Protective Effects of Pretreatment with Ginsenosides on Cardiac and Coronary Vascular Function After Hypothermic Rat Heart Preservation

Jie-Min ZHANG[1], Yuichiro MATSUURA[1], Taijiro SUEDA[1], Kazumasa ORIHASHI[1], Shintaro FUKUNAGA[2] and Masanobu WATARI[1]

1) *First Department of Surgery, Hiroshima University School of Medicine, Kasumi 1–2–3, Minami-ku, Hiroshima, 734–8551, Japan.*
2) *Department of Clinical Engineering, Faculty of Health Sciences, Hiroshima International University, Hiroshima, Japan.*

ABSTRACT

Prevention of cardiac and vascular dysfunction with pretreatment has been accepted as an important factor in heart transplantation. Ginsenosides (GS) have been reported to have some beneficial effects on the cardiac and vascular system. We hypothesized that pretreatment with GS would result in an improvement of functional recovery after a 12 hour (hr) rat heart preservation.

A Langendorff apparatus was applied to estimate the cardiac and vascular function in an isolated rat heart preparation. The hearts were preserved in University of Wisconsin solution at 0°C for 12hr, after pretreatment with 0.9% sodium chloride or GS 100 mg/kg, respectively, in control (n = 9) and GS (n = 14) groups. After storage, the cardiac function, myocardial water content, and coronary vasodilatory response were evaluated.

The GS group showed a significantly higher recovery percentage of cardiac function compared with the control group: aortic flow 81.4 ± 21.4% versus 57.2 ± 11.0% (p = 0.0052); coronary flow 81.4 ± 14.5% versus 57.2 ± 6.0% (p = 0.0001); ±dp/dt max 72.5 ± 16.1% and 66.0 ± 16.1% versus 53.7 ± 4.1% and 51.4 ± 7.1% (p = 0.0027 and p = 0.0189) respectively. The GS group showed a lower increase in myocardial water content. With Langendorff perfusion, the endothelial and vascular smooth-muscle cell function were evaluated by an increasing percentage of coronary flow in response to acetylcholine chloride (0.3×10^{-7} mol/liter) and nitroglycerin (0.5×10^{-5} mol/liter). It was significantly higher in the GS group than that in the control group (19.2 ± 8.8% and 28.0 ± 14.1% versus 9.9 ± 4.7% and 14.7 ± 8.1%, p = 0.008 and p = 0.0187, respectively) at the first minute.
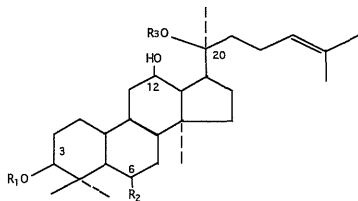
These results suggest a protective effect on ventricular and coronary vascular function in the rats pretreated with Ginsenosides, indicating potential benefits for long-term heart preservation.

Key words: Langendorff perfusion, Heart preservation, Ginsenosides, Reperfusion damage

Prevention of cardiac and vascular dysfunction with suitable pretreatment has been accepted as an important factor in heart transplantation[2,9,21]. An intracellular-type preservation solution, University of Wisconsin (UW) solution, has been used widely as an allograft preservation solution[8,12,32]. However, it contains a high potassium concentration and impairs coronary endothelial function after reperfusion[3,19]. This endothelial damage in early reperfusion leads to chronically increased vascular tone and can also contribute to early atherosclerosis[29]. Therefore, an effective method to prevent the damage to the myocytes and endothelium may still be required for more successful heart preservation.

Ginsenosides (GS), saponins from Panax ginseng, are derivatives with a triterpene dammarane structure (see Fig. 1). Minor components include amino acids, peptides, and minerals. Over 20 Ginsenosides have been extracted from roots. Aglycones of the common ginsenosides are 20 (S)-proropanaxadiol (Rb_1, Rb_2, Rc, and Rd) or 20 (S)-protopanaxatriol (Re, Rf, Rg_1, and Rg_2) structures[27,30]. GS have been documented to possess some suitable cardiac and vascular effects[4,5,10,11,13,15,34–36]. In 1990, An improvement to a myocardial/reperfusion injury was achieved by us in a cardiopulmonary bypass with dogs by perfusing via the aorta with GS added to the cardioplegia[36]. In 1994, the cardioprotective action of GS against ischemia-reperfusion injury was reported in a study of patients undergoing cardiopulmonary bypass for mitral valve surgery[35]. GS may also enhance the cardiac and coronary vascular func-

