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Cardioprotective Effect of the Mixture of Ginsenoside Rg_3 and CK on Contractile Dysfunction of Ischemic Heart

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Abstract : Ginsenosides are one of the most well-known traditional herbal medicines frequently used for the treatment of cardiovascular symptoms in korea. The anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart were investigated through analyses of changes in hemodynamics ; blood pressure, aortic flow, coronary flow, and cardiac output. The subjects in this study were divided into four groups: normal control, the mixture of ginsenoside Rg_3 and CK, an ischemia-induced group without any treatment, and an ischemia-induced group treated with the mixture of ginsenoside Rg_3 and CK. There were no significant differences in perfusion pressure, aortic flow, coronary flow and cardiac output between them before ischemia was induced. The supply of oxygen and buffer was stopped for five minutes to induce ischemia in isolated rat hearts, and the mixture of ginsenoside Rg_3 and CK was administered during ischemia induction. Treatments of the mixture of ginsenoside Rg_3 and CK significantly prevented decreases in perfusion pressure, aortic flow, coronary flow, and cardiac output under ischemic conditions. In addition, hemodynamics (except heart rate) of the group treated with the mixture of ginsenoside Rg_3 and CK significantly recovered 60 minutes after reperfusion compared to the control group (mixture+ischemia vs ischemia - average perfusion pressure: $74.4 \pm 2.97\%$ vs. $85.1 \pm 3.01\%$, average aortic flow volume: $49.11 \pm 2.72\%$ vs. $59.97 \pm 2.93\%$, average coronary flow volume: $58.50 \pm 2.81\%$ vs. $72.72 \pm 2.99\%$, and average cardiac output: $52.47 \pm 2.78\%$ vs. $63.11 \pm 2.76\%$, $p<0.01$, respectively). These results suggest that treatment of the mixture of ginsenoside Rg_3 and CK has distinct anti-ischemic effects in *ex vivo* model of ischemia-induced rat heart.

Key words **:** Ginsenoside Rg_3; Compound K; Langendorff apparatus; working heart; I/R injury; Hemodynamics; Cardiac output.

INTRODUCTION

Panax ginseng has been popularly used as a herbal medicine in Asia for more than 2000 years and currently occupies an important place among the tonic remedies of oriental medicine. Ginseng's genus name *Panax* is derived from the Greek words *pan* (all) and *akos* (cure), meaning cure-all. Ginseng root has been used extensively in Korean and Chinese medicine and has become increasingly popular in the western world for its alleged tonic effect and possible curative and restorative properties. There are increased clinical evidences concerning the potential benefits of ginseng roots in cardiovascular diseases. Administration of ginsenosides, a mixture of saponins extracted from *Panax* ginseng, decreases blood pressure in both hypertensive patients and experimental animals[1,2]. In North America, the ginseng species including *P. quinquefolium* represents an important industry for both domestic and export markets. There are a variety of reports explaining the pharmacological effects of *P.* ginseng against certain chronic disease states and aging, which may result from enhancement of vascular and immune functions. Ginsenosides, which is considered to be a biologically active fraction of *P.* ginseng, is a mixture of triterpene glycosides. The major components of ginsenosides belong either to the protopanaxadiol or protopanaxatriol groups[3]. Ginsenoside Rg_3, Rb_1 and Rc represent protopanaxadiols, whereas ginsenoside Rg_1 and Re are protopanaxatriols. Ginsenosides from *P.* ginseng decrease the blood pressure in both experimental animals and hypertensive patients[2,4-6]. It has also been shown that ginsenosides enhance vasodilatory response to perivascular nerve stim-

