Acta Med. Okayama, 2009
Vol. 63, No. 6, pp. 339-348

Acta Medica
Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Effects of Sulfur Amino Acids on Tyrosyl or Serine/Threonine Phosphorylation and Translocation of Cytosolic Compounds to Cell Membrane in Stimulus-treated Human Neutrophils

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We investigated the effects of various sulfur amino acids on the phosphorylation of proteins and the translocation of cytosolic compounds to cell membrane in stimulus-treated human neutrophils using specific monoclonal antibodies. D,L-homocysteine and D,L-homocysteine-thiolactone enhanced fMLP-induced tyrosyl phosphorylation of proteins and the translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane in a concentration-dependent manner. L-cystathionine, NAc-L-cysteine and carboxymethyl-cysteine suppressed the tyrosyl phophorylation and translocation of cytosolic compounds to the cell membrane. L-cystathionine, L-cysteine and NAc-L-cysteine suppressed PMA-induced serine/threonine phosphorylation and the translocation of cytosolic compounds to the cell membrane. L-cysteine, NAc-L-cysteine and D,L-homocysteine enhanced AA-induced serine/threonine phosphorylation and the translocation of cytosolic compounds to the cell membrane, but L-cystathionine had opposite effects. These results indicated that the effects of sulfur amino acids on tyrosyl or serine/threonine phosphorylation and the translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane in the stimulus-treated human neutrophils were in parallel with those of the stimulus-induced superoxide generation reported in previous paper. L-cysteine, D,L-homocysteine and L-cystathionine weakly inhibited lipid peroxidation, but the other sulfur amino acids tested had no effect.

Key words: sulfur amino acids, phosphorylation, superoxide, cytosolic compounds, human neutrophils

We have reported that, among the cystathionine metabolites found in the urine of 2 patients with cystathioninuria as well as in rat and bovine brain [1-5], cystathionine ketimine significantly enhanced superoxide generation in human neutrophils.

It has been reported that NAc-L-cys reduces superoxide generation response to N-formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 13-acetate (PMA) and partially protects against lipid peroxidation in human polymorphonuclear [6].

Wada *et al.* [7, 8] reported that L-cystathionine significantly scavenges the superoxide radicals derived from the xanthine-xanthine oxidase system and protects the gastric mucosa from acute injury induced by ischemia-reperfusion. After that, it was reported that homocysteine enhances the oxidative stress of neutrophils, which underscores the potential role of phagocytic cells in vascular wall injury through O_2^- release

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Received April 9, 2009; accepted July 17, 2009.
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in hyper-homocysteinemia conditions [9].

It is known that human peripheral neutrophils play a number of critical roles in the defense against microorganisms [10], and that superoxide anion (O_2^-) production in neutrophils is stimulated during phagocytosis by treatment with a variety of stimuli such as certain chemoattractants and activators of protein kinase [11-14]. This phenomenon relies in part on the ability of PMN leukocytes to generate large amounts of superoxide anion and related reactive oxygen species; this is known as the respiratory burst.

The respiratory burst is mediated by the activation of the NADPH oxidase, a multi-component enzyme, localized in the plasma membrane of phagocytic leukocytes. The core enzyme consists of 5 components: $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, $p22^{phox}$, and $gp91^{phox}$. In the resting cell, three cytosolic components remain as a complex ($p40^{phox}$, $p47^{phox}$, and $p67^{phox}$) and the other components; $p22^{phox}$ and $gp91^{phox}$, are located in the membranes of secretory vesicles as heterodimeric flavohemoprotein known as cytochrome b_{558}. When the cell is exposed to stimuli, $p47^{phox}$, together with $p67^{phox}$, migrates to the membrane associating with cytochrome b_{558} under the control of rac via a cytoskeletal scaffold [15-19].

It is also known that the response of neutrophils to an activating stimulus can be potentiated sometimes by prior exposure to a priming agent [20]. A variety of proinflammatory stimuli have been observed to exercise this effect [21-23].

We have reported that D,L-homocysteine and D,L-homocysteine-thiolactone enhanced fMLP-induced superoxide generation by the increased translocation of $p47^{phox}$ and $p67^{phox}$ to the cell membrane; that L-cystathionine and NAc-L-cys suppressed fMLP- and PMA-induced superoxide generation; and that N-acetyl-L-cystathionine also had scavenging activity against DPPH radicals and superoxide anion [24].

Recently, we reported that triterpenoid compounds isolated from root bark of *Aralia elata* suppressed tyrosyl or serine/threonine phosphorylation of proteins and translocation to the plasma membrane of $p47^{phox}$, $p67^{phox}$ and rac in paralled with the effect of stimulus-induced superoxide generation [25, 26].

In the present study, to clarify the mechanisms underlying the effects of sulfur amino acids on stimulus-induced superoxide generations in human neutrophils, we investigated the effects of various sulfur amino acids on tyrosyl and serine/threonine phosphorylation of proteins; on the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane in stimulus-treated human neutrophils; and on lipid peroxidation of erythrocyte membrane ghost by hydroxyl radicals.

