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Alteration of PMN Leukocyte Function by the Change of Sulfhydryl Group and Metabolism of Membrane Components*

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ABSTRACT

In opsonized zymosan activated PMN leukocytes, N-ethylamleiamide and Hg^{++}, penetrable sulfhydryl group inhibitors, inhibited superoxide generation, NADPH oxidase activity and lysosomal enzyme (lactic dehydrogenase and β-glucuronidase) secretion.

P-Chloromercuribenzoic acid and p-chloromercuribenzenesulfonic acid, surface sulfhydryl group inhibitors did not affect superoxide generation but effectively inhibited both NADPH oxidase activity and lysosomal enzyme secretion. During phagocytosis, contents of surface and soluble sulfhydryl groups were gradually decreased with increasing incubation times. N-ethylmaleiamide and Hg^{++} caused a loss of both surface and soluble sulfhydryl groups. P-Chloromercuribenzoic acid and p-chloromercuribenzenesulfonic acid significantly decreased the surface sulfhydryl content but did not after soluble sulfhydryl groups. Cysteine and mercaptopropionylglycine inhibited superoxide generation and lysosomal enzyme secretion. Glutathione had no effect on superoxide generation but remarkably inhibited lactic dehydrogenase release. Suppression of superoxide generation by N-ethylmaleiamide was reversed by cysteine and mercaptopropionyl-glycine but not by glutathione. Inactivation of NADPH oxidase by N-ethylmaleiamide was prevented by glutathione, cysteine or mercaptopropionylglycine. Stimulated superoxide generaion by carbachol was completely abolished by N-ethylmaleiamide and antagonized by atropine. Thus, the expression of PMN leukocyte response to external stimuli may be associated with the change of sulfhydryl groups content. It is suggested that lysosomal enzyme secretion is influenced by both surface and soluble sulfhydryl groups, whereas superoxide generation by intracellular soluble sulfhydryl groups.

Key Words: Sulfhydryl groups, Lysosomal enzyme secretion, Superoxide generation (Human PMN leukocyte)

INTRODUCTION

Some reports suggested that sulfhydryl groups in general and glutathione (GSH) in particulate may be involved in the biochemical events that lead to PMN leukocyte activation (Oliver *et al.*, 1976: Reed, 1980). It is reported that normal GSH levels may be necessary for the transduction of the activation signal from the exterior of the PMN leukocyte (Wedner *et al.*, 1981). The sulfhydryl groups of plasma membrane and an intracellular soluble thiols are probably prerequistite for PMN leukocyte responses to stimulation, such as phagocytosis (Tsan *et al.*, 1976), degranulation (Wedner *et al.*, 1981) and superoxide generation (Curnutte and Babior, 1975) and subsequent maintenance of function. However, The role of surface and soluble sulfhydryl groups in lysosomal enzyme release and superoxide generation is relatively uncertain. Implication of surface sulfhydryl groups in the expression of PMN leukocyte function, particular superoxide generation and changes of soluble sulfhydryl groups content in response to external stimuli is still not clarified.

The lysosomal enzyme release from PMN leukocyte may be modulated by adrenergic and cholinergic agents (Zurier *et al.*, 1974). It has been observed that agents which elevate the levels of cAMP within PMN leukocytes inhibit the release of enzymes during feeding of zymosan particles coated with immune complexes, whereas agents which elevate the levels of cGMP within PMN

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leukocytes enhance the release of enzymes (Weissmann *et al.,* 1975). The previous experiment showed that superoxide generation in activated PMN leukocytes was inhibited by atropine, which action may be associated with autonomic receptors (Lee *et al.,* 1988a).

The α-and β-adrenergic and cholinergic receptors probably possess, at or nearby the recognition site, an sulfhydryl group or groups which can be blocked by sulfhydryl grous alkylating reagents, such as p-chloromercuribenzoate (Aronstam *et al.,* 1978; Stadel and Lefkowitz, 1979; Quennedey *et al.,* 1984). It is suggested that N-ethylmaleiamide interferes with the interaction of cholinergic receptors with a guanine nucleotide binding component. In addition, $HgCl_2$, N-ethylmaleiamide and diamide are found to suppress the chemotactic oligopeptide binding to leukocyte membrne (Lane and Lamkin, 1982) thereby impairing chemotoxis.

