THE FIBROSIS AND OTHER MORPHOLOGIC CHANGES OF RAT LUNG CAUSED BY INTRATRACHEAL INJECTION OF DIFFERENT SIZES OF METALLIC ALUMINUM DUSTS

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INTRODUCTION

The study of relationship between pulmonary biological effect and sizes of dust is the basis for toxicological evaluation of dust and setting up dust sampling curve.[1,2] Although there were a few of this kind of studies in the past, it has not been intensively investigated. Several studies concerning the fibrogenic effect of different sizes of silica dust showed that the strongest fibrogenic size is under 5 μm, and particle size influenced the fibrotic response and other morphological characteristics of animal lung.[3,4,5] There was no study of the fibrogenic effect of different sizes of dust other than silica, such as metal dust. Also the morphological characteristics of animal lung caused by different sizes of dusts has not been evaluated thoroughly. In this paper, we present the experimental result of our studies of biological effects of different sizes of metallic aluminum dusts.

MATERIALS AND METHODS

Aluminum Dusts

Metallic aluminum dusts were obtained from an aluminum company which manufactured aluminum powders with different sizes. The powders were repeatedly separated by the method of sedimentation in ethyl ether. The particles with 1 μm, 5 μm, 10 μm, 15 μm optic diameters were collected, then these particulates were further separated by particulate centrifuge. The optic diameters of the separated aluminum particles were determined under microscope and presented in Table I. The chemical composition of these aluminum particulates were: aluminum, 96%; alumina, 3%; ferric oxides, 0.3%. No toxic heavy metals were detected (atomic absorption spectrometry).

Animal

Wistar outbred strain male rats weighing between 200 and 250 g were used for experiment.

Intratracheal Injection

50 mg of aluminum dust was suspended in 1.0 ml of saline. 5 groups of rats were used. Each group of rats were intratracheally injected respectively with 1.0 ml of 1 μm, 5 μm, 10 μm, 15 μm aluminum dust suspension or saline. The rats were sacrificed 6, 9 months after injection. A total of 100 rats were evaluated in this study and about 10 rats were evaluated in each group at each time point.

Lung Weight and Collagen Content

5 rats in each group were sacrificed with overdose of pentobarbital and their thoracic cavity was opened immediately. After removal of heart and tracheal, the wet lung weight was recorded. The dry weight of the lung was determined after the lung was cut into small pieces and dried in 110°C for 2 hours. The hydroxyproline was analyzed by acid hydrolysis of these dry pieces of lung tissue. The collagen content was estimated from determination of hydroxyproline.

Morphological Studies

About 5 rats in each group were sacrificed and their lungs were instilled drop by drop with 10% formalin. A longitudinal section of the lung, and the lymph node were cut and embedded in paraffin. The sections were stained with hematoxylin and eosin (HE), and also stained for reticulin and collagen.

The histopathologic changes of rat lungs were observed under

Table I

The Particle Sizes of the Experimental Aluminum Dusts

Size (μm)	Size distribution of aluminum particles (%)										
	<1	1-	2.5	2.5-	5	7.5-	10.0	12.5-	15	17.5-	20-
1	5	80	10	4	1						
5			4	10	75	10	1				
10				1	4	8	73	8	3	2	
15						1	2	5	70	18	4

microscope. The degree of nodular fibrosis was evaluated by Belt-King classification. In each lung, left lobe and right lobes were selected to measure the area of nodular fibrosis, area of alveolar wall thickening, area of emphysema. The area of nodular formation and alveolar wall thickening and emphysema were determined under 10 x 10 microscope with a 10 x 10 graticule (3.24 mm^2 in area). 12 locations in each lobe were randomly selected for quantitative observation and 12 lobes were evaluated in each group. The values presented were average of the 144 measurement.

