# ALUMINUM INHALATION REDUCES SILICOSIS IN A SHEEP MODEL

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## INTRODUCTION

Although effective methods of prevention of silicosis have been known for years and implemented in the workplace through legislation, the disease remains of public health interest with some 200 new cases per year from an estimated workforce of 150,000 exposed workers in Canada.[8]

The recent availability of a soluble and inert compound, aluminum lactate (Al) has contributed to the renewed interest of aluminum therapy in silicosis. We have recently documented that Al suppresses the biological activity of quartz up to 10 months after exposure with faster clearance of the Al coated quartz particles.[4] In this study, we evaluate the efficacy of soluble Al aerosol inhalation to alter the biological activity and disease process associated with silica exposure in the sheep tracheal lobe model.

## MATERIALS AND METHODS

### Experimental Design

The flock of 40 sheep was randomly divided in 4 groups of 10 sheep. The first group was exposed to 100 ml phosphate buffered saline (PBS) infusion in the tracheal lobe followed by monthly inhalation of 10 ml PBS (group PBS-PBS). The second group was exposed to 100 ml PBS followed by monthly inhalation of 100 mg Al in 10 ml PBS (group PBS-Al). The third group was exposed to 100 mg Minusil-5® (Pennsylvania Glass Y Sand Co., Pittsburg, PA) in 100 ml PBS followed by monthly inhalation of 10 ml PBS (groups Si-PBS). The fourth group was exposed to 100 mg Minusil-5® in 100 ml PBS followed by monthly inhalation of 100 mg Al in 10 ml PBS (group Si-Al).

Minusil-5® particles have been well characterized,[3] 99.9% of diameter $<5$ $\mu$m and 95% $<1$ $\mu$m.

Exposures were carried out via bronchoscopic catheterization of the tracheal lobe bronchus and slow infusion of the suspension in the lobe. Inhalations were carried out 24 hr after bronchoalveolar lavage (BAL) with the animal intubated and breathing a mist of nebulized 0.01 to 4 $\mu$-sized liquid particles with a Bird Mark 8 pressure ventilator (Bird Corp. Richmond, Ca) set at a maximal pressure of 25 cm $H_2O$ for 20 minutes. Exhaled gases were vented outside the room. BAL were carried out after wedging the distal tip of the bronchoscope in the tracheal lobe bronchus by slow infusion of four 50 ml 39°C aliquots of PBS through a 50-ml syringe attached to the work channel of the bronchoscope and by gentle aspiration of the effluent. BAL were performed prior to exposures and at monthly intervals after. Animals were sacrificed and autopsied at month 6.

### Bronchoalveolar Lavage

The BAL cell differential populations were determined on cytocentrifuge smears stained with Wright-Giemsa. In the supernatant, albumin, IgG and IgM were determined by the immunochemical method (Cappel Lab. Inc., Downington, PA). The activity of lactate dehydrogenase (LDH) was measured by spectrophometric method. BAL phospholipids were measured by the technique of Bartlett[1,2] and contribution of lecithin and phosphatidylglycerol determined on the basis of their $PO_4$ content.

To assess interstitial lung matrix changes we looked at the glycoaminoglycan and fibronectin accumulation in BAL fluid. Oxidant production by alveolar macrophages was evaluated according to methods previously developed.[6]

### Histopathology

The tracheal lobe was identified and 9 samples of the lobe of each sheep were obtained and each evaluated histologically for intensity and profusion of lesions to yield our average pathologic index of disease.

### Determination of Quartz Concentration in Lung Tissue and Lavage

For each sheep in the study, a large fragment of the tracheal lobe was analyzed for quartz concentration using X-ray diffractometry.[7]

## RESULTS

### Lung Lavage Cellularity

The total and differential cell counts per lavage were similar in the group PBS-PBS and the group PBS-Al throughout the study. All silica-exposed sheep demonstrated at month 1 a 3 to 10-fold increase in cellularity which was sustained in the group Si-PBS but significantly attenuated to control levels in the group Si-Al ($p < 0.01$). In the Si exposed sheep, macrophages, lymphocytes and neutrophils were increased but there was no significant change in the eosinophil counts which were less than 4% at all times.

