Mechanisms of Mineral Dust-induced Emphysema

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Mineral dust exposure can result in emphysema and chronic airflow obstruction. We postulated that dust-induced emphysema has a pathogenesis similar to that in cigarette smoke-induced emphysema, namely, excess release of proteolytic enzymes from dust-evoked inflammatory cells, and inactivation of alpha-1-antitrypsin (A1AT) by dust-catalyzed formation of oxidants. To test this theory we examined the antiproteolytic activity of A1AT exposed to quartz in vitro and found that it was decreased in a dose–response fashion. Catalase prevented this effect, which suggested that it was mediated by quartz-generated hydrogen peroxide. We also showed that a variety of dusts could oxidize methionine to methionine sulfoxide in vitro, using either pure amino acid or whole protein. The relative order of activity was coal > quartz > titanium dioxide. Lastly, we used a new high-performance liquid chromatography technique to demonstrate that quartz, coal, and titanium dioxide produced connective tissue breakdown in rat lungs, as determined by the appearance of desmosine and hydroxyproline in lavage fluid after dust instillation. On a particle-for-particle basis, the order of dust potency was similar to that for methionine oxidation. Connective tissue breakdown was associated with elevations of both polymorphonuclear leukocytes and macrophages in lavage fluid, and it is unclear whether one or both of these types of inflammatory cell mediates this process. These observations support our theory that dust-induced emphysema and smoke-induced emphysema occur through similar mechanisms. — Environ Health Perspect 105(Suppl 5):1215–1218 (1997)

Key words: emphysema, airflow obstruction, mineral dusts, coal, silica, asbestos, titanium dioxide

Introduction

Occupational exposure to mineral dusts, particularly coal and silica, is associated with the development of emphysema (1–6) and chronic airflow obstruction. Although the anatomic presence of dust-induced emphysema is established, the mechanisms of emphysema formation are uncertain.

The protease–antiprotease theory of cigarette smoke-induced emphysema (7,8) suggests that in smokers, emphysema develops as a result of excess unopposed proteolytic activity in the lung. This excess proteolytic activity has two sources: a) release of proteolytic enzymes, particularly neutrophil elastase, from smoke-evoked inflammatory cells, with subsequent degradation of the connective tissue matrix of the lung; and b) inactivation of the major anti-proteolytic agent in the lung, alpha-1-antitrypsin (A1AT), by oxidants in the smoke.

Because mineral dusts also evoke a more or less intense and prolonged inflammatory response (9), and because many mineral dusts can catalyze the formation of active oxygen species (AOS) in aqueous media (10,11), we propose that dust-induced emphysema arises on a very similar basis, namely, release of large amounts of proteolytic enzymes from dust-evoked inflammatory cells, and oxidative inactivation of A1AT. This question has not been

extensively investigated. Brown et al. (12) showed that there were increases in lavage proteolytic activity from rats given quartz or titanium dioxide, but Hannothiaux et al. (13) found only small and inconsistent increases in lavage elastase activity from monkeys administered quartz. Rom (14) reported increased levels of elastase complexed to A1AT in lavages from coal miners.

In this paper we report a series of experiments designed to explicitly test our hypothetical mechanisms.

Materials and Methods

These experiments used titanium dioxide (Rutile, Sigma, St. Louis, MO) with geometric mean diameter 0.51 μm; quartz (Minusil-5, U.S. Silica Corp, Clarkstown, WV), geometric mean diameter 0.76 μm (freshly ground); coal (No 1520, Penn State Coal Bank, University Park, PA), geometric mean diameter 1.8 μm; and amosite asbestos (Union Internationale Contre le Cancer, Lyon, France), geometric mean length/width 3.8 × 0.20 μm. Sources of specific chemicals and human A1AT are specified in Li et al. (15,17) and Zay et al. (16).

