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The Translocation and Fate of Sized Man-Made Mineral Fibers following Exposure by Intratracheal Instillation or Inhalation in Rats

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ABSTRACT

A number of studies have suggested that both the length and diameter of glass fibers are important parameters in determining their deposition and translocation in the lung and in the subsequent pathological response by the lung. However, the fibers used in these studies had broad size distributions and were often administered in a highly artificial manner. To better characterize the biological response to glass fibers, a study is being conducted to determine the translocation and ultimate fate of fibers of defined sizes after introduction into the respiratory tract of rats by both instillation and inhalation. The fibers have geometric mean diameters of 1.5 μm (σg = 1.11) and lengths of either 5 μm (σg = 1.49) or 60 μm (σg = 3.76).

Serial sacrifices following intratracheal instillation of either 2 mg or 20 mg doses have shown differences in the response to the two sizes of fibers. The short fibers appear to lie primarily within mononuclear phagocytes in both the lung and lymph nodes. The majority of long fibers, however, cannot be to-

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tally engulfed by macrophages, nor are they cleared to the lymph nodes, although smaller fragments accompanying the long fibers may be so cleared. The long fibers produce a striking foreign body reaction in the lung, particularly when impacted in the bronchi. Significant numbers of long fibers, but few, if any, short fibers are found in the plural cavity.

A "trachea only" inhalation method was used to expose rats to approximately 500 fibers/cc for one hour. Between 30,000 to 50,000 fibers were deposited in the lung of each rat. Serial sacrifices at intervals similar to those in the instillation study will permit comparison of the effects following these two methods of administration.

INTRODUCTION

The length and diameter of glass fibers determine not only their deposition in the lung[2,4] through inhalation but are also thought to be important factors in fiber translocation and subsequent pathological response.[5] Stanton and Wrench[3] have demonstrated that when glass fibers are placed directly into the pleural cavity of rats, they cause mesothelioma. It remains unclear whether fibers actually reach the pleural cavity when administered in a less artificial manner through the tracheal bronchial tree. In the past resolving the relationship of fiber size to these effects has been compounded by the difficulty in separating different lengths of fibers of the same diameter aerodynamically. Using fibers manufactured to specified size distributions, this study has examined the deposition, translocation, and fate of glass fibers after introduction into the respiratory tract of rats.

Three routes of exposure were initially considered: intratracheal instillation of aqueous suspension, nose-only inhalation exposure, and insufflation of a very thick air suspension of fibers. The intratracheal instillation provided a means of administering large quantities of fibers into the lung with relative ease. Rats are obligatory nose breathers, and when inhaled through the nose the majority of the long fibers used in this study were found to deposit in the tortuous ~~nasal pharyngeal~~ nasopharyngeal region of the rat and not get into the lung. Hence, a trachea-only inhalation exposure method was developed to expose animals to fiber aerosols. With the successful development of the trachea-only inhalation methodology, insufflation of a very thick air suspension of fibers was not pursued further.

This report reviews the methods and techniques developed for exposure by intratracheal instillation and presents the glass fiber clearance data and histological results through six months post exposure.

MATERIALS AND METHODS

The fibers were manufactured to either 1.5 x 5 μm or 1.5 x 60 μm in size[1] ( ). The length distribution of each group of fibers is shown in Figure 1 with the shorter fibers having a geometric mean length of 5.1 μm (SD = 1.49) and the longer fibers a geometric mean length of 54 μm (SD = 3.76). Scanning electron micrographs of the fibers are shown in Figures 2 and 3. The fibers were neutron activated, and ^{65}Zn was determined to be the best tracer nuclide to allow quantification of deposition and clearance of the fibers in the rats in vivo.[1]

Animals

These studies were conducted with male Fisher 344 rats purchased from Charles River. The exposures were begun when the animals were approximately 15 weeks old. They were housed in wire cages, allowed food and water ad libitum, and maintained on a 12 hr light cycle at 22 ± 2 C, 50% RH.

Intratracheal Instillation

The techniques for intratracheal instillation of the glass fibers have been described earlier[1] ( ).

Thirty rats per group were instilled with either 1.5 x 60 μm (Group 1) or 1.5 x 5 μm fibers (Groups 2 and 3) that were tagged by activation as outlined in Table I. Rats in group 2 were instilled with approximately the same number of fibers as rats in group 1 and rats in group 3 were given the same weight of fiberglass as rats in group 1. Since the fiber concentration administered to

each rat was determined by radioassay immediately after exposure, only 15 rats could be instilled on any given day. With this limitation and the necessity to radioassay the groups at the same time intervals after exposure, the instillation protocol shown in Table I was necessary.

