**S-allylcysteine mediates cardioprotection in an acute myocardial infarction rat model via a hydrogen sulfide-mediated pathway**

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**Chuah SC, Moore PK, Zhu YZ.** S-allylcysteine mediates cardioprotection in an acute myocardial infarction rat model via a hydrogen sulfide mediated pathway. *Am J Physiol Heart Circ Physiol* 293: H2693–H2701, 2007. First published August 31, 2007; doi:10.1152/ajpheart.00853.2007.—S-allylcysteine (SAC) is an organosulfur-containing compound derived from garlic. Studies have shown that garlic is beneficial in the treatment of cardiovascular diseases. This study aims to elucidate if SAC is responsible for this cardioprotection using acute myocardial infarction (AMI) rat models. In addition, we hypothesized that SAC may mediate cardioprotection via a hydrogen sulfide (H2S)-related pathway. Rats were pretreated with saline, SAC (50 mg·kg−1·day−1), SAC + propargylglycine (PAG; 50 mg + 10 mg·kg−1·day−1) or PAG (10 mg·kg−1·day−1) for 7 days before AMI induction and killed 48 h after. Our results showed that SAC significantly lowered mortality (12.5% vs. 33.3%, P < 0.05) and reduced infarct size. SAC + PAG- and PAG-treated rats had larger infarct sizes than controls (60.9 ± 0.01 and 62.0 ± 0.03%, respectively, vs. 50.0 ± 0.03%; P < 0.05). Pretreatment with SAC did not affect BP, but BP was significantly elevated in SAC + PAG and PAG-treated groups (P < 0.05). In addition, plasma H2S levels and left ventricular cystathionine-γ-lyase (CSE) activities were analyzed to investigate the involvement of H2S. CSE is the enzyme responsible for H2S production in the heart. SAC increased left ventricular CSE activity in AMI rats (2.75 ± 0.34 vs. 1.22 ± 0.27 g protein−1·h−1, P < 0.05). SAC + PAG-treated rats had significantly lower CSE activity compared with the SAC-treated group (1.22 ± 0.27 vs. 2.75 ± 0.34 g protein−1·h−1, P < 0.05). Similarly, SAC-treated rats had higher plasma H2S concentration compared with controls and the SAC + PAG-treated group. Protein expression studies revealed that SAC upregulated CSE expression (1.1-fold of control; P < 0.05), whereas SAC + PAG and PAG downregulated its expression (0.88-fold of control in both groups; P < 0.005). In conclusion, our study provides novel evidence that SAC is protective in myocardial infarction via an H2S-related pathway.

S-allylcysteine; garlic; hydrogen sulfide; myocardial infarction; cardioprotection

**ACUTE MYOCARDIAL INFARCTION (AMI), more commonly known as heart attack is the most prevalent form of cardiovascular death in developed countries. Cardiovascular diseases account for 17 million deaths worldwide every year (34). Myocardial infarction (MI) occurs when one or more of the coronary arteries supplying blood to the heart are occluded, consequently depriving a section of the heart of oxygenated blood and nutrients, inevitably leading to necrosis of the myocardium (12). MI is thus the progression to myocardial necrosis due to the critical imbalance between supply and demand of oxygen to the heart (41).**

Hydrogen sulfide (H2S) is recently discovered to be the third gas neurotransmitter, joining the ranks of the other two gaseous neurotransmitters, namely nitric oxide and carbon monoxide (1, 7, 26). All three have been reported to be present in the cardiovascular system (23). H2S is produced in the heart by the pyridoxal-5-phosphate-dependent enzyme cystathionine-γ-lyase (CSE) with L-cysteine as substrate. H2S is involved in the regulation of vascular tone, myocardial contractility, and neurotransmission in the cardiovascular system (38). Geng and colleagues (16) have demonstrated the protective effects of exogenous H2S in an isoproterenol-induced model of MI. In addition, our group has recently reported that both endogenous and exogenous H2S offers protection to the heart in a coronary artery ligation model of MI (43). Both of these studies serve to validate the role of H2S in mediating cardioprotection during MI.