The antiproteolytic activity of A1AT was determined in vitro by the SLAPN assay using porcine pancreatic elastase (PPE) (16) and levels of hydrogen peroxide generated by quartz by a modification of Sigma method P8250 (16). Methionine sulfoxide levels were assayed in vitro by high-performance liquid chromatography (HPLC) (17). When used with pure amino acids, the solutions were analyzed directly; when used with whole proteins, the proteins were first hydrolyzed in potassium hydroxide (17).

For analysis of lavage desmosine (DES) and hydroxyproline (HP), lavage was performed with distilled water to avoid analytical problems caused by high salt concentrations. The lavageate was hydrolyzed in 6 M hydrochloric acid. Lavage DES and HP analyses were carried out using HPLC methods (15).

Results and Discussion

Oxidative Inactivation of A1AT by Quartz

Figure 1 shows the effects of a 1-hr exposure to quartz on the antiproteolytic potency of pure human A1AT in vitro. Quartz inactivated A1AT in a dose–response fashion, and the degree of inactivation was greater for
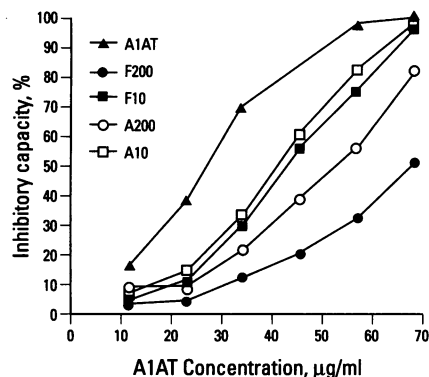
Figure 1. Elastase inhibitory capacity of A1AT determined by SLAPN assay after incubation of A1AT with silica dust for 1 hr. Note the dose-dependent decrease in inhibitory capacity and that at high dose freshly ground silica is more potent than aged silica; the two low-dose treatments (F10 and A10) appear to produce similar results. Abbreviations: A1AT, A1AT without added silica; A10, 10 mg/ml aged silica; A200, 200 mg/ml aged silica; F10, 10 mg/ml freshly ground silica; F200, 200 mg/ml freshly ground silica. Reproduced from Zay et al. (15), by permission.

Figure 2. Catalase provides protection against silica-induced inactivation of A1AT in an elastase inhibitory capacity assay similar to that shown in Figure 1. However, for this experiment only one concentration of A1AT was used. *, inhibitory capacity is significantly reduced by quartz compared to no dust treatment (equal to bars labeled A1AT). Catalase protects against all quartz concentrations. Abbreviations: A20, 20 mg/ml aged quartz; A200, 200 mg/ml aged quartz; F20, 20 mg/ml freshly ground quartz; F200, 200 mg/ml freshly round quartz. Reproduced from Zay et al. (15), by permission.

Figure 3. Demonstration of the relative ability of different mineral dusts to oxidize pure methionine to methionine sulfoxide. Abbreviations: Asb, amosite asbestos dust; Meth, methionine without dust (actual value 0% methionine sulfoxide; the error bar is to show position); Si, silica dust; Ti, titanium dioxide dust.

freshly ground quartz compared to quartz used directly as received (aged quartz). In Figure 2, a single concentration of A1AT was used and catalase or no catalase added to the quartz–A1AT mixture to investigate the role of hydrogen peroxide. Catalase protected A1AT against the effects of all quartz concentrations and against both fresh and aged quartz. Hydrogen peroxide assay showed that quartz liberated hydrogen peroxide into the medium, with greater amounts of hydrogen peroxide produced by fresh compared to aged quartz. Reagent hydrogen peroxide also inactivated A1AT and this effect could be similarly reduced by inclusion of catalase in the incubation mixture (data not shown).

To determine whether quartz interfered with formation of the A1AT–PPE complex, A1AT was treated with quartz or hydrogen peroxide, then mixed with PPE. The supernatants from this mixture were analyzed by sodium dodecyl sulfate gel electrophesis and Western blots prepared using anti-A1AT. This approach showed that the intensity of the band representing the A1AT–PPE complex was diminished by both quartz and hydrogen peroxide treatment.

