Effect of *Acori graminei Rhizoma* on Contractile Dysfunction of Ischemic and Reperfused Rat Heart

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Acori graminei Rhizoma **is one of the best-known traditional herbal medicines frequently used for the treatment of cardiovascular symptoms in Asian countries. The anti-ischemic effect of *Acori graminei Rhizoma* on ischemia-induced isolated rat heart was investigated through analysis of changes in perfusion pressure, aortic flow, coronary flow, and cardiac output. The subjects in this study were divided into two groups, an ischemia-induced group without any treatment (I), and an ischemia-induced group with *Acori graminei Rhizoma* treatment (I+AGR). There were no significant differences in perfusion pressure, aortic flow, coronary flow, or cardiac output between the two groups before ischemia was induced. The supply of oxygen and buffer was stopped for 10 min to induce ischemia in isolated rat hearts, and *Acori graminei Rhizoma* was administered while inducing ischemia. The data showed that *Acori graminei Rhizoma* treatment significantly prevented decreases in perfusion pressure, aortic flow, coronary flow, and cardiac output under an ischemic condition. In addition, hemodynamics (except heart rate) of the AGR-treated group was significantly recovered 60 min after reperfusion compared to the control group, (systolic aortic pressure: 85.5% *vs.* 62.5%, aortic flow volume: 68.1% *vs.* 49.4%, coronary flow volume: 86.8% *vs.* 60.1%, and cardiac output: 73.1% *vs.* 54.1%, $p<0.01$). These results suggest that *Acori graminei Rhizoma* has distinct anti-ischemic effects.**

Key words *Acori graminei Rhizoma*; traditional herbal medicine; anti-ischemia effect

Cardiac ischemia is a condition in which the blood flow and oxygen supply to the heart muscle are lacking. Cardiac ischemia leads to angina pectoris, myocardial infarction, heart failure, and ultimately heart attack. Worldwide, there were an estimated 6.9 million deaths from cardiac ischemia in 2000 (12.4% of all mortality), and predictions indicate that its position as a leading cause of death will be maintained until the year 2020.[1)] Aspirin, beta-blockers, angiotensin-converting enzyme inhibitors, and lipid-lowering agents are currently the backbone of pharmacologic therapy.[2)] Because of the adverse effects associated with these anti-ischemia drugs, many trials have been recently performed to find and develop new anti-ischemic drugs through herbal medicines that would minimize the side effects. Numerous animal and clinical studies with various herbal medicines have been performed, and some studies reported significant improvements in controlling ischemic symptoms without any noticeable adverse effects.[3)] *Acori graminei Rhizoma* (AGR) is one of the best-known traditional herbal medicines frequently used to treat cardiovascular symptoms in Korea. AGR has been used clinically as a traditional oriental medicine against stroke, Alzheimer's disease, and vascular dementia. In particular, AGR, in combination with other herbal drugs, is one of the major components in oriental medical prescriptions for the treatment of stroke. AGR, the dry rhizoma of Acorus gramineus Soland (Araceae), contains volatile oils, which consist mainly of α-asarone (8.8—13.7%) and β-asarone (63.2—81.2%).[4)] Several studies have demonstrated a variety of pharmacological actions of AGR on the central nervous system. Recent studies have shown that AGR and its major component, asarone, have a neuroprotective effect against excitotoxic neural death.[5,6)] In addition, Hsieh *et al.* reported cognitive-enhancing effects of AGR on scopolamine-induced amnesia in rats. AGR in particular, improved both aspects of spatial learning capability and short-term working memory and prevented cell loss in the hippocampal CA1 region produced by ischemia.[7)]

These results suggest that *Acori graminei Rhizoma* may serve as an effective anti-ischemia agent in various tissues including the heart. However, these effects have not been reported for the heart. Thus, we investigated the anti-ischemia effect of *Acori graminei Rhizoma* on ischemia-induced isolated rat heart through changes in perfusion pressure, aortic flow, coronary flow, and cardiac output using the Langendorff system.

MATERIALS AND METHODS

Preparation of *Acori graminei Rhizoma* The spray-dried extracts of *Acori graminei Rhizoma* used in this study were purchased from Sun-Ten Pharmaceutical Company (Taipei, Taiwan).

