Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : August 12, 2022
Accepted : August 29, 2022
Published online : September 30, 2022
doi:10.24659/gsr.9.3_146

Original article

S-allyl cysteine increases blood flow in NO-dependent and -independent manners

Shigekazu Takemura [1,4], Hideshi Ihara [2], Kanako Nakagawa [1], Yukiko Minamiyama [3]

1) Department of Frontier Life-science, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan
2) Department of Biological Chemistry, Graduate School of Science, Osaka Metropolitan University, Sakai, Osaka, Japan.
3) Food Hygiene and Environmental Health Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan
4) Department of Hepato-Biliary-Pancreatic Surgery, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan

Abstract

S-allylcysteine (SAC) is a bioactive substance found in aged garlic and is known to improve liver function, inhibit organ fibrosis, and have anticancer activity. A single oral administration of SAC (10 mg/kg) increased tail vein blood flow in rats by up to 10% over one hour, and a 0.3% SAC mixed diet increased tail vein blood flow compared to the normal diet after 2 months. To investigate the relationship between these increases in blood flow and nitric oxide (NO), a single oral dose of SAC (100 mg/kg) followed by a 3-day administration of L-nitroarginine (LNA, 2 mM), an NO synthase inhibitor, resulted in the disappearance of SAC-induced increases in blood flow. Whereas, when LNA and excess L-arginine were mixed with drinking water, the SAC-induced increase in blood flow was restored. This phenomenon was also observed in plantar blood flow. These results indicate that the increase in blood flow induced by SAC is NO-dependent. To investigate the relationship between these increases in blood flow and NO, a single oral dose of SAC (100 mg/kg) followed by a 3-day administration of LNA (2 mM), resulted in the disappearance of SAC-induced increases in blood flow. Whereas, when LNA and excess L-arginine were mixed with drinking water, the SAC-induced increase in blood flow was restored. This phenomenon was also observed in plantar blood flow. These results indicate that the increase in blood flow by SAC is NO-dependent. Next, Western blotting of vasodilator-stimulated phosphoprotein (VASP) was performed to confirm this in a vascular model. Phosphorylated VASP (pVASP) is characterized as a substrate for both cGMP-dependent and cAMP-dependent kinases. LNA abolished the aortic pVASP band, whereas LNA plus SAC partially recovered. Focusing on the NO-independent vasodilator H_2S, measurements of plasma reactive persulfide showed that LNA treatment significantly reduced thiols, *i.e.*, cysteine, cystine, glutathione (GSH), and H_2S. Of the thiols reduced by LNA treatment, the concurrent SAC application significantly restored the reduction of cysteine and cystine, but not H_2S. These results suggest that SAC may maintain nitrosothiols (compounds of nitric oxide and thiols) that act as NO donors and indirectly increase the bioavailability of NO, resulting in blood flow augmentation through its antioxidant capacity by free radical scavenging.

***KEY WORDS*:** S-allyl cysteine, blood flow, nitric oxide (NO), vasodilator-stimulated phosphoprotein (VASP), nitrosothiol, L-nitro-arginine

Correspondent to: Associate Professor Shigekazu Takemura M.D., PhD
Department of Frontier Life-science, Graduate School of Medicine,
Osaka Metropolitan University,
Abeno-ku, Osaka 545-8585, Japan
TEL & FAX: +81-6-6645-3692 e-mail: take@omu.ac.jp
Co-authors; Ihara H, iharah@omu.ac.jp;
Nakagawa K, k-nakagawa@nichinichi-phar.co.jp;
Minamiyama Y, yukiko-m@kpu.ac.jp

Glycative Stress Research 2022; 9(3): 146-157

Introduction

S-allyl cysteine (SAC) is a biological active organosulfur component of aged garlic extracts. SAC has been extensively studied about tumoricidal effects, anti-inflammatory effects, etc. We have also reported about the improvement of SAC on lung injury [1] and fibrosis [2], liver injury [3] and fibrosis [4, 5], and sperm dysfunction [6]. Thus, SAC has a potential of multifunctional and disease-preventing drug.

SAC decreased H_2O_2-induced ROS production, restored cellular nitric oxide (NO) and cGMP level in human placenta trophoblast cells [7]. In the study, it has been reported that SAC also possesses the ability to increase NO and cGMP level at non-oxidative stress status in the cells. However, it is unknown whether SAC can directly increase blood flow by NO *in vivo*. Furthermore, a gas mediator other than NO, H2S is produced from L-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST) coupled with cysteine aminotransferase (CAT). It has been confirmed that H_2S activates endothelial NO synthase via Akt to increase NO production [8], and this effect is thought to be one of the reasons for the synergistic effect.

Therefore, in the present study, the effects of SAC treatment for blood flow and the mechanisms are studied in rats.

Materials and Methods

Animals

All the rats were taken care of according to the specifications outlined in the Guiding Principles for the Care and Use of Laboratory Animals, and the study was approved by the authorities of the local committee on experimental animal research of Osaka City University Medical School (No.18018).

Single administration of SAC

Male Wistar rats (8 weeks age, 220 ~ 240 g; SLC, Co., Shizuoka) were anesthetized with 1 g/kg urethane. Then, the rats were kept at 37 °C on the heating mat for more than 30 min, and the tail vein or pedal blood flow was measured with MoorLDI (Moor instruments LTD, Devon, UK). The measurement conditions were as follows: Scan Speed: 4 ms/pixel, Scan Distance: 30 cm, Image Resolution: 126 x 54. Changes in blood flow rate were expressed as changes in signal intensity.

To evaluate the effect of single administration of SAC, SAC was orally administered at 10 mg to 200 mg/kg body weight using a gastric tube, and blood flow was measured at 15, 30, 45, 60, 75, and 90 min.

Consecutive administration of SAC

Eight-week-old male Wistar rats were intraperitoneally anesthetized with the following mixed anesthetic agents (2.5 mL/kg) every 4 weeks for 24 weeks. The mixed anesthetic agents were made with total volume 2.5 mL with medetomidine [0.15 mg/0.15 mL], midazolam [2 mg/0.4 mL], butorphanol [2.5 mg/0.5 mL], and saline [1.45 mL]. Rats were maintained on a 37 °C warming mat for at least 30 min to measure tail vein blood flow as described above.

To evaluate NO-dependent blood flow by SAC, 8-week-old male Wistar rats were fed with drinking water containing L-nitro-arginine (LNA; Sigma, N5501) 40 μmol/kg/day for 3 days. In addition, to cancel the effect of LNA, excess L-arginine (Arg; Wako, 019-04611) at 2.4 mmol/kg/day was mixed with LNA solution. SAC (200 mg/kg) was administered orally as a single dose, and then tail vein blood flow was measured over time under urethane anesthesia. The same experiment was performed in rats fed ad libitum with LNA 10 μmol/kg/day and L-Arg 1 mmol/kg/day in drinking water for 3 days. For the measurement of plantar blood flow, rats were placed face down on a plate at 37 °C and the heels were fixed with tape with the soles facing upward.