These observations indicate that quartz can inactivate A1AT and decrease the formation of A1AT–PPE complexes by a mechanism that appears to be mediated largely through hydrogen peroxide formation. It should be noted that the concentration of quartz required is fairly high and that the system by definition ignores the
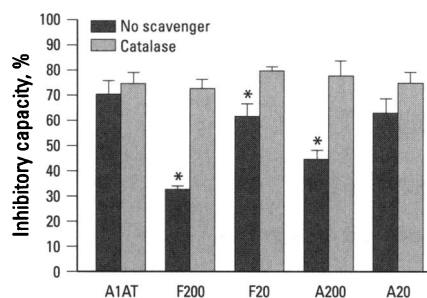
protective effects of proteins that might adsorb to the dust *in vivo*. Nonetheless, the local concentration of mineral dusts and subsequent levels of AOS formation *in vivo* conceivably could be quite high, and the current observations suggest that any dust that can generate AOS in solution may have the same effect.

Oxidation of Methionine Residues by Mineral Dusts

As noted in the introduction, it is believed that cigarette smoke oxidatively inactivates A1AT in the lungs of smokers (7,8,18); the specific mechanism involved appears to be oxidation of methionine residues that are crucial to the protective function of A1AT (18). Proof of this proposition has been difficult, although Carp et al. (18) did report the presence of increased methionine sulfoxide (the oxidation product of methionine) levels in the lavage of smokers. Evans and Pryor (19) addressed this issue by exposing A1AT *in vitro* to aqueous extracts of smoke tar. They found that smoke tar was able to oxidize methionine to methionine sulfoxide when methionine was supplied as pure amino acids or in A1AT, and that the process appeared to be mediated by hydrogen peroxide.

We performed a series of similar *in vitro* experiments using the dusts listed in "Materials and Methods" and either pure methionine or whole protein (human A1AT or human albumin). All the dusts listed were able to oxidize pure methionine (Figure 3), as was reagent hydrogen
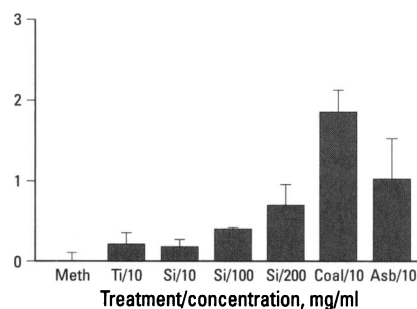
peroxide. This process was dust dose dependent and could be reversed to a great extent by catalase and to a variable extent by scavengers of hydroxyl radical. Coal, silica, and amosite asbestos were also able to oxidize methionine residues in whole proteins (data not shown). The relative order of potency was coal > silica > amosite.

These mineral dust findings are quite similar to those of Evans and Pryor (19) with cigarette smoke and suggest that mineral dusts can potentially inactivate A1AT by oxidizing methionine. The process again appears to be mediated through hydrogen peroxide and less certainly through hydroxyl radical. It is interesting that the relative potency of various dusts appears to parallel their ability to produce emphysema, and it should also be noted that if one uses the mean size data to estimate the relative number of particles present, the differences on a particle-for-particle basis among coal, silica, and titanium dioxide are much more marked than Figure 3 would imply.

Dust-induced Connective Tissue Breakdown

By definition, connective tissue breakdown must occur during the genesis of emphysema. In cigarette smokers the demonstration of connective tissue breakdown has been controversial; older studies failed to find increased levels of urinary DES (a marker of elastin breakdown) in smokers compared to nonsmokers. Newer studies, however, which claim to use more sensitive techniques, have reported increased urinary DES levels in smokers (20).

To investigate this issue with dusts, we administered a single intratracheal dose of quartz, coal, or titanium dioxide to

Sprague-Dawley rats, then analyzed DES and HP in lavage fluid at various times after dust administration. One lung was used for the chemical analyses and the other for lavage inflammatory cell counts.