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Fig. 1. Structures of main ginsenosides of Panax ginseng
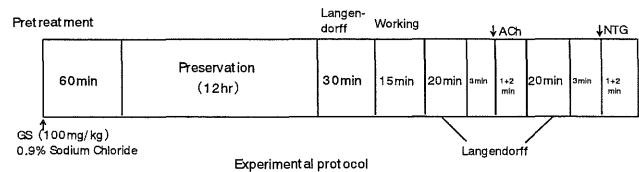

Fig. 2. The hearts were pretreated with GS or saline at 60 min before preservation in UW solution for 12 hr. The hearts were reperfused on L-mode and on W-mode, where hemodynamic indexes were recorded. After perfusion on L-mode for 20 min, CF was measured before and after administration of ACh, followed by measurement before and after administration of NTG.

tion after ischemia-reperfusion with pretreatment. Thus, we investigated the effects of GS on the cardiac and vascular function of isolated rat heart after 12-hour hypothermic preservation with UW solution.

MATERIALS AND METHODS

All animals received human care in compliance with the "Principle of Laboratory Animal Care" formulated by the National Society for Medical Research and, "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH Publication No. 86–23, revised 1985) and the "Guideline for Animal Experimentation" published by the Japanese Association for Laboratory Animal Science (Exp. Anim. 36 (3) 285–288, 1987).

Study protocol (Fig. 2)

Male Wistar rats weighing 300 to 450g were anesthetized by an intraperitoneal injection of sodium pentobarbital (65 mg/kg) and mechanically ventilated (EVM-50, Type 1 Ventilator, Nihon-Akai, Inc., Japan) via a tracheostomy. The heart was exposed by median sternotomy. After receiving systemic heparin (1000 units/kg), the heart was arrested with an intravenous injection of 4°C Young solution (containing potassium citrate, 74 mmol/liter, and magnesium sulfate, 220 mmol/liter), 2.0 to 4.5 ml per rat, then excised and immediately immersed in 4°C Krebs-Henseleit buffer (KHB) solution (consisting of in mmol/liter: NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 3.0, $NaHCO_3$ 25.0, Na_2EDTA 0.5 and glucose 11.0), and quickly perfused from the aorta with 60 ml/kg of UW solution (consisting of in mmol/Liter: hydroxyethyl starch 5g%, raffinose 30, K-lactobionate 100, KH_2PO_4 25, $MgSO_4$ 5, adeno-

sine 5, glutathione 3, allopurinol 1, bactrim 0.5, insulin 100, dexamethasone 16, with osmolarity 320 mOsm/liter, pH 7.4) at 4°C under 38 mmHg pressure continuously. The hearts were stored, and submersed in 50 ml of UW solution and surrounded in crushed ice in a large refrigerated room at 0°C for 12 hr.

The rats were divided into three groups: non-storage (n = 9), control (n = 9) and GS (n = 14) groups. The rats of the control and GS groups were administered intravenously with 0.9% sodium chloride 15 ml/kg or GS 100 mg/kg for 60 min, respectively, before the heart was excised. After cold storage, the heart was mounted on a Langendorff apparatus (UPH-W₂, Unique Medical CO., LTD., Japan) via the aorta and perfused with KHB solution with a constant pressure of 70 mmHg for 30 min in Langendorff perfusion mode (L-mode). KHB solution was filtered (40 micron), equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C. The KHB perfusate in this circuit was not recycled. Meanwhile, the KHB perfusate was monitored by a blood gas analysis apparatus (ABL 510, Nihon Radiometer, Copenhagen, Japan). During preparation, care was taken to cannulate the excised hearts rapidly to minimize the ischemic time. Following cannulation of the left atrium (LA) via the auricle, the heart was switched to working heart perfusion mode (W-mode) with a LA perfusion pressure of 10 mmHg and afterload of 70 mmHg for measurements of the hemodynamic indexes. They included: aortic flow (AF), coronary flow (CF), systolic pressure (SP), left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and rate of left ventricular pressure rise or fall (± dp/dt). The hearts were paced in an atrial mode at 220 beats/min.