Western blotting

For western blot analysis, animals were dissected 60 min after administration of 0.5% CMC or SAC (200 mg/kg, p.o.). Blood was drawn from the abdominal aorta into a heparinized syringe and then into ice-cold saline (50 mL). Blood was drawn from the abdominal aorta into a heparinized syringe and then ice-cold saline (50 mL) was perfused through the abdominal aorta. Thoracic aorta was immediately isolated and frozen in liquid nitrogen. The samples were stored at −80 °C until ready for measurement.

Thoracic aorta was homogenized in RIPA buffer and Western blotting was performed. Antibodies used were VASP (Calbiochem, 676600, 1 : 5,000) and eNOS (endothelial nitric oxide synthase) (BD Biosciences, 610296, 1 : 5,000).

Analysis for Reactive Persulfide Species in plasma

Reactive persulfide species were analyzed by LC-ESIMS/MS (Liquid Chromatograph-Electrospray Ionization Tandem Mass Spectrometer) with β-(4-hydroxyphenyl) ethyl iodoacetamide (HPE-IAM, Molecular Bioscience, Boulder, CO, USA) as a trapping agent for persulfide species [9, 10]. Plasma was diluted 5 times with ice-cold methanol containing 1.25 mM HPE-IAM, and incubated at 37 °C for one hour. The mixture was centrifuged at 15,000 × g at 4 °C for 5 min, and the resultant supernatant was diluted 10 times with 0.1% formic acid containing known amounts of isotope-labeled internal standards, which were synthesized as in our previous work [9, 10]. HPE-IAM adducts with various reactive sulfide species were analyzed with a Waters Alliance HPLC system coupled to a Waters Xevo TQD ESI triple-stage quadrupole mass spectrometer. Separation was achieved by using a Mightysil RP-18 column (50 mm long × 2 mm inner diameter, Kanto Chemical) with a linear 1 ~ 95% methanol gradient for 10 min in 0.1% formic acid at 40 °C. The total flow rate was 0.3 mL/min. Mass spectrometric analyses were performed on line using ESI-MS/MS in the positive ion mode with multiple reaction monitoring (MRM) mode. The MRM parameters were summarized in ***Table 1***.

Statistical Analysis

Unless otherwise stated, data are presented as means ± standard error (SE). Results were considered significant

Table 1. Parameters of Multiple reaction monitoring (MRM) mode.

Analyte	Precursor (m/z)	Product (m/z)	Cone potential (ev)	Collision energy (eV)
CysS-HPE-AM	299.1	121.0	30	29
[^{13}C]CysS-HPE-AM	300.1	121.0	30	29
CysS-S-HPE-AM	330.8	121.0	35	29
CysS-^{34}S-HPE-AM	332.8	121.0	35	29
Bis-S-HPE-AM	388.9	121.0	35	30
Bis-^{34}S-HPE-AM	390.9	121.0	35	30
Bis-S_2-HPE-AM	420.9	121.0	40	23
Bis-$^{34}S_2$-HPE-AM	424.9	121.0	40	23
GS-HPE-AM	484.9	356.3	35	18
[^{13}C]GS-HPE-AM	487.9	359.1	35	18
GSS-HPE-AM	516.9	388.3	40	18
GS^{34}S-HPE-AM	518.9	390.3	40	18

at $p < 0.05$. Statistical analysis was performed by using unpaired Student's t-tests or analysis of variance (ANOVA) for repeated measures followed by Fisher's test for post hoc analysis.

Results

Blood flow after a single administration of SAC

Fig. 1-a shows a representative figure of tail vein blood flow changes. The blue to red color indicates higher blood flow. The results of tail vein blood flow measurement are shown in ***Fig. 1-b***, compared with the control group that received 0.5% carboxymethyl cellulose (CMC) at 2 mL/kg body weight, the blood flow increased in the group that received SAC from 15 min after administration, and the effect was sustained even after 75 min.

Blood flow of tail vein after long-term SAC administration

The results of blood flow measurement are shown in ***Fig. 2***. The blood flow after SAC administration increased significantly at 8, 20, and 24 weeks compared to that of rats fed with normal diet.

Relationship with NO

Fig. 3 shows the results of the study using rats pretreated with LNA, an inhibitor of NO synthase; blood flow in the LNA-treated group was not increased by SAC. Furthermore, the LNA + Arg group did not recover to the SAC alone group (***Fig. 4***). This finding suggests that the increase in blood flow by SAC is due to NO-dependent and NO-independent effects. Similarly, plantar blood flow was also decreased by LNA administration, and the increase in blood flow increased more slowly when SAC was administered than when SAC was administered alone (***Fig. 5***).

The expression of vasodilator-stimulated phosphoprotein (pVASP/VASP), which is associated with the vasorelaxant response, was decreased by LNA treatment and the decrease was ameliorated by SAC treatment (***Fig. 6***). eNOS levels were unchanged.

Involvement of H_2S-related substances

We investigated the involvement of H_2S-related substances as NO-independent vasodilators. The concentrations of cysteine (Cys-SH), cystine, GSH, H_2S, oxidized GSH (GSSH), and H_2S_2 in plasma were measured at 1 hour after a single administration of SAC under various conditions (***Fig. 7***). In the normal rats treated with SAC, only GSSH concentration increased; in the LNA-treated group, Cys-SH, cystine, GSH, and H_2S were all significantly decreased; only Cys-SH and cystine were ameliorated by SAC.

Discussion

The present study showed that SAC treatment increased peripheral blood flow in normal rats in both NO-dependent and NO-independent ways. ***Fig. 8*** shows the estimated pathway of SAC in improving blood flow.

This aspect of the increase in blood flow was found to be consistent with the kinetics [11], which remained high for several hours after oral administration in proportion to the amount of SAC in plasma (***Suppl. 2***). SAC ameliorates ischemic injury in retina by inhibiting excessive elevation of HIF-1α, VEGF, and matrix metalloprotease (MMP)-9 [12]. Inhibition of VEGF inhibits metastasis of hepatocellular carcinoma [13]. However, it has not been reported that SAC increases peripheral blood flow under normal conditions. In humans, 600 mg of garlic was administered for one week, and plasma NOx and IL-6 were measured. As a result, there was a correlation between IL-6 and blood flow, so there is a report that blood flow may be increased via IL-6, but it is not clear [14].