We found that all three dusts produced a dose–response increase in DES and HP at 24 hr, with considerably greater elevations after quartz and coal administration than after titanium dioxide administration. Of particular interest was the time course of events when a single 30-mg dose of dust was administered and animals sacrificed up to 21 days later. Lavage DES returned to control levels within 7 days (Figure 4A) and lavage HP within 14 days. Lavage polymorphonuclear leukocyte(s) (PMN) counts were briefly elevated but dropped rapidly, whereas lavage macrophage(s) (MAC) counts remained elevated over the 21-day period. With quartz and coal the pattern was quite different: both lavage DES and HP remained markedly elevated over 21 days with no evidence of a decrease over time (Figure 4B). With quartz, both PMN and MAC counts also remained elevated, whereas with coal the PMN count was briefly elevated and returned to almost control level by 7 days, while the MAC count remained elevated.

These findings are the first report of connective tissue breakdown after mineral dust exposure. It is important to bear in mind that the doses of dust used here are high and that intratracheal instillation can produce enormous local particle concentrations that would never occur during inhalation exposures. Similarly, the intensity of the inflammatory infiltrate is probably much higher than would be seen with inhalation of dust. Thus the level of connective tissue breakdown seen here may be quite artifactual. Nonetheless, even after inhalation exposures mineral dusts induce a marked inflammatory infiltrate, and thus our system, albeit exaggerated, may serve as a reasonable model for dust-induced connective tissue breakdown.

Different dusts behave quite differently. Titanium dioxide is usually regarded as a prototypical nuisance dust. In our system sufficiently high doses of titanium dioxide produce connective breakdown, but this effect is short lived. Whether this finding applies to humans at any reasonable exposure level is unclear. More important are the findings that coal and silica, dusts that produce emphysema in humans, cause much greater levels of connective tissue breakdown, which persist for long periods of time. It is important to note that, as is true of methionine oxidation, on a particle-for-particle number basis, the effects of coal and quartz are much greater than those of titanium dioxide in producing connective tissue breakdown.

With all three dusts, connective tissue breakdown is accompanied by a fairly intense inflammatory infiltrate, the usual response to any instilled or inhaled dust. However, the correlation of connective tissue breakdown with inflammatory cell counts is unclear. In the silica group PMN levels and MAC levels remain elevated over the entire time course of the experiment, whereas with both coal and titanium dioxide PMN counts rapidly drop and only MAC levels remain elevated. The absolute MAC counts with all three dusts are in fact
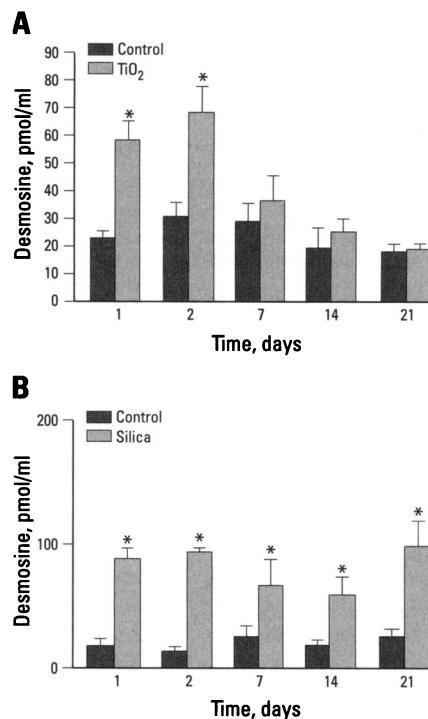

Figure 4. Lavage desmosine levels up to 21 days after a single intratracheal instillation of 30 mg (A) titanium dioxide or (B) silica. *, significantly greater than control. Note that DES levels rapidly return to control after titanium dioxide but remain elevated after silica treatment. Reproduced from Li et al. (14), by permission.

remarkably similar. Interpretation of these findings is difficult but it is possible that some other factor (degree of oxidant generation by dusts and inflammatory cells, actual levels of protease release by inflammatory cells) differs among the dusts. We are currently evaluating this possibility.

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