$ 1.9, 10.5 $\pm$ 1.9 and 10.0 $\pm$ 1.9 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS administered group (A4) were 14.7 $\pm$ 2.9, 14.6 $\pm$ 2.9, 14.8 $\pm$ 2.7, 16.9 $\pm$ 2.8, 17.7 $\pm$ 3.0 and 16.9 $\pm$ 2.9 ml/min at the corresponding post-ischemic times, respectively. On average, coronary flow was substantially decreased to an average of 9.5 $\pm$ 2.4 ml/min in ischemia control (A3) for 60 min compared to an average 22.1 $\pm$ 2.9 ml/min for the normal control group (A1). However, such decreases were inhibited to an average of 15.9 $\pm$ 2.8 ml/min by the administration of 100 mg/kg GS compared to that of the ischemia control for 60 min (\*\* $p$ <0.01, Fig. 7). Especially, the differences between the 100 mg/kg GS control (A2) and the normal control (A1)

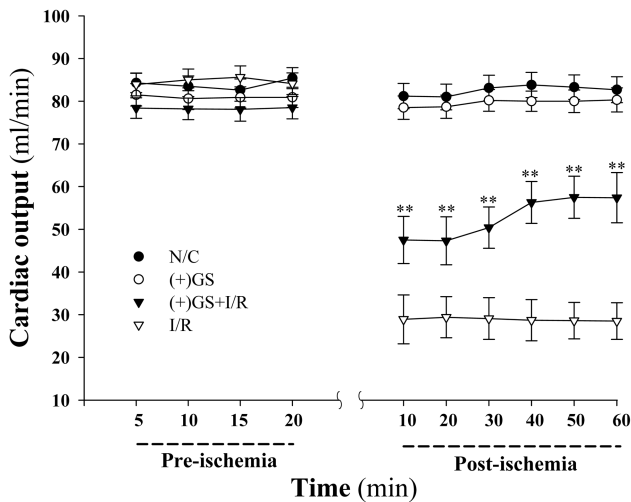


**Fig. 7.** Recovery effect of the GS on coronary flow of ischemia-reperfusion (I/R) induced isolated rat heart. Coronary flow was measured by timed collection of perfusate from pulmonary trunk in each group to detect an anti-ischemia effect. Each symbol represents the mean $\pm$ SEM from twelve rats per group with (●) denoting the normal control group without any treatment and ischemia (A1), (○) the treatment group of GS under normal conditions without ischemia (A2), (▼) the treatment group of GS under ischemic conditions (A3), and (▽) the I/R group without any treatment. Significantly different from the ischemia control group (\* $p$ <0.01 and \*\* $p$ <0.01) based on Student's  $t$ -test.

in coronary flow were not significant during the 0 to 20 min pre-ischemic period and in the 0 to 60 min post-ischemic period (Fig. 7).

#### Effects of GS on cardiac output

The volumes (ml/min) of cardiac output in the normal control (A1) were 81.2 $\pm$ 2.9, 81.0 $\pm$ 3.0, 83.1 $\pm$ 2.9, 83.8 $\pm$ 2.9, 83.3 $\pm$ 2.8 and 82.7 $\pm$ 3.0 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS control (A2) were 78.5 $\pm$ 2.7, 78.7 $\pm$ 2.7, 80.2 $\pm$ 2.5, 80.0 $\pm$ 2.3, 80.0 $\pm$ 2.6 and 80.3 $\pm$ 2.8 ml/min at the corresponding post-ischemic times, respectively (Fig. 8). Similarly, the volumes of cardiac output in the ischemia control (A3) were 28.9 $\pm$ 5.7, 29.4 $\pm$ 4.8, 29.1 $\pm$ 4.9, 28.7 $\pm$ 4.8, 28.6 $\pm$ 4.2 and 28.5 $\pm$ 4.3 ml/min at the post-ischemic times of 10, 20, 30, 40, 50 and 60 min, respectively, while those in the 100 mg/kg GS administered group (A4) were 47.5 $\pm$ 5.5, 47.3 $\pm$ 5.6, 50.4 $\pm$ 4.8, 56.3 $\pm$ 4.9, 57.5 $\pm$ 4.9 and 57.4 $\pm$ 5.8 ml/min at the corresponding post-ischemic times, respectively. On average, under post-ischemic conditions for 60 min, cardiac output of the



