Absence of amosite asbestos in airway mucosa of non-smoking long term workers with occupational exposure to asbestos

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Abstract

There is considerable experimental evidence that asbestos fibres are taken up by epithelial cells, and that uptake of fibres is associated with various deleterious, particularly mutagenic, effects. It is not known, however, if asbestos fibres are taken up by human bronchial epithelial cells in vivo. To investigate this question, the amosite asbestos content of the mucosa of seven different airways and four parenchymal sites supplied by these airways in six necropsy lungs from heavily exposed never-smoking long term shipyard and insulation workers without asbestosis was examined. Amosite asbestos was readily found in moderately high concentration in all parenchymal samples, but 33 of 40 airway samples that could be evaluated showed no amosite fibres. The seven positive airways had fibre concentrations that were always much lower than the parenchymal concentrations, and these very few fibres may have been contaminants from the parenchyma. These data suggest that, at least in non-smokers, amosite asbestos either does not penetrate into or does not accumulate in human airway mucosa. These findings also call into question the idea that asbestos acts as a direct airway carcinogen in humans.

(*British Journal of Industrial Medicine* 1993;50:355-359)

In general asbestos seems to produce disease through various locally mediated rather than systemic effects. The most consistent findings in experimental animal and tissue culture systems have been that epithelial cells take up asbestos fibres, and that fibre uptake is

associated with deleterious effects relating to both fibrogenesis and carcinogenesis¹⁻³ (see discussion).

Although fibre uptake by bronchial epithelial cells is well established in experimental models, it is not known to what degree the same process occurs in humans, or even whether it occurs at all. We have recently devised a technique for analysing the particle content of respiratory bronchial mucosa from human necropsies,⁴ and in this paper we apply the technique to look at airway and parenchymal asbestos retention in workers with substantial occupational exposure to amosite asbestos.

Materials and methods

CASE SELECTION

Cases for this study were selected from persons with occupational exposure to amosite asbestos whose lungs at necropsies were referred to this laboratory for evaluation of asbestos content. These persons had been exposed to asbestos in the Pacific north west in shipyards or as career asbestos insulators and similar trades in which high level asbestos exposure was known to have occurred.

As this study was intended to provide baseline data on fibre burden in the airway mucosa free of interference by variables that might affect fibre retention, we restricted the study to lungs that were essentially pathologically normal. Small amounts of pneumonia were considered acceptable. We also decided to restrict the study to non-smokers because smoking may change fibre uptake.⁵ Because we did not know which airways were likely to accumulate asbestos fibres and because we needed to ensure a broad sample of airways, analyses were restricted to lungs in which several different airways matching the scheme shown could be found. Lastly, to ensure that the population sampled had a reasonably heavy exposure, only cases with greater than 10 years of exposure were used.

TISSUE SAMPLING

Application of the conditions just described resulted in selection of six lungs, all of which turned out to be from patients with mesothelioma. Only the lung

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Table 1 Demographic data

Case	1	2	3	4	5	6	Mean (SD)
Age (y)	50	65	68	76	70	79	68 (10)
Sex	M	M	M	M	M	M	
Exposure (y)	15	35	25	36	22	30	28 (8)
Latency (y)	32	61	48	49	48	49	48 (9)
Years since last exposure	17	26	23	13	26	19	21 (5)

without a tumour was used. All lungs were fixed in formalin by the original pathologist before referral to our laboratory. The lungs were sliced in a sagittal plane, and the listed airways were examined; the sites were designed to sample airways of varying size, distance from the carina, and orientation with respect to gravity. The sample sites were:

Mainstream bronchus (MAIN)

Upper lobe bronchus (ULB)

Apical segmental bronchus upper lobe (APB)

Anterior segmental bronchus upper lobe (ASB)

Lower lobe bronchus (LLB)

Superior segmental bronchus lower lobe (SSB)

Posterior basal segmental bronchus lower lobe (PBB)

The airway mucosa was sampled by isolating a roughly 1 cm long segment of airway⁴; the airway was carefully rinsed with saline to remove adherent mucus, and the mucosa (defined here as all tissue from the epithelium to the top of the cartilage) separated from the underlying cartilage by sharp dissection.

To provide a comparison of parenchymal content of asbestos, samples were also taken from the sites supplied by the segmental airways just listed:

Apical segmental parenchyma upper lobe (APT)

Anterior segmental parenchyma upper lobe (AST)

Superior segmental parenchyma lower lobe (SST)

Posterior basal segmental parenchyma lower lobe (PBT)

The parenchymal samples consisted of wedge shaped pieces of tissue based on the pleura and with

the apex of the wedge extending to the site of the sample of segmental airway.