Garlic (*Allium Sativum*) is used traditionally as a complementary therapy in the treatment of several diseases such as diabetes, several forms of cancer, and neurodegenerative conditions such as ischemic stroke (4, 33). In addition, garlic has been reported to possess a range of cardiovascular effects (32) such as lowering of plasma cholesterol (3), inhibition of platelet aggregation, and lowering of arterial blood pressure (BP; see Ref. 2). S-allylcysteine (SAC), a main bioactive constituent of garlic, is an organosulfur-containing amino acid (22). Similar to garlic extract, SAC is reported to be antioxidative (17, 28), anticancer (10, 39), antihypototoxic (18, 27), and can also reduce the incidence of stroke (21). In the cardiac context, Padmanabhan and Prince (29) have reported that SAC mediates cardioprotection in MI via its antioxidative properties by decreasing lipid peroxide products.

In this study, we have shown an alternative route from the antioxidative pathway put forward by Padmanabhan and Prince in which SAC may mediate cardioprotection. We hypothesized that, in an MI setting, SAC serves as an additional CSE substrate in the damaged myocardium, increasing the bioavailability of substrates for the enzyme. In addition, we also propose that SAC may upregulate the protein expression of CSE. These will in turn result in an increased production of H2S in the myocardium and plasma. The increased generation of H2S may then mediate cardioprotection during AMI.

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MATERIALS AND METHODS

All experiments were undertaken in accordance with regulations as set out by the National University of Singapore (NUS). In addition, experimental protocols involving the use of laboratory animals were approved by the animal ethics committee (Institutional Animal Care and Use Committee) of NUS.

Treatment Groups

One hundred and twenty male adult Wistar rats weighing between 220–260 g were obtained from the Laboratory Animal Centre at NUS. Animals were housed under diurnal lighting conditions and fed standard rat chow and water ad libitum. The rats were randomly assigned to four different treatment groups, namely 1) SAC (5 mg·kg⁻¹·day⁻¹), 2) SAC + propargylglycine (PAG, 50 mg + 10 mg·kg⁻¹·day⁻¹), 3) PAG (10 mg·kg⁻¹·day⁻¹), and 4) vehicle (saline) control group.

Drugs

SAC was purchased from TCI Kyoto Kasei Kogyo (Tokyo, Japan), whereas PAG was purchased from Sigma-Aldrich (St. Louis, MO). Both drugs were dissolved in saline before intraperitoneal injection.

Experimental Induction of Acute Myocardial Infarction

The coronary artery ligation model was used as a model for AMI as we reported previously (15, 42). Within each treatment group, rats were randomly subdivided to a coronary artery ligation operation or sham operation group. The first group (n = 24) was subjected to a permanent ligation of the left anterior descending coronary artery, whereas the second group (n = 6) served as controls and underwent the same operation without ligation of the coronary artery.

Rats were pretreated for 7 days with their respective treatment drugs via intraperitoneal injection once daily. MI was induced on day 8, as described below. Following the surgery, treatment with the respective drugs continued for a further 2 days until the animals were killed 48 h postsurgery. Upon death, blood samples (5 ml) were withdrawn from the apex of the right ventricle for measurement of plasma H₂S concentrations (as described below) using a heparinized syringe. Blood was centrifuged at 4,000 g for 10 min at room temperature, and the plasma was aspirated and stored at −80°C before conducting the assays. In addition, the heart was excised, washed in ice-cold saline, and immerced in liquid nitrogen before storing at −80°C for subsequent experimental assays.

MI was induced by a permanent ligation of the left anterior descending coronary artery (36, 42). The rats were anesthetized with an intraperitoneal injection of ketamine + xylazine (75 mg + 10 mg/kg) mixture. Subsequently, the rats were intubated, connected to a respirator through a tracheotomy, and artificially ventilated. The third and fourth ribs were cut to expose the heart, and a chest retractor was subsequently inserted. The ribs were gently spread to reveal the heart. The left coronary artery was ligated ~3 mm from its origin between the pulmonary artery conus and the left atrium using a sterile 6–0 atraumatic silk suture. The ligation was verified visually by a change in color of the heart apex to a paler shade as reported previously (19, 42). The thorax was then closed using a sterile 4–0 atraumatic silk suture to stitch up the skin and muscle layers individually. The whole surgical procedure took ~10 min. Artificial ventilation was continued for a period of 30 min after the surgery to allow the animals to stabilize.

