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Lung Asbestos Content in Chrysotile Workers with Mesothelioma[1-3]

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SUMMARY The role of chrysotile asbestos in the genesis of mesotheliomas in humans is disputed. We analyzed the asbestos content of the lung in 6 long-term chrysotile miners and millers who had pleural mesotheliomas. In five patients, only chrysotile ore components (chrysotile and tremolite/actinolite/anthophyllite types of amphibole asbestos) were found, while the sixth patient presented both chrysotile ore components and amosite, a type of asbestos that is not derived from the mining process. The mean number of fibers/g dry lung for the 5 patients with mesothelioma containing only chrysotile ore components was higher (chrysotile 64 × 10^6 and tremolite group 540 × 10^6) than in a group of long-term chrysotile miner control subjects who had no asbestos-related disease (chrysotile 23 × 10^6, tremolite group 58 × 10^6), but some patients with mesothelioma had fiber burdens near the mean of the control range. Fiber sizes and aspect ratios in the mesothelioma group were approximately the same as those in the control subjects, and analysis of fiber distribution failed to show any preferential localization in the periphery of the lung. However, the concentration ratio of tremolite in the lungs of the mesothelioma cases compared to the control cases was 9.3, while the ratio of chrysotile was only 2.8. Our findings provide strong evidence that chrysotile mine dust (chrysotile and amphibole components) can produce mesotheliomas in humans; the greater relative amounts of tremolite group amphiboles present in the patients with mesothelioma raise the possibility that these fibers may be important in the pathogenesis of the tumors.
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Introduction

Although the association of mesothelioma with asbestos exposure is well established, there is considerable controversy concerning the relative carcinogenic effects of the different types of asbestos. It is generally agreed that commercial forms of amphibole asbestos, amosite and crocidolite (especially crocidolite), can induce mesothelioma (1–8), but it has been suggested that, in humans (as opposed to experimental animal systems), chrysotile is either very weakly or not at all carcinogenic in this regard (4–8). For example, McDonald and associates (6) found only 11 cases of mesothelioma in a series of 4,547 deaths in Quebec chrysotile industry workers, compared to 9 of 56 deaths (1) in a series of workers who had assembled gas masks incorporating crocidolite asbestos. Berry and Newhouse (8) studied a series of 13,000 asbestos factory workers exposed to chrysotile and crocidolite, and concluded that only those exposed to crocidolite developed mesothelioma. McDonald and Fry (7) found a mesothelioma rate of 0.4/1,000 deaths in a factory processing only chrysotile, compared to 12.6/1,000 deaths in a factory processing chrysotile and amphiboles. Similar conclusions were reached by Acheson and coworkers (4,5) in 2 different exposure groups. However, many of these studies are confounded by uncertainties regarding the exact type of fiber to which individual workers may have been exposed.

Because more than 90% of the asbestos used world-wide is chrysotile, this question is of considerable importance. Mineralogic evaluation of lung tissue is one method of producing exact data regarding lung mineral burden, but to our knowledge, no series of occupationally exposed workers with mesothelioma have been shown to have only chrysotile ore components (see definition subsequent) in their lungs. Wagner and associates (9) attempted such an analysis on lungs from workers in an asbestos factory, and found that, in addition to chrysotile, amosite, and especially crocidolite, were also present, thus ruling out any definitive conclusions. Langer and McCaughey (10) have suggested, based on the observation of chrysotile fibers in a tissue section of lung, that a case of mesothelioma in a brake repair worker was produced by chrysotile asbestos; although this case may indeed represent a chrysotile-induced mesothelioma, the volume of tissue sampled is so small that amosite or crocidolite might have been missed.

Chrysotile miners and millers represent a population with, theoretically, exposure only to chrysotile mine dust; in this paper we analyze the mineral content in the lungs of 6 such workers and demonstrate that chrysotile mine dust can produce mesotheliomas. We also examine the relationship of fiber number, fiber type, and fiber size and shape to the development of mesothelioma.

Throughout this paper, we shall use the term "chrysotile ore components" and "chrysotile mine dust" to signify both chrysotile asbestos and the amphiboles tremolite, actinolite, and anthophyllite, which are integral parts of the chrysotile ore. These latter forms of asbestos are essentially contaminants of the ore, and are not purposely mined. By comparison, the amphibole minerals amosite and crocidolite are forms of asbestos that are completely unrelated to chrysotile and which are mined and used commercially.

