

STUDY ON SUPPRESSION OF SOLUBLE ALUMINUM AEROSOLS ON QUARTZ-INDUCED CYTOTOXICITY —Combined Effects of Both Aerosols in an Artificial Dust Atmosphere

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INTRODUCTION

Since 1930's it has been noted that aluminum (Al) and its compounds could prevent and treat silicosis. A number of studies have shown that Al could resist the cytotoxicity of SiO₂ particles on macrophages and erythrocytes, and suppress or lessen quartz-induced pulmonary fibrosis.^{1-4,6,9,12,18} More recently, it has been found that the mechanism of the effects of Al was the combination of it with SiO₂ particles, which altered some properties of the particle surface, so leading to the decrease of pathogenic activity of SiO₂.^{4,18} And the charges on SiO₂ particles were greatly reduced after the mineral particles were combined with Al, which was quite valuable to aggregation and dropping SiO₂ dusts. Consequently, if Al is used in dust workplace, such as spraying aqueous solution of aluminum compound or adding soluble aluminum into the water for dropping dusts from which the soluble aluminum aerosols are generated and probably combined with the SiO₂ dusts in air and/or inside lungs before or after the dusts are inspired into the worker's respiratory passage, it will give play to the dual effects on both resistance against the pathogenicity of SiO₂ and dropping the SiO₂ dusts in working atmosphere. It is possible to explore a new way to treat dusts in our industries and mines to prevent the development of silicosis.

The present study made an attempt to demonstrate whether or not the soluble aluminum aerosols could combine with SiO₂ particles in the atmosphere, and if they could suppress or lessen the cytotoxicity of the minerals on guinea pig alveolar macrophages and rat erythrocytes.

MATERIALS AND METHODS

Development of a Dynamic Inhalation Exposure System and Collection of SiO₂ Particles Combine with AIAs or DWAs

A dynamic inhalation exposure system was designed and developed to simulate an atmosphere interacting of both aerosols—SiO₂ particles and soluble aluminum aerosols (AIAs) or deionized water aerosols (DWAs). SiO₂ dusts generated by a dust generator (modified F-710 Electromagnetic feeder made in China Jiujiang) were conveyed into the exposure chamber (made of polymethyl methacrylate, 0.8M³ in volume) of the system to form the average concentration of 200mg SiO₂/M³. AIAs or DWAs, on the other hand, nebulized by an ultrasonic nebulizer

(JWC-2A Transistor Ultrasonic Nebulizer made in China Anshan, 0.5–10μm in aerosol diameter) were sprayed into the chamber from another inlet. In the exposure chamber both aerosols interacted on each other, and SiO₂ particles combined with and/or without AIAs or DWAs were sampled on a microfilter (0.45 μm in pore size, made in China Beijing) by WY-1 cascade impactor (7 catch stages, made in Chinese Academy of Preventive Medicine),¹¹ on the basis of which following experiments were done.

Measurement of Aluminum Combined on SiO₂ Particles

The contents of Al and SiO₂ in the sample on the microfilter were directly analyzed and measured by thin film X-ray fluorescence (XRF), based on modification of method of Cui et al.,⁷ with PW-1400 X-Ray Fluorescence Meter (made in Philip Inc.) from which the amounts of Al combined on SiO₂ particles were calculated and expressed in μg Al per cm² specific area of SiO₂, i.e., μgAl/cm² SiO₂. The specific area was calculated from the diameter and density of SiO₂ particles.¹⁶

Cytotoxicity Examination of the SiO₂ Particles Combined with AIAs

Using hemolysis assay and macrophage viability test monitored in vitro the cytotoxicity of AIAs—or DWAs—combined SiO₂ particle samples collected from the 5th stage microfilter of the Impactor, represented by antihemolysis rate (AHR) and macrophage viability index (MVI). The former was based on the hemolysis assay system previously described by Hefner et al.,¹⁰ Briefly, whole blood was taken from rat aorta abdominalis, washed 3 times in normal salt (NS) by centrifugation at 1500 rpm for 10 min., and the pellets, all of which are almost erythrocytes (RBC), were resuspended in NS to 20% (v/v). 1 mg SiO₂ or SiO₂-Al sample was added to 3 ml or the 2% RBC suspension, and the mixture incubated in 37°C water bath for 30 min and gently shook every 5 min. At the end of incubation, with centrifugating the mixtures the supernatants was measured for optical density (OD) at 420nm wavelength. Antihemolysis rate (AHR) was calculated from below formula:

$$\frac{(\text{HR of SiO}_2\text{-H}_2\text{O}) - (\text{HR of SiO}_2\text{-Al})}{\text{HR of SiO}_2\text{-H}_2\text{O}} \times 100\%$$

where HR (hemolysis rate)

$$= \frac{\text{OD of SiO}_2\text{-Al group}}{\text{OD of whole hemolysis group}} \times 100\%$$