The results of ***Figs. 3, 4,*** and ***5*** suggest that vasodilation by SAC involves at least the effect of NO. However, SAC had no effect on plasma NOx, urinary NOx, or plasma renin activity (***Suppl. 1***), nor did it affect blood pressure (data not shown). These suggest that the changes are due to local, or perivascular,

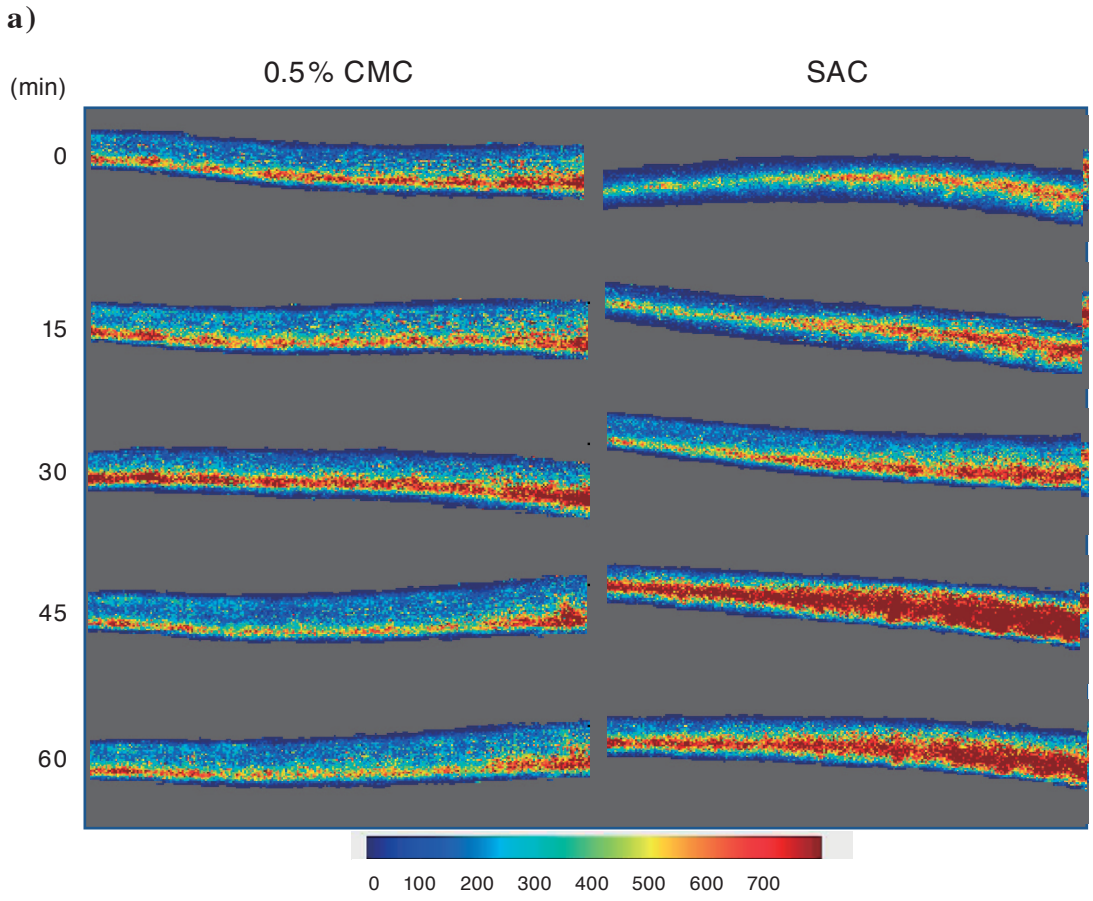


Fig. 1. **Blood flow in the rat tail vein.**

a) Representative figures of blood flow (left vein) after SAC administration. It shows increased blood flow as the color changes from blue to red. **b)** Changes in blood flow. SAC was suspended in 5 mg/mL 0.5% CMC and administered orally at 2 mL/kg. Blood flow in the tail vein of rats was measured under anesthesia. The blood flow signal before treatment was set as one and the change over time was shown. Open circle (○), 0.5% CMC in control rats; closed circle (●), SAC (100 mg/kg) in control rats. Results are expressed as mean ± SE, ** $p < 0.01$ between SAC (n = 6) and control (CMC, n = 17). SAC, S-allylcysteine; CMC, carboxymethyl cellulose; SE, standard error.

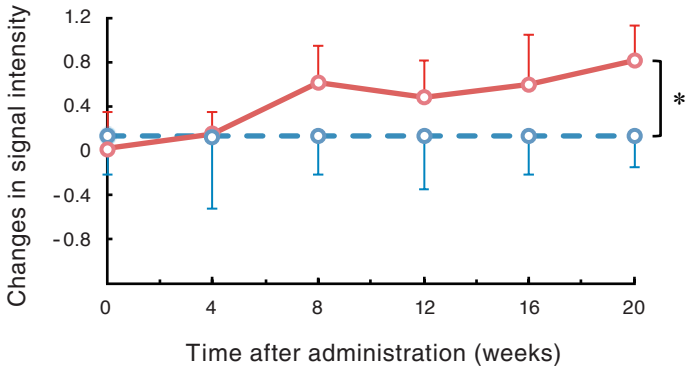


Fig. 2. **Blood flow changes in long-term administration of SAC.**
Blood flow was measured every 4 weeks under triple mixed anesthesia in rats fed a 0.3% SAC mixed diet. * $p < 0.05$, SAC vs. Control, n = 6 each. Red line, SAC; Blue dotted line, Control; SAC, S-allylcysteine; SD, standard deviation.

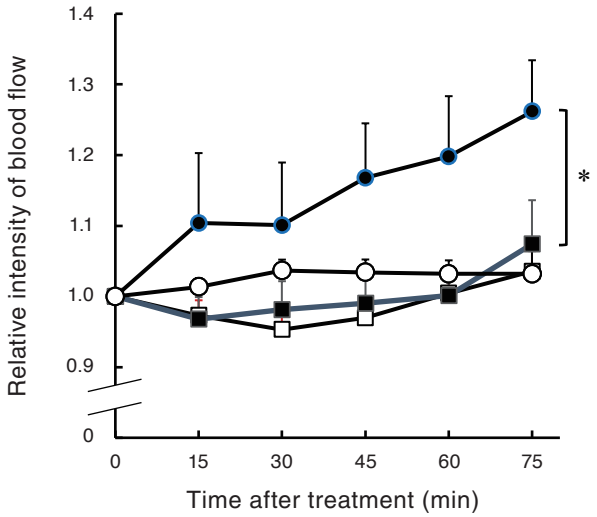


Fig. 3. **Effects of inhibition of NO synthesis on tail vein blood flow.**
LNA (2 mM) was administered to rats as a drinking water *ad libitum* for 3 days. Figure indicates % changes in tail blood flow after SAC (100 mg/kg, p.o.) administration. Open circle (○), 0.5% CMC in control rats; closed circle (●), SAC (100 mg/kg) in control rats; open square (□), 0.5% CMC in LNA treated rats; closed square (■), SAC in LNA treated rats. Results are expressed as mean ± SE, n = 6 ~ 18, * $p < 0.05$. SAC vs. LNA+SAC. SAC, S-allylcysteine; CMC, carboxymethyl cellulose; LNA, L-nitro-arginine; SE, standard error.