Methods

The 6 cases analyzed in this study are derived from a series of approximately 90 sequential autopsies of long-term workers in the Quebec chrysotile industry; the autopsies were performed from late 1980 through the end of 1983 at the Hôpital Général de la Région de l'Amiante at Thetford Mines, Quebec. Cases and exposure histories were obtained through the courtesy of Dr. M. Poulin and Mr. C. Pratte of that institution. All tissues were fixed in formalin.

The 6 patients with mesothelioma represent all the mesotheliomas present in the series of 90 cases. The diagnosis in all cases was confirmed by finding the following: a typical gross appearance of tumor encasing the lung, no evidence of another primary at autopsy, and the typical histologic and histochemical features (11) of mesothelioma. All of the

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TABLE 1

DEMOGRAPHIC DATA

Case	Age/Sex	Employment in Chrysotile Industry	Date of Death	Details of Occupation
1	5[illegible]/M	28 years (19[illegible] to 19[illegible])	19[illegible]	Mechanic in mine, no mill work
2	52/M	10 years (1946 to 1956)	1982	Fiber bagger in mill/mechanic in mine
3	60/M	36 years (1947 to 1983)	1983	Fiber bagger and sorter in mill/miner
4	67/M	42 years (1937 to 1979)	1983	Underground miner only
5	65/M	40 years (1935 to 1975)	1983	Fiber separator, mill
6	68/M	47 years (1928 to 1975)	1981	No details available

tumors in this report were of pleural cavity origin. For comparison purposes, we used 9 previously published long-term chrysotile industry workers from the same autopsy series (12); these 9 patients had neither mesothelioma nor asbestos-induced parenchymal or airway fibrosis.

For mineralogic analysis, we followed the same procedure as previously reported (12). This consists, in brief, of dissolving 3- to 5-g samples of formalin fixed lung in bleach, collecting the mineral particulate sediment on a Millipore filter, and transferring the particles to a coated electron microscope grid. The grid is then scanned in the electron microscope, and every fiber encountered is identified by a combination of morphology, electron diffraction, and energy dispersive X-ray spectroscopy. For the present investigation, a subpleural 0.5-cm deep sample and another piece from 3 cm deep within the lung were obtained and processed in order to compare central and peripheral distribution of fibers. Approximately 100 fibers were examined for each case. Results were calculated using an algorithm relating weight of tissue used and grid area, and expressed as fibers/g dry lung.

A blank control was created for each test sample. This was prepared by running the entire preparatory procedure without tissue, and scanning approximately 50 grid squares in the electron microscope to detect contaminants from solutions and air. In this study, only rare particles of very short fiber chrysotile were found in the blanks, and these values were subtracted from the test values.

Results

Demographic data and occupational details are shown in table 1, and results of mineralogic analysis are shown in table 2. Tremolite, actinolite, and anthophyllite are grouped into 1 category, as we have done previously (12). Five of the 6 patients with mesothelioma had only chrysotile ore components in their lungs. The sixth patient also had substantial levels of amosite, and is excluded from the calculations of mean fiber number, size, etc., in order to deal with a mineralogically homogeneous population. For the 5 patients with mesothelioma, the mean number of chrysotile fibers was 64 ± 65 (mean ± SD)/g dry lung, and of the tremolite group of amphiboles, 540 ± 840 fibers/g dry lung. These values are compared in table 2 to those found in the reference chrysotile miners (12), and a group of 25 men without occupational asbestos exposure (13). Although the patients with mesothelioma have higher mean levels than the control subjects, there is considerable overlap in the 2 groups.

The distribution of fiber sizes in the patients with mesothelioma compared to the control group is shown in table 3. The aspect (length to width) ratios for various fibers is shown in table 4, the ratios of numbers of fibers in central and peripheral sites are listed in table 5, and the fiber size distribution and aspect ratios for fibers in central versus peripheral sites are shown in tables 6 and 7. In all these tables, the mesothelioma group is composed of patients 1 to 5 (those containing chrysotile ore components only). Because distributions of aspect ratios are often skewed, geometric means are included in tables 4 and 7 as well. There were no differences found for fiber size distribution or fiber aspect ratios either between mesothelioma and control groups, or between central and peripheral sample sites. There was no tendency

TABLE 2

NUMBERS OF FIBERS FOR ALL SITES
(ALL COUNTS AS FIBERS × 10^6/G DRY LUNG)

Case	Chrysotile	Tremolite/Actinolite/ Anthophyllite	Amosite or Crocidolite
1	63	244	0
2	8	44	0
3	69	320	0
4	10	62	0
5	169	2023	0
Mean ± SD*	64 ± 65	540 ± 840	0
6	44	54	70 (amosite)
Reference population of 9 chrysotile miners without mesothelioma (12):			
Mean:	23	58	0.02
Reference population of 25 men without occupational asbestos exposure (13):			
Mean:	0.6	0.3	0.01

* Mean values for 5 cases containing only chrysotile ore components.