**Fig. 8.** Recovery effect of the GS on cardiac output of ischemia-reperfusion (I/R) induced isolated rat heart. Cardiac output was calculated by summing the aortic and coronary flows ( $CO=CF+AF$ ). Each symbol represents the mean $\pm$ SEM from twelve rats per group with (●) denoting the normal control group without any treatment and ischemia (A1), (○) the treatment group of GS under normal conditions without ischemia (A2), (▼) the treatment group of GS under ischemic conditions (A3), and (▽) the I/R group without any treatment.  $**p<0.01$  vs. ischemia control group based on Student's *t*-test.

ischemia control (A3) was substantially decreased to an average of  $28.8\pm 4.7$  ml/min compared to an average  $82.5\pm 2.9$  ml/min of normal control (A1). However, such decreases was inhibited to an average of  $52.7\pm 5.3$  ml/min by the administration of 100 mg/kg GS compared to that of the ischemia control for 60 min ( $**p<0.01$ , Fig. 8). Especially, the differences between the 100 mg/kg GS control group (A2) and the normal control (A1) in cardiac output were not significant during the 0 to 20 min pre-ischemic period or in the 0 to 60 min post-ischemic period (Fig. 8).

Many pharmacological actions of ginseng are attributed to its ginsenosides. Some pharmacological studies of ginsenosides on ischemia have been reported. For example, ginsenoside Rg<sub>3</sub> might provide protection against ischemia-induced injury in the rat brain by reducing lipid peroxides, scavenging free radicals, and improving energy metabolism.<sup>40</sup> Ginsenoside Rb<sub>1</sub> protects the cerebral cortex and the hippocampal CA1 neurons against ischemic damage.<sup>41,42</sup> The onset of severe ischemia in the myocardium sets into motion a series of pathological events that result in the death of the tissues. These changes begin seconds after ischemia and they occur due to the insufficient oxy-

gen supply to support oxidative phosphorylation in the cardiac tissue.<sup>43</sup> The isolated perfused small mammalian heart probably represents the optimal compromise in the conflict between the quantity and quality of data that can be acquired from an experimental model of ischemia versus its clinical relevance.<sup>44</sup> The isolated perfused heart provides an excellent test-bed for undertaking carefully controlled dose-response studies of metabolic or pharmacological interventions. The preparation also allows the induction of whole heart ischemia or regional ischemia at various levels of flow. Similarly, in the presence of normal flow, anoxia or hypoxia can be easily imposed at various degrees of oxygen deprivation. The isolated heart preparation can be easily reperfused or reoxygenated at various rates and with multiple perfusate compositions. This system provides a powerful tool for assessing many aspects of I/R injury. The rat heart is by far the best characterized, as well as being the most frequently used, organ for complex perfusion preparations such as working and blood-perfused hearts.<sup>45</sup> In terms of handling ease, the rat has a great advantage over smaller hearts, such as those of the mouse, in which intraventricular pressure recordings are more difficult. Under these ischemic conditions, myocardial oxidative metabolism is suppressed and glycolysis becomes an important source of ATP generation.<sup>46</sup> The increased glycolytic rate, in the face of impaired glucose oxidation, leads to the uncoupling of the two pathways resulting in a buildup of lactate and H<sup>+</sup>. This process may continue during reperfusion. The accumulation of protons leads to the downstream activation of pathways (Na<sup>+</sup>/H<sup>+</sup> exchanger, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger) that result in a Ca<sup>2+</sup> level increase, impaired contractile function, and/or cell death.<sup>46</sup> One of the main indicators of I/R injury is derived from the disturbance of intracellular Ca<sup>2+</sup> homeostasis. Recent reports have shown that hypoxia-induced cellular ATP exhaustion is coupled to intracellular Ca<sup>2+</sup> elevation.<sup>47</sup> The present study further suggests that GS could protect against I/R injury in the isolated rat heart, like that attributed to the intracellular Ca<sup>2+</sup> elevation. The anti-ischemic effects of GS on I/R-induced heart injury were investigated through analyses of perfusion pressure, aortic flow, coronary flow, and cardiac output. To our knowledge, this is the first report providing evidence of the effectiveness of GS on an isolated rat heart model. The administration of GS significantly prevented decreases in perfusion pressure, aortic flow, coronary flow, and cardiac output induced by I/R conditions. Thus, we have demonstrated that the administration of GS exhibited protective effects against I/R injury. We present principal findings on