The HPLC Analysis of Standard Material of *Acori graminei Rhizoma* One thousand milligrams of commercial spray-dried water extract including 34% starch was accurately weighed, placed in test tubes, and dissolved in 10 ml of chloroform (HPLC reagent, J.T. Baker Co. Ltd., U.S.A.). This was filtered using a 0.45 μm syringe filter (PVDF, Waters, U.S.A.). The marker substance used for the quantitative analysis was trans-asarone (Sigma Chemicals, U.S.A.). Ten milligrams of trans-asarone was dissolved in a solution. This solution was then serially diluted at 0.1, 0.5, 1.0, 1.5, and 2.0 mg/ml in order to obtain a standard HPLC chromatogram. The relationship between the concentration and

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the peak-area was measured by the minimum square method (R^2 value). The HPLC apparatus used was a Waters Breeze System (717+ Autosampler, 2487 dual λ absorbance detector, 1525 binary HPLC Pump, Waters Co., Milford, U.S.A.). Another Waters Breeze System (V. 5.00, Waters Co., Milford, U.S.A.) was used for data acquisition and integration. The quantity of trans-asarone solution added to each herbal extract was calculated using the following formula: amount (mg) of trans-asarone=(quantitative amount (mg) of trans-asarone×AT/AS)/n (n=3), where AT is the peak area of the test samples containing the trans-asarone, and AS is the peak-area of trans-asarone. From the results of the standard calibration curve, the R^2 values of all trans-asarone solutions ranged between 0.991 and 0.999. The standard material used for the quantitative analysis of *Acori graminei Rhizoma* was trans-asarone, and its content in *Acori graminei Rhizoma* was 0.19±0.00 mg/g (0.02±0.00%).

Heart Preparation and Perfusion Apparatus Male Sprague–Dawley rats weighing from 250 to 300 g were supplied by Taconic Korea (Taconic Korea, Seoul, Korea). The rats were housed and allowed free access to food and tap water under strictly controlled and pathogen-free conditions (room temperature: 23±1 °C, relative humidity: 50±10%, light cycle: 07:00—19:00). The rats were fed a standard rodent pellet chow and acclimatized to their environment for 2 weeks before commencement of the experiments. Next, the rats were randomly divided into 2 groups (n=10), an ischemia-induced group and ischemia-induced group with *Acori graminei Rhizoma* treatment. The rats were anesthetized with pentobarbital sodium intraperitoneally (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 100 cm 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 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_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. The system recovers function of the heart when the heart is isolated or when ischemia is induced. 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 20 cm above the heart. The left ventricle ejected perfusate *via* the aorta and elasticity chamber (aortic pressure chamber) against a 20 cm 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 operation using the Langendorff system. The system makes it possible for comparing 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 (AF) and coronary flow (CF) were measured by timed collection of perfusate from the aortic and pulmonary trunk cannula, respectively. Cardiac output (CO) was calculated by summing the aortic and coronary flows (CO=CF+AF). Heart rate (HR) 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 (ASP, ADP) 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).

Ischemia Induction of Isolated-Perfused Rat Heart Male Sprague–Dawley rats weighing from 250 to 300 g were anesthetized with pentobarbital sodium intraperitoneally (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 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). 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 the left ventricular pressure. Coronary flow was measured by the coronary flow volume (ml/min). After stabilization (non-working system) to 100 cm H_2O (100 mmHg) for 15 min *via* the aortic cannula, the perfusion pressure was reduced to 20 cm H_2O (20 mmHg) for 20 min at the LA cannula (working system), and then ischemia was induced for 10 min accompanied by AGR injection for 5 min. When ischemia was started, *Acori graminei Rhizoma* extract (50 ml of 3 mg/ml AGR) was dissolved in KH buffer and injected into the aortic line for 5 min to observe the effect of *Acori graminei Rhizoma* on ischemia-induced heart with a 65 mmHg perfusion pressure. Ischemic conditions were maintained for 5 additional min. In the control group, equal volumes of KH buffer were injected into the aortic line for 5 min. Those hearts were retrograde perfused for 15 min according to the Langendorff method as described by Li *et al.* (1996)[8] to recover heart function. Then, the heart was perfused again through the working heart system for 60 min. The functional recovery rates between the ischemia-induced group and ischemia-induced group with *Acori graminei Rhizoma* treatment after ischemia induction were compared through changes in perfusion pressure, aortic flow, coronary flow, and cardiac output to observe the anti-ischemia effect of *Acori graminei Rhizoma*.