Materials and Methods

Chemicals. From Sigma Chemical (St. Louis, MO, USA), we obtained, L-cystathionine, L-cysteine, N-acetyl-L-cysteine (NAc-L-cys), carboxymethylcysteine (CMC), D,L-homocysteine, D,L-homocysteine-thiolactone, NADPH, ferricytochrome *c* (*cyt. c*), superoxide dismutase (SOD), N-formyl-methionyl-leucyl-phenylalanine (fMLP), phorbol 12-myristate 13-acetate (PMA), and arachidonic acid (AA). All other reagents used were of analytical grade and were from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise noted.

Isolation of neutrophils. Polymorphonuclear leukocytes were isolated from human peripheral blood of healthy volunteers by Ficoll-Hypaque (Flow Laboratories, Rockville, MD, USA) density gradient centrifugation [27] and were washed twice with Krebs-Ringer phosphate solution [28]. The cells were resuspended in KRP at a concentration of 1×10^8 cells/ml.

Translocation of $p47^{phox}$, $p67^{phox}$, and rac to neutrophil membrane. The cytosolic components to the cell membrane were translocated as reported elsewhere [29]. Isolated PMNs were preincubated in a phosphate-buffered saline glucose solution containing 4 mM glucose, 1.2 mM $MgCl_2$, 2 mM NaN_3 (for inhibition of O_2 consumption), and 0, 50, 100, 200, 400 μM sulfur amino acid for 6 min at 37℃. Then, PMNs were stimulated by adding stimulus (12.5 nM fMLP, 1 nM PMA or 10 μM AA) for 3 min at 37℃. The cells were spun at 1,500 × g for 5 min at 4℃ and resuspended in buffer A [100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, and 10 mM Pipes (pH 7.3)] after standing on ice for 20 min.

To separate their postnuclear supernatants (PNS), cells were first disrupted by sonication and spun at 500 × g for 5 min at 4℃. PNS fractions were then separated into membrane and cytosol at 200,000 × g for 20 min at 4℃.

The pellet was resuspended in 50 μl of 109 mM

Tris-HCl (pH7.5) containing 3.5% SDS, 0.0087% bromophenol blue, and 17.4% glycerol, and sonicated for 1 h to obtain membrane fractions.

For immunoblot analysis, the membrane fraction was subject to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% gel. The electrophoresed proteins were transferred onto Immobilon-P membrane (Nippon Millipore, Tokyo, Japan) using a semidry blotting apparatus for 90 min at 20 V.

The transferred proteins were probed with a mixture of p47^{phox}, p67^{phox}, rac 1 primary monoclonal antibody (BD Biosciences, Franklin Lakes, NJ, USA) and horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody (E.Y. Laboratories San Mateo, CA, USA) detected by the ECL Western Blotting Detection System (GE Healthcare Bio-Science KK, Tokyo, Japan). EB-1 lysate, as the positive control, was the indicator for the location of p47^{phox}, p67^{phox} and rac.

Detection of tyrosyl and serine/threonine phosphorylation of neutorophils proteins. Neutrophils (1×10^6 cells/ml) were incubated in 1 ml of KRP containing 1 mM $CaCl_2$, 10 mM glucose, and 0–400 μM sulfur amino acid for 3 min at 37℃, after which they were stimulated by 12.5 nM fMLP, 10 μM AA, or 1 nM PMA, and incubated for 3 min at 37℃.

Ice-cold 45% trichloroacetic acid of 0.5 ml (final concentration 15%) containing 1 mM sodium vanadate and phenyl-methylsulfonylfluoride (2 mM) was added to stop the reaction. After incubation for 30 min at 4℃, the mixture was centrifuged at 10,000 × g for 20 min at 4℃. The precipitate was washed twice with diethylether-ethanol (1 : 1, v/v) and then dissolved in 50 μl of 62.5 mM Tris-HCl (pH6.8) containing 2% superoxide dismutase, 0.7M β-mercaptoethanol, and 10% glycerol.

For immunoblot analysis, the sample was subject to SDS-PAGE with a 12% gel. The electrophoresed proteins were transferred onto Immobilon-P membrane (Nippon Millipore) using a semidry blotting apparatus for 90 min at 20 V. Tyrosyl phosphorylated proteins were probed with phosphotyrosine-specific monoclonal antibody (PY-20; ICN Biochemicals) or phosphoserine/threonine-specific monoclonal antibody (BD Biosciences), respectively, then probed with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody (E.Y. Laboratories) and detected by the ECL Western Blotting Detection System (GE Healthcare Bio-Science KK) [30]. The molecular masses of the proteins were determined using prestained molecular weight standards (14,300–200, 000 molecular weight range; Gibco BRL).