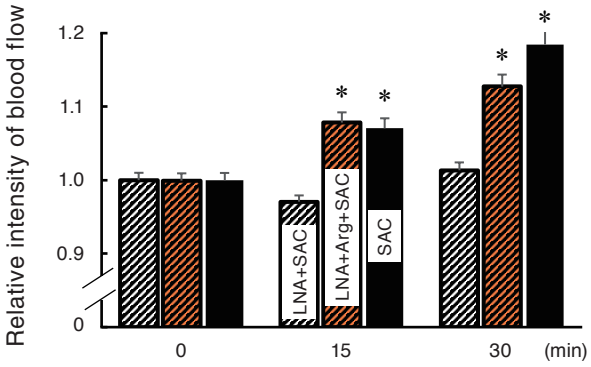


Fig. 4. **Effects of inhibition of NO synthesis on tail vein blood flow.**
LNA (2 mM) and Arg (100 mM) were administered to rats as a drinking water *ad libitum* for 3 days. Figure indicates changes in relative intensity of tail blood flow after SAC (100 mg/kg, p.o.) administration. Open circle (○), 0.5% CMC in control rats; closed circle (●), SAC (100 mg/kg); open square (□), 0.5% CMC in LNA treated rats; closed square (■), SAC in LNA treated rats. Results are expressed as mean ± SE, n = 3 ~ 4, * $p < 0.05$ vs. LNA+SAC. SAC, S-allylcysteine; CMC, carboxymethyl cellulose; LNA, L-nitro-arginine; Arg, L-arginine; SE, standard error.

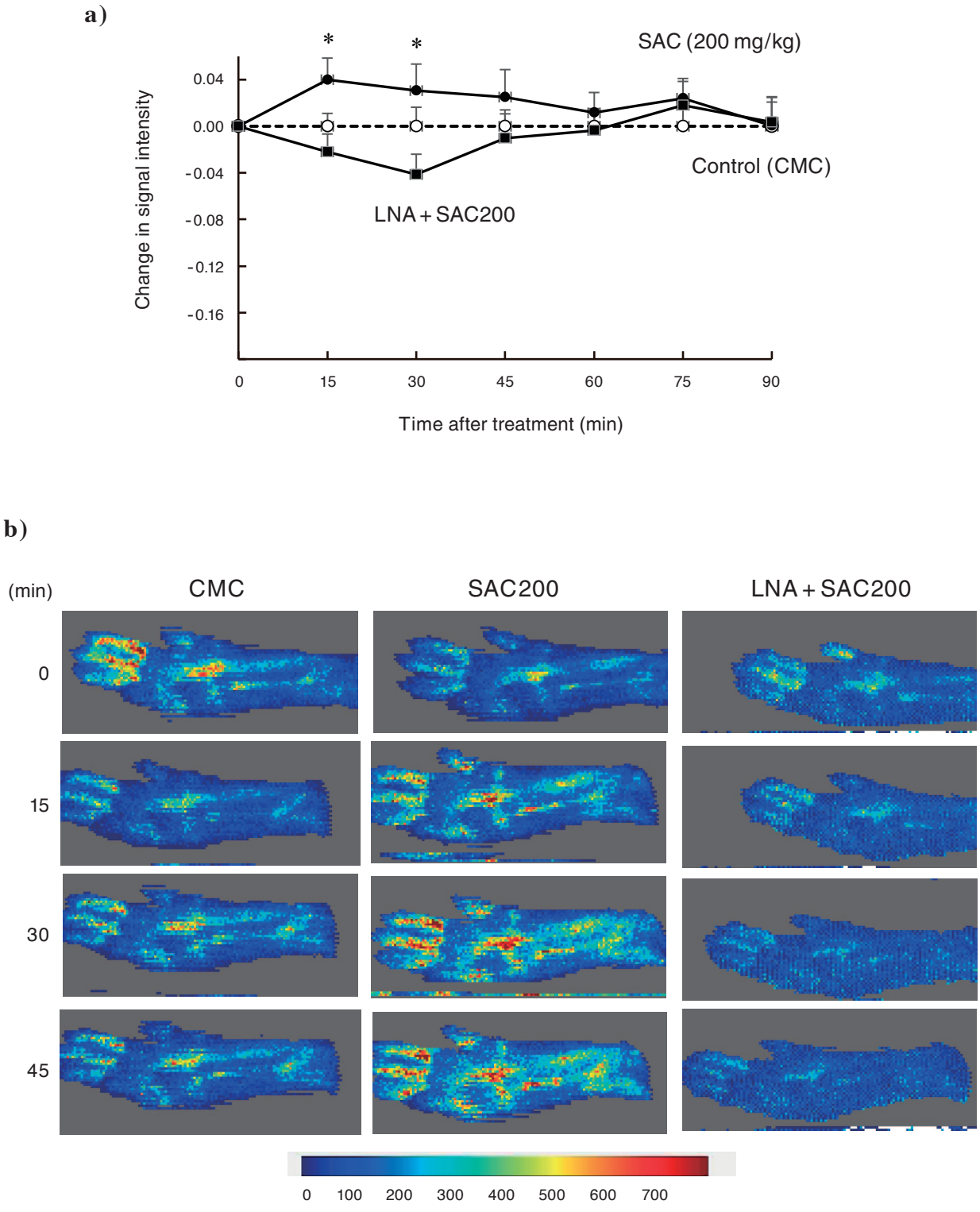


Fig. 5. **Effects of SAC on plantar blood flow.**

a) Changes in blood flow of foot pad after a single oral administration of 200 mg/kg of SAC to rats that received LNA (0.5 mM) for 3 days. Open circle (○), 0.5% CMC in control rats (n = 17); closed circle (●), SAC (200 mg/kg) (n = 7); closed square (■), SAC in LNA treated rats (n = 5). Results are expressed as mean ± SE, * $p < 0.05$, vs LNA + SAC. **b)** Representative changes in plantar blood flow. SAC, S-allylcysteine; CMC, carboxymethyl cellulose; LNA, L-nitro-arginine; Arg, L-arginine; SE, standard error.