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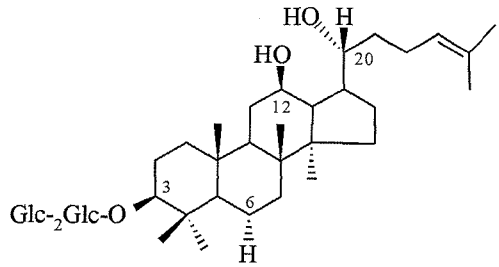
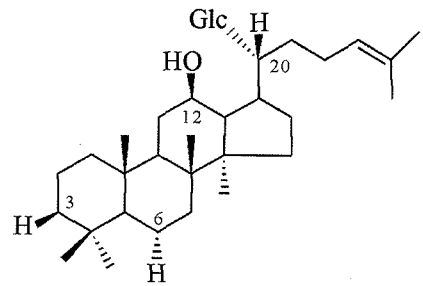
ulation in monkey cerebral arteries[7]. The antihypertensive effects of ginsenosides may result from their ability to inhibit vascular tone. Among the ginsenosides of the protopanaxatriol and protopanaxadiol groups, ginsenoside Rg_3 is the most potent vasodilator. In previous study, it was showed that Rg_3 inhibited Ca^{2+}-induced vascular contraction[8,9]. and that the intracellular accumulation of Ca^{2+} is a crucial factor for the irreversible myocardial injury that occurs after reperfusion following a prolonged period of ischemia[10,11]. According to Bourdillon and Poole-Wilson[12], the accumulation of Ca^{2+} was not due to a decrease in efflux, but due to an increase in influx. Previous studies also have shown that once the myocardium undergoes severe ischemia, restoration of blood flow is a prerequisite for myocardial salvage. However, it may induce deleterious changes, such as enzyme release, decreased myocardial contraction, and arrhythmias. These changes occur at the time of reperfusion, termed as "reperfusion injury" The mechanisms of ischemia/reperfusion (I/R) injury include the production of reactive oxygen species (ROS), abnormal lipid metabolism, and calcium overload[13]. It has been well known that Ca^{2+} overload due to Na^+ -Ca^{2+} exchange and L-type calcium channel opening leads to increase $[Ca^{2+}]_i$[14]. And, it was reported that excessive accumulation of cytosolic Ca^{2+} is taken up by mitochondria during I/R, resulting in an increase in mitochondrial Ca^{2+} level, which may stimulate the opening of the mitochondrial permeability transition and the release of proapoptotic factors[15,16]. Therefore, intracellular Ca^{2+} is referred to as a trigger for myocardial apoptosis. And, I/R injury of the heart can result in arrhythmias, tachycardia, ventricular fibrillation and subsequent death. Because of many functions of ginsenosides, ginsenosides can serve as an effective anti-ischemia agent to improve bad blood circulation induced by abnormal blood coagulation, adjust blood flow to normal, and finally recover dysfunction of heart induced by ischemia. However, it is not reported for any anti-ischemic effect of ginsenoside Rg_3 and CK *ex vivo*. In addition, because of the adverse effects associated with anti-ischemia drugs, many trials have been recently performed to find and develop new anti-ischemic drugs through herbal medicines that would minimize side effects. Numerous studies of various herbal medicines have been performed, and some have reported significant improvements in controlling ischemic symptoms without any noticeable adverse effects[17]. Given those backgrounds, we previously showed that ginsenoside Rg_3 and ginsenoside metabolite compound K (CK) inhibited Ca^{2+} channel subtype[18], providing new insights



(s)-Ginsenoside Rg3



Compound K (CK)

Fig. 1. Chemical structure of (*s*)-ginsenoside Rg_3 and compound K, ginsenoside metabolites. There are various ginsenosides. They differ at three side chains attached the common steroid ring. (A) Side chains for (s)-ginsenoside Rg_3 are R_1, -Glc_2-Glc, R_2, -H and R_3, -OH. (B) Side chains for CK are R_1 and R_2, -H and R_3, -Glc. Abbreviations for carbohydrates are as follows: Glc. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

into one possible mechanism underlying the beneficial effects of ginsenosides in the cardiovascular and nervous systems. However, as activity *in vitro* does not necessarily reflect in organ level activity the current study was conducted to determine whether the apparent activity of ginsenoside in cardioprotective study was reflected *ex vivo* using perfused heart model. In pilot study, we used ginsenoside Rg_3 and CK respectively on ischemia-induced heart for evaluation of cardiac function. But the cardioprotective effects of ginsenoside Rg_3 and CK, one by one, significantly was not obtained by measuring cardiac output, the main factor of heart function, with and without ginsenoside Rg_3 or CK treatment after induction of ischemia while increasing the ginsenoside Rg_3 or CK doses of 1, 3, 10, 30, and 100 μM, (Data not shown). Therefore, we tried to use the mixture of ginsenoside Rg_3 and CK. After all, after we determined the maximum effective dose of ginsenoside Rg_3 and CK mixtures during ischemia reperfusion injury (Fig. 1), we intensively exam-

ined the protective effects of the mixture of ginsenoside Rg_3 and CK using an *ex vivo* Langendorff system. on the ischemia induced rodent heart.

MATERIALS AND METHODS

Drugs

Ginsenoside Rg_3 and CK were kindly obtained from Korea Ginseng Corporation (Taejon, Korea) and other chemicals were of analytical grade and purchased from Sigma (St. Louis, MO).

Animals

Male Sprague-Dawley rats weighing 250 g to 300 g were supplied by Charles River (KFT). The rats were fed a standard rodent pellet chow and housed with free access to commercial food pellets (LSM, Bacutil, Poland), as well as tap water *ad libitum* under strictly controlled pathogen-free conditions (room temperature: 23±1°C, relative humidity: 50±10%, light cycle: 07 : 00-19 : 00). And, the rats were acclimatized to their environment for 2 weeks before commencement of the experi-ments.

All the animals was kept in light (L)-dark (D) conditions L : D=12 : 12. Animal care and handling was in accordance with the highest standards of institutional guidelines.

The animals were divided into four groups:

Group 1 : The group of normal control

Group 2 : The group of the mixture of ginsenoside Rg_3 and CK

Group 3 : The group of I/R

Group 4 : The group of the mixture of ginsenoside Rg_3 and CK + I/R

Each group contained fifteen rats.