Since the activated PMN leukocyte function appears to be partially associated with the change of sulfhydryl grous, in the present study changes of sulfhydryl group contents (of surface and soluble) in PMN leukocytes treated with investigated. Influences of sulfhydryl group inhibitors on superoxide generation, lysosomal enzyme release and sulfhydryl content in activated PMN leukocytes were also examined. In addition protective effects of non-protein sulfhydryl group containing compounds, MPG, cysteine and GSH on the action of sulfhydryl group inhibitors were also studied. The alteration of function due to inhibition of cholinergic agonist binding by sulfhydryl group inhibitors was investigated with respect to the effect of NEM on superoxide generation stimulated by carbachol.

MATERIALS AND METHODS

Chemicals

N-Ethylmaleiamide (NEM), p-chloromercuribenzoic acid (PCMB), p-chloromercuribenzenesulfonic acid (PCMBSA), glutathione (reduced form, GSH), cysteine, N-(2-mercaptopropionyl)-glycine (MPG), carbachol, atropine, NADPH, ferricytochrome c, nitroblue tetrazolium (NBT), NAD, phenolphthalein-glucuronic acid, carboxypyridinedisulfide (CPDS), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), zymosan (from Saccharomyces cerevisiae) and dextran (M.W. 465,000) were purchased from Sigma Chemical Co.. $HgCl_2$ was obtained from Hayashi Pure Chemical Industries, Ltd.; sodium lactate from Junsei Chemical Co., Ltd.; phenolphthalein from Kanto Chemical Co., Inc. Other chemicals were of analytical reagent grade.

Preparation of PMN leukocytes

PMN leukocytes were isolated from heparinized venous blood of healthy donors by dextran (average molecular weight 465,000) sedimentation of erythrocytes and treatment with 0.85% ammonium chloride as described previously (Trush et al., 1978). The purity of PMN leukocyte suspensions averaged 90% as judged by Wright-Giemsa stain method.

Preparation of NADPH oxidase containing granule rich fraction of PMN leukcytes

PMN leukocytes activated by opsonized zymosan at 37℃ for 15 min, or control PMN leukocytes were centrifuged at 1,500 g for 3 min and the pellets were resuspended in 0.25 M sucrose to a concentration of 10^8 cells/ml. The cell suspension was disrupted by sonication for three 15 sec intervals at 25 watts power with a Branson sonifier cell disruptor (Mod. W 185D). Unbroken cells and nuclei were sedimented by centrifugation at 800 g for 5 min. Sucrose was then added to the postnuclear supernatant with constant stirring and the final volume adjusted to the sucrose concentration of 40% (W/V). The suspension was centrifuged at 48,000 g for 1 h in a Beckman L5-50B ultracentrifuge. The supernatant was completely removed and the pellets were resuspended in 0.25 M sucrose. The suspensions were centrifuge at 48,000 g for 1 h and the pellets (granule rich fraction) were suspended in 25% ethylene glycol with a Teflon glass homogenizer (Hohn and Letrer, 1975; Gabig *et al.,* 1982). The protein concentration was determined by the method of Lowry *et al.,* (1951).

Assay of susperoxide radical generation

The superoxide dependent reduction of ferricytochrome c was measured by the method of Markert *et al.,* (1984). Reaction mixtures in plastic microfuge tubes contained 10^6 PMN leukocytes, 75 μM ferricytochrome c, HBSS buffer (or saline) and 2 mg/ml of opsonized zymosan in a volume of 500 μl. The reactions were performed in a 37℃ shaking water bath for the stated times. The reactions

were then stopped by placing the tubes in melting ice and the cells were rapidly pelleted by centrifuging at 1,500 g for 5 min at 4℃. The supernatants were taken and the amount of reduced cytochrome c was measured at 550 nm in a Gilford 260 U. V.-spectrophotometer. The amount of reduced cytochrome c was calculated by using an extinction coefficient of $1.85\times10^{4}\ M^{-1}\ cm^{-1}$ at 550 nm.

The reduction of NBT to purple formazan was also used to measure the generation of superoxide (Baehner, 1975). Reaction mixtures were the same as discribed above. Superoxide induced reduction of NBT was measured at 560 nm.

Assay of NADPH oxidase activity

The activity of NADPH oxidase was measured through reduction of ferricytochrome c by superoxide radicals produced from oxidation of NADPH by NADPH oxidase. The reaction mixture consisted of 0.1 mg/ml granule rich fraction, 100 μM NADPH, 75 μM ferricytochrome c and 50 mM Tris-HCl, pH 7.4 in a total volume of 500 μl. The reaction mixture was preincubated for 5 min at 37℃ and the reaction was initiated by adding NADPH. The reduction rate of ferricytochrome c was measured at 550 nm (Lee *et al.,* 1987).