RESULTS

Lung Weight and Collagen Content

Rats injected with aluminum dust had higher lung weight (wet and dry) and total collagen than control rats. The increase of lung weight and collagen content in 5 μm, 1 μm groups had statistical significance when compared with control group. Although the lung weight and collagen content were higher in 10 μm and 15 μm groups than control group, there was no statistical significance. (Figures 1, 2 and Table II)

Among animals exposed to different sizes of dust, the rats injected with 5 μm, 1 μm dusts have significantly higher lung weight and collagen content than those of rats in 10 μm, 15 μm groups. The order of increase of above parameter was: 5 μm > 1 μm > 10 μm > 15 μm. After statistical analysis of difference between each two groups, we found lung wet weight had significant difference between every two groups except the comparison between 10 μm and 15 μm groups. Dry weight and collagen had significant difference between every two groups except no statistical difference between 10 μm and 15 μm groups, 1 μm and 5 μm groups. (Figures 1, 2 and Table II)

Quantitative Lung Morphology

Six and nine months after intratracheal injection with different sizes of aluminum dusts, the major histological changes of rat lung were nodular fibrosis, interstitial fibrosis (or alveolar wall thickening) and emphysema. The degree of nodular fibrosis of rat lung was evaluated by Belt-King's grading. This result was shown in Table III. At end of experiment (9 months after injection), average degree of nodular fibrosis was 3.5 in 5 μm group, 2.6 in 1 μm group, 1.4 in 10 μm and 1.2 in 15 μm groups.

The extent of nodular fibrosis, alveolar wall thickening and extent of emphysema were determined by counting the number of graticules with positive changes in a total of 100 graticule under microscope. Table IV shows these quantitative results. The area (number of graticule with positive histological changes) of nodular fibrosis of rat lung was largest in 5 μm group, smallest in 15 μm group. The 10 μm group had significant larger area of alveolar wall thickening than those of other groups. The areas of emphysema were larger in 10 μm and 15 μm groups than 1 μm and 5 μm groups.