Heart preparation and perfusion apparatus

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. The hearts were rapidly excised and placed in ice-cold (4°C) Krebs-Henseleit (KH) bicarbonate buffer (NaCl 120.0 mM, $NaHCO_3$ 25 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 1.25 mM, $MgSO_4$ 1.2 mM, and glucose 11.0 mM), which immediately stopped the contractile activity of the heart. Aorta and left atrium cannulation was performed rapidly, and the hearts were perfused in Langendorff mode at a pressure of 100cm H_2O with KH buffer. The buffer was saturated with 95% O_2/ 5% CO_2 at pH 7.4 and thermostatically kept at a constant temperature of 37°C. Global ischemia was achieved by clamping both the aortic and atrial lines for 10 min. In Langendorff perfusion (non-working heart model), perfusion fluid entered the heart via the aorta retrograde from the aortic reservoir located 100cm above the heart. The aortic reservoir, which was the thermostatically maintained oxygenator, carried out a perfusate to the aorta at a 100cm H_2O hydrostatic pressure maintained with the use of a constant head device (CHD). This system maintains the function of the heart, but does not maintain circulation of perfusate to the ventricle. Such a system is used to recover and maintain heart function for 15 min after isolation and ischemia induction. In the working heart model, the left atrium cannula and aortic cannula were open and perfusion fluid entered the heart via the left atrium from an atrial bubble trap located 20cm above the heart. The left ventricle ejected perfusate via the aorta and elasticity chamber (aortic pressure chamber) against a 20cm H_2O hydrostatic pressure to the aortic bubble trap. The same system is used to maintain heart function 20 min before induction of heart ischemia, and to recover heart function for 60 min after ischemia using the Langendorff system. The system makes it possible to compare the recovery of heart function before and after induction of heart ischemia. Aortic and coronary perfusates were not recirculated in the present study. The entire apparatus was thermostatically maintained by a water jacket and coil heat chamber. Aortic flow and coronary flow were measured by timed collection of perfusate from the aortic and pulmonary trunk cannula, respectively. Cardiac output was calculated by summing the aortic and coronary flows. 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 aortic pressures were measured throughout the working heart model perfusion periods in the aortic outflow line with a hemodynamics monitoring system (S & W Medico Teknik A/S, Denmark).

Ischemia induction of isolated-perfused rat heart

Male Sprague-Dawley rats were anesthetized with intraperi-toneal pentobarbital sodium (50 mg/kg). The hearts were rapidly excised and mounted on a Langendorff apparatus (IPH-W, Labo Support, Osaka, Japan) via the aorta, and then perfused at a constant pressure of 65 mmHg with KH buffer. The heart was constantly warmed by a circulating water jacket at 37°C. The buffer was gassed with 95% O_2/ 5% CO_2 at pH 7.4. To measure left ventricular pressure, a pressure transducer was connected

to the aortic cannula. Heart rate was monitored from left ventricular pressure. Coronary flow was measured by coronary flow volume (ml/min). After stabilization (non-working system) to 100 cm H_2O for 15 min via the aortic cannula, the perfusion pressure was reduced to 20cm H_2O for 20 min at the LA cannula (working system), and then ischemia was induced for 5 min accompanied by the injection of 10 μM of the mixture of Ginsenoside Rg_3 and CK for 2 min (flow rate 50 ml/min). When ischemia was started, the stock solutions of ginsenoside Rg_3 or CK (250 mM in DMSO, respectively) was diluted with perfusate before use. Then, the perfusate was injected into the aortic line for 2 min to observe the effects of the mixture of ginsenoside Rg_3 and CK on an ischemia-induced heart. Ischemic conditions were maintained for 3 additional min. The isolated hearts underwent a total 5 minute period of normothermic (37°C) no-flow ischemia. In the control group, equal volumes of KH buffer were injected into the aortic line for 2 min. Those hearts were retrograde perfused for 15 min according to the Langendorff method as described by Li *et al.*[19] to recover heart function. Then, the heart was perfused again through the working heart system for 60 min in the period of post-ischemia. The functional recovery rates between the ischemia group and ischemia group with the treatment of the mixture of ginsenoside Rg_3 and CK after ischemia induction were compared through changes in perfusion pressure, aortic flow, coronary flow, and cardiac output to observe the anti-ischemia effect of the mixture of ginsenoside Rg_3 and CK.

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. Results with a $p<0.05$ were considered statistically significant.

RESULTS

Determination of maximal effective dose of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart

The maximum effective amount of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart was assessed by measuring cardiac output, the direct parameter of heart pump function, with and without the mixture of ginsenoside Rg_3 and CK after induction of ischemia while increasing the doses of ginsenoside Rg_3 and CK mixtures from 1, 3, 10, 30 and 100 μM. As seen in Fig. 2, there was no difference between groups without (normal control; N/C) and with the treatments of 10 μM mixture of ginsenoside Rg_3 and CK under no ischemic conditions [87.92±1.68 (100%, N/C) vs. 86.41±1.53 (98.38% compared to N/C)]. This result suggests that the mixture of ginsenoside Rg_3 and CK itself does not influence cardiac output in normal conditions. In the present studies. the maximum recovery effect for cardiac output after I/R was obtained with the 30 μM mixture of ginsenoside Rg_3 and CK, respectively. The recovery effect on cardiac output after I/R increased with doses of 30 and 100 μM (58.97±3.03 ml/min : 58.76±3.09 ml/min). However, 30.0 and 100 μM of the mixture of ginsenoside Rg_3 and CK, the concentrations of maximum recovery effect for cardiac output, are very high concentrations for application. Also, the recovery for cardiac output after I/R between treatment of 10, 30 and 100 μM of the mixture of ginsenoside Rg_3 and CK was not