Determination of lipid peroxidation of erythrocyte membrane ghosts by hydroxyl radicals. Venous blood from human healthy volunteers was collected in sodium citrate. Erythrocytes were pelleted by centrifugation and washed 3 times in saline. White ghosts were prepared by repeated washing and lysis at 4℃ in 5 mM phosphate buffer [31]. Erythrocyte membrane ghosts were then diluted with saline to obtain a final concentration of 1 mg protein/ml.

Hydrogen peroxide (3 mM) and $FeSO_4$ (5 mM) were added to erythrocyte membrane ghost suspensions (1 ml) with each of the sulfur amino acids (0–600 μM) in 5 separate experiments. The suspensions were incubated for 30 min at 37℃. Hydroxyl radical-induced lipid peroxidation of erythrocyte membrane ghosts was determined by measuring thiobarbituric acid-reactive substances [32].

Results

In the present study, we examined the effect of sulfur amino acids on tyrosyl or serine/threonine phosphorylation of proteins and the translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane in stimulus-induced human neutrophils.

Effects of sulfur amino acids on tyrosyl phosphorylation and cystosolic compounds in fMLP-induced human neutrophils. When neutrophils were incubated with fMLP, tyrosyl phosphorylation of 99.5, 77.6, and 70.7 -kDa proteins was induced. D,L-homocysteine and D,L-homocysteine-thiolactone increased tyrosyl phosphorylation in a dose-dependent manner (Fig. 1A–B). Conversely, L-cystathionine suppressed tyrosyl phosphorylation in a dose-dependent manner (Fig. 1C).

D,L-homocysteine or D,L-homocysteine-thiolactone increased the translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane in a concentration-dependent manner, as shown in Fig. 2A and B. On the other hand, L-cystathionine and NAc-L-cys decreased the translocation of cytosolic p47^{phox}, p67^{phox}, and rac in a concentration-dependent manner (Fig. 2C and D).

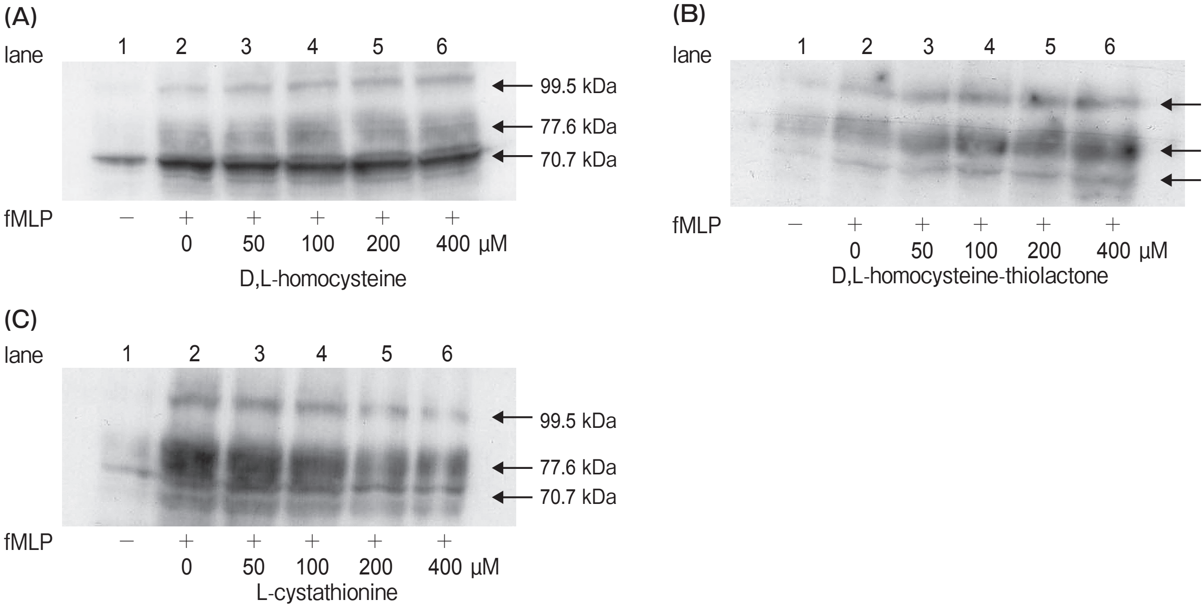

Fig. 1 Effects of D,L-homocysteine, D,L-homocysteine-thiolactone, and L-cystathionine on fMLP-induced tyrosyl phosphorylation of human neutrophil proteins. The tyrosyl-phosphorylated-proteins were detected by immunoblotting using phosphotyrosine-specific monoclonal antibodies. Lane 1, without compound; lane 2, 12.5 nM fMLP; lanes 3–6, 12.5 nM fMLP and 50, 100, 200, 400 μM compound. (**A**) D,L-homocysteine, (**B**) D,L-homocysteine-thiolactone, (**C**) L-cystathionine.