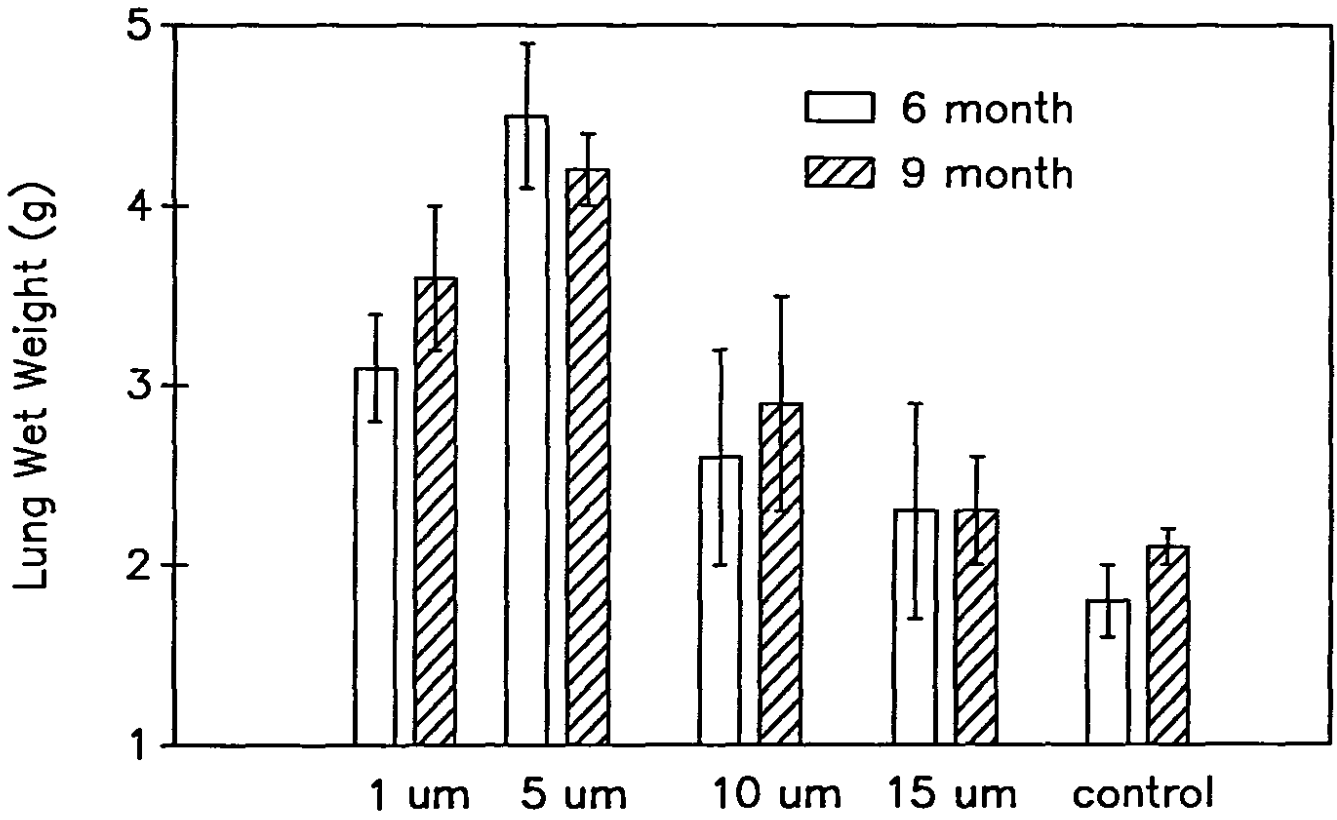

Figure 1. The changes of lung wet weights among rats intratracheally injected with different sizes of aluminum dusts.