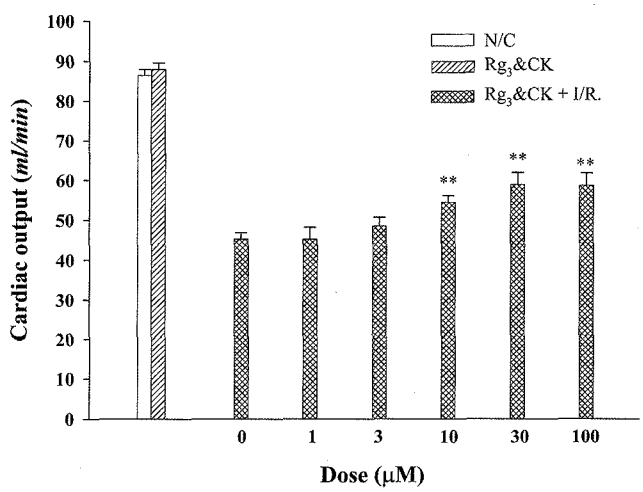


Fig. 2. Determination of maximum dose of the mixture of ginsenoside Rg_3 and CK that results in maximum anti-ischemic effect. Cardiac output was measured throughout the working heart model perfusion periods in the aortic flow plus coronary flow with a hemodynamic monitoring system in both groups to detect the maximal anti-ischemia effect according to the mixture dose (0-100 μM). White histograms represent the mean±SEM from fifteen rats per control group without any treatment under no ischemia conditions. Striped histograms represent the mean±SEM from fifteen rats per treatment group of the mixture of ginsenoside Rg_3 and CK under no ischemia conditions. Web histograms represent the mean±SEM from fifteen rats per treatment group of the mixture of ginsenoside Rg_3 and CK under post ischemia conditions according to dose of the mixture of ginsenoside Rg_3 and CK (0-100 μM).

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

Group	Pre-ischemia (beats/min)	Post-ischemia (beats/min)		
		10 min	30 min	60 min
I/R	275.1±18.8 (100.0±6.7%)	283.5±22.7 (101.3±8.3%)	276.1±23.9 (99.7±9.1%)	268.6±24.5 (98.3±9.4%)
Rg_3&CK+I/R	283.2±25.3 (100.0±5.3%)	268.6±27.6 (96.8±7.6%)	260.7±22.3 (94.6±6.4%)	256.8±18.8 (93.7±6.2%)

Table 2. Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat hearts.

Time (min)	Perfusion pressure for post-ischemia (mmHg)			
	N/C	Rg_3& CK	I/R	Rg_3&CK+I/R
10	104.8±2.23	105.3±1.25	82.7±3.94	88.8±3.62
20	106.1±1.87	104.7±1.47	80.2±3.97	90.4±3.70*
30	104.3±1.94	106.1±1.83	79.2±2.43	89.5±3.39**
40	104.1±2.29	105.6±1.65	75.2±3.17	88.4±2.58**
50	103.9±2.51	104.8±1.82	73.5±3.92	87.1±2.10**
60	105.1±2.45	102.9±2.38	73.8±2.19	86.0±2.47**

significantly different (54.51±1.61 ml/min with 10 μM, 58.97±3.03 ml/min with 30 μM and 58.76±3.09 ml/min with 100 μM). Thus, 10 μM was determined to be the appropriate dose of the mixture of ginsenoside Rg_3 and CK, respectively to optimize the anti-ischemic effect on ischemia-induced isolated rat heart.

Heart rate in ischemia-induced isolated rat heart

Since it is well-known that heart rate does not significantly change under ischemic conditions[20,21], the heart rate of I/R-induced isolated rat heart was assessed. As shown in Table 1, the heart rate between pre-ischemic and post-ischemic conditions was not significantly different. Also, the heart rate between the control and the mixtures groups under post-ischemic conditions was not significantly different. These results indicate that heart rate does not change in I/R-induced isolated rat heart regardless of treatment.

Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart

The degree of ischemic injury was assessed by measuring the extent of perfusion pressure, aortic flow, coronary flow, and cardiac output, all of which are basic assessments of cardiac function. All four parameters were substantially decreased by induction of ischemia to an average of 74.4±2.97%, 49.11±2.72 %, 58.5±2.81%, and 52.47±2.78%, respectively compare to normal control in the post-ischemic periods (100% being normal control values, Fig. 3). However, the treatment of the mixture of ginsenoside Rg_3 and CK significantly recovered ischemic conditions to an average of 85.1±3.01%, 59.97±2.93 %, 72.72±2.99%, and 63.11±2.76%, respectively, compare to normal control considered as 100% in the post-ischemic

