EFFECT OF MINERAL DUST ON GENERATION OF SUPEROXIDE RADICALS AND HYDROGEN PEROXIDE BY ALVEOLAR MACROPHAGES, GRANULOCYTES, AND MONOCYTES

V. A. Gusev, E. V. Danikovskaya, and O. E. Vatolkina

UDC 613.633-07:612.24-008.953.3/.4-008.939.15-39

KEY WORDS: macrophages, granulocytes, mineral dust, superoxide radicals, hydrogen peroxide.

The pathogenesis of pneumoconiosis and, in particular, of silicosis has been the subject of numerous investigations, but the causes of the high aggressiveness of chemically inert silica-containing dust and the trigger mechanisms leading directly to the stimulation of collagen formation remain unexplained. An extremely important role in the cleansing of the lungs from dust particles deposited in them is ascribed to the alveolar macrophages. The phagocytosis of quartz particles quickly leads to their death, and this is regarded as a key stage in the development of pneumoconiosis [2]. The development of the pathological process in the lungs in pneumoconiosis is also accompanied by a sharp increase in the number of polymorphonuclear leukocytes in bronchopulmonary washings from patients and experimental animals [13, 15]. Meanwhile the concrete mechanisms of the cytotoxic action of fibrogenic dusts on phagocytes have not been adequately studied. Recent investigations have shown that phagocytosis of microorganisms is accompaned by a sharp increase in generation of toxic active forms of oxygen, such as the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical [13]. Stimulation of generation of active forms of oxygen during phagocytosis of mineral dusts has been recorded by the chemiluminescence method [14] and also by recording an increase in oxygen consumption [4].

The aim of the present investigation was to study the effect of quartz dust and of low-fibrogenic alumina dust on generation of various forms of active oxygen by alveolar macrophages, granulocytes, and monocytes with the aim of elucidating the biochemical mechanisms of participation of these cells in the pathological processes in the lungs in pneumoconiosis.

EXPERIMENTAL METHOD

Thirty male chinchilla rabbits and blood from 45 healthy blood donors were used in the experiments. Alveolar macrophages were isolated from endobronchial washings of the rabbit lung [9]. The cells were washed five times with 40 ml of Hanks' solution. Granulocytes and the fraction of mononuclear cells were isolated from human blood by the method described by [3]. Mononuclears were isolated from the rabbit blood by the method described in [7]. Since lymphocytes cannot undertake phagocytosis or generate active forms of oxygen [8], their presence in the mononuclear fraction did not affect the experimental results. The purity of the cellular fraction was verified by morphological study of a film of a cell suspension in plasma, stained by Pappenheim's method. Total generation of active forms of oxygen by the cells was investigated by substrate-free reduction of nitro-blue tetrazolium (nitro-BT) to formazan [6]. The cells ($3 \cdot 10^6$) were incubated for 15 min at 37°C in 1 ml of Krebs–Ringer solution containing 0.5 mM $CaCl_2$, 10 mM glucose, and 40 mM nitro-BT (Reanal). Quartz dust or alumina dust was added to the samples. The particle size of the dust in all the experiments was ≤5 μ. The reaction was stopped by the addition of 10 ml of 0.5 M HCl. The formazan thus formed was extracted from the residue with 4 ml pyridine during heating for 15 min in a boiling water bath. Absorption was measured at 515 nm. The intensity of

Clinical Laboratory, Research Institute of Work Hygiene and Occupational Diseases, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. F. Izmerov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 372-375, October, 1990. Original article submitted December 2, 1989.