Statistical Analysis The results are presented as the mean±S.E.M. 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 AGR on Ischemia-Induced Isolated Rat Heart The maximum effective amount of AGR on ischemia-induced isolated rat heart was assessed by measuring cardiac output, the direct parameter of heart pump function, with and without AGR treatment after induction of ischemia while increasing the AGR dose from 0.1 mg/ml to 30 mg/ml. As seen in Fig. 1, there is no difference between groups with and without 3 mg/ml of AGR treatment under pre-ischemic condition [86.6±2.3 (95%) *vs.* 91.6±2.9 (100%)]. This result suggests that AGR itself does not influence cardiac output in normal condition. The maximum recovery effect for cardiac output after ischemia was obtained with 3.0 mg/ml of AGR. The recovery effect of AGR on cardiac output after ischemia decreased continuously with doses over 3.0 mg/ml (65.8±2.0 ml/min with 10 mg/ml and 61.7±3.4 ml/min with 30 mg/ml). Thus, 3.0 mg/ml was determined to be the appropriate AGR dose to obtain the optimal anti-ischemic effect on ischemia-induced isolated rat heart.

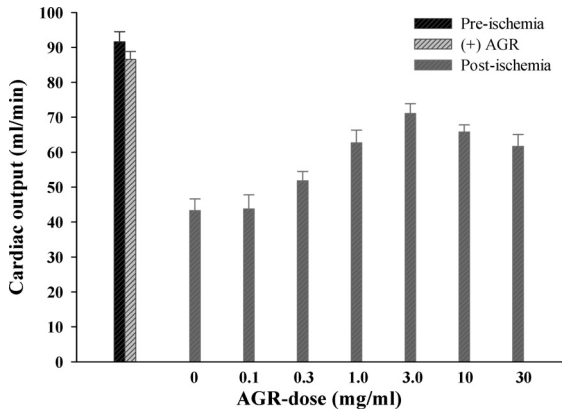

Fig. 1. Determination of Maximum AGR Dose That Results in Maximum Anti-ischemic Effect

Perfusion pressure was measured throughout the working heart model perfusion periods in the aortic outflow line with a hemodynamic monitoring system in both groups to detect the maximal anti-ischemia effect according to AGR dose (0—30 mg/ml). Black histograms represent the mean±S.E.M. from ten rats per control group without any treatment in pre-ischemia condition. Striped histograms represent the mean±S.E.M. from ten rats per AGR treatment in pre-ischemia condition. Gray histograms represent the mean±S.E.M. from ten rats per AGR treatment group in post-ischemia condition according to AGR dose (0—30 mg/ml).

Heart Rate in Ischemia-Induced Isolated Rat Heart Since it is well-known that heart rate does not significantly change under ischemic conditions,[9,10] the heart rate of ischemia-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 [285.7±24.1 (100%) *vs.* 258.6±10.8 (90.5%)]. Also, the heart rate between the control and AGR treatment groups in post-ischemic condition was not significantly different [258.6±10.8 (90.5%) *vs.* 260.7±15.5 (92.6%)]. These results indicate that heart rate does not change in ischemia-induced isolated rat heart regardless of AGR treatment.

Overall Anti-ischemic Effects of AGR on Ischemia-Induced Isolated Rat Heart The degree of ischemic injury was assessed by measuring the extent of perfusion pressure (PP), aortic flow (AF), coronary flow (CF), and cardiac output (CO), all of which are basic assessments of cardiac function. Perfusion pressure (PP), aortic flow (AF), coronary flow (CF), and cardiac output (CO), all were substantially decreased by induction of ischemia to an average of 62.5±1.3%, 49.4±1.6%, 60.1±0.7%, and 51.4±1.6%, respectively after inducing ischemia (100% being pre-ischemic condition values, Table 2 and Fig. 2). However, AGR treatment recovered such decreases to an average of 85.1±1.3%, 68.1±2.9%, 86.8±2.0%, and 73.1±3.1%, respectively, compared to pre-ischemic conditions ($p<0.01$, Table 2, Fig. 2). These recovery rates correspond to average increases of 36%, 38%, 44%, and 42%, respectively (PP, AF, CF and CO, respectively) compared to control under post-ischemic conditions ($p<0.01$, Fig. 2). These results indicate that AGR treatment significantly recovered the heart dysfunction induced by ischemia.

