Chapter 14

Freshly Fractured Silica Exerts Greater Toxicity To Lung Macrophages Than Aged Dust: Organosilane (Prosil 28) Coating Markedly Reduces Dust Toxicity to Cells

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Previously we reported crushing or grinding crystalline silica results in silica-based free radicals on the particulate surface which can generate hydroxyl radicals in aqueous solution (1). Now we find that freshly ground silica is more cytotoxic to and a more potent activator of alveolar macrophages than similarly sized aged silica.

Since the United States Department of Labor estimates that over 1.25 million American workers may be exposed to freshly ground crystalline silica and nearly 60,000 may be at risk of developing some degree of silica-based occupational lung disease, then this is hardly a trivial problem. Occupations of concern include workers who come in contact with silica including miners of soft and hard minerals, stone quarry workers, glass workers and even millers who stone grind flour. Acute silicosis is manifested by inflammation, alveolar lipo-proteinosis, and the rapid development of respiratory disability that depending on the dose of silica can produce chronic silicosis which takes 20 - 40 years to develop. It is demonstrated by the appearance of concentric hyalinized nodular lesions and progressive development of dyspnea(3).

Certainly the free radicals produced on the surface of freshly ground crystalline silica play a most important role in producing the acute and chronic symptoms of silicosis. When respirable-size freshly- ground silica (0.1 -- 5 microns in diameter) enters the deep alveolar spaces in the lung and contacts an aqueous environment, hydroxyl radical are generated and cellular damage is bound to occur. We have found that freshly ground silica can cause lipid peroxidation and lysis of erythrocytes(1).

The objective of this investigation was to further elucidate the enhanced toxicity of freshly ground versus aged silica. It can be deduced from the data that not only is freshly ground silica more cytotoxic to alveolar macrophages but it is a more potent stimulant of these phagocytes.

However the mere characterization of toxicity is not entirely helpful if there is not a useful means to detoxify the cause of the damage. We have identified an aqueously soluble substance that can be used in high dilution to detoxify the SiO(·) and Si(·) type of free radicals. This is the organosilane known as Prosil 28(R). We have found that coating of the freshly ground silica can effectively reduce its toxicity. The importance of this observation is that such a coating could be used with water cooled drills. This may partially detoxify silica at the point of generation of the free radical, i.e., the generation of the fresh silica dust itself. Such a coating could be used in great dilution $1:10^3$ or $1:10^4$. The coating would be long lasting, inexpensive and should be non-toxic because of the great dilution.

Methods

a.Silica Preparation

The detailed method of preparation of the freshly ground silica has been described elsewhere (4). Crystalline silica 0.2 - 5.0 mm in diameter was ground for 30 min using a ball mill equipped with agate balls. The dust was sieved through a 20 micron mesh filter. Purity was checked using X-ray analysis and morphometric analysis indicated a mean diameter of 3.7 μ meters.

b.Coating of silica

Silica prepared as above was coated using Prosil 28® according to manufacturer's directions (PCR Inc., Gainsville, FL). The coated particles were washed in physiological solution as previously described(4).

c.Preparation of macrophages

Cells were harvested from Sprague -- Dawley rats by bronchialveolar lavage as described previously(4).

d.Measurement of cell viability

Effect of silica on cell viability was determined by monitoring trypan blue exclusion, propidium fluorescence using a fluorescence activated cell sorter, or hemolysis as previously described(4).

e.Macrophage activation

Silica induced activation of alveolar macrophages was determined by monitoring hydrogen peroxide release or generation of lucigenin dependent chemiluminescence as described previously(4).

Results

The cytotoxic effect of freshly ground or aged silica on alveolar macrophages was determined by monitoring the silica-induced decline in membrane integrity. Viability of unexposed cells was 94 ± 2%. Both freshly ground silica and ground silica aged for 2 weeks caused dose-dependent membrane damage. However, freshly ground silica was significantly more cytotoxic than aged silica; i.e., following exposure to fresh silica (15mg/m), viability was 41 ± 9% compared to 83 ± 1% after treatment with aged silica (15 mg/ml).

Freshly ground silica was also a more potent stimulant of alveolar macrophages than ground silica aged for 2 weeks. Both fresh and aged silica caused significant activation of hydrogen peroxide secretion from alveolar macrophages. However, fresh silica was 50% more potent than aged silica.

Measurements of cellular chemiluminescence also support the conclusion that freshly ground silica was a more potent stimulant of alveolar macrophages than aged silica. Data indicate that both fresh and aged silica activated macrophages. However, the ability of ground silica to induce chemiluminescence generation from alveolar macrophages quickly declined with storage. That is, the potency of ground silica aged for 1 or 2 days was only 39% or 18% as great, respectively, as that for fresh dust.

Coating freshly ground silica with Prosil 28, an organo-silane material, was effective in preventing the cytotoxicity of this dust. Coating silica resulted in a significant decline (75%) in the ability of silica to lyse red blood cells. Similar results were also obtained with alveolar macrophages. These data indicate fresh silica caused a time-dependent decrease in membrane integrity; i.e., a 1 hr exposure of alveolar macrophages to silica decreased viability by 17% while viability decreased by 60% after a 5 hr treatment. At all exposure times, coating fresh silica substantially decreased its cytotoxic effect; i.e., coating decreased the cytotoxicity of fresh silica by 53% at 1 hr and 73% after 5 hrs.

Coating freshly ground silica with an organo-silane material was also effective in decreasing the ability of silica to activate the secretion of reactive products from alveolar macrophages, i.e.,

chemiluminescence generated from silica-exposed alveolar macrophages was decreased by 58% with coated dust.

Summary

Freshly cleaved silica is clearly more toxic to erythrocytes and macrophages than aged silica. This is judged monitoring silica-induced decreases in membrane integrity. Freshly ground silica is also a more potent activator of alveolar macro phages as determined by hydrogen peroxide release and generation of lucigenin-dependent chemiluminescence. Organosilane coating of freshly cleaved silica inhibits hemolysis of erythrocytes, causes protection of viable alveolar macrophages assayed by propidium fluorescence, and decreased the degree of silica-induced chemiluminescence.

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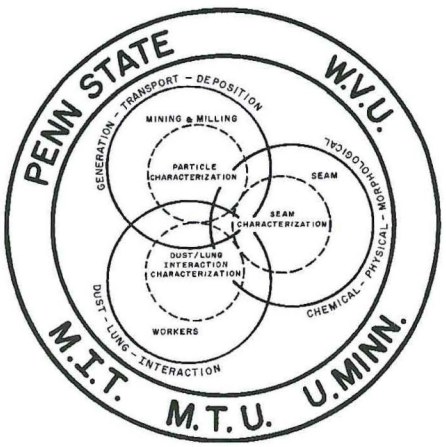

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