 0007-4888/90/0010-1348$12.50

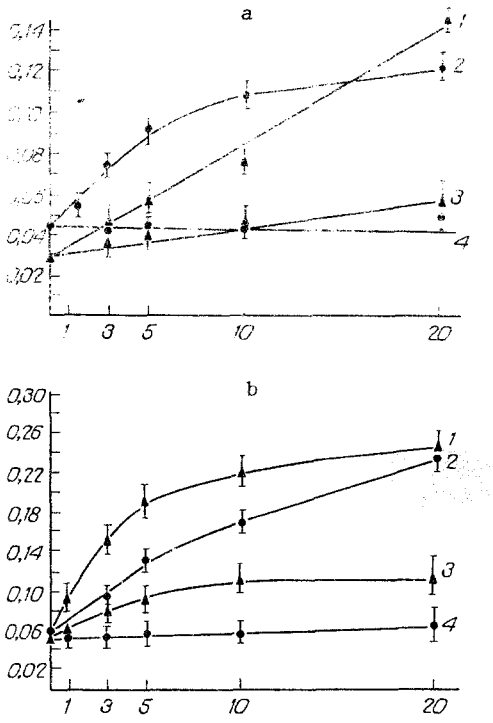

Fig. 1. Effect of quartz dust (1, 2) and alumina dust (3, 4) on reduction of nitro-BT to formazan by alveolar macrophages and monocytes from rabbit blood (a) and granulocytes and monocytes from human blood (b). Abscissa, quantity of dust (in mg); ordinate, optical density (in relative units). 1, 3) Rabbit alveolar macrophages; 2, 4) Rabbit blood monocytes; 1, 3) human blood granulocytes; 2, 4) human blood monocytes.

superoxide radical generation by the cell was assessed from the quantity of reduced cytochrome C inhibited by superoxide mutase [5]. The incubation medium consisted of Hanks' solution containing cytochrome C (Sigma) in a concentration of 50 μM, and in the case of granulocytes, human serum albumin (Reanal) in a concentration of 1 mg/ml. The granulocytes were incubated ($1.5 \cdot 10^6$ cells) for 15 min in 1.5 ml of medium at 37°C and the alveolar macrophages and monocytes for 90 min. The reaction was stopped in an ice bath, and after centrifugation the optical density was measured at 550 nm in the presence of 50 μg of superoxide dismutase (Sigma) or in its absence. The quantity of reduced cytochrome was calculated by means of an extinction coefficient of 21.0 $M^{-1} \cdot cm^{-1}$. H_2O_2 generation was determined by a method based on peroxidase-mediated oxidation of phenol red [10]. The incubation medium consisted of Hanks' solution containing 0.28 mM phenol red (Sigma), horseradish peroxidase (Reanal) in a concentration of 50 μg/ml, and 1 mM sodium azide. Altogether $3 \cdot 10^6$ cells were incubated in 3.5 ml of medium at 37°C for 15 min. The reaction was stopped by the addition of 35 μl of 1 N NaOH. Optical density was measured at 610 nm.

EXPERIMENTAL RESULTS

The experimental results show that incubation of rabbit alveolar macrophages and monocytes and also of human monocytes and granulocytes with various quantities of quartz dust leads to a considerable increase in the total generation of active forms of oxygen (Fig. 1). The less fibrogenic and cytotoxic alumina dust, in the same concentrations as quartz dust, increased the total generation of active forms of oxygen by a much lesser degree than silica dust. The much higher level of response of the granulocytes than of other cells will be noted. Superoxide dismutase, added in a concentration of 50 μg/ml to the incubation medium did not affect reduction of nitro-BT by macrophages and monocytes, but inhibited this process in granulocytes on average by 50%. In the course of these experiments we were unable to record any exogenous generation of superoxide radicals and H_2O_2 by alveolar macrophages or monocytes, whether from rabits or man, during phagocytosis of quartz dust. In-

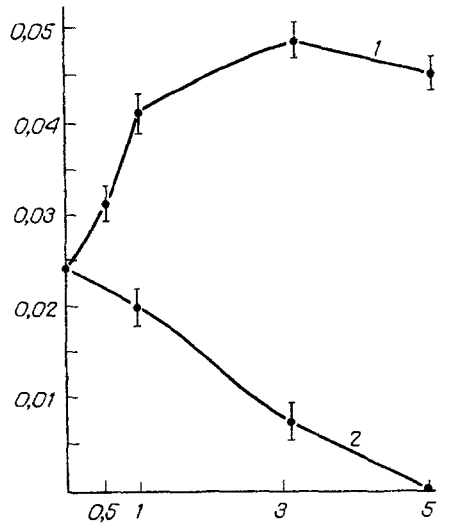

Fig. 2. Effect of mineral dust of superoxide anion-radical generation by human blood granulocytes. Abscissa, quantity of dust (in mg); ordinate, concentration of O_2^- (in nmoles/10^6 cells/min). 1) Silica dust; 2) alumina dust.