TABLE 3

FIBER SIZE DISTRIBUTION FOR ALL SAMPLE SITES

	Percent of fibers in size range		
	0.5 to 5 μ	5 to 10 μ	10 + μ
Chrysotile			
Mesothelioma Patients 1 to 5	89	9	2
Mining control group (12)	85	8	7
Tremolite/actinolite/anthophyllite			
Mesothelioma Patients 1 to 5	87	11	2
Mining control group (12)	94	6	0

TABLE 4

MEAN FIBER ASPECT RATIOS FOR ALL SAMPLE SITES*

	Mesothelioma Patients 1 to 5	Control Group (12)
Chrysotile	92 ± 103 (60)	96 ± 120 (53)
Tremolite/actinolite anthophyllite	14 ± 12 (11)	13 ± 12 (9)

* Values are mean ± SD; geometric mean in parentheses

TABLE 5

MEAN VALUES OF RATIO OF NUMBERS OF CENTRAL TO PERIPHERAL FIBERS

	Chrysotile	Tremolite/Actinolite/Anthophyllite
Mesothelioma patients		
Patient 1	7.5	5.9
Patient 2	1.0	0.5
Patient 3	0.4	0.1
Patient 4	0.6	0.5
Patient 5	3.6	1.1
Mean ± SD	2.6 ± 3.0	1.6 ± 2.4
Control group: Mean ± SD	2.3 ± 3.1	2.0 ± 1.1

TABLE 6

DISTRIBUTION OF FIBER SIZES IN PERIPHERAL VERSUS CENTRAL SAMPLES OF MESOTHELIOMA PATIENTS 1 TO 5

	Percent in Size Range		
	0.5 to 5 μ	5 to 10 μ	10+ μ
Chrysotile			
Central	87	12	1
Peripheral	95	4	1
Tremolite/Actinolite/Anthophyllite			
Central	88	11	1
Peripheral	86	12	2

TABLE 7

ASPECT RATIOS FOR FIBERS IN CENTRAL VERSUS PERIPHERAL SITES IN MESOTHELIOMA PATIENTS 1 TO 5*

	Central	Peripheral
Chrysotile	92 ± 101 (57)	92 ± 106 (62)
Tremolite/Actinolite/Anthophyllite	13 ± 12 (11)	14 ± 12 (10)

* Values are mean ± SD; geometric mean in parentheses.

for fibers to concentrate in the periphery of the lung (table 5).

The ratios of numbers of fibers of each type in mesothelioma patients 1 to 5 versus the reference cases are shown in table 8. The ratio of tremolite (9.3) between the groups is considerably higher than that of chrysotile (2.8).

Discussion

As noted in INTRODUCTION, epidemiologic studies suggest that chrysotile is a relatively weak mesothelial carcinogen in man; however, it is clear that, even in many factories that processed largely chrysotile, amphibole was also used at one point (5,8,9), and hence, actual tissue analysis is required to demonstrate that only chrysotile or chrysotile ore components are present before a given case can be definitely ascribed to chrysotile dust inhalation. The requirement for such analysis is well illustrated by our Patient 6, a long-term worker in the chrysotile industry, whose lungs contained large amounts of amosite asbestos as well as chrysotile ore dust. In this instance, it is likely that the worker was employed in one of the asbestos processing factories in Quebec that used amosite at various times (14), but we were unable to obtain enough occupational details to verify this possibility. Amosite in chrysotile miners has been demonstrated previously: Rowlands and associates (15) found it in the lungs of 2 of 47 Quebec chrysotile workers, an observation that emphasizes again the usefulness of confirming epidemiologic studies with mineralogic analysis. Nonetheless, our data from the analysis of Patients 1 to 5 provide strong evidence that chrysotile mine dust components (as defined) can induce mesotheliomas.