Measurement of Infarct Size and Hypertrophy Index

Three rats from each treatment group that underwent the MI procedure were randomly chosen for the measurement of infarct size. Upon killing the animal, the isolated heart was washed in saline to clear the ventricles of any residual blood. The heart was immersed in 1% triphenyl tetrazolium chloride solution for ~45 min. This technique relies on the ability of dehydrogenase enzymes and cofactors in the noninfarcted tissue to react with terazolium salts to form a formazan pigment. Infarcted myocardium lacks dehydrogenase activity and fails to stain (14). Ischemic myocardium remained pale, whereas the nonischemic zone was stained deep purple. At the end of the 45 min, the left ventricle was separated from the rest of the heart and placed between two microscope slides that were secured with clips. The stained left ventricle was then scanned, and the image was stored digitally. Total left ventricular area and the infarct area were determined by computerized planimetry (Scion Image). Infarct size was expressed as a percentage of the infarct area to the left ventricle area (42).

In addition, the animals was weighed before their death and recorded in grams. Isolated rat heart was also weighed upon death, and the wet weight of the heart was recorded in milligrams. Hypertrophy index, an indicator of ventricular hypertrophy, was calculated as a ratio of the whole heart weight in milligrams to body weight in grams.

Hemodynamic Measurements

BP and electrocardiogram (ECG) readings were measured on three occasions throughout the animal model study. First, BP and ECG readings were recorded before the start of treatment (day 1). Subsequently, readings were taken on day 7 after 1 wk of drug pretreatment. The last pair of readings was measured 48 h post-MI before the death of the animal (day 10).

Blood Pressure

BP was measured by the tail-cuff method using the noninvasive BP system (ML125/R; ADInstruments Powerlab System), which uses a specialized tail cuff and pulse transducer to measure BP at intervals based on the periodic occlusion of tail blood flow. Triplicate readings of BP were obtained on each occasion.

Electrocardiogram

ECG was recorded in the anesthetized animal for a period of 1 min using the Animal BioAmp amplifier (ML 136; ADInstruments Powerlab System), as described previously (9). The amplifier amplifies and filters the small bioelectrical signals associated with nerve and muscle activity. In addition, heart rate of the anesthetized rat was also measured for a period of 1 min.

Morphological Examination

After the animal was killed, hearts obtained from all treatment groups were isolated and washed immediately in saline. Transverse sections from three rats of each treatment group were obtained and stored individually in histology cassettes that were immersed in 10% buffered neutral formalin solution. Following fixation, the heart tissue was processed and embedded in paraffin. The heart tissue was then sectioned at 5 µm and mounted on a microscopic slide. The section was stained with hematoxylin and eosin (H&E) and subsequently examined under a high-power microscope at a magnification of ×200.

Measurement of CSE Activity in the Left Ventricle

H₂S-synthesizing activity of myocardial tissue in the left ventricle was assayed as described by Stipanuk and Beck (37) with slight modifications. Briefly, 0.1 g of left ventricular myocardial tissue was thawed on ice and homogenized in 2 ml of 100 mM ice-cold potassium phosphate buffer (pH 7.4). The tissue homogenate was subsequently centrifuged at 24,000 g for 5 min at 4°C, and the supernatant was used for the assay. The reaction mixture contained 20 µl of 10 mM l-cysteine, 20 µl of 2 mM pyridoxal-5-phosphate, 30 µl of saline, and 430 µl tissue homogenate. The reaction mixture was prepared in 2-ml microtubes and sealed with a double layer of parafilm. The catalytic reaction was initiated by transferring the tubes
from ice to a 37°C water bath where the tubes were subjected to 30 min incubation. Two hundred fifty microinents of 1% zinc acetate were then added to the tubes using a syringe to trap the evolved H2S. Two hundred fifty microinents of 10% trichloroacetic acid (TCA) were next added to quench the enzymatic reaction. N,N-dimethyl-p-phenylenediamine dihydrochloride (NNDPD, 133 μl) in 7.2 M HCl and 133 μl of FeCl3 in 1.2 M HCl were next added in sequence. The absorbance of the final reaction mixture was measured at 670 nm using a 96-well microplate reader (Tecan Systems). For every sample, the background H2S concentration was subtracted from the absorbance of the reaction mixture. Enzymatic activity of CSE was stopped immediately by the addition of 10% TCA before addition of l-cysteine and pyridoxal phosphate. This served as the basal H2S level in the tissue homogenate. Similarly, all samples were assayed in duplicate, and the H2S concentration for each sample was calculated against a calibration curve for NaHS (3.125–250 μM). Results are expressed as micromoles per gram protein per hour. Protein quantitation was determined using Bradford assay (Bio-Rad).