MVI is complex indicators of damage effects of SiO₂ particles on macrophages, including rate of macrophage viability (RMV), intracellular K⁺ contents and lactate dehydrogenase (LDH) activity in the conditioned medium of macrophages cultured with the SiO₂ samples. Alveolar macrophages (7×10⁶/ml) harvested from male guinea pig lungs with bronchopulmonary lavage as described earlier⁹ were incubated with 150μg/mg SiO₂ (SiO₂-Al or SiO₂-H₂O samples) or normal salt (NS, as negative control) in medium RPMI1640 at 37°C in 5% CO₂-95% air for 4 hours, RMV(%) was assayed by trypan blue exclusion test, intracellular K⁺ contents of macrophages by Flame Atomic Absorption Spectroscopy and LDH activity in the conditioned medium by a colorimetric process.^{14,18} According to these parameters above, MVI was obtained from the formula:

$$\left(\frac{\text{RMV in SiO}_2\text{-Al group}}{\text{RMV in NS group}} + \frac{\text{K}^+ \text{ in SiO}_2\text{-Al group}}{\text{K}^+ \text{ in NS group}} \frac{\text{LDH in NS group}}{\text{LDH in SiO}_2\text{-Al group}} \right) \times 300\%$$

and the rate of AIs-suppressed toxicity of SiO₂ dusts to macrophages (RAST) was calculated from:

$$\frac{(\text{MVI in SiO}_2\text{-Al}) - (\text{MVI in SiO}_2\text{-H}_2\text{O})}{(\text{MVI in NS}) - (\text{MVI in SiO}_2\text{-H}_2\text{O})} \times 100\%$$

RESULTS AND DISCUSSIONS

Combination of Soluble Aluminum Aerosols with SiO₂ Particles

1) *The amounts of Al combined on SiO₂ particles at nebulization with various aluminum compounds.* Four soluble aluminum compounds, Al-I, Al-II, Al-III and Al-IV (synthesized and supplied by the Beijing Medical University School of Pharmacy) were prepared to 0.5mgAl/mg solution in deionized water. Experiments were respectively done with each of them under the consistent conditions such as nebulizing, sampling and measuring described above. As shown in Figure 1, all but Al-I aqueous solution were found to significantly increase the amounts of Al combined on

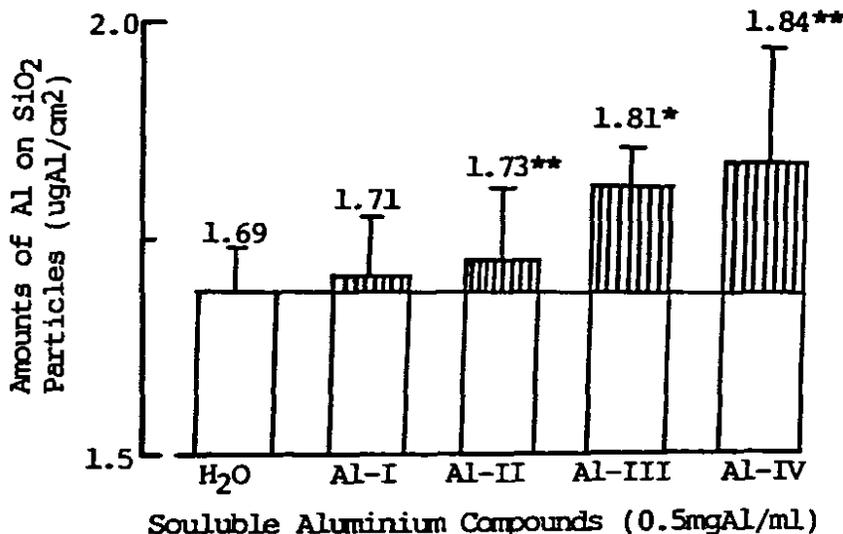


Figure 1. The amounts of Al combined on SiO₂ particles at nebulization using four soluble aluminum compounds (Al-I, Al-II, Al-III and Al-IV aqueous solution) and deionized water (H₂O). Mass Mean Aerodynamic Diameter (MMAD) of SiO₂ particles in these samples was 5.14μm. *P<0.01; **P<0.05 t-test with H₂O group; expresses the net amounts of Al combined on SiO₂ particles.

