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Effect of Alveolar Lining Material-Coated Silica on Rat Alveolar Macrophages

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The disparity between *in vitro* silica cytotoxicity toward macrophages and their *in vivo* resistance to injury following inhalation of silica at physiologic concentrations is unresolved. It is probable that inhaled silica particles absorb a variety of biological substances including proteins and alveolar lining material (ALM) thus altering the *in vivo* response of the macrophage to these particles.

Silica (SI) particles coated with rat ALM and uncoated SI particles were studied for their ability to injure rat alveolar macrophages (AM) *in vitro*. Suspensions of particles were tested at concentrations from 0 to 400 μg per 2×10^6 cells. Cytotoxicity was assessed by the percent of total cellular lactate dehydrogenase (LDH) released by AM into the culture medium during incubation. Comparable physical association by ALM-coated and uncoated SI particles with AM was shown by scanning electron microscopy combined with X-ray energy spectrometry. These data show that SI coated with ALM is effectively phagocytosed by AM *in vitro* but is much less cytotoxic than uncoated SI. The surfactant lipids which presumably coat inhaled SI particles in the lung may reduce or delay their toxicity for AM.

Introduction

Alveolar macrophages (AM) from rats exposed to sublethal doses of alpha-quartz silica (SI) aerosols remain viable and functional immediately after dusting (1 hr-6 weeks) and are ultimately activated (3-12 months) despite large amounts of intracellular SI (*1*). The paradox between rapid SI-mediated cytotoxicity *in vitro* (*2*) and tolerance of intracellular SI *in vivo* might be explained if alveolar lining material (ALM) surfactant lipids coated SI particles following inhalation, which in turn ameliorated AM membrane injury following phagocytosis. Delayed *in vitro* cytotoxicity has been demonstrated when silica particles are coated with serum, phosphatidyl choline or poly-2-vinylpyridine-1-oxide (PVPNO) (*2*). This preliminary study was carried out to examine the cytotoxic effects of silica coated with ALM obtained from the cell-free bronchopulmonary lavage supernatant of rats whose AM were subsequently used in the assay.

Materials and Methods

Alveolar macrophages were obtained by bronchopulmonary lavage of respiratory disease-free Fischer 344 rats. Cells were cultured as monolayers on plastic cover slips (2×10^6 cells/mL medium) in Dulbecco's Modified Eagles Medium (DMEM) + 10% heat in activated fetal calf serum (FCS) and antibiotics. ALM was obtained from the cell-free lavage supernatant and concentrated 100-fold by a 40,000*g* centrifugation for 30 min. Biochemical analysis of the lavage fluid was performed as described by Low et al. (*3*) Respirable SI particles (Thermal American Fused Quartz, Montville, NJ) were incubated for 2 hr in the concentrated ALM prior to being added to the macrophage monolayers. Control SI particles were incubated in DMEM + FCS only. Suspensions of ALM coated and uncoated SI particles were tested at concentrations from 0-400 μg per 2×10^6 cells following a 24-hr incubation, and at 4 and 24 hr using 200 μg per 2×10^6 cells. Cytotoxicity was assessed by the percent of total cellular lactate dehydrogenase (LDH) released by AM into the culture medium during incubation (*4*). Lavage macrophage monolayers were fixed in buffered glutaraldehyde and examined with a JEOL JSM-35 scanning electron microscope equipped for KEVEX X-ray spectrometry for elemental analysis of SI particles.