Measurement of Plasma H2S Concentration

The concentration of H2S in plasma was determined as described previously (23). Briefly, 75 μl of plasma were added to a 1.5-ml microtube containing 0.25 ml of 1% zinc acetate and 0.425 ml of distilled water. H2S is chemiosorbed by zinc acetate and transformed into stable zinc sulfide. The sulfide is then recovered by extraction with water. Next, 20 mM of NNDPD in 7.2 M HCl and 30 mM FeCl3 in 1.2 M HCl were next added to the microtube. The solution was then incubated at room temperature for 10 min. In contact with FeCl3 in a strongly acid solution, the extracted sulfide reacts with N,N-dimethyl-p-phenylenediammonium ion to yield methylene blue. Next, 0.25 ml of 10% TCA were added to remove the proteins present. The microtubes were briefly shaken after each addition. This final solution was then centrifuged at 24,000 g for 5 min at 4°C. The optical absorbance of the resulting solution was measured at 670 nm using a 96-well microplate reader (Tecan Systems). Each plasma sample was assayed in duplicate. The absorbance of the final reaction mixture was measured at 670 nm using a 96-well microplate reader (Tecan Systems). For every sample, the background H2S concentration was subtracted from the absorbance of the reaction mixture. Enzymatic activity of CSE was stopped immediately by the addition of 10% TCA before addition of l-cysteine and pyridoxal phosphate. This served as the basal H2S level in the tissue homogenate. Similarly, all samples were assayed in duplicate, and the H2S concentration for each sample was calculated against a calibration curve for NaHS (3.125–250 μM). Results are expressed as micromoles per gram protein per hour. Protein quantitation was determined using Bradford assay (Bio-Rad).

Protein Extraction and Expression

CSE protein expression in the left ventricle was quantified using Western blot. Three each of MI-operated and sham-operated tissue samples were selected from each treatment group for the assay. Briefly, 0.1 g left ventricular tissue from each rat was weighed and homogenized in 1 ml of CellLytic lysis buffer. The ventricular homogenate was then centrifuged at 24,000 g for 10 min at 4°C. The supernatant was aspirated and stored at −80°C until use. Protein concentration from the supernatant was determined using the bicinchoninic acid method. Protein samples (50 μg) were boiled at 95°C for 5 min before loading for 10% SDS-PAGE. Electrophoresis was performed at 120 volts for 75 min, and the proteins were subsequently transferred from the gels to polyvinylidene difluoride membranes. Protein transfer was carried out at 100 volts for 120 min at 4°C. Subsequently, the membranes were blocked in 5% nonfat milk in 1× PBS containing 0.1% Tween 20 at room temperature for 60 min. After being blocked, the membranes were incubated with their respective primary antibodies CSE (1:200) and α-tubulin (1:4,000; Santa Cruz), which was used as a housekeeping control. The incubation was performed at 4°C overnight on a rocking platform. The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (1:20,000; Santa Cruz) for 60 min. Protein bands were visualized using an enhanced chemiluminescence detection system (Pierce). Protein quantification was done by measuring band intensities using the Bio-Rad QuantityOne software. CSE protein amounts are expressed by normalizing CSE band intensities from the various treatment groups to their corresponding α-tubulin band intensities. Results are reflected as the degree of difference in protein expression of different treatment groups to that of the saline control group.

Statistical Analysis

Results shown are means ± SE. Statistical comparisons between groups were analyzed using one-way ANOVA. The statistical significance of difference between two groups was determined using Student’s t-test. The Chi square test was employed for calculating the significance of mortality data. A probability value of <0.05 was taken to indicate statistical significance.

RESULTS

Mortality

Mortality of the animals that died within 48 h after the induction of MI is shown in Fig. 1. Rats in the saline-treated vehicle control group had a mortality of 33.3%. SAC-treated rats showed a significant improvement in mortality of 12.5% (P < 0.05 vs. vehicle). In addition, SAC + PAG- and PAG-treated groups displayed comparable mortality of 34.8 and 37.5%, respectively. When the SAC + PAG-treated group was compared with the SAC-treated group, there was a significant improvement in mortality (P < 0.05). Rats that died before regaining full consciousness after the surgery were excluded from the study. These rats were assumed to have died because of surgical complications.