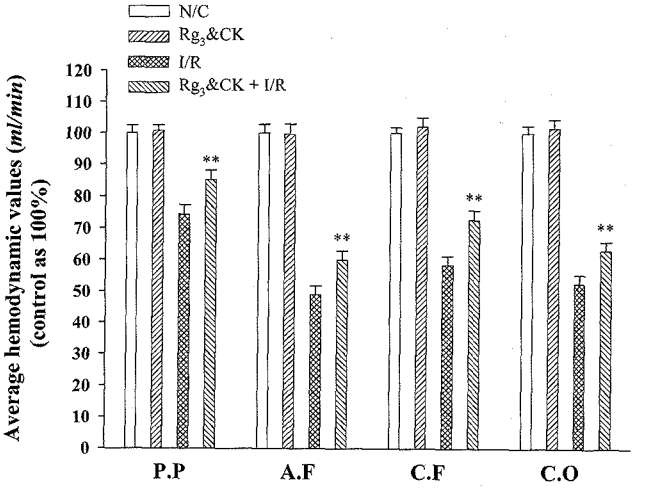


Fig. 3. Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart. Perfusion pressure (PP), aortic flow (AF), coronary flow (CF), and cardiac output (CO) were measured by timed collection of perfusate from the aortic and pulmonary trunk cannula of both groups to detect an anti-ischemia effect. Each histogram represents the mean±SEM from fifteen rats per group, the control group without any treatment (first histogram, N/C), the treatment group of the mixture of ginsenoside Rg_3 and CK (second histogram, Rg3&CK), the ischemia-reperfusion group without any treatment (third histogram, I/R) under post-ischemic conditions, and the treatment group of the mixture of ginsenoside Rg_3 and CK (fourth histogram, Rg_3&CK-I/R) under post-ischemic conditions. **Significantly different from control group ($p<0.01$) based on Student's *t*-test.

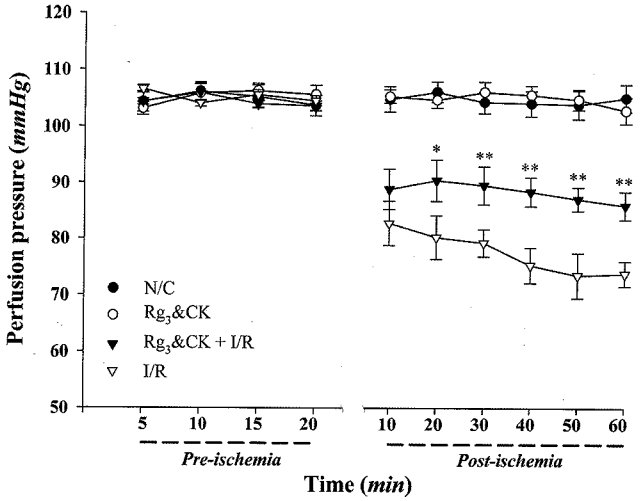


Fig. 4. Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased perfusion pressure (PP) of ischemia-induced isolated rat heart. Perfusion pressure was measured throughout the working heart model perfusion periods in the aortic outflow line with a hemodynamic monitoring system in the control and treatment groups of the mixture of ginsenoside Rg_3 and CK to detect an anti-ischemia effect. Each symbol represents the mean ± SEM from fifteen rats per group with (●) denoting the normal control group without any treatment and ischemia, (○) the treatment group of the mixture of ginsenoside Rg_3 and CK under normal conditions without ischemia, (▼) the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions, and (▽) the ischemia-reperfusion group without any treatment. **Significantly different from the control group without any treatment under ischemic conditions ($p<0.01$) compared to the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions based on Student's *t*-test.

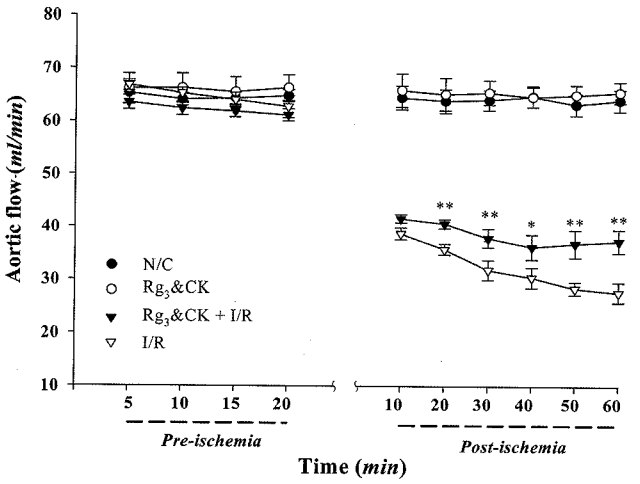


Fig. 5. Recovery effect of the mixture of ginsenoside Rg_3 and CK on the decreased aortic flow (AF) of ischemia-induced isolated rat heart. AF was measured by timed collection of perfusate from the aortic cannula in each group to detect an anti-ischemia effect. Each symbol represents the mean±SEM from fifteen rats per group with (●) denoting the normal control group without any treatment and ischemia, (○) the treatment group of the mixture of ginsenoside Rg_3 and CK under normal conditions without ischemia, (▼) the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions, and (▽) the ischemia-reperfusion group without any treatment. **Significantly different from the control group without any treatment under ischemic conditions ($p<0.01$) compared to the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions based on Student's *t*-test.

periods. (**$p<0.01$, Fig. 2). These recovery rates of the group of mixture correspond to average increases of 15.1% in perfusion pressure, 23.3% in aortic flow, 25.7% in coronary flow, and 20.7% in cardiac output compared to ischemia group under post-ischemic periods (**$p<0.01$, Fig. 2). These results indicate that treatment of the mixture significantly recovered heart dysfunction induced by ischemia.

Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased perfusion pressure of ischemia-induced isolated rat heart

Perfusion pressure was substantially decreased by ischemia induction to an average of 74.4±2.97% of normal control under post-ischemic conditions (Fig. 3).

Table 3. Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat hearts.

Time (min)	Aortic flow for post-ischemia (ml/min)			
	N/C	Rg_3& CK	I/R	Rg_3&CK + I/R
10	64.5±2.22	65.9±3.18	38.7±1.11	41.6±0.78
20	63.9±2.23	65.2±3.04	35.7±1.03	40.6±0.89**
30	64.1±1.88	65.5±2.29	31.8±1.85	37.9±1.79**
40	64.8±1.97	64.7±1.87	30.5±1.89	36.2±2.38**
50	63.3±2.01	65.1±1.85	28.5±1.19	36.8±2.62**
60	64.1±2.03	65.6±1.97	27.7±1.85	37.3±2.22**

However, such decreases were recovered by treatments of the mixture of ginsenoside Rg_3 and CK to an average of 85.1±3.01% of normal control (*p<0.05, **p<0.01, Fig. 3). These anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on perfusion pressure (mmHg) were continuously observed for 10 to 60 min during the post-ischemic period in each group (Table 2 and Fig. 4). Thus, treatment of the mixture of ginsenoside Rg_3 and CK significantly recovered the perfusion pressure reduced by ischemia for 60 min post-ischemia (*p<0.05, **p<0.01, Table 2 and Fig. 4). In addition, any effects of the mixture of ginsenoside Rg_3 and CK on perfusion pressure were not significantly observed in corresponding times with ischemia group, namely 5 to 20 min during the pre-ischemic period and 10 to 60 min during the post-ischemic period (normal control vs. Rg_3 & CK, p>0.05, Fig. 4).

Taken together, these results suggest that the mixture of ginsenoside Rg_3 and CK does not influence perfusion pressure under no ischemic conditions and does recover decreased per-fusion pressure induced by ischemia specifically.

Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased aortic flow of ischemia-induced isolated rat heart

Aortic flow was substantially decreased by I/R to an average of 49.1±2.72% of normal control under post-ischemic conditions (Fig. 3). However, such decreases were recovered by treatments of the mixture of ginsenoside Rg_3 and CK to an average of 59.9±2.93% of normal control (**p< 0.01, Fig. 3). These anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on aortic flow (ml/min) were continuously observed for 10 to 60 min during the post-ischemic period in each group (Table 3 and Fig. 5). Thus, treatment of the mixture of ginsenoside Rg_3 and CK significantly recovered the aortic flow reduced by ischemia for 60 min post-ischemia (*p<0.05, **p<0.01, Table 3 and Fig. 5). Also, any effects of the mixture of ginsenoside Rg_3 and CK on aortic flow were not significantly observed in corresponding times with ischemia group alike perfusion pressure (normal control vs. Rg_3 & CK, p>0.05, Fig. 5).

Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased coronary flow of ischemia-induced isolated rat heart

Induction of ischemia elicits a substantial decrease in coronary flow up to 58.5±2.81% compared to control (Fig. 3). However, treatment of the mixtures dramatically recovered coronary flow to 72.7±2.99% of normal control values under no ischemic conditions. Such recovery continued from 10 min to 60 min in the working heart model after ischemia was induced (**p<0.01, Table 4 and Fig. 6). As well as aortic flow, any difference between normal control and the group of mixtures on coronary flow were not significantly observed in corresponding times with ischemia group for 5 to 20 min during the pre-ischemic

Table 4. Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat hearts.

Time (min)	Coronary flow for post-ischemia (ml/min)			
	N/C	Rg_3& CK	I/R	Rg_3&CK + I/R
10	21.5±0.83	22.5±1.31	14.2±1.32	17.0±0.82
20	22.6±0.77	23.0±1.03	13.2±1.16	15.7±1.52
30	22.3±0.68	22.9±0.92	13.5±1.11	16.2±1.41
40	22.0±0.64	22.8±0.89	12.2±1.62	16.0±1.34**
50	21.7±0.75	21.5±0.76	11.6±0.95	15.2±1.02**
60	22.0±0.81	21.3±1.10	11.7±0.93	15.0±0.95**

Table 5. Overall anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat hearts.

