

Persistence of Long, Thin Chrysotile Asbestos Fibers in the Lungs of Rats

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The distribution of inhaled mineral fibers in the lung determines the site and severity of disease caused by the fibers. Some of our recent work has described the fate of inhaled asbestos fibers in rodents. After a brief inhalation exposure, asbestos fibers are deposited primarily at the first alveolar duct bifurcations, and fibrotic lesions are initiated. These sites of deposition occur as close to the visceral pleura as 220 μm . Several studies have suggested that short fibers are cleared from the lung more efficiently than long ones, and our data support this view. Our laboratory has shown that aerosolized chrysotile fibers longer than 16 μm can be deposited in the peripheral lung parenchyma of rats, and the measured clearance rate of these fibers is not significantly different from zero. Chrysotile, but not amphibole, fibers split longitudinally, so that the number of retained chrysotile fibers $\geq 16 \mu\text{m}$ in length increases over time. We have not observed significant changes in chemical composition of chrysotile fibers up to 30 days post-deposition in the rat. Nor have we observed translocation of chrysotile fibers from the "central" regions of the lung toward the subpleural regions. However, 1 month after a single 3-hr exposure to chrysotile asbestos, the longest, most pathogenic fibers persist throughout the lung parenchyma. These retained fibers have the potential to cause disease in both parenchyma and pleura. — *Environ Health Perspect* 102(Suppl 5):197-199 (1994)

Key words: asbestos, chrysotile, pulmonary clearance, particle translocation, pleural disease

Introduction

Pulmonary deposition, clearance, alteration (leaching and splitting), and translocation of mineral fibers play important roles in determining the sites and severity of disease caused by these fibers. In this report, we review some of our recent findings on the fate of inhaled chrysotile asbestos in the lungs of rats.

Deposition

After brief inhalation exposures in rats and mice, asbestos fibers are deposited primarily at alveolar duct bifurcations, and fibrotic lesions are confined to these sites (1,2). Recently our laboratory has been investigating the mechanisms of asbestos-related pleural disease. We have sought to study the differential deposition of chrysotile aerosols in peripheral (subpleural) and central regions of the rat lung (3). This study showed no differential deposition of fibers in central versus

peripheral regions. Indeed, even the longest fibers classified ($\geq 16 \mu\text{m}$) were deposited abundantly in the peripheral region within 1 mm of the visceral pleura. We attempted to reconcile this somewhat surprising finding with previous observations showing that deposition occurs largely at the first alveolar duct bifurcations (1). Thus, we measured the distance from first alveolar duct bifurcations to the nearest visceral pleural surface in tissue sections from the caudal half of the left lung of rats (Figure 1). Roughly half of all first-duct bifurcations are found within 1 mm of the visceral pleural surface, which corresponds to the peripheral tissue resected and digested in our previous study (3). The minimum bifurcation/pleural distance measured was just over 200 μm . These findings show that fibers of the putatively most carcinogenic sizes ($\geq 10 \mu\text{m}$ length, $< 1.5 \mu\text{m}$ diameter (4)) can be deposited readily within a few hundred microns of the visceral pleura of the rat. The significance of this finding is discussed below.

Clearance

Several laboratories have shown that, for modest exposures, a large proportion of the mass of inhaled asbestos is cleared from the lungs of rodents within 30 days (5-7). Given the well-known role of fiber

dimensions in both pulmonary fibrosis and pleural carcinogenesis (4,8-10), description of pulmonary clearance kinetics in terms of fiber mass is not adequate.

There is now sufficient experimental data to describe the differential clearance of asbestos fibers in terms of fiber length. Several workers have noted that the average length of retained asbestos fibers increases after an inhalation exposure (5,6,9-11). These experiments suggest that, qualitatively, short asbestos fibers are cleared from the lung more effectively than long fibers. However, few workers have been able to quantify the dimensions of "short" versus "long" fibers. Morgan et al. (12), employing monodisperse fiberglass, showed that fibers greater than 30 μm in length are not cleared readily from the lung. Recently, a novel statistical sampling scheme has been employed to show that asbestos fibers greater than 16-20 μm in length are not cleared effectively from the lungs of rats or hamsters (3,13).

