Effect of Some Xenobiotics on Oxidative Metabolism of Human Blood Neutrophils

V. M. Mushtakova, V. A. Fomina, and V. V. Rogovin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 3, pp. 315-317, March, 2007
Original article submitted July 14, 2006

The effect of SO_3^{2-}, S^{2-}, NO_3^-, and NH_4^+ on activity of the peroxidase-hydrogen peroxide system in human peripheral blood neutrophils was studied by the cytochemical method. We showed that the effect of these xenobiotics on neutrophils is similar to that on plants.

Key Words: *immunity; neutrophil; xenobiotics*

Air pollution with sulfur dioxide, nitrogen dioxide, hydrogen sulfide, and ammonia is an urgent problem of industrial cities, because these xenobiotics directly affect the major physiological processes in plant, animal, and human cells. Oxidative activity of cells is of particular importance for evaluation of the damaging effect of xenobiotics during air pollution, since oxygen-dependent nonspecific immunity reflects survival capacity of the organism. The molecular basis of nonspecific immunity is the myeloperoxidase system in neutrophilic granulocytes, which includes enzyme myeloperoxidase, hydrogen peroxide, and cofactors chloride, thiocyanate, bromide, and iodide. Cofactor chloride plays a key role in antimicrobial activity of the myeloperoxidase system. Oxidation of chloride with hydrogen peroxide results in the formation of hypochlorite, a potent nonradical oxidizing agent. In human neutrophils about 70% hydrogen peroxide is spent for HOCl formation [6]. A general mechanism for the bactericidal effect of hypochlorite is damage to the ATP synthase complex (*i.e.*, alteration of energy transformation system in the cell) [1].

A convenient method was developed for evaluation of activity of the peroxidase—hydrogen peroxide system in animal and human neutrophils using peripheral blood smears. This method allows us to evaluate total oxidative activity of neutrophils. In the present work this method was used to study the *in vitro* effect of SO_3^{2-}, S^{2-}, NO_3^-, and NH_4^+ on activity of the peroxidase—hydrogen peroxide system in human peripheral blood neutrophils.

MATERIALS AND METHODS

Peripheral blood smears from 10 healthy donors were dried (2 h) and fixed with alcohol and formalin (9:1) for 1 h. Blood smears of treatment group 1 were washed with water to remove fixative, dried, and placed for 1 h in solutions of Na_2SO_3, $NaNO_3$, $Na_2S\times 10\ H_2O$, and NH_4Cl. The concentration of solutions decreased from 10^{-1} to 10^{-6} M. Control blood smears were maintained in water. After treatment with salts, blood smears were washed with water and incubated in a medium containing 3-3'-diaminobenzidine tetrahydrochloride (DAB) in 0.2 M Tris buffer (pH 7.6, 8 mg DAB per 10 ml buffer) at 37°C for 1 hour and 15 minutes. Blood smears of treatment group 2 were fixed and incubated with salts at the specified concentrations. Smears were then washed with water, dried, and stained with 1% methyl green. Washing of blood smears and preparation of the buffer and salt solutions were performed using bidistilled water.

RESULTS

Published data show that the incidence of diseases of the upper airways and lungs in humans and ani-

N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow. ***Address for correspondence:*** karl2255@orc.ru. V. M. Mushtakova