Infarct Size

Infarct size is defined as a ratio of left ventricular infarct area to whole left ventricle area. All rats in the four treatment groups subjected to the infarction procedure developed MI. The results obtained (n = 3/treatment group) are shown in Fig. 2. The infarct size of the saline-treated control group was 50.0 ± 0.03%. Rats treated with SAC had a smaller infarct size.
of 43.9 ± 0.03% compared with control. In addition, SAC + PAG- and PAG-treated groups developed significantly larger infarct sizes compared with the saline-treated group (60.9 ± 0.01 and 62.0 ± 0.03% respectively, P < 0.05 vs. control). In addition, there was considerable reduction in the infarct size (P < 0.005) when rats in the SAC-treated group were compared with those in SAC + PAG treatment group. No statistically significant difference in infarct size was observed between SAC + PAG- and PAG-treated groups. This result suggests that SAC offers cardioprotection via a CSE-related pathway, since inhibition of CSE by PAG in the SAC + PAG group exacerbated the infarct development.

**Ventricular Hypertrophy**

The ventricular hypertrophy index is indicative of the extent of cardiac remodeling in the left ventricle. Following induction of MI, occlusion of blood vessels causes the heart to be enlarged, since the remaining healthy cardiac muscles have to pump harder to supply the myocardium with sufficient oxygenated blood. Six hearts from each treatment group (n = 3 from MI-operated rats; n = 3 from sham-operated rats) were randomly selected for the evaluation of ventricular hypertrophy. The results of MI-operated rats from all treatment groups are shown in Fig. 2. Although the results are not statistically significant, it was observed that the SAC-treated group had lower hypertrophy index (3.13 ± 0.001) compared with the saline-treated group (3.23 ± 0.086). SAC + PAG- and PAG-treated groups also had larger hypertrophy indexes (3.51 ± 0.189 and 3.29 ± 0.052, respectively) than the control group. Hypertrophy indexes for rats that underwent sham operation in all four treatment groups were also measured. The results were similar in all groups, with hypertrophy indexes ranging between 2.71 ± 0.026 and 2.82 ± 0.050. When compared within each treatment group, the sham data were significantly smaller (P < 0.01) compared with their MI counterparts.

**Table 1. Blood pressure of rats in saline, SAC, SAC + PAG, and PAG-treated groups**

<table>
<thead>
<tr>
<th>BP, mmHg</th>
<th>Saline</th>
<th>SAC</th>
<th>SAC + PAG</th>
<th>PAG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td>122.5 ± 1.6</td>
<td>117.3 ± 1.0</td>
<td>120.2 ± 1.8</td>
<td>114.4 ± 1.6</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>122.5 ± 3.6</td>
<td>121.5 ± 1.5</td>
<td>130.4 ± 1.9*</td>
<td>120.7 ± 2.3*</td>
</tr>
<tr>
<td><strong>Day 10</strong></td>
<td>113.3 ± 6.8</td>
<td>109.9 ± 4.4</td>
<td>116.8 ± 3.9</td>
<td>118.9 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; SAC, S-allylcysteine; PAG, propargylglycine. BP was measured on day 1 (before start of treatment), day 7 (1 wk of treatment), and day 10 (48 h postsurgery). *P < 0.05 when compared with respective blood pressure readings on day 1.

**Histological Changes**

The morphology of myocardial tissues in all treatment groups subjected to both sham and MI operations were visualized by H&E staining. Myocardium of rats in all treatment groups that underwent the sham operation had normal healthy cardiac fibers without any infarction, edema, and inflammatory cells, as shown by a representative diagram in Fig. 4a. In the saline-treated control group that underwent MI, edema was evident, and inflammatory cells were observed in the infarct area as shown in Fig. 4b. The myocardium of the SAC-treated MI group showed mild edema with few inflammatory cells (Fig. 4c). In addition, the morphology of the myocardial tissue was better preserved compared with the other treatment groups. Edema was seen in the myocardium of SAC + PAG-treated group that was subjected to MI operation. Inflammatory cells were seen throughout the infarct area and the area at risk (Fig. 4d). The PAG-treated MI group showed severe edema in the infarct zone, with inflammatory cells distributed throughout. Signs of necrosis were also observed.