Recovery Effect of AGR on Decreased Perfusion Pres-

Table 1. Heart Rate in Ischemia-Induced Isolated Rat Heart

Group	Pre-ischemia (beats/min)	Post-ischemia (beats/min)		
	15 min	10 min	30 min	60 min
Control	285.7±24.1 (100%)	264.6±8.7 (92.6%)	260.2±12.9 (91.1%)	258.6±10.8 (90.5%)
AGR treatment	281.3±18.5 (100%)	263.6±11.5 (93.7%)	255.6±16.3 (90.8%)	260.7±15.5 (92.6%)

Table 2. Overall Anti-ischemic Effects of AGR on Ischemia-Induced Isolated Rat Heart

Time (min)	Control				AGR Treatment			
	PP	AF	CF	CO	PP	AF	CF	CO
Pre-ischemia								
5	93.7±1.0	70.3±1.2	22.9±0.8	92.2±1.5	92.1±1.2	70±2.5	22.3±2.3	92.3±2.4
10	93.5±0.9	67±1.3	23.3±0.8	90.3±1.5	93.3±0.6	68.9±2.0	23±1.5	91.9±2.0
15	93.8±1.1	66.7±0.9	22±1.0	88.7±1.3	93.03±0.5	71±1.8	21.7±0.9	92.7±1.8
20	92.3±1.2	66.2±1.2	22.8±0.4	89±1.3	93.1±0.7	70.1±2.4	23.6±0.9	93.7±2.4
Post-ischemia								
10	64.3±1.1	34.7±1.0	13.8±0.5	48.5±1.1	82*±1.2	39.3±3.0	18.9*±2.3	58.2*±3.0
20	63.7±1.2	34.9±1.2	13.9±0.8	48.8±1.3	80.8*±1.5	50.7*±2.7	19.7*±1.8	70.4*±2.7
30	59±1.3	32.4±1.4	12.7±0.7	45.1±1.6	79.8*±1.5	51.0*±4.4	18.0*±1.8	69.0*±4.3
40	58.6±1.5	31.7±1.1	13.4±1.1	45.1±1.7	79.3*±1.4	51.3*±3.2	20.8*±2.1	72.1*±3.2
50	54.1±1.1	32.4±1.4	12.9±0.7	45.3±1.6	79.2*±1.5	48.7*±2.3	19.6*±0.7	68.3*±2.3
60	51.6±1.0	31.7±1.6	13.8±0.9	45.5±1.6	77.6*±1.9	50.8*±2.0	21.5*±0.9	72.3*±2.0

* Significantly different from control group ($p<0.01$) based on Student's *t*-test. PP indicates perfusion pressure, AF aortic flow, CF coronary flow and CO cardiac output.

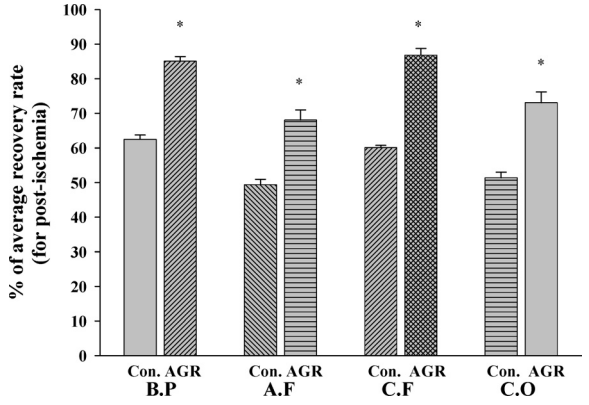

Fig. 2. Overall Anti-ischemic Effects of AGR on Ischemic Induced Isolated Rat Heart

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±S.E.M. from ten rats per group, the control group without any treatment (left histogram, Con), and the AGR treatment group (right histogram, AGR). * Significantly different from control group ($p<0.01$) based on Student's *t*-test.