PREPARATION OF TISSUE FOR ASBESTOS AND PARTICLE IDENTIFICATION

Airway or parenchymal samples were dissolved in bleach and the mineral particles collected on millipore filters and then transferred to coated electron microscope grids as previously described.⁶ Preparations were examined in a Phillips 400T electron microscope equipped with a Kevex energy dispersive x ray spectrometer. For the parenchymal samples, 50 randomly selected grid squares were evaluated and all amosite fibres identified and measured. For the airway samples, 50 grid squares were initially evaluated, but, because this examination failed to find amosite fibres in any of the airway sites, a further 250 grid squares for a total of 300 squares was examined for each sample. This number was chosen both to provide low detection limits (see below) and as a practical matter because of the extensive time involved in counting this many squares.

When it became apparent that even examination of 300 grid squares failed to show asbestos in most airway sites, a decision was made to count total mineral particles using the same grids. This count was intended as a positive control to indicate whether the preparations were faulty, or whether in fact the asbestos content of the airways was extremely low.

Results were calculated using an algorithm that relates weight of tissue dissolved and number of grid squares examined.⁶ All results were expressed in terms of fibres or particles/g dry tissue. The detection limit for each sample is defined as the number of fibres/g dry tissue that would be represented by the finding of 1 actual fibre in the counted grid squares (this indicates the minimum sensitivity of the technique (see discussion)). Because many of the distributions shown in tables 2 and 4 are skewed, as is common with particle counts, both arithmetic and geometric mean values were calculated for presenta-

Table 2 Amosite asbestos and total particle concentration in airways

Site	Case											
	1		2		3		4		5		6	
	A	P	A	P	A	P	A	P	A	P	A	P
MAIN	<11	54	<13	200	<30	100	<14	18	<11	140	<14	140
ULB	<13	48	<29	28	<26	48	<60	180	<33	180	<28	56
APB	35	110	<15	120	<45	140	<52	150	73	290	<35	140
ASB	<40	240	69	110	ND	ND	120	200	<12	360	<45	280
LLB	<21	86	<15	170	17	98	<36	32	<19	56	<19	26
SSB	<19	58	<24	260	<81	160	<18	260	<21	48	ND	ND
PBB	<90	156	36	140	61	72	<12	210	<91	180	<39	460

Amosite concentration in thousands of fibres/g dry tissue; Total particle concentrations in millions of particles/g dry tissue; Symbol <: less than indicated detection limit, ND = not analysed; A = amosite; P = particles.

Table 3 Arithmetic mean and geometric mean for amosite asbestos and total particle concentration in airways

Site	Arithmetic mean		Geometric mean	
	Amosite	Particles	Amosite	Particles
MAIN	0	110	0	85
ULB	0	66	0	54
APB	18	160	0	150
ASB	38	240	0	220
LLB	3	78	0	65
SSB	0	160	0	120
PBB	17	200	0	180

Amosite concentration in thousands of fibres/g dry tissue; Total particle concentrations in millions of particles/g dry tissue.

tion in tables 3 and 5. For this purpose the airways with no amosite fibres were treated as value zero. Standard deviations have been omitted from tables 3 and 5 for clarity.

Results

DEMOGRAPHIC DATA

Table 1 shows age, sex, years of exposure, latency, and time since last exposure for each case. All of the patients were non-smokers. Exposure was moderately heavy, with exposures varying from 15 years to 36 years, mean (SD) 28 (8) years.

FIBRES AND PARTICLES IN AIRWAY EPITHELIUM

Table 2 shows these data for each case. The symbol < indicates that no fibres were found, and that the possible concentration (if any) was therefore less than the indicated detection limit. In two cases one airway site was not usable so that the total number of sampled airways was 40. Amosite fibres were detected in only seven of the 40 sample sites; no particular airway was consistently positive for asbestos fibres. When present airway amosite concentrations ranged from 35 000 to 120 000 fibres/g dry tissue. Non-asbestos mineral particles were present in all airways in concentrations ranging between 20 and 400 million particles/g dry tissue. Table 3 shows arithmetic and geometric mean summary data. Treating the airways with no detectable fibres as concentration

Table 5 Arithmetic mean and geometric mean for amosite asbestos and total particle concentration in parenchyma

Site	Arithmetic mean		Geometric mean	
	Amosite	Particles	Amosite	Particles
APT	590	340	470	320
AST	1000	310	490	240
SST	400	210	350	190
PBT	370	260	280	250

Amosite concentration in thousands of fibres/g dry tissue; Total particle concentrations in millions of particles/g dry tissue.

values of zero, the overall arithmetic mean concentration of asbestos fibres in airways was roughly 11 000 fibres/g, and the geometric mean concentration 0 fibres/g.

FIBRES AND PARTICLES IN PARENCHYMA

Tables 4 and 5 show similar data for the parenchymal sites. No parenchymal samples were prepared for the two sites with unusable airways. Amosite fibres were easily identified in every site, so that there are no entries (<) indicating less than the detection limit. Concentration values ranged from 100 000 to 3 900 000 fibres/g dry tissue. Particle counts were of the order of 100 to 800 million particles/g dry tissue.