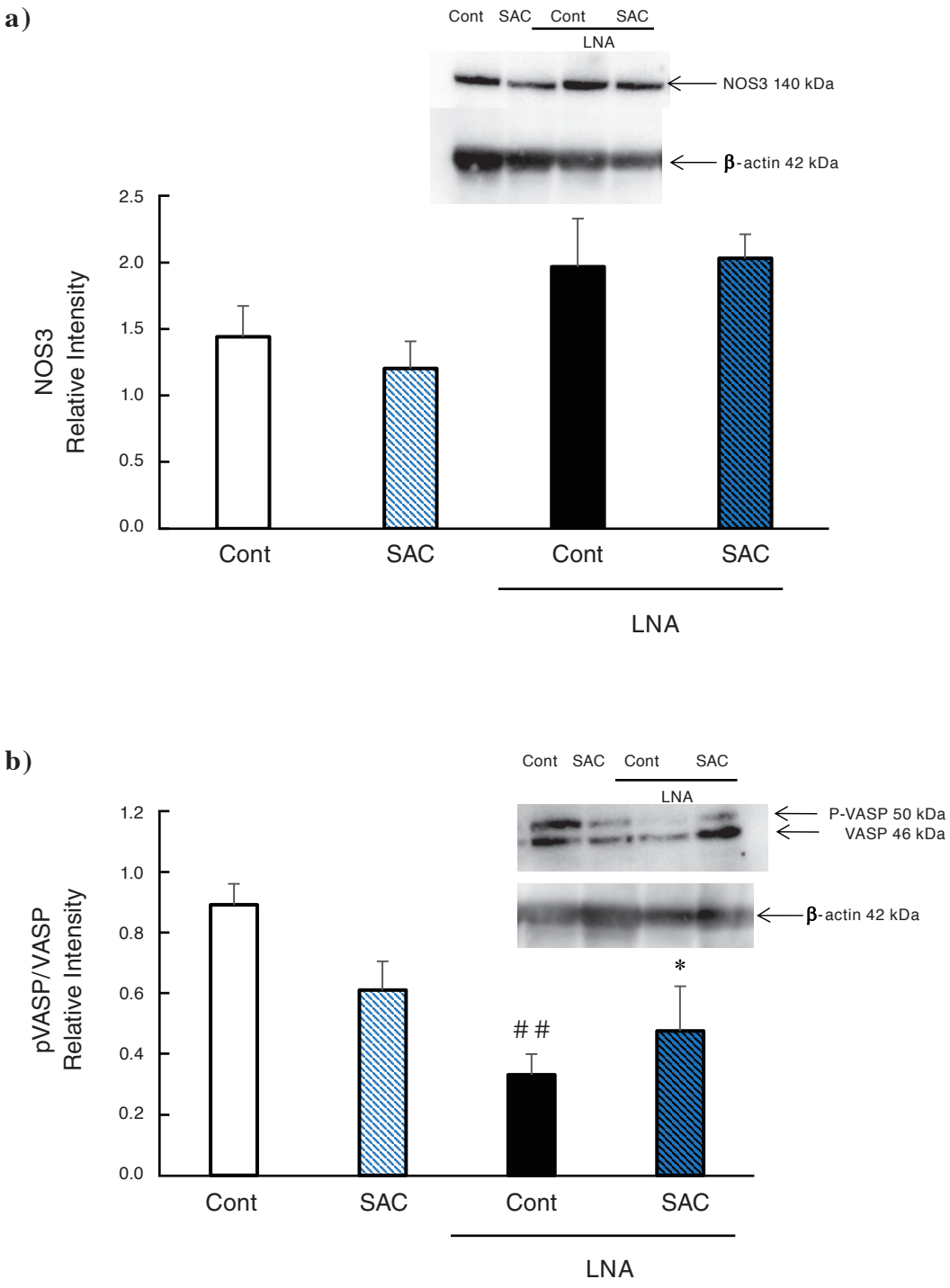


Fig. 6. **Western blot analysis of thoracic aorta.**

a) Expression of eNOS protein, **b)** Expression of pVASP. Rats were treated with 0.5 mM LNA for 3 days and 0.5% CMC (Control) or 200 mg/kg SAC orally. Results are expressed as mean ± SE, n = 3 ~ 4, ## $p < 0.01$ vs. vehicle control, * $p < 0.05$ vs. LNA. eNOS, endothelial nitric oxide synthas; VASP, vasodilator-stimulated phosphoprotein; pVASP, phosphorylated VASP; SAC, S-allylcysteine; CMC, carboxymethyl cellulose; LNA, L-nitro-arginine; SE, standard error.