**Left Ventricular CSE Activity**

Left ventricular CSE activity was analyzed in the tissue homogenates from six rats (n = 3 from MI-operated rats; n = 3 from sham-operated rats) from each treatment group. Sham-operated rats had higher levels of CSE activity compared with their MI counterparts (Fig. 5). Among rats that were subjected to the infarction procedure, the SAC-treated group had significantly higher CSE activity compared with the saline-treated control group (2.75 ± 0.34 vs. 1.23 ± 0.16 μmol·g protein⁻¹·h⁻¹; P < 0.01). The PAG-treated group had the
highest CSE activity at 0.85 μmol·g protein⁻¹·h⁻¹. In addition, the SAC + PAG-treated group had significantly lower CSE activity compared with the SAC-treated group (1.22 ± 0.27 vs. 2.75 ± 0.34 μmol·g protein⁻¹·h⁻¹; P < 0.05). Sham-operated rats in saline and SAC- and SAC + PAG-treated groups had comparable CSE activity (3.27 ± 0.52 vs. 3.42 ± 0.27 μmol·g protein⁻¹·h⁻¹), whereas PAG-treated rats had a significant decrease in its activity compared with the saline control (2.27 ± 0.16 vs. 3.42 ± 0.27 μmol·g protein⁻¹·h⁻¹, P < 0.05).

**Plasma H₂S Concentration**

Plasma H₂S concentration was measured in 12 rats (n = 6 from MI-operated rats; n = 6 from sham-operated rats) from each treatment group. In general, MI-operated rats had a slightly higher plasma H₂S concentration compared with their sham counterparts. In rats that underwent the MI operation, the SAC-treated group had higher H₂S concentration compared with the saline-treated group. The PAG-treated group had significantly lower H₂S concentration (26.3 ± 1.4 μM; P < 0.05) compared with the control group, since PAG inhibited the H₂S-synthesizing activity of CSE. The plasma H₂S concentration of the SAC + PAG-treated group was significantly lower than that of SAC-treated group (33.3 ± 3.1 vs. 43.4 ± 3.6 μM; P < 0.01). This result indicates that SAC is involved in the production of H₂S via a CSE-dependent pathway during MI. In rats that underwent the sham operation, plasma H₂S concentrations were similar in saline- and SAC-treated groups. SAC + PAG- and PAG-treated groups had significantly lower H₂S concentration (25.5 ± 1.2 and 22.9 ± 1.2 μM, respectively, P < 0.05) compared with the saline-treated group (27.1 ± 0.6 μM). The results are illustrated in Fig. 6.

**Protein Expression**

CSE protein expression was measured in the left ventricles of MI-operated and sham-operated rats. This is shown in Fig. 7. In all treatment groups, MI-operated rats had signifi-
significantly less CSE protein expression compared with their sham-operated counterparts. This is indicated by the darker CSE band intensities of sham-operated rats. Among rats that underwent the MI procedure, SAC-treated rats showed a slight but significant increase (1.1-fold of control; \( P < 0.05 \)) in CSE protein expression compared with controls. In addition, SAC + PAG- and PAG-treated rats showed a significant downregulation of protein expression to 0.88-fold (\( P < 0.005 \)) of control in both groups. On the other hand, CSE protein was expressed similarly in sham-operated rats of all treatment groups. Treatment with the different drugs seemingly did not affect CSE protein expression in the myocardium of healthy rats.

**DISCUSSION**

Because of the complex pathways, mechanisms, and the myriad range of molecules involved in the pathophysiology of MI, a complete understanding of this condition has yet to be achieved.

The recent discovery of H\(_2\)S as a novel gasotransmitter has sparked interest in several areas in the research field. H\(_2\)S has been reported to be involved in several inflammation models such as septic and hemorrhagic shock (24, 25), caerulein-induced pancreatitis (6), as well as hypertension (11), diabetes mellitus (20), and Alzheimer’s disease (13). In the cardiovascular context, H\(_2\)S has been reported to be involved in stroke (31), ischemic injury (43), and isoproterenol-induced MI (16). When rats were given an intravenous bolus injection of NaHS, a H\(_2\)S donor, a dose-dependent decrease in mean arterial pressure was observed (40). The role of H\(_2\)S as a vasodilator is also substantiated in a separate study conducted by Cheng and co-workers (8). They showed that H\(_2\)S induced the relaxation of rat resistance mesenteric arteries. In addition, our group has recently demonstrated that H\(_2\)S plays a beneficial role in the ligature-induced MI rat model. We showed that NaHS improved mortality and infarct size, whereas PAG, an inhibitor of CSE, showed the reverse. In vitro studies also revealed that higher H\(_2\)S levels in the culture medium improved cell viability after exposure to hypoxic conditions (43).