The aortic flow was recorded with an electromagnetic flow meter (Flow 0.1 liter/min, Model FF-030T, Nihon Koden Inc., Japan). The LVDP, LVEDP and LV ±dp/dt (mmHg/sec) were measured by puncturing the left ventricular apex with a pressure transducer (AP-641G Type and EQ-

601G Type, Nihon Koden Inc., Japan). The vascular dilatory function of the endothelial and vascular smooth-muscle cells was assessed by the increasing percentage of coronary flow in response to ACh (0.3×10^{-7} mol/liter) and NTG (0.5×10^{-5} mol/liter). After the cardiac functional indexes were evaluated, the hearts were then perfused with the KHB solution on L-mode for 20 min for stabilization, and the CF was measured for 3 min after stabilization. The hearts were perfused with KHB solution containing acetylcholine chloride (ACh) 0.3×10^{-7} mol for 3 min. The CF was measured for 1 min, and total content (of 2 and 3 min) during the infusion of the drug. The hearts were perfused with KHB solution on L-mode for the next 20 min to reestablish the basal CF. The hearts were subsequently perfused with KHB solution containing nitroglycerin (NTG) 0.5×10^{-5} mol for 3 min with the same method. An increased percentage of CF after perfusion with KHB solution containing ACh and NTG was calculated and expressed per minute.

A ventricular specimen was weighed after the experiment was finished, and reweighed after the specimen dried at 80°C for 24 hr. The myocardial water content was calculated using the following formula:

myocardial water content (%)
= (wet weight - dry weight)/wet weight × 100

Preparation of Chemicals and Reagents

Ginsenosides, extracted from Chinese Panax ginseng of root C.A. Meyer[28] provided by the Chemical Department of Norman Bethune University of Medical Sciences, China; University of Wisconsin solution from the Netherlands Production Laboratory for Blood Transfusion Apparatus and Infusion Fluids, Inc., Holland (imported by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan); acetylcholine chloride from The Daiichi Pharmaceutical, Inc., Japan; nitroglycerin from Nihon-Kayaku, Inc., Japan.

Statistical analysis

Results for each group are expressed as the mean ± standard deviation (SD). The three groups were compared with a Fisher's Protected Least Significant Difference (Fisher's PLSD) test and the two groups were compared with a Student's t-test by using statistic software StatView 4.5. Statistical significance was accepted at P values less than 0.05.

RESULTS

Cardiac Function and Recovery

The myocardial hemodynamic indexes measured in the GS and control groups were assessed as a recovery percentage and compared with those in the non-storage group. Cardiac function is summarized in Table 1. The hearts in the GS group showed a significantly higher recovery percentage of cardiac function compared with those in the control group: aortic flow 81.4 ± 21.4% versus 57.2 ± 11.0% (p = 0.0052); coronary flow 81.4 ± 14.5% versus 57.2 ± 6.0% (p = 0.0001); systolic pressure 83.6 ± 12.7% versus 71.4 ± 6.6% (p = 0.0148); left ventricular developed pressure 81.7 ± 13.2% versus 65.8 ± 5.6% (p = 0.0027); left ventricular end-diastolic pressure 111.9 ± 16.6% versus 135.6 ± 7.9% (p = 0.0007); ± dp/dt max 72.5 ± 16.1% and 66.0 ± 16.1% versus 53.7 ± 4.1% and 51.4 ± 7.1% (p = 0.0027 and p = 0.0189), respectively.

Myocardial water content (Table 2)

The myocardial water content was 83.8 ± 0.6% in the non-storage group, 86.0 ± 0.8% in the GS group, and 87.1 ± 0.6% in the control group. A lower increase in myocardial water content was achieved in the GS group than that in the control group (p = 0.0004).