SiO₂ particles as compared with the DWAs-sprayed SiO₂ samples. The net amounts of Al on SiO₂ particles ranged from 0.09 to 0.15 μgAl/cm² SiO₂. Although the amounts of Al at the Al-III-nebulized aerosols group were situated between Al-II and Al-IV, considering that Al-III was in great resource and easy to be produced in our country, our investigations were mainly done on the interaction of Al-III aqueous solution aerosols with SiO₂ dusts as follows.

2) *Combination of SiO₂ particles with the AIs nebulized with the various concentrations of Al-III aqueous solution.* Al-III aqueous solution was diluted into 0.1, 0.3, 0.5 and 1.0 mgAl/ml with deionized water, and respectively nebulized into the exposure chamber with SiO₂ dusts at same conditions. Significant linearity between the Al-III aqueous solution concentrations and the amounts of Al combined on SiO₂ particles was observed in Figure 2, i.e., the amounts of Al on SiO₂ particles increased with increasing the concentrations of Al-III solution. It was noted that in the presence of nebulization by the lower concentrations of Al-III solution the amounts of Al on SiO₂ particles was 0.16 ± 0.03 μgAl/cm² SiO₂ and reached an effective level reducing pathogenicity of the mineral dusts, as the previous experiment in which the interaction of Al with SiO₂ was in test tube had suggested that while the amounts of Al on SiO₂ were 0.15 μgAl/cm² or so, the cytotoxicity of SiO₂ was obviously suppressed. When the SiO₂ samples combined with AIs or DWAs were washed 4 to 5 times in deionized water by centrifugation at 4000 rpm for 20 min., respectively resuspended and filtered on microfilter by suction for XRF

analysis, it was found that amounts of Al on the post-washed SiO₂ particles were yet more enough (data not shown) to lessen the SiO₂-induced cytotoxicity on the basis of preliminary experiments in test tube.⁴ This suggested that the combination of AIs with SiO₂ particles was quite firm and relatively stable.

3) *Combination of various SiO₂ dusts in diameter with AIs.* With nebulizing 0.5 mgAl/ml of Al-III aqueous solution into the SiO₂ dust atmosphere of the exposure chamber, the SiO₂ samples were collected on four stage microfilters from 2.54 to 6.97 μm MMAD (2.54, 3.68, 5.14 and 6.97 μm MMAD, respectively) by the cascade impactor (10L/min sampling flow rate), and measured by XRF to calculate the amounts of Al combined on various SiO₂ particles in diameter. The results shown that the higher the SiO₂ particle-size distribution was, i.e. the smaller SiO₂ particles, the more AIs could be combined with them (Figure 3). The net amounts of Al on the 2.54 μm SiO₂ MMAD were 9 times as many as that on the 6.97 μm SiO₂. In accordance with the deposition curve of SiO₂ particles in lungs, the 2 to 3 μm MMAD SiO₂ particles were of maximum deposition in lungs.¹⁷ Therefore, no doubt will the AIs in dusty workplace be much more significant as a preventive measure to silicosis.

Anti-cytotoxic Effect of Soluble Aluminum Aerosols on SiO₂ Dusts

To estimate the preventive effects of AIs on silicosis, the changes in cytotoxic effects (AHR and MVI) of the SiO₂