Enzyme assays

1) Lactic dehydrogenase: Released amount of lactic dehydrogenase from activated PMN leukocytes was spectrophotometrically measured at 340 nm by reduction of NAD. Reaction mixtures contained 2×10^{6} PMN leukocytes, 2 mg opsonized zymosan, 1 mM NAD, 54 mM sodium lactate, pH 7.0, 50 mM sodium phosphate buffer, pH 8.8 and other compounds in a total volume of 500 μl. After 5 min of preincubation at 37℃, the reaction was initiated by addition of opsonized zymosan and NAD. Released lactic dehydrogenase is expressed as absorbance at 340 nm/2×10^{6} cells (Wacker *et al.,* 1956).

2) Beta glucuronidase: Released β-glucuronidase in reaction mixtures consisted of 2×10^{6} PMN leukocytes, 2 mg opsonized zymosan, 1 mM phenolphthalein-glucuronic acid, pH 7.0, 60 mM ctate buffer, pH 4.6 and other compounds in a total volume of 500 μl. After 18 h of incubation at 37℃, reaction was stopped by adding 2 ml of 0.2 M ice-cold glycine buffer, in 0.2 M NaCl, pH 10.4 and absorbance was read at 500 nm. β-Glucuronidase activity is expressed as μg phenolphthalein/18 h/2×10^{6} cells (Brittinger *et al.,* 1968).

Assay of sulfhydryl group content

1) Surface sulfhydryl groups: The thione formed in reaction between cell surface sulfhydryl groups and carboxypyridinedisulfide (CPDS) was spectrophotometrically measured. Reaction mixtures (1.0 ml) contained 10^{7} PMN leukocytes, 5 mg opsonized zymosan, 100 μM CPDS, HBSS buffer, pH 7.4 and other compounds. After the stated time of incubation, the mixtures were centrifuged at 800 g for 10 min, and the absorbance of the supernatant was read at 344 nm. Content of sulfhydryl groups was expressed as nmol/10^{7} cells using the molar extinction coefficient of 1×10^{4}/M/cm for the thione (Grassetti *et al.,* 1969; Mehrishi and Grassetti, 1969).

2) Soluble sulfhydryl groups: After CPDS treatment, the cell pellets obtained were washed with 1.0 ml HBSS and centrifuged at 800 g for 10 min. Pellets were resuspended in 1.0 ml of deionized water for 20 min at room temperature and followed by adding 0.5 ml of 0.2 M phosphate buffer, pH 6.8. Suspension was centrifuged at 800 g for 10 min and 1.0 ml of supernatant was mixed with 75 μl of 10 mM DTNB. The absorbance was read at 412 nm and the content of sulfhydryl groups was estimated from the molar extinction coefficient of p-nitrothiophenol anion, 1.36×10^{4}/M/cm (Ellman, 1959).

RESULTS

Effect of sulfhydryl group inhibitors on superoxide generation

Effects of NEM and Hg^{++} which are known to inhibit both intracellular and surface sulfhydryl groups (Vansteveninck *et al.,* 1965; Giordano and Lichtman, 1973) and those of PCMB and PCMBSA which inhibit only surface sulfhydryl groups (Tsan *et al.,* 1976) on superoxide generation in activated PMN leukocytes were investigated. The amount of superoxide generated in opsonized zymosan activated PMN leukocytes was 29.80 nmol/10^{6} cells/15 min. Table 1 shows that at a concentration of 10 μM, NEM and Hg^{++} remarkably inhibit superoxide generation, whereas both PCMB and PCMBSA at concentrations up to 100 μM do not affect it.

Since it is well established that superoxide

Table 1. Effects of sulfhydryl group inhibitors on superoxide generation

Compounds		Superoxide nmol/ 10^6 cells/15 min
None		29.80±0.31
NEM	100 μM	4.41±0.61
	10 μM	8.03±1.85
Hg^{++}	10 μM	1.43±0.29
	5 μM	11.23±0.15
PCMB	100 μM	28.31±0.39
	100 μM	28.64±0.29
PCMBSA	100 μM	25.98±0.47
	10 μM	27.49±0.48

Superoxide generation by resting PMN leukocytes was 3.46 nmol/10^6 cells/15 min. PMN leukocytes were preincubated with compound for 5 min at 37℃. Superoxide generated from activated PMN leukocytes was measured by the reduction of ferricytochrome c. The value represents mean±S.E. of 6 experiments.

