Effect of quartz and alumina dust on generation of superoxide radicals and hydrogen peroxide by alveolar macrophages, granulocytes, and monocytes

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Abstract
Phagocytosis of quartz particles by rabbit alveolar macrophages and monocytes and human granulocytes and monocytes was accompanied by stimulation of substrate free reduction of nitroblue tetrazolium to formazan. This reflects activation of an oxygen dependent bactericidal system of phagocytes and total (exogenic and endogenic) generation of active oxygen species. Low fibrogenic and cytotoxic alumina dust tended to increase formazan production by comparison with quartz dust. During phagocytosis of quartz dust by alveolar macrophages and monocytes there was no exogenic generation of superoxide radicals and hydrogen peroxide by these cells. By contrast, incubation of human granulocytes with quartz dust caused a significant increase in exogenic generation of superoxide radicals and hydrogen peroxide. Under such conditions, low fibrogenic alumina dust had no effect on hydrogen peroxide generation and substantially decreased the level of superoxide radical generation by human granulocytes. During incubation of rabbit granulocytes with quartz dust, an increase in the level of superoxide radical generation was also detected. It is considered that the differences between alveolar macrophages and granulocytes in their response to quartz dust are important from a physiological point of view. Alveolar macrophages are permanently present in pulmonary alveolae in large quantities; therefore their uncontrolled generation of superoxide radicals and hydrogen peroxide might immediately cause damage to pulmonary parenchyma. At the same time, destruction products from alveolar macrophages that died during phagocytosis of quartz particles contain a factor attracting granulocytes. Presence of a significant number of granulocytes in bronchopulmonary lavage fluid in cases of silicosis indicates development of a pathological process. This agrees well with the data obtained on exogenic generation of superoxide radicals and hydrogen peroxide by granulocytes, and on stimulation of this process due to phagocytosis of quartz dust.

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Phagocytosis of quartz particles rapidly causes death of alveolar macrophages, which is considered to be an essential step in the pathogenesis of silicosis. Progress of the pathological process in lungs in cases of pneumoconiosis is also accompanied by a prominent increase in number of granulocytes in bronchopulmonary lavage fluid from patients and laboratory animals. Our present study was undertaken to examine the effects of quartz dust and low fibrogenic alumina dust on generation of various active oxygen species by rabbit and human alveolar macrophages, granulocytes and monocytes. The aim was to ascertain the biochemical mechanisms involved in the cells’ participation in the pathological process of silicosis.

Materials and methods
Forty male chinchilla rabbits and blood from 45 donors were used for the experiments. Alveolar macrophages were obtained from a rabbit lung by endobronchial washing. The cells were washed five times with 40 ml of Hanks’ solution. Mononuclear cells were obtained from rabbit blood by the method described previously. Granulocytes were obtained from rabbit blood by methods modified by us as described in previous studies. Blood (40 ml) taken from a rabbit ear artery was mixed with heparin. The blood was diluted by half with 0-9% sodium chloride solution and mixed with...
Effect of centrifugation, were removed and the precipitate of cells with and treated at Percoll (Pharmacia, Sweden), were determined by pH solution, tetrazolium to medium for human and rabbit granulocytes, the method of substrate medium for 50 minutes. The released granulocytes of 0-28 mM caused active oxygen increase in total generation of exogenic radicals and hydrogen peroxide. On the other hand, generation of hydrogen peroxide by cells was determined by the method based on phenol red oxidation in the presence of peroxidase.11 The incubation medium was Hanks’ solution containing 0-28 mM phenol red, horse radish peroxidase at a concentration of 50 μM/ml, and 1 mM sodium azide. The cells (3 × 10⁶) were incubated in 3-5 ml medium at 37°C for 15 minutes.

Results

Incubation of rabbit alveolar macrophages and monocytes, as well as human monocytes and granulocytes with different amounts of quartz dust caused a significant increase in total generation of active oxygen (fig 1). By contrast with silica dust, the less fibrogenic and cytotoxic alumina dust at the same concentrations as those of quartz dust caused much less increase in active oxygen production. There was a considerably higher level of granulocyte response in comparison with other cells. Superoxide dismutase added to the incubation medium had no effect on reduction of nitroblue tetrazolium by alveolar macrophages and monocytes, but inhibited this process by 50% on average in the case of granulocytes. We did not find exogenic generation of superoxide radicals and hydrogen peroxide by rabbit and human alveolar macrophages and monocytes during phagocytosis of quartz dust. On the other hand, incubation of human granulocytes with quartz dust caused a significant increase in exogenic generation of superoxide radicals, and especially hydrogen peroxide in

Figure 1 Effect of quartz dust (1,2) and alumina dust (3,4) on reduction of nitroblue tetrazolium by rabbit alveolar macrophages and monocytes (A) and human granulocytes and monocytes (B). For A, 1,3 rabbit AM; 2,4 rabbit monocytes; for B, 1,3 human granulocytes; 2,4 human monocytes. Optical density in optical units.
comparison with intact cells (figs 2 and 3). Low fibrogenic alumina dust had no influence on the generation of hydrogen peroxide by human granulocytes and caused a pronounced decrease in the intensity of superoxide radical generation by these cells. Incubation of rabbit granulocytes with quartz dust also caused a considerable increase in exogenic generation of superoxide radicals (fig 4); however, the stimulating effect of quartz dust, by contrast with human granulocytes, was not seen for each rabbit, but only in 60% of the experiments performed.

Discussion

The method of substrate free reduction of nitroblue tetrazolium to formazan by phagocytes makes it possible to assess the generation of active oxygen species both inside and outside phagosomes.¹² The inability of alveolar macrophages and monocytes to generate superoxide radicals and hydrogen peroxide exogenically when stimulated by quartz dust, along with enhanced generation of active oxygen species as a result of interaction with quartz dust, indicate intracellular development of the generation process in the cells. Such a conclusion is confirmed by data showing the absence of any effects of superoxide dismutase added to the incubation medium on nitroblue tetrazolium reduction by alveolar macrophages and monocytes. The difference found between alveolar macrophages and granulocytes in their response to quartz dust is important from a physiological point of view. Active oxygen species released by phagocytes possess not only bactericidal properties but exert a powerful damaging action on phagocytes and surrounding tissues.¹³ ¹⁴ Alveolar...
macrophages are permanently present in pulmonary alveolae in large quantities. When quartz dust gets into the lungs, uncontrolled exogenic generation of superoxide radicals and hydrogen peroxide by macrophages might immediately cause damage to pulmonary parenchyma. At the same time, products of destruction of alveolar macrophages after phagocytosis of quartz particles contain a factor that attracts granulocytes.

Presence of a significant number of granulocytes in bronchopulmonary lavage fluid indicates development of a pathological process. This agrees well with the data obtained on exogenic generation of superoxide radicals and hydrogen peroxide during phagocytosis of quartz dust. The fact that rabbit granulocytes stimulated by quartz dust showed enhanced generation of superoxide radicals in only 60% of all experiments cannot be accounted for adequately so far. In our opinion, this might be related to individual sensitivity of granulocytes of various rabbit species to quartz dust.

The important role of superoxide radicals and hydrogen peroxide in pathogenesis of silicosis is also shown by our data previously obtained on inhibition of the progress of pneumofibrosis in rat lungs in experimental silicosis by exogenic superoxide dismutase and catalase inhaled in the form of aerosol two to five times a week for two and a half months.15-17

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