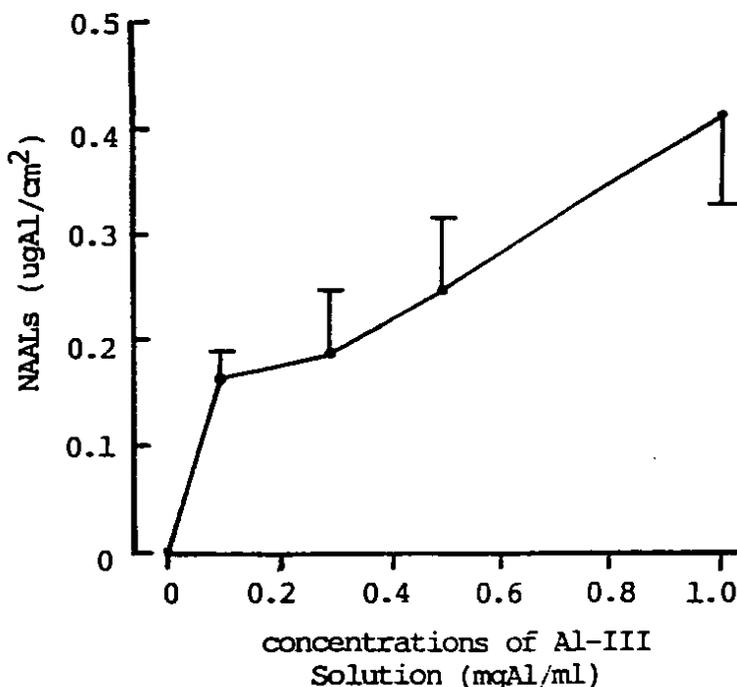


Figure 2. The combination of SiO₂ dusts with AIs nebulized by various concentrations (0.1, 0.3, 0.5 and 1.0 mgAl/ml) of Al-III aqueous solution. NAALs represents the net amounts of Al combined on SiO₂ particles, i.e., the amounts of Al on AIs-treated SiO₂ minus the ones on DWAs-treated SiO₂. The number of samples was 4 per group.

particles combined with aerosols of Al-III aqueous solution of various concentrations on guinea pig alveolar macrophages and rat erythrocytes were examined, which is rapid, sensitive and common marks to test cytotoxicity of mineral dusts and also an important evidence to reflect pathogenicity of them.⁵ The greater AHR and MVI are, the lower the cytotoxicity of SiO₂ particles is and the more effectively the AIs could resist SiO₂-induced pulmonary damage.

As seen in Figure 4, the AIs-combined SiO₂ particles greatly increased the AHR and MVI with statistical significance as compared to control group. To some extent the increases were correlated with the amounts of Al on SiO₂. When the amount of Al was $0.18 \pm 0.06 \mu\text{gAl}/\text{cm}^2$ (in the nebulizing group of 0.3mgAl/ml Al-III aqueous solution), AHR and MVI reached maximum level, 66.23% and 64.3%, respectively. At nebulization with the lower concentration

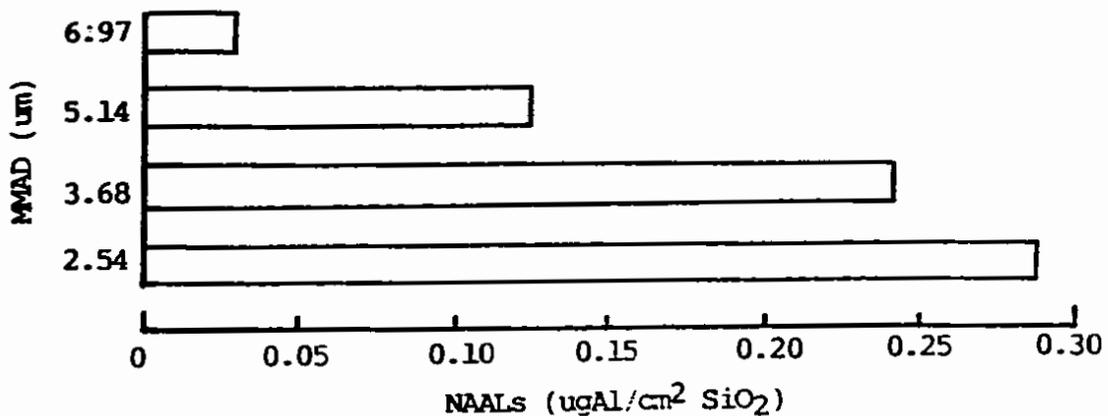


Figure 3. The net amounts of Al (NAALs) combined on the SiO₂ particles of various diameter (MMAD 2.54 to 6.97μm) sampled by cascade impactor (10L/min sampling flow rate).