Coronary vasodilatory response

The baseline coronary flow after reperfusion with Langendorff was not significantly different between the control and GS groups. An administration of vasodilatory agents resulted in a reproducible increase in CF without causing any significant changes in the systolic pressure in either groups. The vasodilatory response to ACh and NTG is shown in Figs. 3 and 4. The increased

Table 1. Cardiac Function

Group	Time	AF (ml/min)	CF (ml/min)	SP (mmHg)	LVDP (mmHg)	LVEDP (mmHg)	+dp/dt max (mmHg/S)	-dp/dt max (mmHg/S)
Non (n=9)	0	40.2±3.2†	10.9± .9††	91.5± 4.4††	107.2± 5.3††	5.95±.4	2354.9±377.8†††	1755.9±179.6†††
Con (n=9)	12	24.1±4.1	6.2± .7	65.3± 6.0	70.5± 6.1	8.1 ±.5	1265.0± 95.3	902.3±125.1
GS (n=14)	12	32.7±8.6**	8.8±1.6***	75.9±11.1*	87.6±14.2	6.6 ±.98***	1707.6±379.3**	1158.5±282.9*

Non: Non-storage group; Con: control group (pretreatment with 0.9% Sodium Chloride 15 ml/Kg); GS: Ginsenosides group (pretreatment with Ginsenosides 100 mg/Kg). Time: preservation time of heart; AF: aortic flow; CF: coronary flow; SP: systolic pressure; LVDP: left ventricular developed pressure; LVEDP: left ventricular end-diastolic pressure; ±dp/dt max: maximum rate of left ventricular pressure rise or fall. Values are mean ± SD. * p <.05, ** p <.01, *** p <.001, as absolute values of GS group compared with control group. †p <.05, ††p <.001, †††p <.0001 as absolute values of GS group compared with non-storage group.

Table 2. Myocardial Water Content

Group	Water content (%)
Non-storage (n=9)	83.8±.6[†††]
Control (n=9)	87.1±.6
GS (n=14)	86.0±.8[***]

Water content (%) = (wet weight - dry weight)/wet weight × 100. Values are mean ± SD. [***] p = 0.0004 as GS group compared with control group. [†††] p < .0001 as GS group compared with Non-storage group.
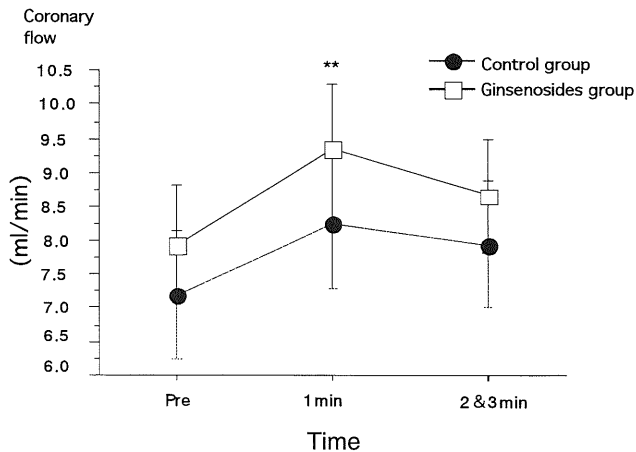

Fig. 3. Coronary vasodilatory response to Acetylcholine. Acetylcholine chloride: 0.3×10^{-7} mol/liter; Pre: mean values (ml/min) of all contents for 3 min before administration of ACh. 1 min: the contents of the first minute after the drugs were administered. 2 and 3 min: mean values (ml/min) of contents of the second and third minutes after the drugs were administered. Values are mean ± SD. **p < .01 as increased percentage of GS group compared with control group.

Fig. 4. Coronary vasodilatory response to Nitroglycerin. Nitroglycerin: 0.5×10^{-5} mol/liter; Pre: mean values (ml/min) of all contents for 3 min before administration of NTG. 1 min: the contents of the first minute after the drugs were administered. 2 and 3 min: mean values (ml/min) of contents of the second and third minutes after the drugs were administered. Values are mean ± SD. *p < .05 as increased percentage of GS group compared with control group.
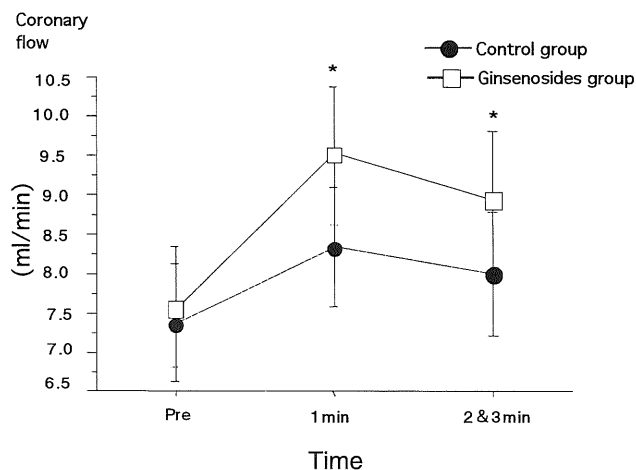
percentage in the coronary flow was significantly higher in the GS group than that in control group (19.2 ± 8.8% and 28.0 ± 14.1% versus 9.9 ± 4.7% and 14.7 ± 8.1%, p = 0.008 and p = 0.0187, respectively) at 1 min.