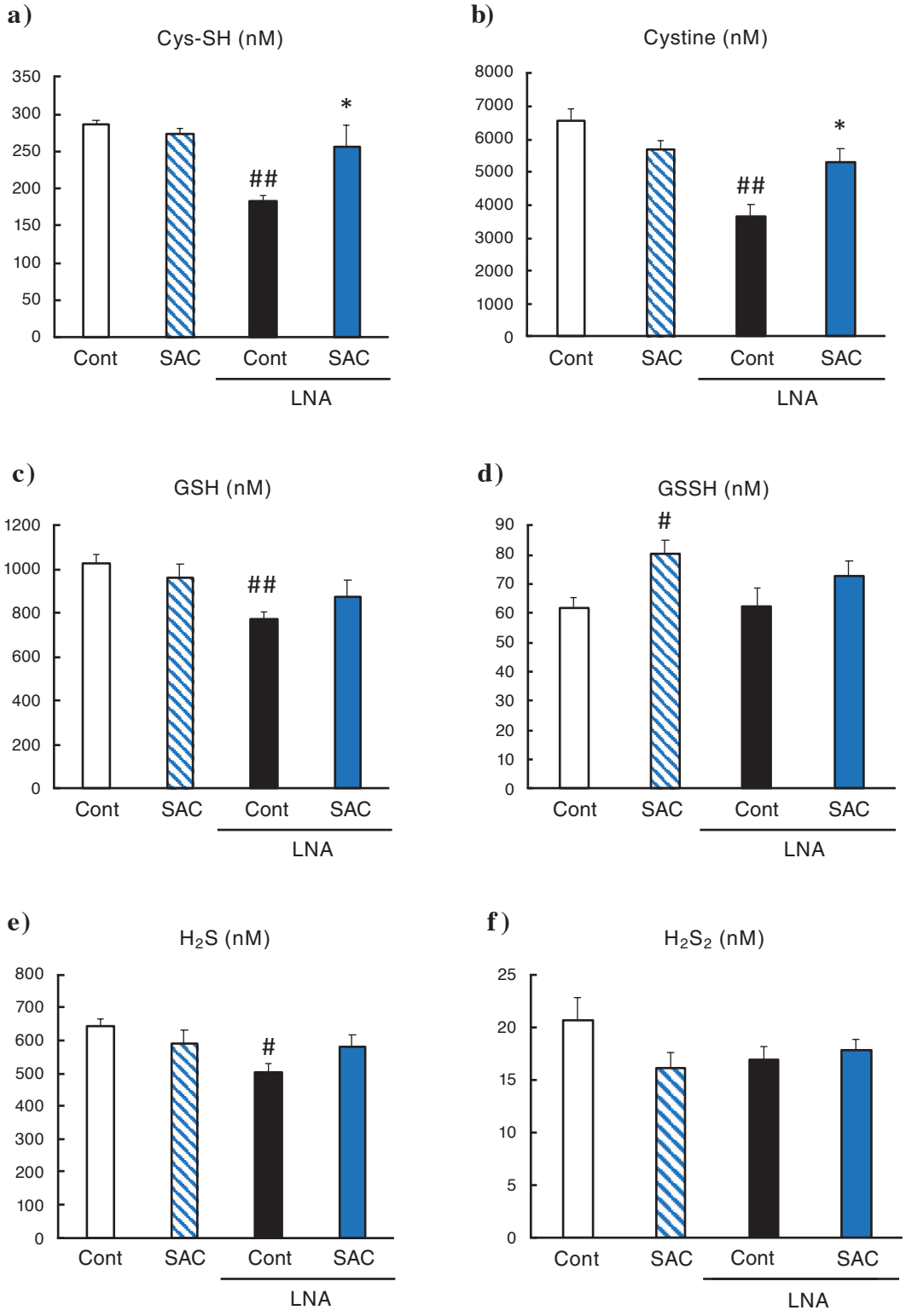


Fig. 7. **Analysis for reactive persulfide species by LC-ESI-MS/MS in plasma.**

a) Cys-SH, **b)** Cystine, **c)** GSH, **d)** GSSH, **e)** H_2S, **f)** H_2S_2. Rats were treated with 0.5 mM LNA for 3 days and 0.5% CMC (Control) or 200 mg/kg SAC orally. Results are expressed as mean ± SE, n = 3 ~ 4, # $p < 0.05$, ## $p < 0.01$ vs. vehicle control, * $p < 0.05$ vs. LNA. Cys-SH, cysteine; GSH, glutathione; GSSH, oxidized glutathione; SAC, S-allylcysteine; CMC, carboxymethyl cellulose; LNA, L-nitro-arginine; SE, standard error.

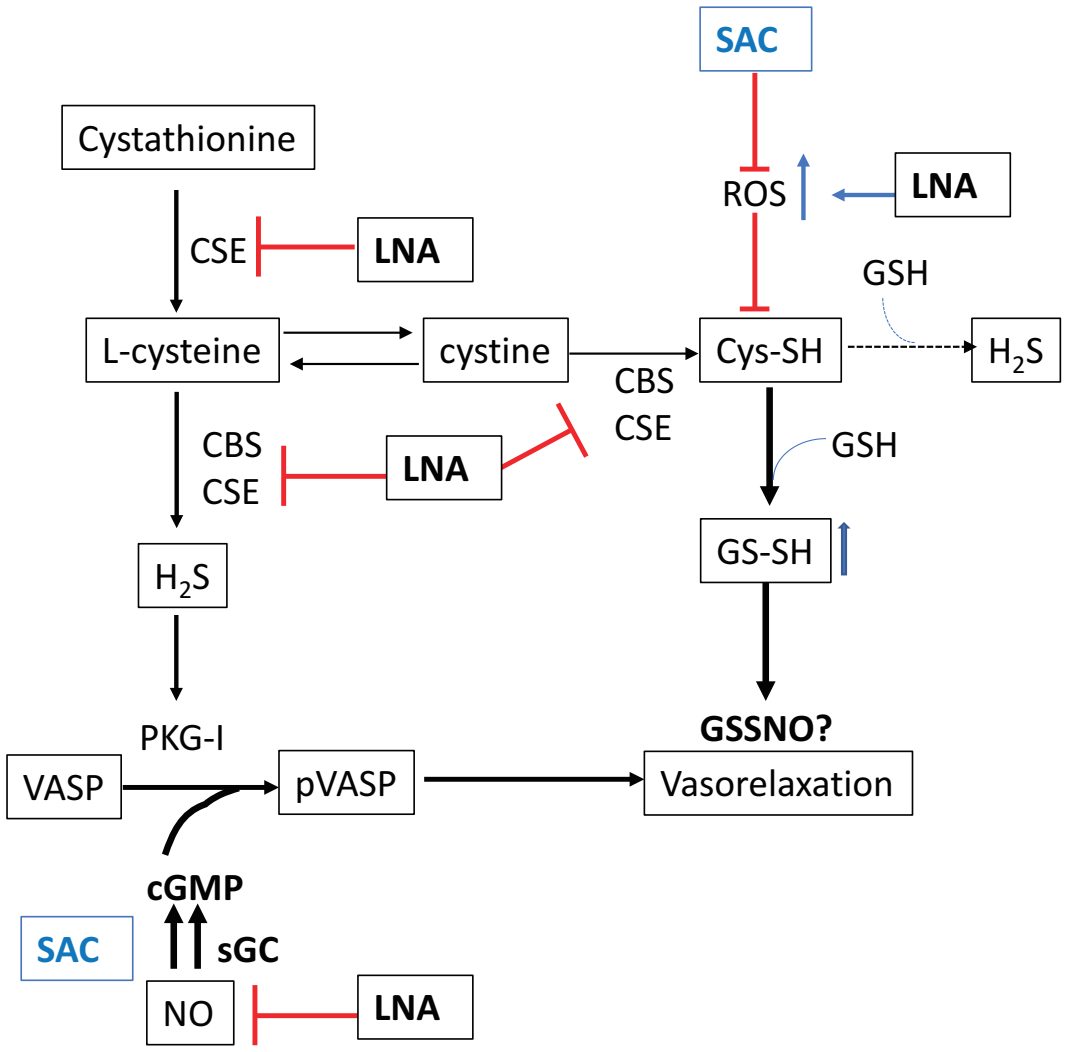
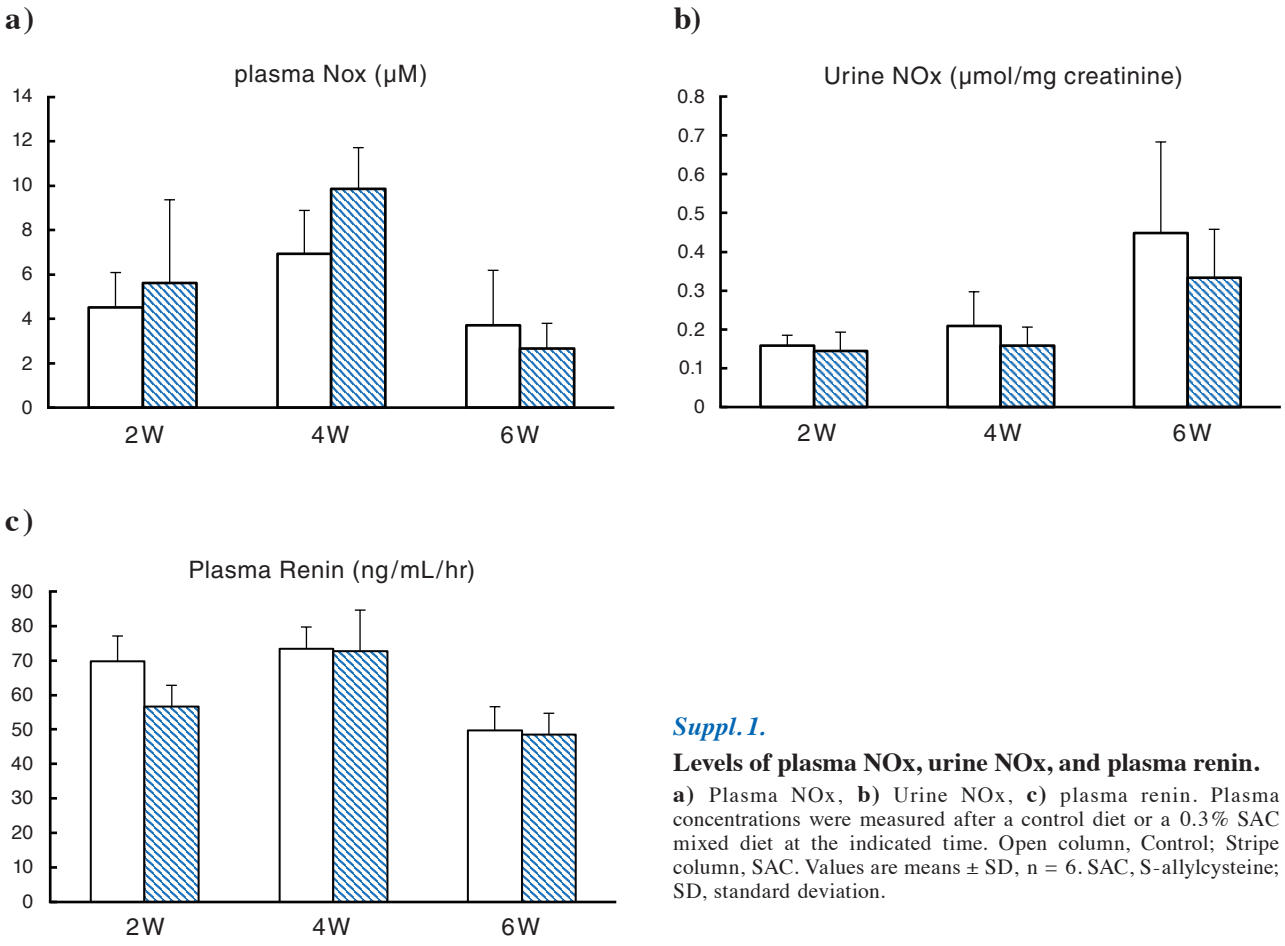