Time (min)	Cardiac output for post-ischemia (ml/min)			
	N/C	Rg_3& CK	I/R	Rg_3&CK + I/R
10	86.3±1.72	88.0±2.15	53.5±1.42	58.9±0.81**
20	86.1±1.58	89.3±1.96	49.1±1.28	56.7±1.23**
30	87.3±1.32	88.5±1.71	45.3±1.93	54.2±1.70**
40	86.9±1.45	87.5±1.35	43.3±1.76	53.0±1.95**
50	85.2±1.52	86.0±1.28	40.2±1.33	52.3±1.94**
60	85.8±1.63	86.3±1.63	39.5±1.96	51.9±1.78**

N/C: normal control, I/R: ischemia-reperfusion
*, **indicates significantly different from control group (*p<0.05, **p<0.01) based on Student's t-test

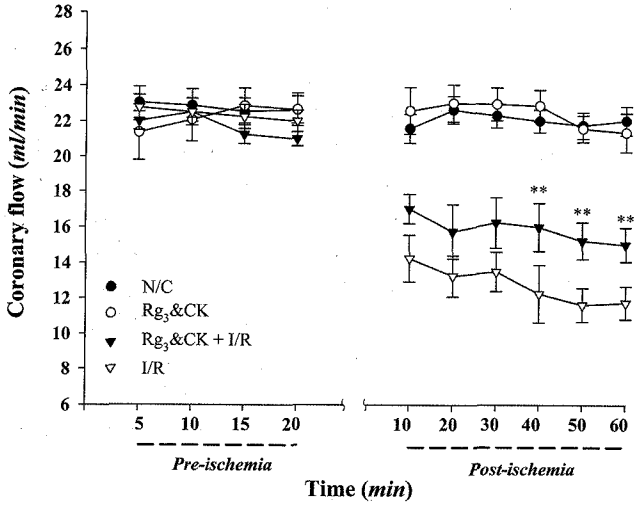


Fig. 6. Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased coronary flow (CF) of ischemia-induced isolated rat heart. Coronary flow (CF) was measured by timed collection of perfusate from pulmonary trunk in each group to detect an anti-ischemia effect. Each symbol represents the mean ± SEM from fifteen rats per group with (●) denoting the normal control group without any treatment and ischemia, (○) the treatment group of the mixture of ginsenoside Rg_3 and CK under normal conditions without ischemia, (▼) the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions, and (▽) the ischemia-reperfusion group without any treatment. **Significantly different from the control group without any treatment under ischemic conditions ($p<0.01$) compared to the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions based on Student's *t*-test.

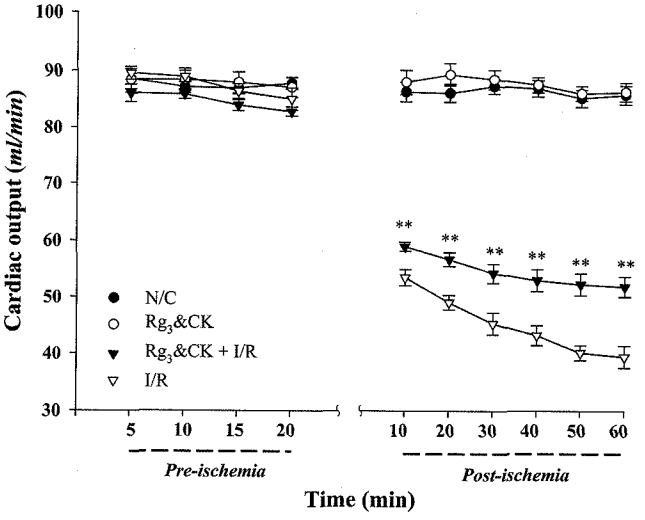


Fig. 7. Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased cardiac output (CO) of ischemia-induced isolated rat heart. Cardiac output (CO) was calculated by summing the aortic and coronary flows (CO=CF+AF). Each symbol represents the mean±SEM from fifteen rats per group with (●) denoting the normal control group without any treatment and ischemia, (○) the treatment group of the mixture of ginsenoside Rg_3 and CK under normal conditions without ischemia, (▼) the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions, and (▽) the ischemia-reperfusion group without any treatment. **Significantly different from the control group without any treatment under ischemic conditions ($p<0.01$) compared to the treatment group of the mixture of ginsenoside Rg_3 and CK under ischemic conditions based on Student's *t*-test.

period and 10 to 60 min during the post-ischemic period (normal control vs. Rg_3 & CK, $p>0.05$, Fig. 6).

Recovery effect of the mixture of ginsenoside Rg_3 and CK on decreased cardiac output of ischemia-induced isolated rat heart

Cardiac output was substantially decreased by induction of ischemia to an average of 52.47±2.78% of control (Fig. 3). However, such decreases were significantly recovered by treatment of the mixture of ginsenoside Rg_3 and CK to an average of 63.11±2.76% of normal control under no ischemic conditions. Thus, in the working heart model during the post-ischemic period, the mixture of ginsenoside Rg_3 and CK significantly recovered decreases in cardiac output (**$p< 0.01$, Table 5 and Fig. 7). Also, any effects of the mixture of ginsenoside Rg_3 and CK on cardiac output were not observed in corresponding times with ischemia group for 5 to 20 min during the pre-ischemic period and 10 to 60 min during the post-ischemic period (normal control vs. Rg_3 & CK, $p>0.05$, Fig. 7).