Results and Discussion

Biochemical data for unconcentrated normal rat alveolar lavage cell-free supernatants are shown in

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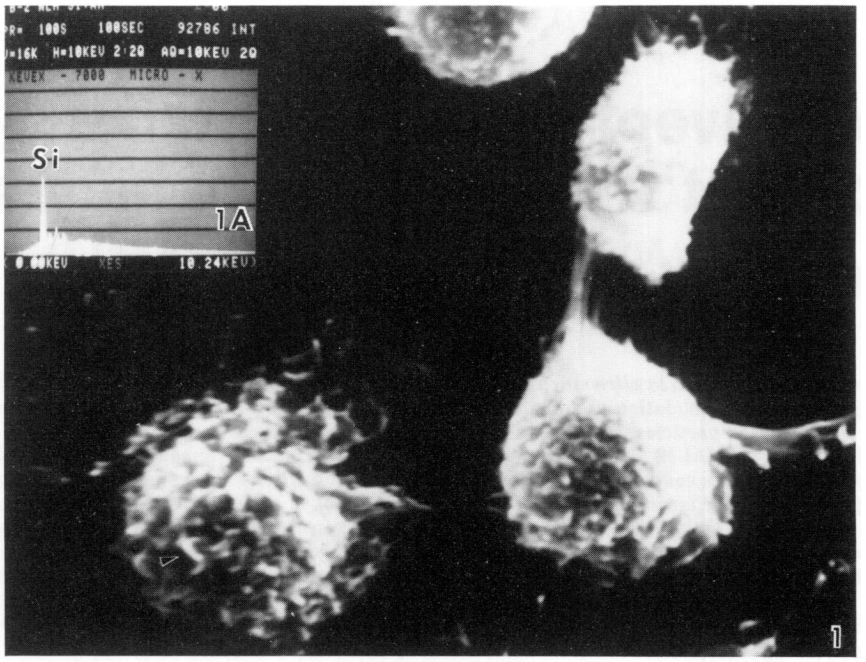

FIGURE 1. Scanning electron micrograph (SEM) of alveolar macrophages containing alveolar lining material-coated silica (arrow); and (*A*) X-ray energy spectrum of a particle demonstrating a high silicon peak. × 3300.

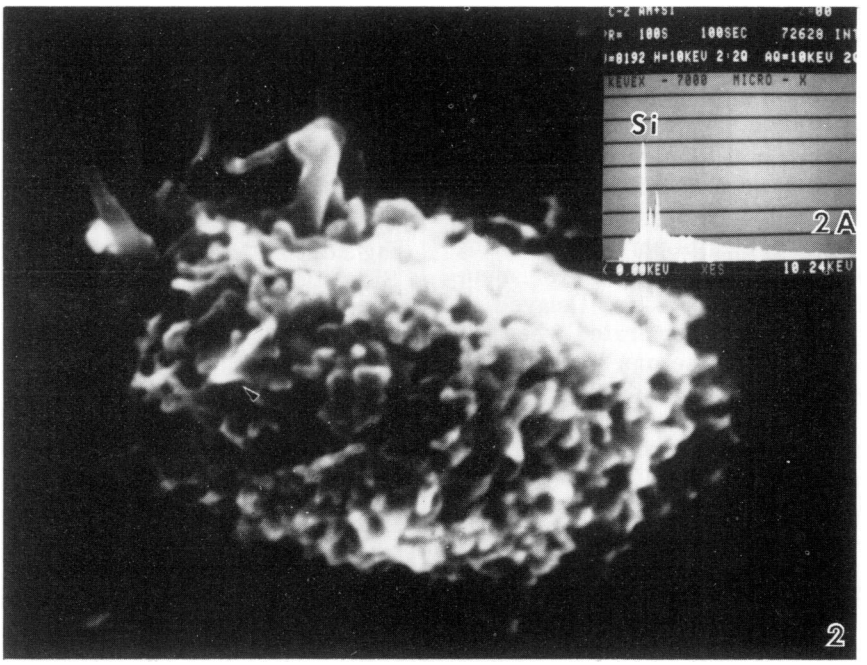

FIGURE 2. SEM of alveolar macrophage containing uncoated silica (arrow); and (*A*) X-ray energy spectrum of the particle demonstrating a high silicon peak. × 6500.

Table 1. Lavage biochemical data.

	Concentration in original lavage fluid, μg/mL[a]
Protein	44.8
Carbohydrates	4.6
Nonpolar lipid	6.15
Polar lipid	42.0

[a]Total ($N = 16$)/mean.