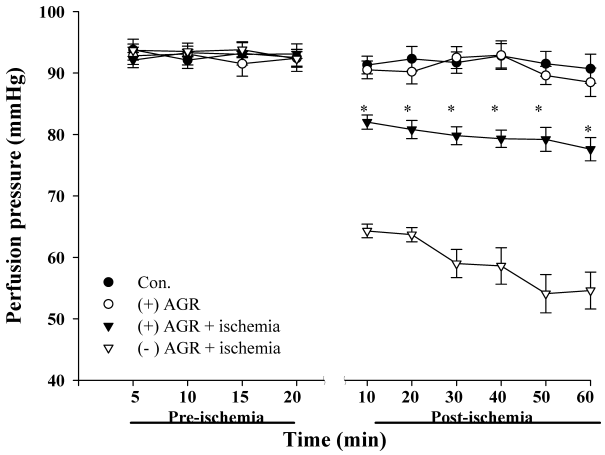

Fig. 3. Recovery Effect of AGR on Decreased Perfusion Pressure (PP) of Ischemia-Induced Isolated Rat Heart

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 AGR treatment groups to detect an anti-ischemia effect. Each circle presents the mean±S.E.M. from ten rats per group with denoting (●) the control group without any treatment, (○) the AGR treatment group under normal condition, and (▽) the control group without any treatment and (▼) the AGR treatment group under ischemic condition. * Significantly different from the control group without any treatment under ischemic condition ($p<0.01$), compared to the AGR treatment group under ischemic condition based on Student's *t*-test.

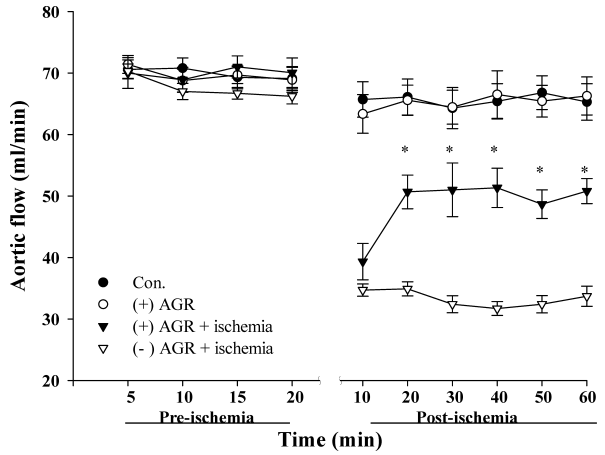

Fig. 4. Recovery Effect of AGR on the Decreased Aortic Flow (AF) of Ischemia-Induced Isolated Rat Heart

Aortic flow (AF) was measured by timed collection of perfusate from the aortic and pulmonary trunk cannula in the control and AGR treatment groups to detect an anti-ischemia effect. Each circle presents the mean±S.E.M. from ten rats per group with denoting (●) the control group without any treatment, and denoting (○) the AGR treatment group under normal condition, and (▽) the control group without any treatment and (▼) the AGR treatment group under ischemic condition. * Significantly different from the control group without any treatment under ischemic condition ($p<0.01$), compared to the AGR treatment group under ischemic condition based on Student's *t*-test.

sure (PP) and Aortic Flow (AF) of Ischemia-Induced Isolated Rat Heart Perfusion pressure (PP) was substantially decreased by ischemia induction to an average of 62.5±1.3% of control under pre-ischemic conditions (Table 2, Fig. 2). However, such decreases were recovered by AGR treatment to an average of 85.1±1.3% of control before ischemia was induced ($p<0.01$, Table 2, Fig. 2). These anti-ischemic effects of AGR on perfusion pressure (mmHg) were continuously observed for 10 to 60 min during the post-ischemic period; control 64.3±1.1 *vs.* AGR 82.0±1.2, $p<0.01$ (10 min); control 63.7±1.2 *vs.* AGR 80.8±1.5, $p<0.01$ (20 min); control 59±1.3 *vs.* AGR 79.8±1.5, $p<0.01$ (30 min); control 58.6±1.5 *vs.* AGR 79.3±1.4, $p<0.01$ (40 min); control 54.1±1.1 *vs.* AGR 79.2±1.5, $p<0.01$ (50 min); control 51.6±1.0 *vs.* AGR 77.6±1.9, $p<0.01$ (60 min) (Fig. 3). However, any effects of AGR on perfusion pressure (mmHg) under normal condition were not observed for 5 to 20 min during the pre-ischemic period and 10 to 60 min during the post-ischemic period (control *vs.* AGR, $p>0.05$, Fig. 3). Taken together, these results suggest that AGR does not influence perfusion pressure and do recover decreased perfusion pressure induced by ischemia specifically.