These findings are interesting in light of work suggesting that longer mineral fibers are more fibrogenic than short fibers (9,10,14,15). Our findings show that the longest, most fibrogenic fibers are retained in the lung for extended periods. The slower clearance of the longer fibers may partly explain their enhanced fibrogenicity.

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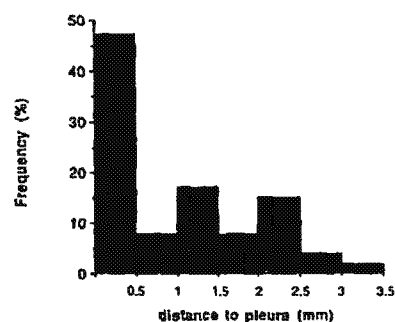


Figure 1. Distribution of alveolar duct bifurcation/pleural distances. Distances from first-duct bifurcations to the nearest visceral pleural surface were measured on tissue sections of the caudal half of the left lung lobes of 11 adult rats. A total of 106 bifurcations was measured. Lungs were fixed intratracheally, giving comparable inflation to that of the lungs used for peripheral/central digests (3). Approximately 50% of first-duct bifurcations are within 1 mm of the visceral pleura. The minimum bifurcation/pleural distance measured was 0.22 mm. Therefore, resected "peripheral" tissue (3) should contain substantial numbers of asbestos fibers deposited at first duct bifurcations.

Alteration

Asbestos fibers can undergo longitudinal and transverse splitting in the lung. The former causes a decrease in fiber diameter, while the latter causes a decrease in fiber length. Chrysotile fibers readily undergo both types of splitting in the rodent lung, while amphibole fibers do not (3,5,6,16, 17). We have found that long ($\geq 16 \mu\text{m}$) chrysotile fibers are not cleared at a significant rate up to 30 days after deposition in the rat, but that they do split (3). The number of retained long fibers increased over time, and the average diameter decreased (Table 1). The mass of retained long fibers decreased slightly over time while the surface area increased slightly, though neither trend was statistically significant (Table 1). These findings suggest that, in our model, long fibers undergo longitudinal splitting but little transverse splitting. In contrast, Churg et al. (17) found that

chrysotile split both longitudinally and transversely within 30 days of instillation into the lungs of guinea pigs. Our findings are not necessarily at odds with those. We did find evidence of transverse splitting of fibers shorter than $16 \mu\text{m}$ (3). In addition, Churg et al. classified fibers only up to a maximum $>10 \mu\text{m}$ in length, and they did not compensate for longitudinal splitting within a length category as we did (3). Our previous work has shown that there is a marked change in the fate of fibers $\geq 16 \mu\text{m}$ in length as compared to shorter fibers, probably due to the inability of pulmonary macrophages to phagocytize these fibers (3). This failure of phagocytosis may play a role in splitting of the longest fibers.

Leaching of magnesium from chrysotile fibers has not been observed consistently in the lungs of experimental animals. We have found no significant leaching of long chrysotile fibers after 30 days residence in the rat lung (Table 1). Similarly, Churg et al. (17) did not observe leaching of chrysotile after 30 days residence in the guinea pig lung. Others have observed leaching of chrysotile fibers after 1 month to 2 years of residence in the lungs of hamsters and rats (11,16). Bellmann et al. (16) found a decrease of 1 to 2% per day in the Mg/Si ratio of chrysotile fibers resident in the rat lung for 30 days. Calculations of statistical power show that our experiment had an 80% chance of detecting a decrease in Mg/Si of 1.3% per day. It is possible that the discrepancy arises from the restriction of our analysis to fibers $\geq 16 \mu\text{m}$ in length, which are not likely to be phagocytized completely by macrophages (3) and undergo leaching in the acidic environment of phagolysosomes (18). Bellmann et al. (16) did not specify the lengths of the six fibers selected for chemical analysis in each sample, but 90% of the fibers in the instilled suspension were $<3 \mu\text{m}$ in length.