DISCUSSION

The results from this study show that the mixture of ginsenoside Rg_3 and CK significantly recovered the hemodynamics functions after I/R injury. (decreased perfusion pressure, aortic flow, coronary flow and cardiac output), but without influencing heart rate. One of the main indicators of ischemia-induced cardiac cell death is derived from the disturbance of intracellular Ca^{2+} homeostasis. Recent reports showed that under ischemic conditions, myocardial oxidative metabolism is suppressed and glycolysis becomes an important source of ATP genera-

tion[22]. The increased glycolytic rate in the face of impaired glucose oxidation leads to uncoupling of the two pathways and a buildup of lactate and H^+, a process that may continue during reperfusion. This accumulation of protons leads to downstream activation of pathways (Na^+/H^+ exchanger, Na^+/Ca^{2+} exchanger) that result in Ca^{2+} overload, impaired contractile function, and/or cell death[22]. Also, reactive oxygen species and metabolites are known to play important roles in the pathogenesis of I/R and anoxia/reoxygenation injury[23]. The reduction of O_2 results in the production of superoxides as well as hydrogen peroxide (H_2O_2). H_2O_2 is highly diffusible and induces cell damage. H_2O_2 appears to affect not only lipids but also transmembrane proteins. The hydroxyl radical (OH) also participates in lipid hyperoxidation[23]. It was reported that in ischemia-reperfused heart, generation of reactive oxygen species and Ca^{2+} overload may correlate cardiac dysfunction[23-25]. Besides, in ischemia-reperfused cardiomyocytes, intracellular Ca^{2+} overload and reactive oxygen species production are suggested to be involved in the progression of apoptosis or necrosis, and may mediate signals to the nucleus, leading to altered gene transcription[26]. And, in the pathophysiology of ischemic contractile dysfunction, reactive oxygen species and intracellular Ca^{2+} overload modulate cardiomyocyte contractility through phosphorylation of various kinases, especially PKC and p38 kinase[27,28]. In addition, reactive oxygen species have been shown to interfere with Ca^{2+} transport systems across the sarcolemmal membrane[29,30]. Therefore, in present studies, the protective effects of the mixture of ginsenoside Rg_3 and CK might be due to modulation of any number of different physiological processes that are involved in the development of I/R injury. So, what is the mechanism underlying the protective effect of ginsenoside Rg_3 and CK against ischemia-caused cardiac dysfunction *ex vivo*? One possibility is that these ginsenosides-induced protection against myocardial ischemia might be derived from the inhibition on ischemia-induced Ca^{2+} influx via L- and other types of Ca^{2+} channel since panaxadiol saponins and panaxatriol saponins block Ca^{2+} channel in the cultured cardiac cells[31] and ginsenoside block Ca^{2+} channel and anti-free-radical actions[32]. Also, we have shown that ginsenoside inhibit L-, N-, and P/Q-types of Ca^{2+} channels[33-36] and ginsenoside Rg_3 and CK inhibit voltage-dependent Ca^{2+} channel subtypes[18]. The second possibility is that myocardial protection of the mixtures against cardiac I/R might be derived from the attenuation of oxidative stress caused by hypoxia, since ginsenosides inhibit overproduction of NO and malonyldiadehyde and prevented a decrease of superoxide dismutase activity[34,37,38]. Given that ginsenosides possesses vasodilative properties[39,40], this also may be a potential benefit of this agent against the 'no-reflow' in ischemic heart. In this study, the protective effects of the mixtures of ginsenoside Rg_3 and CK lasted for at least 60 min in post-ischemic conditions. It is too early to say whether the pre-treatment of the mixture would continue to provide ongoing protection beyond this time point. Clearly, further studies are required to clarify these issues. Nevertheless, in present study we showed that the mixture of ginsenoside Rg_3 and CK is a compound without toxicity because of no difference in hemodynamic parameters between normal control and mixture treatment, that provide protective effects in myocardium IR injury. These results demonstrate that the mixture of ginsenoside Rg_3 and CK have distinct anti-ischemic effects, and preventing Ca^{2+} overload in cardiac myocytes may be one action mechanism of this mixture. However, the molecular mechanism of the mixture of ginsenoside Rg_3 and CK with respect to its anti-ischemic effects should be studied further before firm conclusions are drawn.

In summary, using a rodent Langendorff model system that shows a mydiocardial I/R injury, we obtained results suggesting that the mixture of ginsenoside Rg_3 and CK has cardioprotective effects against myocardial I/R injury.

CONCLUSIONS

The anti-ischemic effects of the mixture of ginsenoside Rg_3 and CK on ischemia-induced isolated rat heart were investigated through analyses of changes in hemodynamic functions (perfusion pressure, aortic flow, coronary flow, and cardiac output). Treatment of the mixture significantly prevented decreases in hemodynamics under ischemic conditions. These results suggest that the mixture of ginsenoside Rg_3 and CK has distinct anti-ischemic effects. Also, these results support the development of a novel anti-ischemia agent based on the pharmacological actions of the mixture of ginsenoside Rg_3 and CK.

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