Table 2. Percent total LDH released into culture medium after a 24-hr incubation

Silica μg 2×10^6 macrophages	LDH released %	
	ALM coated	Uncoated
0	18 ± 1	18 ± 3
50	28 ± 2	27 ± 2
100	28 ± 2	40 ± 3
200	30 ± 4	61 ± 5
400	39 ± 3	74 ± 5

Table 1. This 800*g* supernatant contains protein, carbohydrates and a variety of lipids. The lipid rich fraction of this material was obtained following a 40,000*g* centrifugation for 30 min.

A comparable physical association by ALM-coated and uncoated SI particles with AM was shown by scanning electron microscopy which revealed intracellular particles in many of the macrophages (Figs. 1 and 2). X-ray energy spectrometry of these particles demonstrated an analytical spectrum identical to the silica particles which were added to the cell monolayers (Fig. 1*A*, 2*A*).

The percent total LDH released by macrophages exposed to ALM-coated and uncoated SI particles demonstrated a dose-response relationship (Table 2). The uncoated silica was highly cytotoxic at doses from 100 to 400 μg per 2×10^6 cells. This cytotoxic effect was significantly reduced when the particles were coated with ALM. The time for macrophage injury (LDH release) varies; uncoated SI caused the greatest release of LDH both at 4 and 24 hr. Although the response was similar for ALM-coated SI, the cytotoxic effects were significantly delayed and never reached the values obtained for uncoated particles (Fig. 3).

The mechanism by which SI particles cause AM death appears to depend upon injury to cell membranes especially those surrounding the phagolysosomes. This effect can be ameliorated by coating the SI particles with such substances as PVPNO, aluminum and phosphatidylcholine (*5*). Surfactant, a major constituent of ALM, is primarily a phosphatidylcholine.

Our data show that SI coated with ALM is effectively phagocytosed by AM *in vitro* but is much less

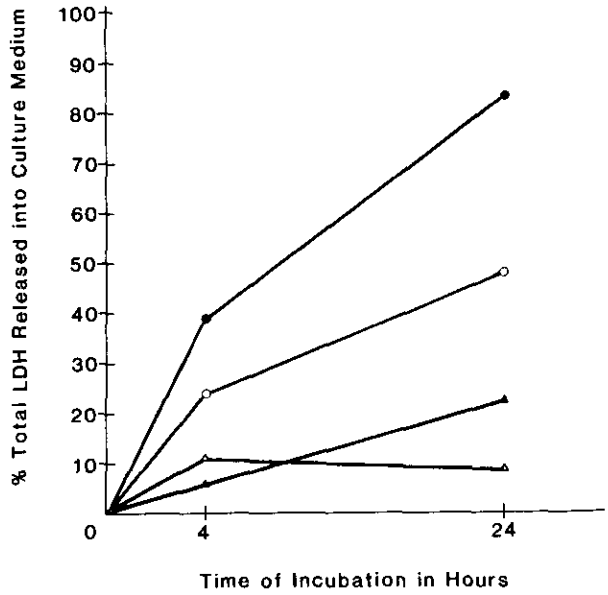

FIGURE 3. Influence of time on the release of lactate dehydrogenase (LDH) from cultures of rat alveolar macrophages (AM) exposed to (●) uncoated silica, (○) alveolar lining material coated silica, (▲) alveolar lining material and (△) untreated AM cultures. Cultures were exposed to 200 μg silica per 2×10^6 cells. Values represent the means of 6 separate cultures; the standard deviations are < 6% of the means and for clarity have not been shown.

cytotoxic than serum-uncoated SI. It is reasonable to assume that inhaled SI particles will become coated with a variety of material, including ALM. Consequently, inhaled SI particles that become coated may reduce or delay their toxicity for AM. Recent data suggest that it is not the dead or dying macrophage but the viable activated AM that may be responsible for releasing factors which promote pulmonary fibrosis (*1*, *6*).

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