Table 2. Effects of sulfhydryl group inhibitors on NADPH oxidase activity

Compounds		Superoxide nmol/ mg protein/10 min
None		31.05±0.81
NEM	10 μM	14.04±1.62
Hg^{++}	1 μM	18.09±1.08
PCMB	1 μM	16.13±1.32
PCMBSA	1 μM	13.80±0.84

Activity of NADPH oxidase from resting PMN leukocytes was 3.62 nmol/mg protein/10 min. Granule rich fraction which obtained from activated PMN leukocytes was preincubated with compound for 5 min at 37℃ and the reaction was initiated by addition of NADPH. Reduction of ferricytochrome c by interaction of NADPH and NADPH oxidase was measured at 550 nm. The value represents mean±S.E. of 3-5 experiments.

generation in PMN leukocytes in accomplished by activation of NADPH oxidase system located in the plasma membrane (Fantone and Ward, 1982), the influence of sulfhydryl group inhibitors on NADPH oxidase activity was examined. As can be seen in Table 2, NEM, Hg^{++}, PCMB and PCMBSA at the stated amount significantly inhibited NADPH oxidase activity. Thus, it is postulated that when inside active site of the plasma membrne is inhibited, NADPH oxidase dependent superoxide generation may be suppressed.

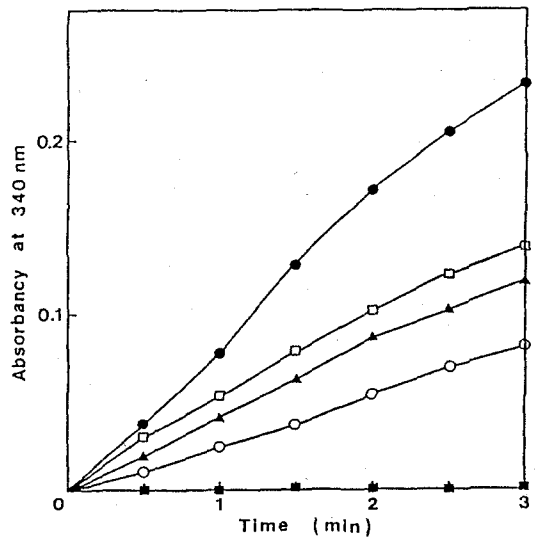

Fig. 1. Inhibition of lactic dehydrogenase release by sulfhydryl group inhibitors. Lactic dehydrogenase activity of resting PMN leukocytes was 0.153 of △ OD/4×10^6 cells/3 min. PMN leukocytes (4×10^6/ml) were preincubated for 5 min at 37℃ with sulfhydryl group inhibitor. Lactic dehydrogenase release was initiated by addition of opsonized zymosan. Activity was expressed as △ OD/4×10^6 cells. The points represent an average absorbancy of 5 experiments. ●, None; ○, 500 μM NEM; ■, 50 μM Hg^{++}; ▲, 50 μM PCMBSA; □, 50 μM PCMB.

Inhibition of lysosomal enzyme release from activated PMN leukocytes by sulfhydryl group *inhibitors*

After PMN leukocytes were preincubated for 10 min with sulfhydryl group inhibitors, lysosomal enzyme release was initiated by opsonized zymosan. As shown in Fig. 1 and Table 3, NEM, Hg^{++}, PCMB and PCMBSA effectively inhibited lactic dehydrogenase release, and in the presence of Hg^{++}, this enzyme release was not produced. On the other hand, sulfhydryl group inhibitors slightly inhibited β-glucuronidase. Accordingly, the inhibitory action of surface active sulfhydryl group inhibitors on enzyme release suggests that an enzyme secretion is mediated by a certain metabolic processes other than superoxide generation.

Table 3. Inhibition of β-glucuronidase release by NEM and Hg^{++}

Compounds		μg phenolphthalein/ 4×10^6 cells/18 h
None		27.30±0.36
NEM	100 μM	22.92±0.39
Hg^{++}	10 μM	23.61±0.88

β-Glucuronidase activity of resting PMN leukocytes was 0.68±0.01 μg phenolphthalein/4×10^6 cells/18 h. β-Glucuronidase release was initiated by addition of opsonized zymosan in the presence of NEM or Hg^{++}. The value represents mean±S.E. of 5 experiments.