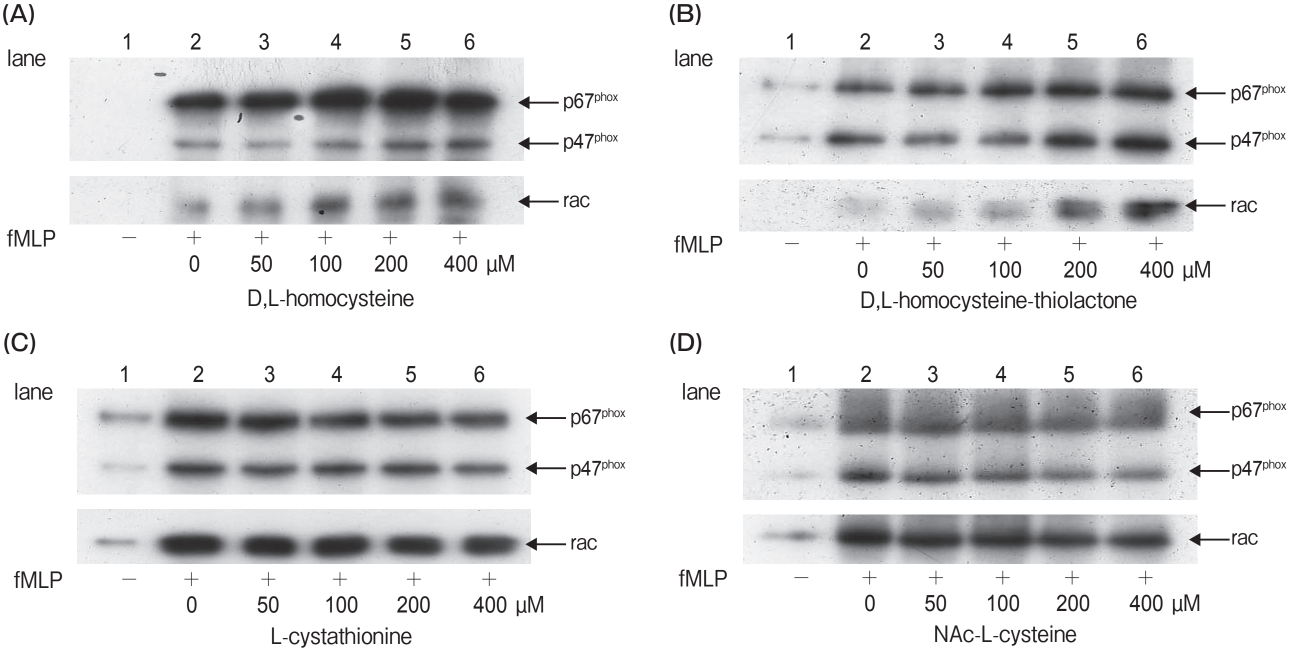

Fig. 2 Effects of D,L-homocysteine, D,L-homocysteine-thiolactone, L-cystathionine, and NAc-L-cysteine on translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac in fMLP-stimulated neutrophils. The translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac was detected by immunoblotting using $p47^{phox}$, $p67^{phox}$, and rac1-specific monoclonal antibodies as described in Materials and Methods. Lane 1, without compound; lane 2, 12.5 nM fMLP; lanes 3–6, 12.5 nM fMLP and 50, 100, 200, 400 μM compound. (**A**) D,L-homocysteine, (**B**) D,L-homocysteine-thiolactone, (**C**) L-cystathionine, and (**D**) NAc-L-cysteine.

Effect of sulfur amino acids on serine/threonine phosphorylation and cystolic compounds in PMA-induced human neutrophils. When neutrophils were incubated with PMA, serine/threonine phosphorylation of 63.5 and 37.3 kDa was increased. L-cysteine, NAc-L-cys and L-cystathionine suppressed serine/threonine phosphorylation in a concentration-dependent manner. (Fig. 3A–C).

However, serine/threonine phosphorylation did not decreased in the presence of D,L-homocysteine (data not shown).

The translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane decreased slightly in a dose-dependent manner in the presence of L-cysteine, NAc-L-cys and L-cystathionine, but D, L-homocysteine did not decreased (Fig. 4A–D).

Effect of sulfur amino acids on tyrosyl phosphorylation and cystolic compounds in AA-induced human neutrophils. When neutrophils were incubated with AA, tyrosyl phosphorylation of 31.1 and 29.3 kDa was induced. The tyrosyl phosphorylation increased in the presence of L-cysteine, NAc-L-cys and D,L-homocysteine (Fig. 5A–C).

Conversely, tyrosyl phosphorylation was suppressed in the presence of L-cystathionine in a concentration-dependent manner (Fig. 5D), but serine/threonine phosphorylation did not decrease in the presence of L-cystathionine (data not shown).