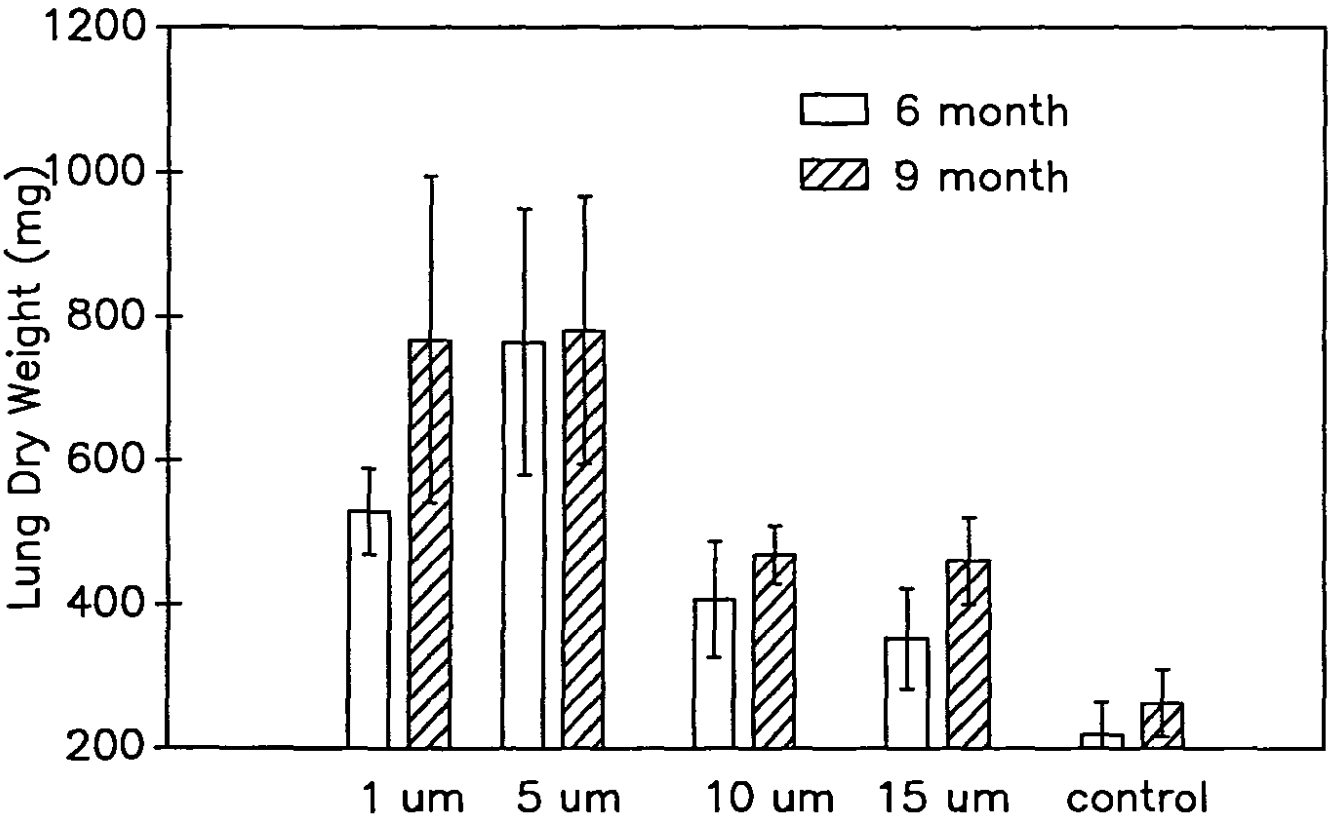

Figure 2. The lung dry weight among rats intratracheally injected with different sizes of aluminum dusts.

Table II

The Lung Collagen of the Rats Administered with Different Sizes of Aluminum Dusts

Group	Time (month)	No.of rats	Collagen (mg) mean	SD
1 μm	6	5	55.0	14.6**
	9	5	65.4	28.4*
5 μm	6	5	74.0	16.2**
	9	4	76.4	16.8**
10 μm	6	5	25.2	7.0
	9	5	34.6	13.2
15 μm	6	5	23.8	4.8
	9	5	23.2	12.1
control	6	5	15.8	3.6
	9	5	22.4	2.6

*: comparing with control group, P<0.05.
**:comparing with control group, P<0.01.

Table III

The Lung Fibrosis of Rats Administered with Different Sizes of Aluminum Dusts

Group	Time (month)	No.of rats	Degree of fibrosis#					Average
			I	II	III	IV	V	
1 μm	6	5	1	2	2	0	0	2.2
	9	5	0	2	3	0	0	2.6
5 μm	6	5	0	2	3	0	0	2.6
	9	4	0	0	2	2	0	3.5
10 μm	6	6	5	1	0	0	0	1.2
	9	5	3	2	0	0	0	1.4
15 μm	6	5	5	0	0	0	0	1.0
	9	5	4	1	0	0	0	1.2

#:Belt-King's grading.

Table IV

The Quantitative Measurement of Areas* of Nodular Fibrosis, Alveolar Wall Thickening, Emphysema

Groups	Time (month)	No.of lung	Area of nodular fibrosis		Area of alveolar wall thickening		Area of emphysema	
			mean	SD	mean	SD	mean	SD
1 μm	6	12	1.0	0.04	22.7	3.5	4.2	1.7
	9	12	2.8	0.10	15.7	3.3	8.4	1.5
5 μm	6	12	7.2	0.41	24.2	4.1	1.4	0.4
	9	12	6.6	0.22	18.0	4.3	7.2	1.8
10 μm	6	12	2.5	0.21	36.2	3.9	10.1	3.9
	9	12	1.6	0.11	27.3	5.2	17.2	2.9
15 μm	6	12	0.2	0.01	26.5	4.5	8.0	1.9
	9	12	0.3	0.04	17.8	5.6	16.4	3.3
control	6	12	--	--	--	--	2.3	0.7
	9	12	--	--	--	--	2.5	1.2

*: Number of graticule with positive pathologic changes in a total of 10 * 10 graticule (3.24 mm^2) under microscope.