Similarly, AGR treatment successfully recovered the AF reduced by ischemia to 68.1±2.9%, of the control value ($p<0.01$, Table 2, Fig. 2). In the working heart model, AGR treatment continuously recovered decreases in aortic flow 20 to 60 min after ischemia was induced (Fig. 4).

Recovery Effect of AGR on Decreased Coronary Flow (CF) of Ischemia-Induced Isolated Rat Heart Induction of ischemia elicits a substantial decrease in coronary flow (CF) up to 60.1±0.7% compared to control (Table 2, Fig. 2). However, AGR treatment dramatically recovered coronary flow to 86.8±2.0%, of control values under pre-ischemic conditions ($p<0.01$, Table 2, Fig. 2). Such recovery continued from 10 min to 60 min in the working heart model after ischemia was induced (Fig. 5).

Recovery Effect of AGR on Decreased Cardiac Output (CO) of Ischemia-Induced Isolated Rat Heart The anti-ischemic effects of AGR were also determined by examining cardiac output (CO). Cardiac output (CO) was substantially decreased by induction of ischemia to an average of 51.4±1.6% of control (see Table 2, Fig. 2). However, such decreases were recovered by AGR treatment to an average of 73.1±3.1%, of control under pre-ischemic conditions ($p<0.01$, see Table 2, Fig. 2). Also, such decreases were significantly increased by AGR treatment to an average of 42% of control under post-ischemic conditions ($p<0.01$, see Table 2, Fig. 2). In the working heart model during the post-ischemic period, AGR treatment significantly recovered decreases in cardiac output (Fig. 6).

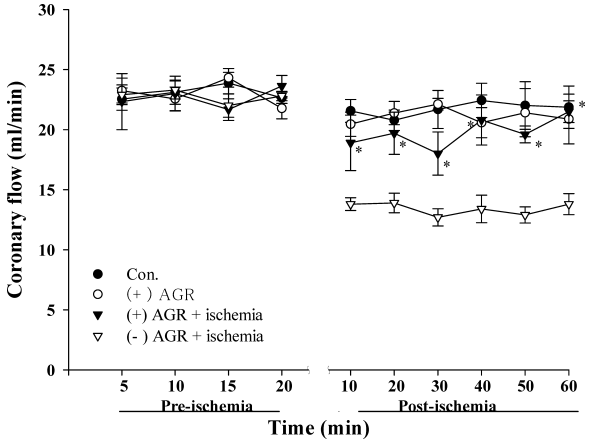

Fig. 5. Recovery Effect of AGR on Decreased Coronary Flow (CF) of Ischemia-Induced Isolated Rat Heart

Coronary flow (CF) was measured by timed collection of perfusate from the aortic and pulmonary trunk cannula in the control and AGR treatment groups to detect an anti-ischemia effect. Each circle presents the mean±S.E.M. from ten rats per group with denoting (●) the control group without any treatment, and denoting (○) the AGR treatment group under normal condition, and (▽) the control group without any treatment and (▼) the AGR treatment group under ischemic condition. * Significantly different from the control group without any treatment under ischemic condition ($p<0.01$), compared to the AGR treatment group under ischemic condition based on Student's *t*-test.