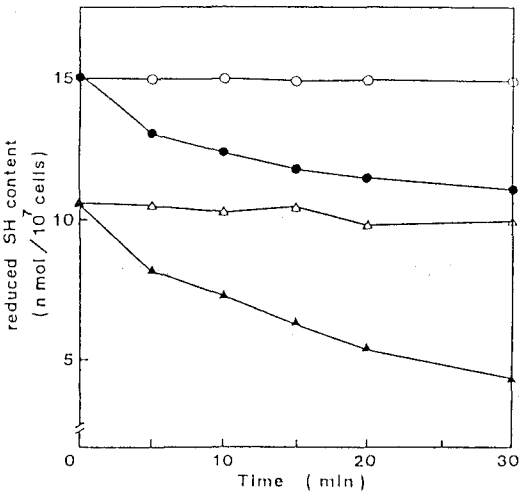

Fig. 2. Change of sulfhydryl group content in activated PMN leukocytes. 10^7/ml of PMN leukocytes were incubated with 5 mg/ml of opsonized zymosan at 37℃ for the stated time. Contents of surface and soluble sulfhydryl groups were measured as described in Materials and Methods. The value represents mean of 6 experiments, in nmol/10^7 cells. Surface slfhydryl groups of resting △, cells and activated cells ▲, : soluble sulfhydryl groups of resting cells and ○, activated cells ●.

Changes of sulfhydryl group contents as influenced by sulfhydryl group inhibitors and duration of incubation

Sulfhydryl groups of reduced form appear to be required for the expression of PMN leukocyte function and a during phagocytosis of opsonized zymosan was observed. Thus, the present study confirmed the changes of surface and soluble sulfhydryl groups in opsonized zymosan activated PMN leukocytes with increasing incubation time. The amounts of surface and soluble sulfhydryl groups in resting PMN leukocytes were 10.55 and 15.01 nmol/10^7 cells. After PMN leukocytes were treated with opsonized zymosan, the amounts of surface and soluble sulfhydryl groups were gradually decreased with increasing incubation time (Fig. 2).

Since the change of sulfhydryl groups in activated PMN leukocytes and the inhibitory effect of sulfhydryl group inhibitors on superoxide generation were demonstrated, effects of sulfhydryl group inhibitors on the sulfhydryl group content were examined. Table 4 shows that NEM, Hg^{++}, PCMB and PCMBSA caused a loss of surface sulfhydryl groups. However, soluble sulfhydryl groups were decreased by NEM and Hg^{++} but not by PCMB and PCMBSA.

Table 4. Effects of sulfhydryl group inhibitors on sulfhydryl groups (SHG)

Compounds		Surface SHG	Soluble SHG
		nmol/10^7 cells/10 min	
None		7.48±0.09	12.36±0.14
NEM	10 μM	6.21±0.08	9.06±0.16
Hg^{++}	5 μM	6.03±0.14	8.48±0.13
PCMB	10 μM	5.27±0.19	13.34±1.62
PCMBSA	10 μM	3.64±0.43	11.91±0.41

10^7/ml of PMN leukocytes were preincubated with sulfhydryl group inhibitor for 5 min and then reaction was initiated by addition of opsonized zymosan (5 mg/ml). The value represents mean±S.E. of 6 experiments.

Inhibition of superoxide generation and lysosomal enzyme release by sulfhydryl group containing compounds

Sulfhydryl group containing compounds which are known to act as an effective source of oxidizable sulfhydryl groups as well as to scavenge the oxygen free radicals (Rajkovic and Williams, 1984) were investigated with regard to their effects on PMN leukocyte function. Cysteine and MPG at a concentration of 100 μM significantly inhibited

Table 5. Effects of sulfhydryl group containing compounds on superoxide generation

Compounds		△A of NBT reduction (in 10^6 cells/15 min)
None		0.095±0.006
GSH	100 μM	0.088±0.004
Cysteine	100 μM	0.066±0.002
MPG	100 μM	0.064±0.004

Reduction of NBT by superoxide generated was spectrophotometrically measured at 560 nm. The value represents the mean absorbancy of 6 experiments.

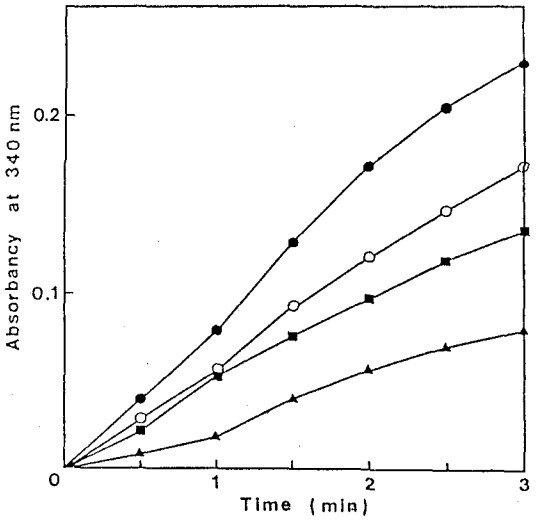

Fig. 3. Inhibition of lactic dehydrogenase release by sulfhydryl group containing compounds. PMN leukocytes were preincubated with sulfhydryl group containing compound of 1 mM for 5 min. Activity was expressed as △ OD/4×10^6 cells. The points represent an average absorbancy of 3 experiments. ●, None; ○, Cysteine; ■, MPG; ▲, Glutathione.

superoxide generation (Table 5) and lactic dehydrogenase release from activated PMN leukocytes (Fig. 3), and they also slightly inhibited β-glucuronidase release (Table 6). On the other hand, glutathione did not show any significant effect of superoxide generation, but it inhibited lactic dehydrogenase release remakably.