The translocation of p47^{phox}, p67^{phox}, and rac to the cell membrane increased dose-dependently in the presence of L-cysteine, NAc-L-cys or D,L-homocysteine (Fig. 6A–C).

L-cystathionine decreased the translocation of cytosolic compounds to the cell membrane, as shown in Fig. 6D.

Effect of sulfur amino acids on lipid peroxidation. The effects of sulfur amino acids on lipid peroxidation were also investigated. The effect of sulfur amino acids on hydroxy radical-induced lipid peroxidation of erythrocyte membrane ghosts are shown in Fig. 7.

L-cysteine, D,L-homocysteine, and L-cystathionine reduced weakly the lipid peroxidation level (thiobarbituric acid-reactive substances) of erythrocyte membrane ghosts at a concentration of 600 mM, but NAc-L-cys, CMC, and D,L-homocysteine-thiolactone did not reduce lipid peroxidation levels.

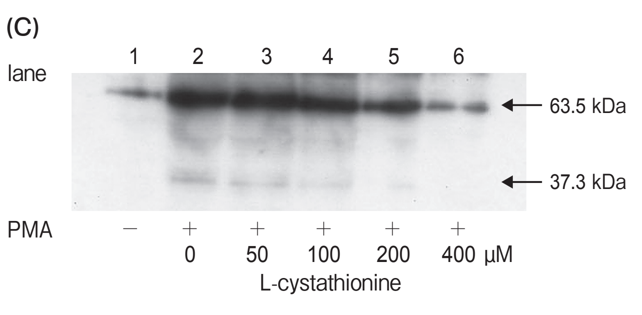

Fig. 3 Effects of L-cysteine, NAc-L-cysteine, and L-cystathionine on PMA-induced serine/threonine phosphorylation of human neutrophil proteins. The serine/threonine phosphorylation proteins were detected by immunoblotting using phosphoserine/threonine-specific monoclonal antibodies. Lane 1, without compound; lane 2, 1 nM PMA; lanes 3–6, 1 nM PMA and 50, 100, 200, 400 μM compound. (**A**) L-cysteine, (**B**) NAc-L-cysteine, and (**C**) L-cystathionine.

Discussion

A previous paper investigated the effects of sulfur amino acids, L-cysteine, NAc-L-cys, CMC, L-cystathionine, D,L-homocysteine, and D,L-homocysteine-thiolactone on superoxide generation in stimulus-induced human neutrophils and on the scavenging of free radicals [24].