Recent studies have demonstrated that garlic plays a beneficial role in the heart in isoproterenol-induced MI by reducing
oxidative stress. Banerjee and colleagues (5) reported that rats fed with raw garlic homogenates showed a significant preservation of myocardial superoxide dismutase activity and improvement in myocardial morphology upon MI induction with isoproterenol. In a separate study, Saravanan and Prakash (35) further verified the antioxidant role of garlic in MI. It was shown that garlic oil preserved the activities of a range of antioxidant enzymes, including superoxide dismutase and catalase. They also demonstrated that garlic oil lowered lipid peroxide levels in the serum and heart (35).

More recently, similar studies investigating the cardioprotective potential of a specific active component of garlic-SAC have been launched. Padmanabhan and Prince (29) showed that SAC exerted antioxidative effects in an isoproterenol-induced MI rat model by decreasing lipid peroxide products and improving the antioxidant status of the infarcted myocardium. In a follow-up study, the same group reported that SAC improved isoproterenol-induced cardiac damage by stabilizing cardiac and lysosomal enzymes to non-MI levels (30).

In this study, we established that SAC is protective in MI via a H2S-related pathway. Functional studies such as mortality, infarct size, and hypertrophy index are used as indicators. In addition, BP measurement, ECG recordings, and histological examination were performed. We also conducted biochemical assays to verify the involvement of SAC in the H2S pathway.

We showed that SAC improved mortality to 12.5% in a ligation-induced MI rat model compared with controls (33.4%). Rats in SAC + PAG- and PAG-treated groups had similar mortality (34.8 and 37.5%) with each other (Fig. 1). Infarct size, a key indicator of the ischemia extent, is important for the prognosis of MI. In agreement with our hypothesis, SAC-treated rats had smaller infarct sizes compared with control, suggesting that SAC is beneficial to the heart. SAC + PAG-treated rats had comparable infarct sizes to the PAG-treated group, and infarct sizes of both groups were significantly larger compared with controls. Thus it can be deduced that PAG abrogated the protective effects of SAC in the SAC + PAG-treated group, suggesting that SAC mediates cardioprotection in a CSE-related pathway.

Hypertrophy index is used to indicate the extent of ventricular remodeling. As shown in Fig. 2, all rats subjected to MI induction developed hypertrophy of the left ventricle. SAC-treated MI rats had smaller hypertrophy indexes compared with the other groups that were subjected to the MI operation. Although the result is not statistically significant, it suggests that rats in this group experienced an improvement in left ventricular remodeling, which resulted in less hypertrophy developed. As expected, the hypertrophy indexes of sham-operated rats in the various treatment groups were low and comparable to each other. Sham-operated rats did not have MI; thus, no ventricular remodeling and enlargement of the myocardium would have taken place.

Our results demonstrated that SAC did not affect BP after 1 wk of drug treatment (Table 1). However, both SAC + PAG- and PAG-treated groups showed a slight but significant increase in BP after 1 wk of injection with the respective drugs. This indicated that, when PAG is given, the blockade of endogenous H2S production resulted in an elevation in BP. On the other hand, when an exogenous source of H2S-SAC is given, the BP was not affected. This suggests that endogenous H2S plays a greater role than exogenous H2S sources in the regulation of BP. ECG recorded 48 h after the induction of MI showed obvious ST-segment elevations and pathological Q waves in all rats. SAC-treated rats had lower elevation of the ST-segment, whereas SAC + PAG- and PAG-treated rats had a greater elevation in the same segment. This further substan-
tiates that SAC is effective in improving the pathological conditions of MI.

Examination of the infarcted left ventricle showed a significant improvement in the morphology of SAC-treated rats. Ventricular tissue of this group displayed only mild edema and was similar to the morphology of the sham-operated rats, which had healthy cardiac fibers. Morphological examination of the saline, SAC + PAG-, and PAG-treated groups revealed edema in the infarct zone and fraying of the cardiac fibers. In addition, infiltration of inflammatory cells was also seen in the infarct area. To add on, sections from the sham-operated rats in all treatments showed normal myocardial tissues, indicating that the various treatments did not pose adverse effects under normal circumstances.