### Lung Lavage Biochemistry

Albumin averaged 70 ± 8 μg/ml in the group PBS-PBS and did not vary significantly over time. In the group PBS-Al, albumin levels were comparable ($p > 0.05$). In the Si-PBS group, there was a transient increase to 200% control level at month 1 with gradual return to control levels by month 5. In the Si-Al group, albumin remained at control level after Al inhalation. Lactate dehydrogenase levels in the PBS-PBS group averaged 6 ± 1 ml U/ml and did not vary significantly; in the PBS-Al group, levels were comparable ($p > 0.05$). In the Si-PBS group, there was a significant sustained 6 to 8-fold increase but in the group Si-Al, after initial increase, the levels of lactate dehydrogenase returned to the PBS-PBS group levels. Surfactant showed patterns of response similar to that of lactate dehydrogenase in the 4 groups.

### Fibronectin in Macrophage Supernatant

The production of fibronectin by alveolar macrophages in culture at month 6 was undetectable in groups PBS-PBS, PBS-Al and Si-Al but significantly increased at 2.1 ± 1 ng/$10^6$ cells per 24 hours in the SI-PBS group ($p < 0.01$).

### Oxidant Production and Glutathione

Lung cells of the PBS-PBS group at time 0 spontaneously released low amounts of superoxide (1.77 ± 0.55 nmol cytochrome-C reduced/$10^6$ cells-hr) and hydrogen peroxide (0.67 ± 0.34 μM/$10^6$ cells-hr), and the release of oxidants did not change during the study period in any of the groups. Glutathione in the bronchoalveolar lavage fluid of the PBS-PBS group at time 0 was 0.23 ± 0.05 μM and did not differ between groups throughout the study period.

### Lung Silica Content

The concentration of quartz in the lung parenchyma of the tracheal lobe of the sheep 6 months after initial exposure was as follows: in the group PBS-PBS and in the group PBS-Al, it was undetectable. In the group Si-PBS, it was 2.83 ± 0.98 μg/mg and in the group Si-Al it was 1.01 ± 0.74 ($p < 0.05$).

### Pathological Scores of Disease

The lung morphology of the sheep in the group PBS-PBS and PBS-Al remained normal. In the group Si-PBS, we found early nodular silicotic lesions composed largely of macrophages and lymphocytes with no evidence of collagen deposition comparable to those reported earlier,[3-5] with a pathological score of disease of 2.9 ± 1.0. In marked contrast, the group Si-Al had milder histological changes and a significantly lower score of 1.0± 0.3 ($p < 0.05$). In the Si-Al group, there was significant reduction of both the profusion and the severity scores ($p < 0.05$). Whereas well-defined silicotic nodules were seen in 8/10 sheep in the Si-PBS group, they were seen in only 1/10 of Si-Al sheep.

## DISCUSSION

This study documents that soluble aluminum lactate aerosol inhalation does not alter the normal biological processes in the bronchoalveolar milieu and does not produce significant pathological lung damage. In this study, we have observed that Al inhalation at monthly intervals significantly suppresses the alveolitis of silcosis, reduces the intensity and profusion of the disease process, and accelerates the clearance of quartz particles from the lung tissue.

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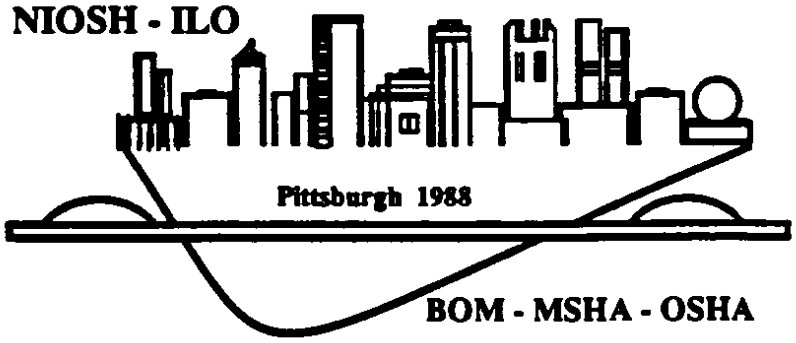


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