In addition, to insure that if long term effects were noted that these effects were from the fibers and not the small amount of radioactivity present in them, 50 rats/group were exposed to non-activated (or cold) fibers. This number was chosen from statistical considerations to permit detection of a ~~two~~ true tumor difference between the activated and non-activated exposure groups at the end of two years.

Necropsy

The rats were sacrificed by anesthesia with pentabarbatol and subsequent exsanguination via the descending aorta. The lungs were then perfused in situ with heparized isotonic saline through the pulmonary artery, removed, and inflated with glutaraldehyde vapor using an endotracheal tube (20 cm water); and then infused with glutaraldehyde fixative through the pulmonary vasculature (20 cm water).

After the lungs were fixed, the hylar lymph nodes, thymus, and adipose tissue were resected. In addition, the diaphragm, gastrointestinal tract, liver, spleen, kidney, brain, and femur were removed for histological examination.

Tissues were embedded in parafin, sectioned (10 μm), mounted on a glass slide, and stained with either hemotoxylin and eosin (H&E), reticulum, or trichrome blue stains. Histoclad mounting media was used for all sections.

Microscopy

The standard histological sections were viewed by light field microscopy for histopathological evaluation and by a pseudo dark field technique for evaluation of glass fiber numbers and location in the tissue sections. In addition, scanning electron microscopy was performed on fixed tissues prepared by a critical point drying technique. The size distributions of the glass fibers were determined by microscopic measurement with the aid of a Zeiss MOP-3 image analyzer.

RESULTS

ANIMAL WEIGHTS

Rats were weighed in each group at equal intervals after exposure. Since the time of exposure was offset as shown in Table II and the rats were all of the same age when received, the weight curves are shifted reflecting this offset in exposure. The growth curves for each group are not significantly different as determined by an F test on the slopes and shown by the parallel curves in Figure 4.

GLASS FIBER CLEARANCE

The clearance of glass fibers from the rats was determined by *in vivo* radioassay of each animal. It is important to note, however, that this technique only accounts for whole body clearance of fibers and is not sensitive to translocation of fibers within the rat.

The clearance curves for each of the three exposure groups through ~300 days are shown in Figures 5, 6, and 7. The low dose short fiber clearance is significantly different $P < 0.01$ (as determined by an analyses of variance) from

either the long or short high dose group. However, the two high dose groups are not significantly different. This is reflected in the percent remaining at ~300 days as shown in Table II. If the data is evaluated by a three component exponential model in terms of short, intermediate, and long term phases of clearance (Table III), significant differences ($P < 0.01$) are noted in the short and intermediate phases of the two high dose groups.

SCANNING ELECTRON MICROGRAPHS

To examine by electron microscopy the lungs' response to the two types of fibers, rats instilled with either 20 mg of long or 20 mg of short fibers were sacrificed two weeks after exposure. The lungs of rats exposed to long fibers are shown in Figures 8 and 9. Macrophages attempting to phagocyte the long fibers are easily noted (Figure 8). In addition, other areas are seen with clumps of fibers present associated with a dense cellular response (Figure 9).

This is in contrast with the response to the short fibers seen in Figures 10 and 11. The short fibers are found in large numbers in macrophages and for the most part are completely engulfed by these cells. Some fibers are seen in free airspaces (Figure 11). Little of the dense cellular response noted with the long fibers is evident.

HISTOLOGICAL RESULTS

The histological results reported here are from the first serial sacrifice at six months post exposure involving four animals/group. In addition, a few animals died under anesthesia for the radioassay procedure. These animals were sacrificed as described above and the histological results presented as well.

To illustrate the numbers and locations of fibers and their associations with the histological observations, photographs for many of the light field H&E

stained sections are accompanied with a dark field image of the same field which highlights the fibers. It should also be noted that with 10 μm sections of the lung, it is likely that a 60 μm fiber would be cut in the sectioning process. This, together with the fibers being somewhat randomly orientated in the lung may give the appearance that the long fibers are somewhat shorter than 60 μm.

1.5 x 60 μm Fibers, 20 mg Exposure

At four days post-exposure a notable cellular response in association with large numbers of fibers is observed (Figure 12). The initial stages of granuloma formation is evident at 10 days (Figure 13), however, no giant cells are observed. Many fibers can be seen either free in the alveolar space or in association with macrophages. At 40 days (Figure 14) the granulomas have become more defined and giant cells within these granulomata are easily seen.