DISCUSSION

Effect of GS on Cardiac Function

This study clearly demonstrates that GS have beneficial effects on preserving cardiac and coronary vascular function after hypothermic heart preservation for 12 hr. It is expected that the mechanism of this effect in reperfusion injury will be clarified. A lot of studies have shown that the hypothesis that the mechanism explains the cellular events involved in reperfusion damage is oxygen-derived free radical damage and calcium overload[22]. Free radicals probably exert their damaging effects by disrupting membrane lipids or membrane-bound proteins. Impaired cell membranes cause an increase in the intracellular free calcium that may lead to reperfusion injuries such as reperfusion arrhythmias, vascular damage and no-reflow, and myocardial stunning[22,33].

In our experiment, the hearts in the GS group showed a better recovery of cardiac function at reperfusion. Both the systolic and diastolic function of the left ventricle as well as coronary flow were better in the GS group, suggesting that GS prevented myocardial ischemia/reperfusion damage after hypothermic heart preservation. The mechanism may be that GS increase 6-keto-PGF1α and decrease lipid peroxidation to protect against myocardial ischemia-reperfusion damage[5]. It is a key condition that stable cardiac function and higher coronary flow are preserved in the donor in heart transplantation.

Effect of GS on Coronary Vascular Function

The endothelium is an important regulator of vasomotor tone in the coronary microvasculature, which is important in the regulation of myocardial perfusion[20,26]. A key concept derived from experimental work is that reperfusion injury is triggered by dysfunction of the reperfused endothelium[17,18,37].

Reperfusion injury may be viewed as an imbalance between endogenous forces that promote and those that inhibit cell adherence and activation. Two potent endogenous inhibitors of cell adherence and activation are prostacyclin and nitric oxide (NO)[1,16,23,24,31]. NO has a wide spectrum of effects, loss of which may contribute to the development of ischemia-reperfusion injury. NO is a powerful vasodilator; this effect is mediated through increased levels of cyclic guanosine monophosphate (cGMP) in vascular smooth-muscle cells. NO inhibits platelet aggregation and adhesion to the endothelium, again through stimulation of cGMP[7]. Kang et al reported that GS induced endothelium-dependent relaxation and an

increase in the tissue content of cGMP in the rat aorta[13].

Our experiment has shown that GS have a remarkable effect on coronary artery vasodilation and cause an increase of coronary flow in the endothelium-dependent vasodilatory response to ACh. It indicates that GS protect the coronary endothelium and prevent the coronary vascular dysfunction which is induced by reperfusion injury after hypothermic rat heart preservation. GS might attenuate the endothelial damage induced by a high concentration of potassium in the UW solution. It is possible that more successful allografts can be applied in heart transplantation. GS not only protect the coronary endothelium, but also attenuate reperfusion damage of the vascular smooth-muscle cells after cold storage. It is a favorable sign that the coronary flow of the GS group remarkably increased in the endothelium-independent vasodilatory response to NTG.

A possible mechanism of dilating the coronary artery with GS is that the cGMP content increases in the vascular smooth-muscle cells of the coronary artery, and NO induces coronary vasodilatation through increased levels of cGMP and protects the endothelial function in reperfusion after hypothermic rat heart preservation. Evidence has been offered that ginseng enhances formation of citrulline from added arginine, implying an enhanced synthesis of NO[5,6,14,25]. When used, arginine reverses the action of NO synthase inhibitors, and when measured, tissue cGMP shows an increase by GS[11,13,14,15]. Studies clarifying its mechanism will be performed during experiments of heart preservation in the future.

In conclusion, the experiment demonstrated that pretreatment with GS improved the preservation of the cardiac and vascular function in the early phase of reperfusion after a 12-hour hypothermic ischemic period. We therefore consider GS to be a potentially promising agent for heart preservation.

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