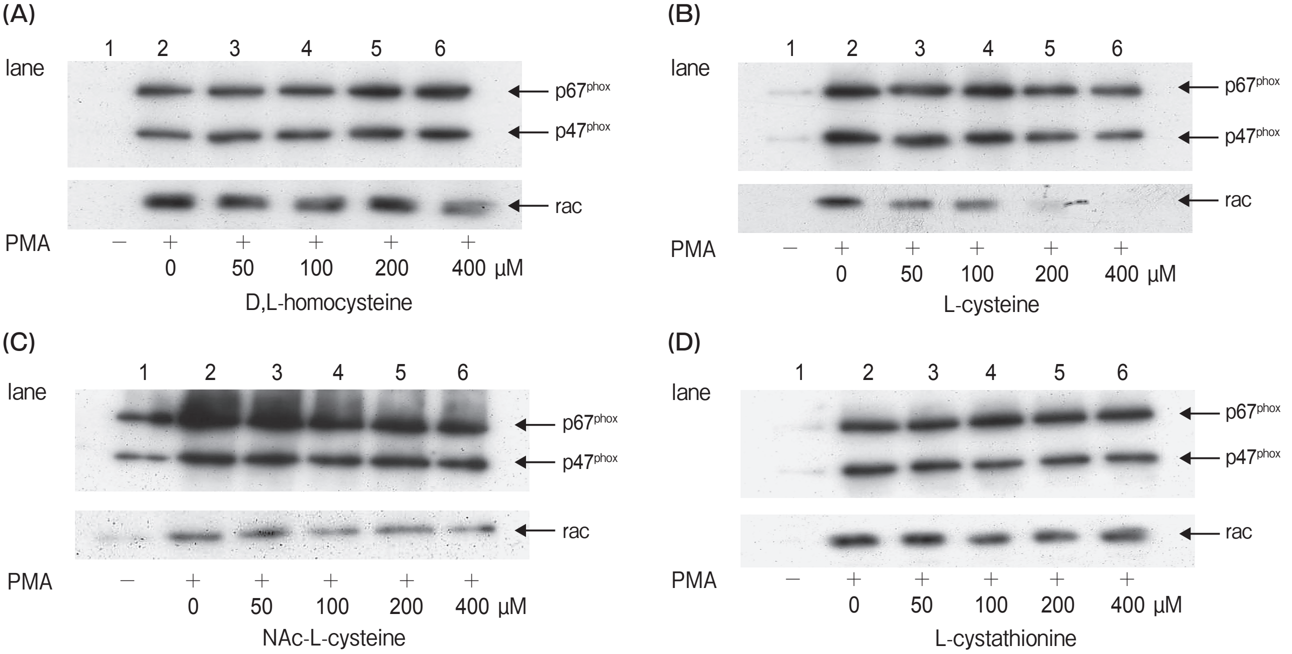

Fig. 4 Effects of D,L-homocysteine, L-cysteine, NAc-L-cysteine, and L-cystathionine on translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac in PMA-stimulated neutrophils. The translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac was detected by immunoblotting using $p47^{phox}$, $p67_{phox}$, and rac1-specific monoclonal antibodies, as described in Materials and Methods. Lane 1, without compound; lane 2, 1nM PMA; lanes 3-6, 1nM PMA and 50, 100, 200, 400 μM compound. (**A**) D,L-homocysteine, (**B**) L-cysteine, (**C**) NAc-L-cysteine, and (**D**) L-cystathionine.

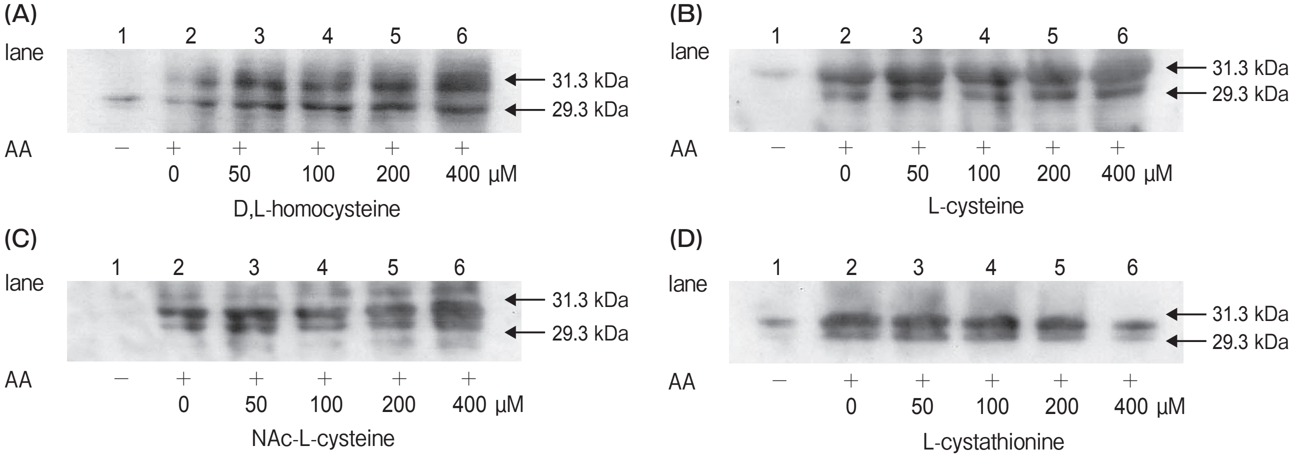

Fig. 5 Effects of D,L-homocysteine, L-cysteine, NAc-L-cysteine, and L-cystathionine on AA-induced tyrosyl phosphorylation of human neutrophil proteins. The tyrosyl-phosphorylated proteins were detected by immunoblotting using phosphotyrosine-specific monoclonal antibodies. Lane 1, without compound; lane 2, 10 μM AA; lanes 3-6, 10 μM AA and 50, 100, 200, 400 μM compound. (**A**) D,L-homocysteine, (**B**) L-cysteine, (**C**) NAc-L-cysteine, and (**D**) L-cystathionine.