Microscopic examination of the six month sacrifice of four rats/group showed that the lungs contained numerous granulomata which were well developed, containing epitheloid cells and giant cells, and which contained large numbers of fibers (Figure 15). These granulomata were often of peribronchiolar in orientation (Figure 16); fairly uniformly distributed throughout the sections, and sharply demarcated at their margins. Moderate numbers of fibers occur singly or in small groups outside the granulomata (Figure 17), often in the interstitum, however, relatively few fibers are seen within the alveolar spaces. Hemosiderin is present in association with the fibers. No 60 μm fibers were seen in the lymph nodes although occasional smaller fibers or fragments of larger ones were present (Figure 18).

1.5 x 5 μm Fibers, 2 mg Dose

No animals in this group died prior to the six month sacrifice.

At six months, animals exposed to 2 mg of the short fibers revealed a generally heterogenous distribution of the residual fibers throughout the pulmonary tissue (Figure 19). There were few or no fibers in most areas. Where found, the fibers are present in association with variable sized aggregates of mononuclear cells, small groups of which often appear to be interstitial (Figure 20). There are, however, some larger fiber-cell aggregates with components that are within air spaces, air spaces now largely obscured by the abundance of cells. The mononuclear aggregates are not arranged into structured granulomata and no giant cells are present. Small amounts of hemosiderin are found in association with fiber aggregates.

Lymph nodes were found to contain fibers occurring in dense aggregates of macrophages. There was wide interanimal variability in the extent of lymph node involvement and the size of lymph nodes within each exposure group.

1.5 x 5 μm fibers, 20 mg dose

From the single animal that died at 40 days post-exposure we see a response that is greater in intensity but very similar to that which is described below.

Microscopic evaluation of the lungs of the animals from the six month sacrifice reveals a distinct, usually peribronchiolar granulomatous reaction to fibers in some areas, while in other areas fibers occurred in dense aggregates in association with a relatively sparse cellular response. No giant cells were seen within these granulomata. Fibers were clearly evident within interstitial macrophages (Figure 21) and in some areas could be seen in alveolar macrophages

(Figure 22). In still other areas, lymphocytes rather than histiocytes were the predominant cell seen in relation to the fibers. Granulomata containing dense aggregates of fibers were seen in paratracheal lymphoid tissue (lymph sumps) (Figure 23). There appeared to be a 2 to 3 fold range in amount of residual fiber and associated cellular reaction in this exposure category. In the lymph nodes large numbers of fibers were seen in dense aggregates associated with a granulomatous response (Figure 24). Again, there was a large amount of interanimal variability in the extent of lymph node involvement and size of lymph nodes.

DISCUSSION AND CONCLUSION

In both the 2 and 20 mg dose 1.5 x 5 μm exposure group, the fibers appear to lie primarily within mononuclear phagocytes in both the lung and lymph nodes. The process of engulfment of the fibers by the macrophages seems to take place for the majority of fibers within a few days after exposure. At ~290 days after exposure 80-90% of the fibers deposited in the lungs of the rats are cleared from the animal with the low-dose (2 mg) short fiber group having a significantly faster clearance than the high-dose (20 mg) group.

In contrast, the majority of long fibers cannot be totally engulfed by macrophages, nor are they cleared to the lymph nodes, although smaller fragments accompanying the long fibers may be so cleared. The long fibers produce a striking foreign body reaction in the lung, particularly when impacted in the bronchi. Long fibers are found in the pleural cavity with histological examination revealing (Figure 17) single fibers near the pleural surface of the lung.

The whole-rat clearance curves for the long fiber exposed animals are different from those for the short-fiber exposed animals only in the early stages

of clearance (<60 days). The majority of both types of fibers are thought to be cleared by way of the cilliated mucous escalator; however, short fibers are also translocated to the lymph nodes while long fibers do not reach the lymph nodes in significant numbers. Instead, long fibers appear to be bound in foreign body-type granulomas. While these differences are apparent from the histological findings the clearance curves are not sensitive to fiber translocations within the rat.

The formation of large granulomas as opposed to a more diffuse response to the long fibers may in part be due to the single 20 mg dose administered. To circumvent this, another group of rats ~~are~~ is being exposed by intratracheal instillation repeatedly over several weeks to low doses of ? sized fibers. Comparison of the fiber distributions in the lungs from these two studies as well as the distribution from our inhalation study will help evaluate the validity of these exposures.