the GS protective actions against I/R injury using *in vitro* studies. The finding was that GS-induced cardioprotection against I/R injury might be derived from the inhibition of ischemia-induced  $\text{Ca}^{2+}$  influx via an L-type calcium channel, as well as other types of  $\text{Ca}^{2+}$  channels. A previous report showed that GS suppresses L-type  $\text{Ca}^{2+}$  channel in neuron.<sup>48)</sup> Thus, GS might be able to attenuate the I/R-induced  $[\text{Ca}^{2+}]_i$  elevation in cardiomyocyte. Our study also showed that GS inhibited the  $[\text{Ca}^{2+}]_i$  increase (Fig. 3), consistent with the results of a previous report (Wang et al., 2008). This finding indicates that GS protects the cardiomyocytes through the inhibition of  $[\text{Ca}^{2+}]_i$  elevation. Clinically, therapeutic plasma concentrations of GS in humans are unknown. However, in our experiments, we observed cardioprotection after the administration of GS (100 and 300 mg/kg). Concentrations below 30 mg/kg may have no protective effects against I/R injury, since we did not observe any effect of GS ( $\leq 30 \mu\text{M}$ ) on I/R injured rat hearts. Therefore, the concentrations of GS that may exert cardioprotection against I/R injury in the rat are those over 100 mg/kg. Further studies are needed to explore the effective concentration *in vivo*. The observation that GS can protect *ex vivo* ischemic and reperfused hearts expands the spectrum of the potentially favorable effects of GS. This finding may have profound implications for the early administration of GS in a variety of clinical conditions in which the heart is subjected to ischemia followed by reperfusion (acute coronary syndrome, coronary angioplasty, thrombolytic therapy, and cardiopulmonary bypass surgery). The main contributing factors of GS-induced protection against I/R injury may be due to the inhibitory effects on the  $[\text{Ca}^{2+}]_i$  elevations that can be caused by  $\text{Ca}^{2+}$  channel. In summary, using the isolated I/R injury rat heart model, we obtained results suggesting that GS provides cardioprotection against I/R injury. The present findings further suggest that administration of GS might be a novel preventive strategy against I/R injury. However, the molecular mechanism of GS with respect to its cardioprotective effects should be studied further before firm conclusions are drawn.

## CONCLUSION

The anti-ischemic effects of GS on the ischemia-induced isolated rat heart were investigated through analyses of changes in perfusion pressure, aortic flow, coronary flow, and cardiac output. Administration of GS under ischemic conditions significantly prevented decreases in perfusion pressure, aortic flow, coronary flow, and cardiac

output. These results suggest that G-Re has distinct anti-ischemic effects. Also, in our *in vitro* studies, the inhibitory effects on  $[\text{Ca}^{2+}]_i$  elevations were investigated. In conclusion, the administration of GS has cardioprotective effects on the rat heart after I/R injury

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