Translocation

Postdepositional movement (translocation) from parenchyma to pleura is believed to be important in the genesis of asbestos-induced pleural disease (19), but there have been few experimental studies on the subject. Morgan et al. (20) exposed rats briefly to aerosols of radiolabeled amphibole asbestos and found that the label was concentrated in "hot spots" adjacent to the visceral pleura, 100 or more days post-exposure. They suggested that these concentrations were due to translocation of fibers from the central regions of the lung toward the peripheral (subpleural) regions, a so-called "pleural drift." In contrast, we have found no evidence for pleural drift after inhalation of chrysotile asbestos in the rat (3). However, there were important differences between the two studies. Morgan et al. (20) employed amphibole asbestos, and the subpleural accumulations of labeled fibers were observed more than 100 days postexposure, three times longer than the follow-up of our study, which may not have given time to detect a slow translocation process. In addition, neither our study (3), nor that of Morgan et al. (20) can distinguish between translocation from central to peripheral regions, and slower clearance from the peripheral relative to the central region (3).

Extensive and rapid translocation of asbestos may not be necessary for the development of pleural disease. Our studies of deposition and clearance show that the longest, possibly most pathogenic fibers are deposited near the visceral pleura and retained there for long periods of time. These fibers could be translocated to the pleura by slow processes not detectable in our study (3). In addition, asbestos fibers in the pulmonary parenchyma cause the release of growth factors and other mediators (21), which could have an effect on the nearby pleura (22,23). Asbestos deposited at alveolar duct bifurcations has a mitogenic effect on endothelial and smooth muscle cells of small pulmonary vessels.

Table 1. Alteration of chrysotile fibers $\geq 16 \mu\text{m}$ in length.

	Days postexposure				Correlation, <i>r</i>	Statistical significance
	1	8	15	29		
Fiber number	3.1×10^5 ^a	3.2×10^5	6.6×10^5	5.1×10^5	+0.56	$p < 0.02$ ^a
Average fiber diameter, μm	0.191	0.150	0.133	0.125	-0.82	$p < 0.01$ ^a
Fiber surface area, mm^2	4.4	3.6	6.6	4.8	+0.23	$p = 0.36$
Fiber mass, μg	0.65	0.44	0.69	0.47	-0.17	$p = 0.50$
Mg/Si mean	0.75 ± 0.04	Not measured	Not measured	0.67 ± 0.08		$p > 0.80$ (t-test)

^aValues in the table are geometric means for fibers $\geq 16 \mu\text{m}$ length in digests of the entire left lung of four or more animals. See Coin et al. (3) for experimental details. For determination of Mg/Si ten fibers $\geq 0.2 \mu\text{m}$ in diameter were examined from each of four animals at 1 and 29 days postexposure. Arithmetic mean and standard error are shown in the table. The Mg/Si ratios were compared between time points with a t-test. ^bThese parameters show a statistically significant ($p \leq 0.05$) trend with time.

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and this effect presumably is mediated by cytokines or other diffusible factors (24).

Conclusions

In our model, inhaled asbestos fibers are deposited largely at first alveolar duct bifurcations, many of which are within a few hundred microns of the visceral pleura. The deposited fibers include many >16 μm

in length and <1 μm in diameter, within the range considered most pathogenic. These fibers are cleared slowly, if at all. Long chrysotile fibers undergo longitudinal splitting in the lung, so that their number actually increases over time, possibly increasing their potential for biologic effects. Even though extensive splitting of chrysotile fibers occurs, we have not

observed substantial leaching of magnesium from chrysotile fibers up to 30 days after deposition. Translocation of chrysotile from deep parenchymal regions toward the subpleural regions of the lung does not occur in our model. Extensive translocation, however, may not be necessary for the development of asbestos-related pleural disease.

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