While there are clear differences in the lung's response to the different size fibers, there is no evidence thus far to indicate that either type of fiber produces fibrosis. These studies are continuing, however, with serial sacrifices of four rats/group scheduled at six month intervals.

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TABLE I

Fiber Glass Instillation Schedule*

Exposure Group	Fiber Size and Dose	Number of Rats Instilled	Date of Instillation
Group I	1.5 x 60 μm	14	2/26/79
	20 mg	16	2/28/79
Group II	1.5 x 5 μm	14	3/26/79
	2 mg	17	3/28/79
Group III	1.5 x 5 μm	15	3/12/79
	20 mg	15	3/14/79
Group IV	Saline	15	4/9/79
	Control	15	4/11/79

*The instillation schedule was staggered as shown to enable radioassay of each rat following exposure.

TABLE II

FIBER CONCENTRATION

Fraction Remaining at ~300 days after Exposure

Exposure Group	N	Mean	(Std. Dev.)
20 mg 1.5 x 60 µm	21	0.15	(0.04)
2 mg 1.5 x 5 µm	24	0.09	(0.04)
20 mg 1.5 x 5 µm	22	0.18	(0.02)

TABLE III

FIBER GLASS CLEARANCE

THREE COMPONENT EXPONENTIAL MODEL

Exposure Group	Clearance Half Times (days) 1-10 days	11-60 days	61-300 days
20 mg 1.5 x 60 μm	20	58	173*
2 mg 1.5 x 5 μm	14	43	115*
20 mg 1.5 x 5 μm	29	63	173*

*Not significantly different ($P \leq 0.01$). All other half times are significantly different from one another ($P \leq 0.01$).

FIGURE CAPTIONS

Figure 1 Length distribution of 1.5 x 5 μm and 1.5 x 60 μm glass fibers.

Figure 2 Scanning electron micrographs of 1.5 x 60 μm fibers at 500 and 5000 X. The spheres are latex beads of 2.02 μm.

Figure 3 Scanning electron micrographs of 1.5 x 5 μm fibers at 500, 2000, and 10,000 X.

Figure 4 Mean rat weights for each exposure group as a function of time.

Figure 5 Whole rat clearance of glass fibers as a function of time. 1.5 x 60 μm fibers, 20 mg dose.

Figure 6 Whole rat clearance of glass fibers as a function of time. 1.5 x 5 μm fibers, 2 mg dose.

Figure 7 Whole rat clearance of glass fibers as a function of time. 1.5 x 5 μm fibers, 20 mg dose.

Figures 8,9 Scanning electron micrographs of lungs from rats exposed to 20 mg of 1.5 x 60 μm fibers approximately 2 weeks after exposure.

Figures 10,11 Scanning electron micrographs of lungs from rats exposed to 20 mg of 1.5 x 5 μm fibers approximately 2 weeks after exposure.

Figure 12 H&E stained 10 μm section of lung from 1.5 x 60 μm 20 mg exposure group 4 days after instillation, 3X.

Figure 13 H&E stained 10 μm section of lung from 1.5 x 60 μm 20 mg exposure group, 10 days after instillation, 10X.

Figure 14 Light and dark field (L&D) of 1.5 x 60 μm, 20 mg exposure group at 40 days.

Figure 15 1.5 x 60 μm, 20 mg exposure group at 6 months (L&D).

Figure 16 Peribroncheal granulomas, 1.5 x 60 μm, 20 mg, 6 months (L&D).

Figure 17 Single long fibers near the pleural surface of lung, 6 months (L&D).

Figure 18 Lymph nodes of 1.5 x 60 µm, 20 mg group at 6 months showing fiber fragments.

Figure 19 1.5 x 5 µm, 2 mg exposure group, 6 months, 3X.

Figure 20 1.5 x 5 µm, 2 mg exposure group, fibers in macrophages, 6 months (L&D).

Figure 21 Interstitial macrophages containing 1.5 x 5 µm fibers, 20 mg exposure group, 6 months (L&D).

Figure 22 Alveolar macrophage containing 1.5 x 5 µm fibers, 20 mg exposure group, 6 months (L&D).

Figure 23 Lymph sumps with aggregates of macrophages with 1.5 x 5 µm fibers, 20 mg exposure group, 6 months (L&D).

Figure 24 Lymph nodes with dense aggregates of macrophages with 1.5 x 5 µm fibers, 20 mg, 6 months (L&D).