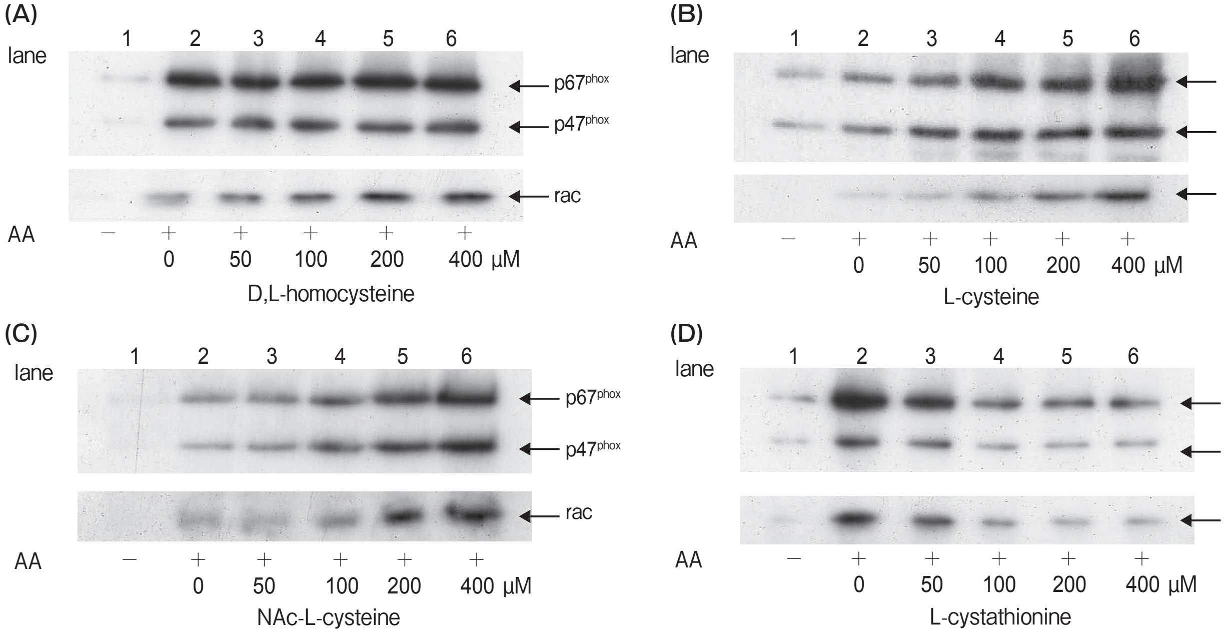

Fig. 6 Effects of D,L-homocysteine, L-cysteine, NAc-L-cysteine, and L-cystathionine on translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac in AA-stimulated neutrophils. The translocation to the cell membrane of $p47^{phox}$, $p67^{phox}$, and rac was detected by immunoblotting using $p47^{phox}$, $p67^{phox}$, and rac1-specific monoclonal antibodies as described in Materials and Methods. Lane 1, without compound; lane 2, 10μM AA; lanes 3–6, 10μM AA and 50, 100, 200, 400μM compound. (**A**) D,L-homocysteine, (**B**) L-cysteine, (**C**) NAc-cysteine, and (**D**) L-cystathionine.

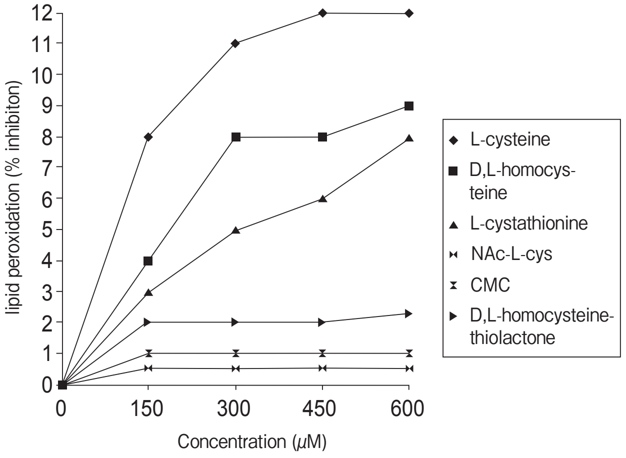

Fig. 7 Effects of the sulfur-amino acids on hydroxyl radical-derived lipid peroxidation of erythrocyte membrane ghosts were determined by measuring thiobarbituric acid-reactive substances as described in Materials and Methods. Results are expressed as means ± SD (n = 3) of the inhibition of lipid peroxidation.

In the present study, D,L-homocysteine and D,L-homocysteine-thiolactone enhanced fMLP- and AA-induced superoxide generation in a concentration dependent manner, but had no effect on PMA-induced superoxide generation. In the previous paper, on the other hand, L-cystathionine inhibited fMLP-, AA-, and PMA-induced superoxide generation. L-cysteine and NAc-L-cys enhanced AA-induced superoxide generation, but inhibited fMLP- and PMA-induced superoxide generation.

In our studies on superoxide generation and inflammation, we found that the various compounds affected tyrosyl or serine/threonine phosphorylation, and the phosphorylation of neutrophil proteins occurred in parallel with stimulus-induced superoxide generation in PMN [32, 34]. Therefore, we proposed that these compounds affect stimulus-induced superoxide generation by affecting the tyrosyl or serine/threonine phosphorylation of PMN proteins.

To gain insights into the mechanism underlying the suppression or enhancement of stimulus-induced superoxide generation by the sulfur amino acids, we here investigated the effects of these sulfur amino acids on tyrosyl or serine/threonine phosporylation of proteins, as well as the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane in human neutrophils using fMLP, AA, and PMA as the stimuli.

FMLP, AA, and PMA were used as, the inducer of receptor-mediated activation, a membrane perturber, and an activator of Ca^{2+}- and phospholipids-dependent protein kinase C, respectively.

L-cystathionine suppressed the tyrosyl phosphorylation of proteins induced by fMLP and AA, as well as the serine/threonine phosphorylation of proteins with PMA in a concentration-dependent manner, as shown in Fig. 1, 3, 5. D,L-homocysteine increased the tyrosyl phosphorylation of proteins induced by fMLP and AA in a concentration-dependent manner, but had no effect on PMA-induced serine/threonine phosphorylation. L-cysteine and NAc-L-cys dose-dependently suppressed the serine/threonine phosphorylation of proteins induced by PMA. L-cysteine and NAc-L-cys enhanced the tyrosyl phosphorylation of proteins induced by AA.