Thus, the results suggest that oxidation process of sulfhydryl groups may be necessarily for the expression of PMN leukocyte function.

Table 7 shows that the inhibitory action of

Table 6. Inhibition of β-glucuronidase release by sulfhydryl group containing compounds

Compounds		μg phenolphthalin/4×10^6 cells/18 h
None		27.29±0.42
GSH	1 mM	22.52±0.35
Cysteine	1 mM	22.05±0.31
MPG	1 mM	21.83±0.32

β-Glucuronidase release was initiated by addition of opsonized zymosan in the presence of sulfhydryl group containing compounds. The value represents mean±S.E. of 5 experiments.

Table 7. Effects of sulfhydryl group containing compounds on the inhibited superoxide generateion by NEM

Compounds		Without NEM	With NEM
		Superoxide nmol/10^6 cells/15 min	
None		30.90±1.09	4.64±0.98
GSH	10 μM	30.86±0.02	4.86±0.26
Cysteine	10 μM	27.66±0.18	8.29±1.05
MPG	10 μM	28.16±0.62	10.30±0.73

Concentration of NEM is 100 μM. PMN leukocytes were preincubated with sulfhydryl group containing compounds for 5 min in the presence of NEM. Superoxide genearted was measured by the reduction of ferricytochrome c. The value represents mean±S.E. of 6 experiments.

NEM on superoxide generation was interfered with cysteine or MPG but not by GSH. The inactivation of NADPH oxidase by NEM was prevented by GSH, cysteine and MPG (Table 8).

Inhibition of carbachol stimulated superoxide generation by atropine and NEM

Since sulfhydryl group inhibitors are known to inhibit cholinergic agonist binding to the plasma membrane, effect of NEM on enhanced superoxide generation by membrane, effect of NEM on enhanced superoxide generation by carbachol was studied. As shown in Table 9, the stimulatory effect of carbachol was completely abolished by NEM and antagonized by atropine.

Surface sulfhydryl group contents in activated PMN leukocytes were slightly reduced by carbachol but slightly increased by atropine (Table 10).

Table 8. Effects of sulfhydryl group containing compounds on the inactivated NADPH oxidase by NEM

Compounds	Without NEM	With NEM
	Superoxide nmol/mg protein/10 min	
None	31.05±0.81	14.04±1.62
GSH	31.59±0.54	22.41±1.35
Cysteine	31.32±0.27	17.39±1.89
MPG	33.21±1.35	18.09±1.08

Concentraction of NEM and sulfhydryl group containing compounds is 10 μM. Granule rich fraction was preincubated with sulfhydryl group containing compounds for 5 min in the presence of NEM. The value represents mean±S.E. of 3 experiments.

Table 9. Effects of atropine and NEM on carbachol stimulated superoxide generation

Compounds		Superoxide nmol/ 10^6 cells/15 min
None		30.82±0.53
Carbachol	100 μM	34.96±0.38
+Atropine	100 μM	25.12±1.76
+NEM	10 μM	8.29±0.42
Atropine	100 μM	24.56±0.27
NEM	10 μM	8.32±0.75

PMN leukocytes were preincubated with compounds for 5 min. Superoxide generated was measured by the reduction of ferricytochrome c. The value represents mean±S.E. of 6 experiments.

DISCUSSION

Sulfhydryl groups take part in forming intermediates in many enzyme recactions, play a part in intermediate stages of electron transport processes and participate in binding substrates and cofactors to enzymes (Torchinskii, 1974a). thus, conformational changes of enzyme structure by sulfhydryl group inhibitors may produce either alteration or loss of enzyme ativity.