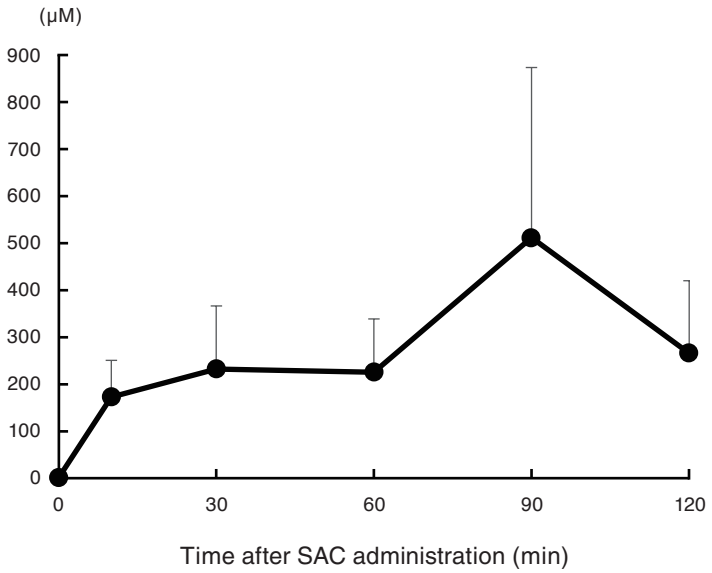
Fig. 8. **Estimated pathway of SAC in improving blood flow.**
In addition to improving blood flow by increasing the bioavailability of NO through free radical scavenging, SAC may also be a source of cysteine, which increases SH compounds and increases NO releaser by increasing nitroso compounds, resulting in increased blood flow. It is also thought that the increase in NO releaser due to the increase in nitroso compounds may cause increased blood flow. Cys-SH, cysteine; GSH, glutathione; SAC, S-allylcysteine; LNA, L-nitro-arginine; ROS, reactive oxygen species; GSH, glutathione; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; VASP, vasodilator-stimulated phosphoprotein; pVASP, phosphorylated VASP; PKG-1, protein kinase G-1; cGMP, cyclic guanosine monophosphate; sGC, soluble guanylate cyclase.



Suppl. 1.

Levels of plasma NOx, urine NOx, and plasma renin.

a) Plasma NOx, **b)** Urine NOx, **c)** plasma renin. Plasma concentrations were measured after a control diet or a 0.3% SAC mixed diet at the indicated time. Open column, Control; Stripe column, SAC. Values are means ± SD, n = 6. SAC, S-allylcysteine; SD, standard deviation.



Suppl. 2. **Effects of SAC concentration after the oral administration.**

SAC (200 mg/kg) was orally treated to rats. At the indicated times, heparinized blood was collected via the femoral vein. Values are means ± SE, n = 3 ~ 6. SAC, S-allylcysteine; SE, standard error.

physiological fluctuations rather than systemic changes.

H_2S has recently attracted much interest as a potent vaso-relaxative substance that may establish itself alongside another gaseous signal molecule, NO. In contrast to NO, the major source of H_2S in blood may be production by red blood cells[15] or by vascular smooth muscle cells[16]. H_2S is produced from Cys-SH, involving the enzymes cystathionine beta-synthase and cystathionine gamma-lyase (CSE). The importance of CSE was recently demonstrated in a mouse lacking CSE which showed reduced H_2S levels and developed hypertension and reduced endothelium-mediated vasorelaxation. These data establish H_2S as a new and important biological signal molecule and as a new regulator of vascular blood flow and blood pressure[17]. Furthermore, H_2S up-regulated the effect of NO/cGMP/sGC/PKG signaling pathways. Partially, the dilating effect of H_2S is realized via activation of ATP-sensitive K(+) channels in plasmalemma of smooth muscle cells. In the brain, ischemia/reperfusion injury degrades the ability of pial arteries to dilate via inhibition of NO-mediated signaling pathway[18].