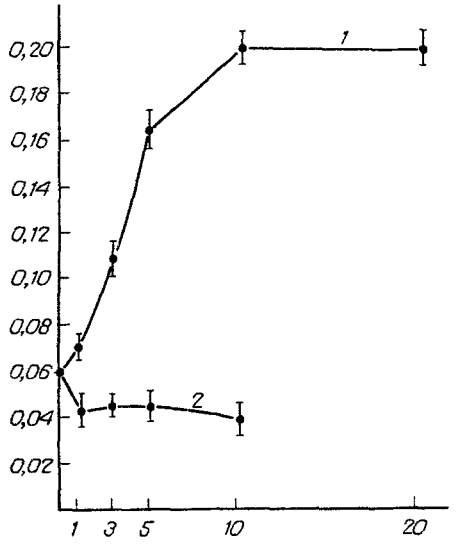

Fig. 3. Effect of mineral dust on H_2O_2 generation by human blood granulocytes. Abscissa, quantity of dust (in mg); ordinate, content of H_2O_2 (in mmoles/10^6 cells/min); 1) silica dust, 2) alumina dust.

cubation of granulocytes with quartz dust, on the other hand, led to a marked increase in exogenous generation of O_2^- and, especially, of H_2O_2 compared with intact cells (Figs. 2 and 3).

The method of substrate-free reduction of nitro-BT can be used to judge generation of active forms of oxygen both inside and outside phagosomes [12]. The inability of mononuclear phagocytes to undertake exogenous generation of O_2^- and H_2O_2, together with the more intensive generation of active forms of oxygen recorded during interaction with mineral dusts, is evidence of the intracellular course of the regeneration processes, and also of the great similarity of the biochemical processes in alveolar macrophages and monocytes. This conclusion is confirmed by the observed absence of any effect of superoxide dismutase on reduction of nitro-BT by mononuclear phagocytes. The differences discovered between mononuclear phagocytes and granulocytes in their response to dust evidently have great physiological importance. Active forms of oxygen, released by phagocytes, not only possess bactericidal properties but they also have a powerful damaging action on the phagocytes them-

selves and on surrounding tissues [11, 13]. Alveolar macrophages are present constantly in large numbers in the alveoli of the lungs, and for that reason the uncontrolled exogenous generation of superoxide radicals and H_2O_2 by them on the entry of dust into the lungs could quickly lead to damage to the lung parenchyma. Meanwhile breakdown products of alveolar macrophages contain a factor which attracts granulocytes. The presence of granulocytes in the bronchopulmonary washings is evidence of the development of a pathological process. This conclusion is in good agreement with results showing exogenous generation of O_2 and H_2O_2 by granulocytes and stimulation of this process during phagocytosis of quartz dust. Since mineral dusts, unlike microorganisms, cannot be degraded by phagocytes, fibrosis of the lung tissue can be regarded as a protective reaction to the development of the inflammatory process. Further evidence of the important role of superoxide radicals and H_2O_2 in the pathogenesis of pneumoconiosis also is given by our previous observations of inhibition of the development of pneumofibrosis in the lungs in experimental silicosis by exogenous superoxide dismutase and catalyase, administered by inhalation two to five times a week for 2.5 months [1].

LITERATURE CITED

1. O. G. Arkhipova, V. V. Yaglov, V. A. Gusev, et al., Bioantioxidants [in Russian], Vol. 2, Chernogolovka (1986), p. 195.
2. B. T. Velichkovskii, Fibrogenic Dusts [in Russian], Gor'kii (1980).
3. L. B. Kheifets and V. A. Abalakin, Lab. Delo, No. 10, 579 (1973).
4. A. V. Yakh''ev, A. N. Osipov, O. A. Azizova, et al., Byull. Éksp. Biol. Med., No. 4, 443 (1985).
5. B. M. Babior, R. S. Kipnes, and J. T. Curnutte, J. Clin Invest., 52, 741 (1973).
6. R. L. Baechner, J. A. Boxer, and I. J. Davis, Blood, 48, 309 (1976).
7. D. L. Feldman and T. C. Mogelsky, J. Immunol. Meth., 102, 243 (1987).
8. K. B. Johnston, J. L. Loyce, and L. A. Guthrie, J. Exp. Med., 141, 1151 (1976).
9. Q. N. Myrvik, E. E. Leoke, and S. B. Faris, J. Immunol., 86, 128 (1961).
10. E. Pick and Y. Keisari, J. Immunol. Meth., 38, 161 (1980).
11. M. L. Salin and T. M. McCord, J. Clin. Invest., 56, 1319 (1975).
12. R. F. Schopf, J. Mattar, W. Meynburg, et al., J. Immunol. Meth., 67, 109 (1984).
13. H. V. Thommasen, Clin. Invest. Med., 8, 185 (1985).
14. V. Vilim, I. Welhelm, P. Brzak, and I. Hurich, Immunol. Lett., 8, 69 (1984).
15. A. Xaubet, K. Rodriges-Rosin, J. A. Bombi, et al., Am. Rev. Resp. Dis., 133, 848 (1986).