0007-4888/07/1433-0344

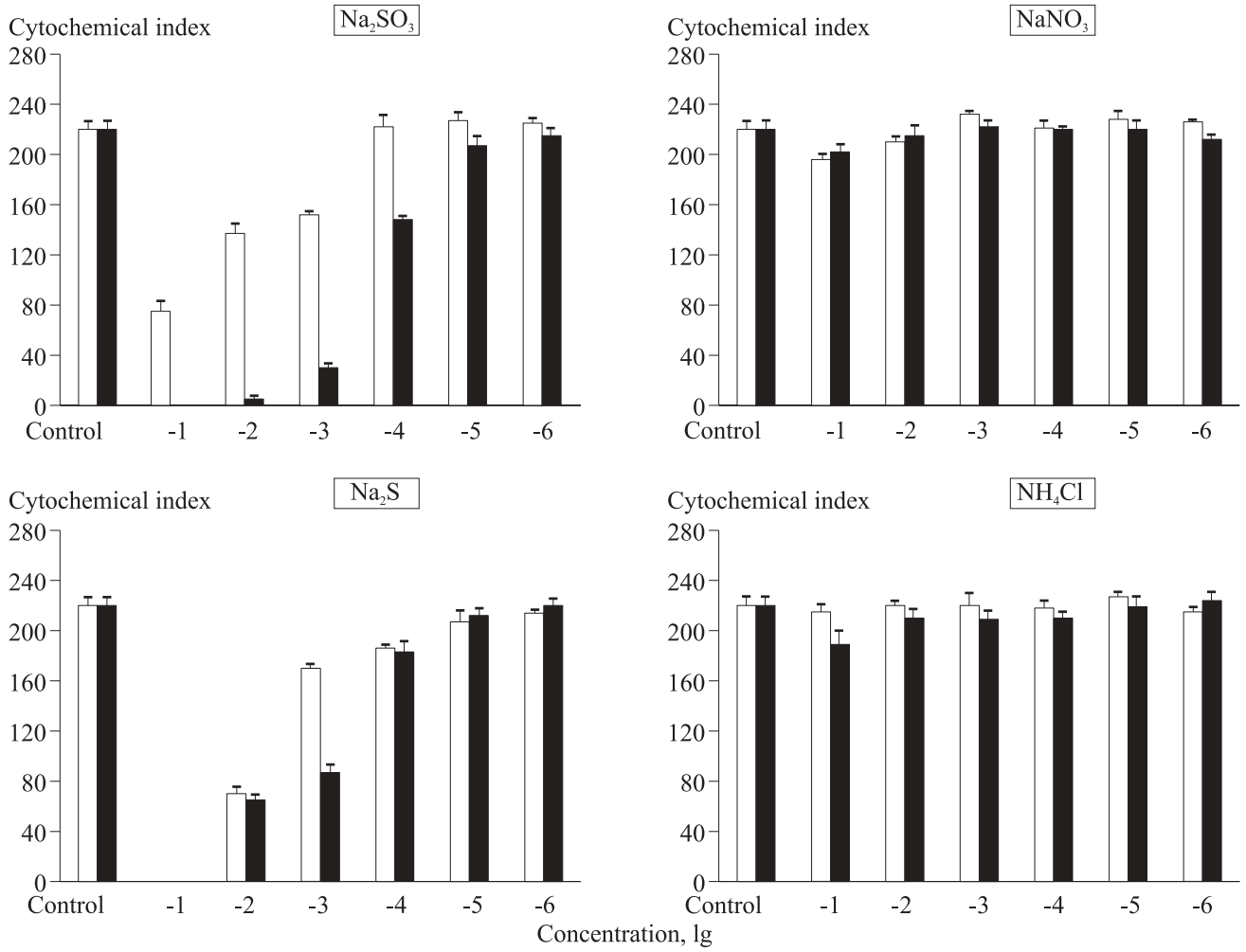

Fig. 1. Effect of xenobiotics on activity of the peroxidase—hydrogen peroxide system in neutrophilic leukocytes from human peripheral blood. Light bars: preincubation in xenobiotic solutions; dark bars: addition of xenobiotics to the incubation medium. Each bar of the diagram represents the mean of 10 measurements.

mals depends on air pollution with gas wastes, which are formed during combustion of black oil, diesel oil, coal, and benzene [5]. Immune disorders are associated with oxidative dysfunction of neutrophilic leukocytes. Study by the method of luminol-dependent chemiluminescence showed that aqueous suspension of carbon (400 mg) and sulfite particles (5×10^{-4} M), components of smog, decreased reactive oxygen generation by human polymorphonuclear leukocytes [5].