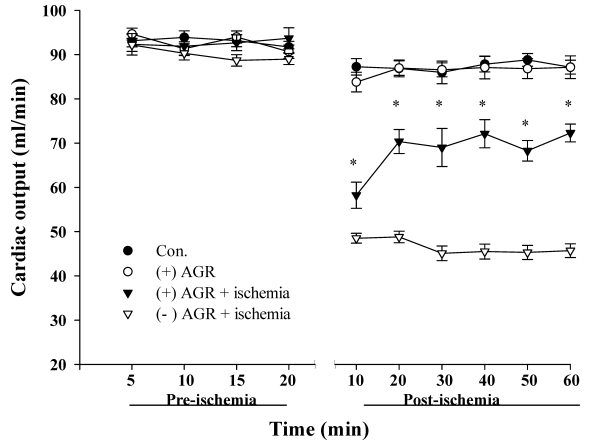

Fig. 6. Recovery Effect of AGR on Decreased Cardiac Output (CO) of Ischemia-Induced Isolated Rat Heart

Cardiac output (CO) was calculated by summing the aortic and coronary flows (CO=CF+AF). Each circle presents the mean±S.E.M. from ten rats per group with denoting (●) the control group without any treatment, and denoting (○) the AGR treatment group under normal condition, and (▽) the control group without any treatment and (▼) the AGR treatment group under ischemic condition. * Significantly different from the control group without any treatment under ischemic condition ($p<0.01$), compared to the AGR treatment group under ischemic condition based on Student's *t*-test.

DISCUSSION

Under ischemic conditions, myocardial oxidative metabolism is suppressed and glycolysis becomes an important source of ATP generation. 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.[11] It is known that AGR contains various chemicals (Table 3), and of these components, eugenol and β-asarone have been most widely recognized as having Ca^{2+} antagonist effects that could potentially prevent Ca^{2+} overloads or intakes by the cell.[12—14] In more detail, it has been reported that the extract of AGR significantly decreased Aβ-induced cell death. Further, eugenol and β-asarone were isolated and identified as the major active principles. Both purified eugenol and β-asarone protected PC-12 cells from the toxic effect of Aβ. Both eugenol and β-asarone inhibited Ca^{2+} intake by PC-12 cells: β-asarone mainly inhibited basal Ca^{2+} intake, whereas eugenol inhibited Aβ-induced Ca^{2+} intake preferentially.[12] Thus, it is suggested that AGR may work as a Ca^{2+} antagonist, resulting in recovery of Ca^{2+} overload-relevant cardiac dysfunction induced by ischemia. Reactive oxygen species and metabolites are known to play important roles in the pathogenesis of ischemia/perfusion and anoxia/reoxygenation injury. 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.[11] Some chemicals in AGR including eugenol, isoeugenol, astragole, and anethole are recognized as antioxidants (Table 3) capable of reducing reactive oxygen species (ROS).[15—17] However, it was reported that the amounts of those components in AGR except asarones are very small.[4] Thus, it is thought that asarones in AGR mainly work as anti-ischemic agent. Based on the previous report and this assumption, we investigated the effect of AGR on intracellular calcium overload induced by homocystein and glycine in cultured cardiac myocytes from neonatal rats. Our preliminary data show that AGR prevented homocystein and glycine-induced increase in $[Ca^{2+}]_i$ (data not shown). These results implicate that *Acori graminei Rhizoma* has distinct anti-ischemic effects and preventing calcium overload in heart myocytes may be the one of action mechanism of AGR. However, the molecular mechanism of AGR with respect to its anti-ischemic effects should be studied further before a firm conclusion is drawn.

Table 3. The Known Components of AGR

trans-Isoeugenol methyl ether	*trans*-Isoelemicin
Eugenol	*cis*-Isoelemicin
Isoeugenol	1,2,4-Trimethoxy-5-(*E*-3′-methyloxiranyl)benzene
Anisaldehyde	Estragole
Safrole	*p*-Methoxycinnamaldehyde
γ-Asarone	Terpinen-4-ol
Asarone	1,8-Cineole
Chavibetol	δ-Cadinene
Elemicin	α-Selinene
1,2-Dimethoxy-4-(*trans*-3′-methyloxiranyl)benzene	(−)-Calamenene
Isoeugenol methyl ether	*p*-Cymene
cis-Isoeugenol methyl ether	1,3,11-Elematriene
cis-Isoeugenol methyl ether	3(15),6-Caryophylladiene
γ-Asarone	Menthol
β-Asarone	Ylangene
α-Asarone	(+)-Borneol
Anethole	Camphor
Acoradin	Camphene
Methyl eugenol	Geranyllinalool

This table was referred from www.tradimed.com.

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