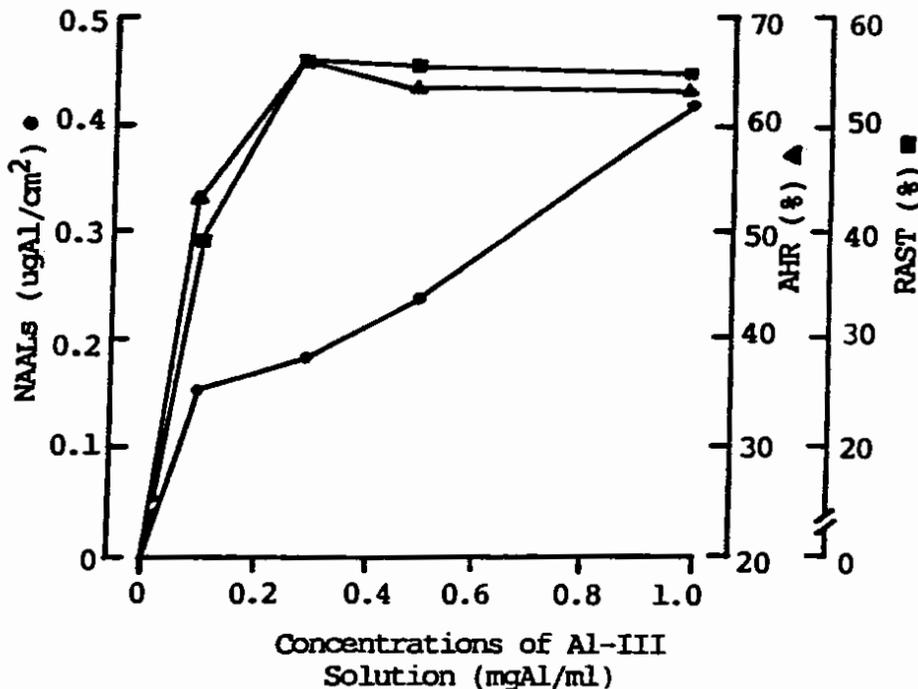


Figure 4. The suppressing effects of soluble aluminum aerosols on SiO₂-induced cytotoxicity to macrophages and erythrocytes. NAALs: net amounts of Al combined on SiO₂ particles; AHR: antihemolysis rate; RAST: rate of AIs-suppressed toxicity of SiO₂ dusts to macrophages.

of Al-III aqueous solution (0.1mgAl), the cytotoxicity of the SiO₂ particles was also suppressed by more than 40% (AHR by 48.32% and RAST by 52.1%).

CONCLUSIONS

The experimental results indicated that AIAs could not only stably combine with SiO₂ dusts in an atmosphere, but also effectively suppress the cytotoxicity of the minerals of macrophages and erythrocytes, which is consistent with the previous investigations in water system.^{4,18} Although the present study did not examine the change of surface charges of SiO₂ particles, according to the preliminary experiments in which the surface charges of SiO₂ particles significantly decreased once the SiO₂ particles were combined with Al, it may be speculated that AIAs could reduce the charges of SiO₂ surface and promote aggregating and dropping of the SiO₂ particles in a dusty atmosphere.

It must be pointed out that in this study the AIAs combined with SiO₂ dusts were only involved, but the other AIAs combined without SiO₂ in the air can be inspired into lungs together with SiO₂ dusts and interact with the minerals in respiratory passage, by which the AIAs could in the same way make resistance against the cytotoxicity of SiO₂ particles. Therefore, though in the nebulizing group with 0.1mgAl/ml Al-III aqueous solution the amounts of Al combined on SiO₂ particles and the suppression of Cytotoxicity were not maximum, the effective amounts of Al will greatly increase if it is considered that the AIAs can combine with SiO₂ dusts in respiratory passage. It is suggested that 0.1mgAl/ml Al-III aqueous solution or lower may be suitable for further experiments in lab and dust working sites. However, the optimal dosage of soluble aluminum compounds used in workplace remain to be further experimented.

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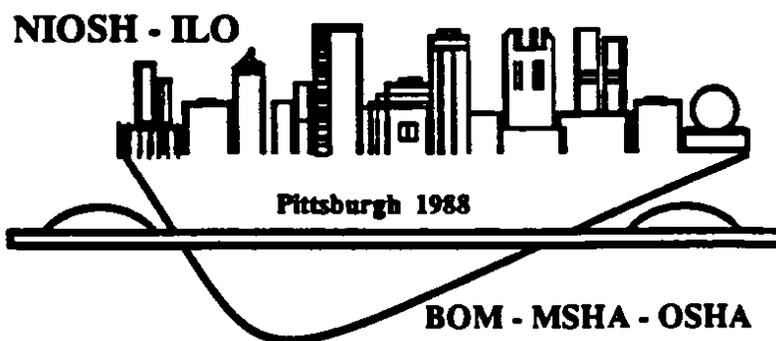
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