PMN leukocytes stimulated by chemotactic or phagocytic stimuli undergo a complex series of biochemical events that begin within a minute after exposure to stimuli (Babior, 1978; Simchowitz *et al.*, 1980; Lew *et al.*, 1985). The change of sulfhydryl groups in PMN leukocytes treated with opsonized material may also be implicated in the events that lead to PMN leukocyte activation (Wedner *et al.*, 1981). Fig. 2 shows that after exposure to opsonized zymosan, the amounts of surface and soluble sulfhydryl groups in activated PMN leukocytes are gradually decreased with increasing incubation time, and at 15 min incubation time, surface and soluble sulfhydryl group contents are decreased to 37.3% and 21.4%, respectively. These changes of soluble sulfhydryl groups in experiments are somewhat less than those in previous reports.

Table 10. Effects of carbachol and atropine on sulfhydryl groups (SHG)

Compounds	Surface SHG	Soluble SHG
	nmol/10^7 cells/10 min	
None	7.56±0.16	12.29±0.06
Carbachol	6.67±0.32	11.79±0.25
Atropine	8.79±0.35	13.22±0.13

PMN leukocytes were preincubated with carbachol or atropine of 100 μM. The value represents mean±S.E. of 6 experiments.

The normal level of reduced form of sulfhydryl groups may be necessarily for the proper function of PMN leukocyte. Several experiments reported that in PMN leukocytes, oxidation of sulfhydryl groups occurs during phagocytosis (Mendelson *et al.*, 1977; Voetman *et al.*, 1980). Thus, substances which can cause loss of sulfhydryl groups probably affect PMN leukocyte responses to stimuli. As can be seen in Fig. 1 and Table 1, 2 and 3, NEM and Hg^{++}, which are cell penetrable sulfhydryl group inhibitors, caused a loss of both surface and soluble sulfhydryl groups, significantly inhibited superoxide generation, NADPH oxidase activity and lactic dehydrogenase release and slightly inhibited β-glucuronidase release in opsonized zymosan activated PMN leukocytes.

On the other hand, PCMB and PCMBSA which do not penetrate the plasma membrane caused only a loss of surface sulfhydryl groups, but did not affect superoxide generation. They markedly inhibited NADPH oxidase activity and lactic dehydrogenase release. GSH had no effect on the inhibitory action of NEM on superoxide generation in activated PMN leukocytes but effectively reversed inactivation of NADPH oxidase caused by NEM. Accordingly, the results suggest that when active site at the inside of the plasma

membrane which may be connected with NADPH oxidase is stimulated, superoxide generation may be attained, and activation of superoxide generation system appears to be controlled by soluble sulfhydryl groups. The lysosomal enzyme secretion is probably mediated by both extracellular and intracellular metabolic events including the change of sulfhydryl groups. In addition, the findings that phorbol myristate acetate induced superoxide generation in PMN leukocytes is not accompanied by the enhanced intracellular calcium concentration (Gabig *et al.,* 1982; Lew *et al.,* 1984) and lysosomal enzyme secretion is closely correlated with Ca^{++} dependent processed (Takenawa *et al.,* 1985) also suggest that releases of above two metabolic products are probably mediated by different events.

Sulfhydryl groups are sensitive to oxidative attack and if oxidized, PMN leukocyte function is reduced. When PMN leukocytes are exposed to a phagocytic stimulus or activated immune complements, a burst of oxidative metabolism occurs and reactive oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical are produced (Weissmann *et al.,* 1980; Fantone and Ward, 1982). Oxygen free radicals are implicated in the tissue damage in various pathological conditions (McCord, 1974; Demoupoulos *et al.,* 1980). Consequently, PMN leukocyte and the surrounding environment can be under heavy oxidative stress, potentially resulting in depletion of sulfhydryl groups of structurally and functionally important proteins (Jeon *et al.,* 1986; Lee *et al.,* 1988b). Sulfhydryl containing compounds may protect the oxidative damage of PMN leukocytes by a scavenging effect on oxygen free radicals and also compensate for lost sulfhydryl groups caused by either metabolic activation or reactive oxygen species (Rajkovic and Williams, 1984; Lee *et al.,* 1988b). On the basis of aforementioned action of sulfhydryl group containing compounds, effects of GSH, cysteine and MPG on superoxide generation and lysosomal enzyme release were studied. Cell penetrable compounds, cysteine and MPG effectively inhibited the respons of activated PMN leukocyte (Fig. 3, Table 5 and 6). On the other hand, GSH, a thiol that cannot cross the plasma membrane (Bannai and Tsukeda, 1979) did not affect on superoxide generation but significantly inhibited lactic dehydrogenase release. These results indicate that superoxide generation may be accomplished by intracellular metabolic events which include Ca^{++} immobilization, activation of protein kinase C and probably oxidoreduction of soluble sulfhydryl groups. Inhibitory effects of cysteine and MPG on superoxide generation are probably not attributable to their direct action on superoxide radical, because GSH which is known to play a protective role in oxidative injury to cellualr macromolecules have no effect on superoxide generation. In PMN leukocytes, superoxide is liberated from the cell surface and endogenously produced superoxide radicals are dismutated to hydrogen peroxide, thereby initiating the myeloperoxidase catalyzed reaction (Fantone and Ward, 1982). At any rate, exogenously supplemented sulfhydryl groups reduced the response of PMN leukocytes to external stimuli rather than enhancing it. In addition, cysteine and MPG except GSH effectively reversed inhibitory action of NEM on superoxide generation (Table 7 and 8). Thus, it is also suggested that the oxidation process may be required for the expression of PMN leukocyte function.