These results for sulfur amino acids on the tyrosyl or serine/threonine phosphorylation of proteins in stimulus-induced human neutrophils well coincided with the sulfur amino acid-mediated suppression or enhancement of superoxide generation in stimulus-treated human neutrophils, as reported in a previous paper [24].

It is generally accepted that upon activation of the respiratory burst oxidase in stimulated human neutrophils, cytosolic $p47^{phox}$, $p67^{phox}$, and rac move to the cell membrane and associate with cytochrome b_{558}, forming an electron-transport chain responsible for the reduction of molecular oxygen to superoxide. Therefore, we also investigated the effect of these sulfur amino acids on the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane in fMLP-, AA- , and PMA-stimulated human neutrophils.

When neutrophils were incubated with fMLP, AA, or PMA, the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane was increased.

L-cystathionine suppressed the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane in fMLP-, AA-, and PMA-stimulated human neutrophils; NAc-L-cys suppressed the translocation of $p47^{phox}$, $p67^{phox}$, and rac in fMLP-, and PMA-stimulated human neutrophils, but increased the translocation of cytosolic compounds to the cell membrane in AA-stimulated human neutrophils. D,L-homocysteine increased the translocation of cytosolic compounds to the cell membrane in fMLP-, and AA-stimulated neutrophils, but PMA-induced neutrophils did not affect the translocation of cytosolic compounds to the cell membrane. L-cysteine and NAc-L-cys enhanced the translocation of cytosolic compounds to the cell membrane in AA-stimulated human neutrophils.

These results well coincided with the effects of these sulfur amino acids on tyrosyl phophorylation induced by fMLP and AA or on serine/threonine phosphorylation induced by PMA.

The effects of the sulfur amino acids on tyrosyl or serine/threonine phosphorylation and translocation of cytosolic $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane also well coincided with the effects of these sulfur amino acids on stimulus-induced superoxide generation.

It has also been noted that NAc-L-cys reduced superoxide anion generation of proteins and phorbol myristate in a concentration-dependent manner [6].

The effect of these sulfur amino acids on serine/threonine phosphorylation of protein and the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane paralleled that of PMA-induced superoxide generation reported previously by us and Villagrase *et al.* in previous papers. The same parallel effect was found in two other compounds; CMC, and D,L-homocysteine-thiolactone (data not shown).

We also investigated the effects of sulfur amino acids on tyrosyl phosphorylation and translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane in AA-stimulated human neutrophils. These results coincided well with the effect of sulfur amino acids on stimulus-induced superoxide generation.

These data indicated that the process involves the migration of cytosolic compounds $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane and the tyrosyl or serine/threonine phosphorylations of some neutrophil proteins by affecting tyrosine kinase or protein kinase C.

Wada *et al.* [7, 8] reported that L-cystathionine significantly scavenged superoxide radicals derived from the xanthine-xanthine system and protected the gastric mucosa from acute injury-induced ischemia-reperfusion.

It was reported that NAc-L-cys reduced superoxide generation of the response to fMLP and PMA, and partially protected against lipid peroxidation in human neutrophils [6].

Thereafter, it was reported that homocysteine enhanced the oxidative stress of neutrophils. This underscores the potential role of phagocytic cells in vascular wall injury through O_2^- release in hyperhomocystinemic conditions [9].

Previous results [6–9] well coincided with the effects of sulfur amino acids on tyrosyl or serine/threonine phosphorylation and the translocation of cytosolic compounds to the cell membrane in stimulus-treated neutrophils.

It is well known that the sulfur amino acids, L-cystathionine and NAc-L-cys exhibit scavenging functions against superoxide radicals and hypochlorous. Therefore, in the present study, the effects of the sulfur amino acids on lipid peroxidation were also investigated. L-cysteine, D,L-homocysteine, and L-cystathionine reduced weakly the lipid peroxidation level (thiobarbituric acid - reactive substances) of erythrocyte membrane ghosts at the concentration of 600 mM, but NAc-L-cys, CMC, and D,L-homocysteine-thiolactone did not reduce lipid peroxidation levels.

These results suggest that sulfur amino acids suppress superoxide generation induced by stimulus-treated neutrophils rather than scavenging the generated superoxide anions.

Our present results demonstrate clearly that sulfur amino acids involve superoxide generation mainly via tyrosyl or serine/tyreonine phosphorylation, and the translocation of $p47^{phox}$, $p67^{phox}$, and rac to the cell membrane.

Further studies on the relationships between pharmaceutical function and their effects on stimulus-induced superoxide generation may be helpful in the development of clinical applications.

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