To investigate if the cardioprotective potential of SAC is mediated through an H2S-related pathway, left ventricular CSE enzyme activity and plasma H2S concentration were measured. Our results revealed that MI-operated rats had lower CSE enzyme activities in the left ventricle compared with their sham-operated counterparts. This may be partially explained by a reduction in enzyme activity in the injured myocardium. Among rats that underwent the MI procedure, the SAC-treated group demonstrated the highest level of CSE activity, which was significantly higher compared with rats treated with saline. SAC is suggested to increase CSE activity in the infarcted myocardium either by increasing the enzyme efficiency through an increase in substrate bioavailability or via an upregulation of enzyme expression. SAC + PAG-treated rats had significantly lower levels of CSE activity compared with the SAC-treated group. This is attributed to the additional treatment of this group with PAG, which is an irreversible inhibitor of CSE, thus lowering the amount of H2S produced. SAC-treated rats had significantly lower CSE activity than controls, but the inhibition of CSE is not complete, since low quantities of H2S were still produced in the tissue. Surprisingly, in sham-operated rats, CSE activities were similar in saline, SAC, and SAC + PAG-treated groups. This led us to hypothesize that SAC is only capable of stimulating CSE activity in the injured myocardium when the basal CSE activities are lower. Only sham-operated rats treated with PAG showed a notable reduction in CSE activity compared with the saline control.

Plasma H2S levels were also measured, since it will better reflect the overall H2S amount in the whole body. Generally, plasma H2S concentrations were higher in rats that were subjected to the MI procedure compared with sham-operated rats. This trend was different from that observed about the left ventricular CSE activity. It is suggested that the reduced enzyme activities in the injured myocardium stimulated healthy tissues elsewhere to generate more H2S so as to maintain the overall concentration of H2S in the body in check. This compensatory H2S production may account for the higher plasma H2S concentration observed in MI rats. Among MI-operated rats, the SAC-treated group showed the highest concentration of H2S in plasma compared with control, and its levels were significantly higher compared with the SAC + PAG-treated group. The elevated H2S level in the SAC-treated group is blocked by PAG in rats treated with SAC + PAG. Also, PAG-treated rats had significantly lower plasma H2S levels than controls. Both trends are similar to that observed about the left ventricular CSE activity. For rats that underwent the sham operation, SAC treatment only increased plasma H2S levels marginally. On the other hand, SAC + PAG and PAG treatments significantly lowered plasma H2S levels in sham-operated rats. It may be suggested that the body can regulate the amount of H2S present. Exogenous sources of CSE substrates such as SAC can only increase H2S levels when their levels are lowered in conditions such as MI.

CSE protein expression was measured to determine whether the higher levels of H2S produced in SAC-treated rats was because of an upregulated expression of CSE or rather because of an increase in efficiency of CSE, since SAC increased the availability of CSE substrates. Our results revealed that SAC indeed upregulated CSE protein in MI rats albeit slightly. However, this phenomenon was not observed in non-MI rats. SAC may only induce an increase in CSE expression when there is a drop in basal H2S levels in the injured myocardium during MI. Rats that underwent MI in SAC + PAG and PAG groups had significantly lower CSE expressions compared with controls. Notably, CSE was downregulated in both groups to the same levels, indicating the PAG is responsible for the lowered expression of CSE in these tissues. On the other hand, this trend was not revealed in sham-operated rats, suggesting that PAG at our given dose did not affect CSE levels in the healthy myocardium.

Conclusion

Our study demonstrated that SAC is cardioprotective in MI by lowering mortality as well as a reduction in infarct size and ventricular hypertrophy. In addition to the antioxidative role of SAC as reported by other groups, we propose that this cardioprotection is also mediated by a novel H2S-related pathway. This is because SAC served to upregulate CSE expression and activity in the infarcted myocardium and significantly increased plasma H2S concentration. This increase in left ventricular CSE expression and activity together with plasma H2S levels were abrogated when rats were treated concurrently with PAG. This new finding serves to substantiate that SAC is beneficial to the ischemic myocardium and is promising as a drug in the treatment of MI.

GRANTS

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