The cholinergic agonist, carbachol enhances release of lysosomal enzyme from PMN leukocytes and this action of carbachol can be blocked by atropine (Zurier *et al.,* 1974). The similar finding is also observed in superoxide generation, as shown in Table 9 (Lee *et al.,* 1988a). A rise in the cytosolic calcium concentration is considered to be an important factor in the stimulation of PMN leukocyte response (Goldstein *et al.,* 1975; Lee *et al.,* 1987). It has been postulated that interaction of cholinergic agonists with muscarinic receptors lead to the activation of a phosphatidyl inositol specific phospholipase C at the plama membrane (Doughney *et al.,* 1987). The resulting breakdown in phosphatidyl inostitol is postulated to cause an opening of calcium gates in the plasma membrane and the release of calcium from intracellular storage sites (Michell, 1975). Thus, PMN leukocyte response to external stimuli appears to be regulated by cholinergic system. And cholinergically regulated PMN leukocyte response may also probably involve changes of sulfhydryl groups. On the other hand, cholinergic receptor binding in the neuronal plama membrane appears to be regulated by sulfhydryl groups (Aronstam *et al.,* 1978). PCMB reacts with a group (s) within or under the allosteric control of the receptor binding site to inhibit both agonist and antagonist binding. This interaction is also manifested in the abolition of carbachol stimulated superoxide generation by NEM.

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= 국문초록 =

Sulfhydryl기와 세포막 구성성분의 대사 변화에 따른 다형핵 백혈구 기능의 변경

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면역 보체가 결합되어 있는 zymosan에 의하여 활성화된 다형핵 백혈구에서 세포 투과성 물질인 N-ethylmaleiamide과 Hg^{++}은 superoxide 라디칼 생성, NADPH oxidase 활성도 및 lysosomal enzyme (lactic dehydrogenase, β-glucuronidase)의 유리를 억제하였다. 세포막 단백에 특이적인 p-chloromercuribenzoic acid와 p-chloromercuribenzenesulfonic acid는 superoxide 라디칼 생성에 영향을 주지 않았으나 NADPH oxidase 활성도와 lysosomal enzyme의 유리를 억제하였다. 식작용 중에 세포막과 세포내의 sulfhydryl기는 반응시간에 따라 점진적으로 감소하였다. N-ethylmaleiamide와 Hg^{++}은 세포막과 세포내의 sulfhydryl기를 모두 감소시켰다. P-Chloromercuribenzoic acid와 p-chloromercuribenzenesulfonic acid는 세포막의 sulfhydryl기를 유의하게 감소시켰으나 세포내 용해성 sulfhydryl기에는 영향을 주지않았다. Cysteine과 mercaptopropionylglycine는 superoxide 라디칼의 생성과 lysosomal enzyme의 유리를 억제하였다. Gluthathione은 superoxide생성에 영향을 주지 않았으나 뚜렷하게 lactic dehydrogenase의 유리를 억제하였다. N-ethylmaleiamide에 의한 superoxide 생성의 억제는 cysteine과 mercaptopropionyl-glycine에 의하여 반전되었으나 gluthathione의 영향은 없었다. N-ethylamleiamide에 의한 NADPH oxidase의 비활성화는 gluthathione, cysteine과 mercaptopropionylglycine에 의하여 저해되었다. Carbachol에 의하여 항진된 superoxide 라디칼 생성은 N-ethylamleiamide에 의하여 완전히 억제되었고, atropine에 의하여 길항되었다.

그러므로, 외부 자극에 대한 다형핵 백혈구 반응의 표현은 sulfhydryl기의 양의 변화와 연관이 있을 것으로 시사되었다. Lysosomal enzyme 유리는 세포막과 세포내의 sulfhydryl기에 의하여, 이에 반하여 superoxide 생성은 세포내 sulfhydryl기에 의해서 영향받을 것으로 추정되었다.