Other Major Morphological Changes

9 months after intratracheal injection, rat lung and lymph nodes in different size groups had their own futures. In rats injected with 1 μm dust,there were slight thickening of alveolar wall with reticulin and slight collagen staining, inflammatory cells infiltration with reticulin and collagen proliferation around bronchiole, dust foci with reticulin staining within lymph nodes. In rats administered with 5 μm aluminum dust, there was significant alveolar wall fibrosis with intensive collagen staining, a large amount of inflammatory cell infiltration and collagen fiber proliferation around bronchiole and many dust-cell foci with collagen staining in lymph nodes. While in the 10 μm group, significant alveolar wall cell proliferation with only mild reticulin increase was observed, there was only slight increase of reticulin around bronchiole and a few dust foci in lymph nodes. The rat lung treated with 15 μm aluminum particles showed only slight alveolar wall thickening or dust deposition and no pathological changes around bronchiole and within lymph nodes. Lung and lymph nodes of control rats were normal.

DISCUSSION

According to the few past experimental studies on silica, the strongest fibrogenic size was below 5 μm. King et al studied 4 kinds of different sizes of silica. He found that the fibrogenicity was strongest in 1–2 μm silica, 0.5–1.0 μm and 2.0–4.0 μm silica were less fibrogenic, 4.0–8.0 μm silica only

produced slight fibrosis.[3] Kysela et al investigated 9 kinds of different silica dusts ranging from 0.7 to 35 μm. He reported that 1 μm silica caused strongest fibrosis response in animal lung, 7–10 μm silica caused cell nodule, 35 μm silica only produced alveolar wall thickening.[4] Goldstein and Webster reported that 2–5 μm silica had stronger fibrosis response than 1–2 μm silica.[5] All these results show that there is a strong fibrogenic size of dust.

In our study, we selected metallic aluminum dust to study the relationship between particle sizes and lung biological response. The metallic aluminum dust was selected because it is a known strong fibrogenic dust.[6,7,8,9] Metallic aluminum dust could cause nodular lung fibrosis, interstitial lung fibrosis and emphysema, which include nearly all chronic pathological changes from inhalation of toxic dust.

After 6, 9 months of intratracheal injection of 50 mg dust, we found 5 μm, 1 μm dusts were more fibrogenic than 10 μm, 15 μm dust. 5 μm and 1 μm aluminum dusts caused grade III even grade IV lung nodular fibrosis, intensive staining of collagen fiber in alveolar wall and higher collagen content than control animals; while 10 μm and 15 μm aluminum dusts produced only grade II or less than grade II lung nodule, increase of reticulin in alveolar wall and no significant increase of lung collagen protein. These results demonstrated that the strong fibrogenic size of metallic aluminum dust was smaller than 5 μm. 10 μm particles were less fibrogenic and 15 μm particles were non fibrogenic. Our results were consistent with those findings in silica.

Between 1 μm and 5 μm aluminum dust, we found 5 μm dust was more fibrogenic. This is similar to Goldstein and Webster's results. The strongest fibrogenic particle of aluminum dust is between 1 μm and 5 μm.

We also found that 10 μm aluminum dust produced significant alveolar wall thickening (cell proliferation) and slight emphysema. Snipes et al reported that 10–13 μm particle was removed from animal lung slower than 7–9 μm particles and even much slower than 3 μm particles.[10–11] Because a considerable amount of particles between 10 μm and 15 μm were found in the lung of pneumoconiosis workers, we should not overlook the biological effect of particles between 10 μm and 15 μm.

We concluded from our study that metallic aluminum particles between 1 μm and 5 μm caused strong fibrosis response and particles larger than 15 μm were not fibrogenic. Although 10 μm particle had slight fibrogenic effect, it produced alveolar wall thickening and slight emphysema. The biological effect of 10 μm particles should not be overlooked.

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Proceedings of the VIIth International Pneumoconioses Conference *Part*
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses *Tome*
Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis *Parte*

II

Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
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November 1990

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DHHS (NIOSH) Publication No. 90-108 Part II