N-Nitro-L-arginine methylester (L-NAME) inhibited CSE activity and H_2S generation. Dysfunction of the vascular H_2S synthase/H_2S pathway was found in L-NAME-induced hypertensive rats. Exogenous H_2S effectively prevented the development of hypertension induced by L-NAME. These findings suggest that the H_2S synthase/H_2S pathway participates in hypertension[19].

However, SAC had little effect on H_2S concentrations, suggesting that LNA inhibits the synthesis of Cys-SH from cystathionine and that NO is not synthesized, which may lead to a decrease in Cys-SH by increasing ROS. The bioavailability of NO is decreased by O_2^-. The bioavailability of NO was reduced by O_2^-, but the enzymes that scavenge it, SOD1 and SOD2, were unchanged (data not shown). Whereas, it has been reported that S-propargyl-cysteine (SPRC), an H_2S donor, is a structural analogue of SAC. It was investigated for its potential anti-cancer effect on gastric cancer cells[20]. Therefore, the possibility that non detectable H_2S is produced around the microvasculature of SAC administration cannot be denied, but it is not clear.

Nitrosothiols are compounds produced by the reaction of NO_2 or NO_2^- with thiols (compounds indicated by R-SH) and have vasorelaxant activity *in vivo*. The S-nitroso forms of Cys-SH and GSH, as nitrosothiols, act as NO providers (donors) *in vivo* to relax vascular smooth muscle and exert vasodilation. In the present experiment, LNA administration decreased Cys-SH, cystine, GSH, and H_2S, while SAC improved Cys-SH and cystine; it is possible that SAC maintains Cys-SH and cystine, which react with NO to produce nitrosothiols.

In addition, in a double blind random clinical trial of SAC in humans, it was reported that administration of 2 mg capsules for 4 weeks improved fatigue[21]. Therefore, we would like to study the effect of the capsules on improving blood flow in humans.

Conclusion

In this study, we examined the mechanism of action of SAC to increase blood flow, suggesting that SAC may increase blood flow by indirectly increasing the bioavailability of NO through the maintenance of nitrosothiols, which act as NO donors, and antioxidant capacity through free radical scavenging activity.

References

1) Mizuguchi S, Takemura S, Minamiyama Y, et al. S-allyl cysteine attenuated CCl_4-induced oxidative stress and pulmonary fibrosis in rats. *Biofactors*. 2006; 26: 81-92.
2) Tsukioka T, Takemura S, Minamiyama Y, et al. Attenuation of bleomycin-induced pulmonary fibrosis in rats with S-allyl cysteine. *Molecules*. 2017; 22: 543.
3) Kodai S, Takemura S, Minamiyama Y, et al. S-allyl cysteine prevents CCl(4)-induced acute liver injury in rats. *Free Radic Res*. 2007; 41: 489-497.
4) Kodai S, Takemura S, Kubo S, et al. Therapeutic administration of an ingredient of aged-garlic extracts, S-allyl cysteine resolves liver fibrosis established by carbon tetrachloride in rats. *J Clin Biochem Nutr.* 2015; 56: 179-185.
5) Shinkawa H, Takemura S, Minamiyama Y, et al. S-allylcysteine is effective as a chemopreventive agent against porcine serum-induced hepatic fibrosis in rats. *Osaka City Med J*. 2009; 55: 61-69.
6) Takemura S, Ichikawa H, Naito Y, et al. S-allyl cysteine ameliorates the quality of sperm and provides protection from age-related sperm dysfunction and oxidative stress in rats. *J Clin Biochem Nutr.* 2014; 55: 155-161.
7) Yu J, Feng L, Hu Y, et al. Effects of SAC on oxidative stress and NO availability in placenta: Potential benefits to preeclampsia. *Placenta*. 2012; 33: 487-494.
8) Predmore BL, Julian D, Cardounel AJ. Hydrogen sulfide increases nitric oxide production from endothelial cells by an akt-dependent mechanism. *Front Physiol*. 2011; 2: 104.
9) Akaike T, Ida T, Wei FY, et al. Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat Commun*. 2017; 8: 1177.
10) Ihara H, Kasamatsu S, Kitamura A, et al. Exposure to electrophiles impairs reactive persulfide-dependent redox signaling in neuronal cells. *Chem Res Toxicol*. 2017; 30: 1673-1684.
11) Amano H, Kazamori D, Itoh K, et al. Metabolism, excretion, and pharmacokinetics of S-allyl-L-cysteine in rats and dogs. *Drug Metab Dispos*. 2015; 43: 749-755.
12) Chen YQ, Pan WH, Liu JH, et al. The effects and underlying mechanisms of S-allyl l-cysteine treatment of the retina after ischemia/reperfusion. *J Ocul Pharmacol Ther.* 2012; 28: 110-117.
13) Ng KT, Guo DY, Cheng Q, et al. A garlic derivative, S-allylcysteine (SAC), suppresses proliferation and metastasis of hepatocellular carcinoma. *PLoS One.* 2012; 7: e31655.

14) Anim-Nyame N, Sooranna SR, Johnson MR, et al. Garlic supplementation increases peripheral blood flow: a role for interleukin-6? *J Nutr Biochem*. 2004; 15: 30-36.
15) Vitvitsky V, Yadav PK, An S, et al. Structural and mechanistic insights into hemoglobin-catalyzed hydrogen sulfide oxidation and the fate of polysulfide products. *J Biol Chem*. 2017; 292: 5584-5592.
16) Kanagy NL, Szabo C, Papapetropoulos A. Vascular biology of hydrogen sulfide. *Am J Physiol Cell Physiol*. 2017; 312: C537-C349.
17) Wagner CA. Hydrogen sulfide: A new gaseous signal molecule and blood pressure regulator. *J Nephrol*. 2009; 22: 173-176.
18) Lobov GI, Sokolova IB, Gorshkova OP, et al. Contribution of hydrogen sulfide to dilation of rat cerebral arteries after ioschemia/reperfusion injury. *Bull Exp Biol Med*. 2020; 168: 597-601.
19) Zhong G, Chen F, Cheng Y, et al. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. *J Hypertens*. 2003; 21: 1879-1885.
20) Ma K, Liu Y, Zhu Q, et al. H2S donor, S-propargyl-cysteine, increases CSE in SGC-7901 and cancer-induced mice: Evidence for a novel anti-cancer effect of endogenous H2S? *PLoS One*. 2011; 6: e20525.
21) Takayanagi M, Eguchi A, Ooe K, et al. Effects of S-allylcysteine on reducing physical fatigue: A randomized, double-blind, placebo-controlled crossover study. *Pharmacology and Therapy*. 2019; 47: 607-619. (in Japanese)