Which of the minerals in the chrysotile ore is producing the tumors is a matter of considerable importance. As in our previous study of chrysotile miners (12) and the study of Rowlands and associates (15), we found more amphibole (tremolite actinolite anthophyllite) derived from the ore body than chrysotile in these lungs, an observation that reflects the failure of chrysotile, originally present in the ore in much larger quantities than amphibole, to accumulate in lung tissue. Whether this phenomenon reflects chemical leaching of the fibers (16), enhanced clearance of chrysotile compared to amphibole, or low pulmonary deposition of chrysotile compared to amphibole, is not clear. Our subsequent comments must, therefore, be interpreted in light of the fact that we cannot determine what chrysotile load may have been present in these lungs, although the study of Rowlands and associates (15) suggests that estimates of fiber burden derived from mineralogic analysis correlate well with estimates of dust exposure in these workers.

The types, sizes, and distribution of fibers that induce mesothelioma have been the subject of considerable discussion based on animal experiments (17,18), measurements of mine dust size, and theoretical calculations of fiber deposition in the lung (see Harington (19) for review). Animal studies (17,18) have shown that mesothelioma can be induced by virtually any insoluble mineral fiber if it is sufficiently small (particularly, diameter less than 0.25 μ and length greater than 8 μ) and has a high aspect (length to width) ratio. In contrast to the situation in humans, chrysotile appears to be just as effective as crocidolite in inducing mesothelioma in animals (18). It has also been postulated that, in humans who are inhaling asbestos dust, mesothelioma is induced by dust that penetrates far into the periphery of the lung (19). Hence, it is reasonable to suppose that patients with mesothelioma might have either higher aspect ratio fibers, or greater numbers of fibers, or more fibers in the periphery of the lung than patients with roughly comparable exposure but no mesotheliomas.

In fact, we were unable to find any consistent differences in fiber size or fiber aspect ratio between our 5 patients with mesothelioma and 9 control subjects (12). There was no evidence of a trend for peripheral deposition of fibers in the patients with mesothelioma (table 5). One

TABLE 8

RATIOS OF NUMBERS OF FIBERS OF DIFFERENT MINERALS IN MESOTHELIOMA PATIENTS 1 TO 5 AND CONTROL GROUPS

Mineral	Ratio Mesothelioma Reference (12)
Chrysotile	2.8
Tremolite/actinolite/anthophyllite	9.3

might also suspect, a priori, that because milling the ore separates the long fibers, mesothelioma should be found in millers rather than miners. But 2 of the 5 patients for whom we have detailed occupational histories appear to have worked only in the mines.

However, we did find differences in total fiber numbers and in the ratios of types of fibers between the groups (tables 2 and 8). The mean values of fiber load for the mesothelioma group were considerably higher than those for the control group, but 2 of the patients with mesothelioma (Patients 2 and 4) had fiber concentrations at or below the mean for the reference group. Hence one cannot easily ascribe the mesotheliomas only to high overall numbers of fibers.

Of particular interest is the fact that the patients with mesothelioma having only chrysotile ore components had a much higher ratio of tremolite group amphiboles (9.3) than chrysotile fibers (2.8) compared to the control group (table 8), and that this was not true of Patient 6, in whom amosite was found. Stanton and associates (17) and Wagner and coworkers (20) have shown that long, thin, high aspect ratio tremolite induces mesothelioma in animals, whereas short thick fiber tremolite is less effective or ineffective, an observation that agrees with the theory of Stanton and associates (17) that tumor frequency is proportional to fiber length and aspect ratio (aspect ratio greater than 32 in the study of Stanton and associates). But as Harington (19) points out, the animal studies also show that the requirement for high aspect ratio or long length is not absolute, and that large numbers of short lower aspect fibers can induce mesothelioma. Our chrysotile miners would presumably fall into the latter category because the mean aspect ratio for tremolite amphiboles in their lungs is about 13 to 14 (table 4).

Our observations in chrysotile mine industry workers thus raise the possibility that the amphibole component of the chrysotile ore is important in the genesis of mesothelioma in this group. This situation may be similar to that reported in mesothelioma patients exposed to mixtures of chrysotile and the commercial amphiboles amosite and crocidolite (21,22), where the amphibole appears to play a major role in tumor induction. Additionally, because elevated levels of tremolite and actinolite amphiboles have been found in the lungs of some secondary chrysotile industry workers, which we (23, and Churg A, unpublished data) and others (9) have analyzed, and in particular, because some of that amphibole is of relatively long length (23), the same conclusion might apply to textile workers and others exposed to processed chrysotile ore.

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