We took into account that dissolution of SO_2, NO_2, H_2S, and NH_3 in water drops at neutral pH results in their transformation into SO_3^{2-}, NO_3^-, S^{2-}, and NH_4^+. Figure 1 illustrates the effect of the corresponding salts on activity of the peroxidase—hydrogen peroxide system. Ions into S^{2-} and SO_3^{2-} were most active both after maintenance of blood smears in salt solutions and during addition of salts to the incubation medium. The effect of sulfite on oxidative activity of neutrophils was most significant during addition of Na_2SO_3 to the incubation medium. Sulfite in a concentration of 10^{-4} M decreased activity of the peroxidase—hydrogen peroxide system by 30%. Our results are consistent with published data that sulfite in a concentration of 5×10^{-4} M decreases chemiluminescence of zymosan-activated neutrophils by 30% [5].

We previously studied the effect of SO_3^{2-}, NO_3^-, S^{2-}, and NH_4^+ on peroxidase-dependent plant immunity by the method of luminol-dependent chemiluminescence [9]. These data were compared with the results of our present work. Xenobiotics had a similar effect on oxidative activity of plant cells and human neutrophils. Sulfite in a concentration of 5×10^{-4} M decreases chemiluminescence of damaged pea roots [9], which is consistent with published data on human neutrophils [5] and results of our study.

In our experiment, chemiluminescence of pea roots was induced by damage [9]. The cascade of protective processes induced by damage or other elicitors in plant cells (formation of signal polypeptides, activation of lipoxygenase and NADPH oxidase complex with subsequent transcriptional expression of protease inhibitors) is similar to that in animal cells [10,11]. The superoxide radical in plant cells is generated by the enzyme complex similar to the NADPH oxidase complex in human neutrophils [2,7,13]. According to the results of immunoassay, plant cells contain components of the NADPH oxidase complex [4,12] and other proteins participating in the function of the oxidase complex in human neutrophil membranes [3]. Superoxide generation was modeled by mixing of the cytosolic fraction from human neutrophils with the plant cell membrane [4]. Diphenyliodonium, an inhibitor of flavocytochrome B_{558} playing a key role in the oxidase complex of neutrophils, inhibits hydroxymetabolite formation in plant cell membranes by preventing electron transfer from NADPH to O_2 [8]. These data illustrate similarity of oxidative metabolism in plant and animal cells, which partially explains similar effect of some xenobiotics on these cells.

REFERENCES

1. P. G. But, R. A. Murav'ev, V. A. Fomina, and V. V. Rogovin, *Izv. Ros. Akad. Nauk. Ser. Biol*., No. 3, 266-270 (2002).
2. K. Apel and H. Swift, *Ann. Rev. Plant Biol*., **55**, No. 2, 373-399 (2004).
3. I. Couchy, Z. Minic, J. Laporte, *et al*., *Exp. Bot*., **49**, No. 327, 1647-1659 (1998).
4. R. Desikan, J. T. Hancock, M. J. Coffey, and S. J. Neill, *FEBS Lett*., **382**, No. 1, 213-317 (1996).
5. S. Hippeli and E. F. Elstner, *Oxidative Stress: Oxidants and Antioxidants*, New York *et al*. (1991), pp. 3-55.
6. A. J. Kettle, C. A. Cedyl, and C. C. Winterbrourn, *Biochem. Pharmacol*., **45**, No. 19, 2003-2011 (1993).
7. B. M. Lamb and R. A. Dixon, *Ann. Rev. Plant Physiol. Plant Molec. Biol*., **48**, No. 1, 251-275 (1997).
8. A. Mithofer, A. Daxberger, D. Fromhold-Tren, and J. Ebel, *Phytochem*., **45**, No. 6, 1101-1107 (1997).
9. V. V. Rogovine, V. M. Mushtakova, and V. A Fomina, *Water Qual. Res. J. Canada*, **32**, No. 4, 829-837 (1997).
10. C. A. Ryan, *Biochim. Biophys. Acta*, **1477**, No. 1, 112-121 (2000).
11. C. A. Ryan and G. Pierce, *Ann. Rev. Cell Rev. Biol*., **14**, No. 1, 1-17 (1998).
12. M. A. Torres, H. Onouchi, S. Hamada, *et al*., *Plant J*., **14**, No. 3, 365-370 (1998).
13. P. Van Gestelen, H. Asard, and R. J. Caubergs, *Plant Physiol*., **115**, No. 4, 343-350 (1997).