Discussion

As noted in the introduction, there is considerable experimental evidence that pulmonary epithelial cells, including bronchial epithelial cells, take up asbestos fibres, and that this process is generally deleterious.^{1-3 5 7-11} Fibres that penetrate through the bronchial epithelium to the interstitium may cause release of fibrogenic mediators from interstitial macrophages and other interstitial cells.^{3 12} Fibres within epithelial cells produce various potentially mutagenic effects including DNA strand breaks and interference with the mitotic apparatus.¹³⁻¹⁵ As well, in these systems, asbestos fibres increase the transport of adsorbed carcinogens such as benzo(a)pyrene into the cells and also enhance metabolic formation of active carcinogens.¹⁶ The potential consequences of

Table 4 Amosite asbestos and total particle concentration in parenchyma

Site	Case											
	1		2		3		4		5		6	
	A	P	A	P	A	P	A	P	A	P	A	P
APT	210	350	160	470	480	260	540	560	340	200	360	200
AST	380	180	390	730	ND	ND	270	340	390	260	190	84
SST	230	300	63	100	140	130	430	330	590	190	ND	ND
PBT	140	160	67	160	100	260	260	270	270	260	790	240

Amosite concentration in thousands of fibres/g dry tissue; Total particle concentrations in millions of particles/g dry tissue; ND = not analysed; A = amosite; P = particles.

the penetration of asbestos fibres into epithelial cells are thus clear, but despite extensive experimental work in this area, there are no data available on whether asbestos actually enters human respiratory epithelial cells in vivo.

It is known from animal model systems that inhaled mineral particles not removed by macrophages may penetrate into the respiratory epithelium and be transported through the cell to end up in the underlying interstitial tissue, from which site they are probably cleared poorly or not at all.^{17,18} Thus numbers of particles found in the respiratory mucosa (all tissues between the cartilage of the large airways and the epithelial surface) could potentially be used to determine patterns of particle retention in humans. In a study of airway mucosa from non-smoking members of the general population,⁴ we found that the airway mucosa contained exogenous mineral particles of the order of 10^7 to 10^8 /g dry tissue, and that the pattern of retention in terms of airway size and distance from the carina matched that predicted from theoretical models of particle deposition,¹⁹ suggesting that in fact particles retained in the airway walls are probably excellent indicators not only of retention but also of initial deposition patterns.

We have now applied this technique to examine the question as to whether asbestos fibres enter the epithelium and walls of the larger conducting airways in human lungs. The population we have used was one with fairly substantial historic exposure to asbestos, exposure sufficient to cause mesotheliomas, and sufficient to produce readily detectable fibre concentrations in the parenchyma. We found that no fibres could be detected in 83% of the bronchial sample sites despite the presence of large numbers of non-asbestos particles in the same samples. In fact the non-asbestos particle concentrations in these airways showed the same pattern that we have previously reported⁴—namely, higher concentrations in segmental compared to mainstem or upper and lower lobe bronchi (table 3). These findings indicate that the technique was working properly and that the apparent absence of asbestos fibres from the airways is not an analytical artifact. Even in the positive airway samples only one or at most two fibres were found in 300 grid squares, and it is entirely possible that these few fibres are contaminants from the much larger number of fibres present in the parenchyma (the actual number of fibres counted in 50 squares in the parenchymal sites varied from 14 to 50, mean 35).

It should be noted that the word "absence" is not strictly correct in this context; the more accurate conclusion is that amosite fibres, if present, might be present at any level below the detection limit. But in terms of fibre burdens found in patients with occupational exposure to amosite exposure, the potential maximum burdens represented by the detection limits (average about 40 000 fibres/g) or by

the actual number of fibres (average about 11 000 fibres/g, treating the airways with no detectable fibres as value 0) in the airway mucosa are extraordinarily low. This fact can be best appreciated by noting that the average fibre concentration in the parenchymal samples supplied by these same airways was about 600 000 fibres/g dry lung, a value typical of that we found in workers with mesothelioma.²⁰ By contrast, members of the general population usually carry no more than 10 000 fibres of amosite/g of parenchyma, values fairly similar to the detection limits just mentioned.

It thus seems that the airway mucosa in humans may not accumulate many amosite asbestos fibres, but this conclusion must be made with considerable caution. For one thing, the number of cases examined is obviously small, although the fairly large number of sampled airways partly makes up for this problem. Although the persons in our study have had substantial exposure, groups of workers with considerably higher exposure do exist: in our laboratory lungs with amosite induced asbestosis typically have 10 to 100 times the fibre load found in lungs with mesothelioma, the lungs used in this study.²⁰ The possibility that fibres do accumulate in the airway mucosa at high fibre exposures still needs to be ruled out. It is also possible that cigarette smokers accumulate more fibres in their airways than the non-smokers examined here. Smoking is believed to increase retention of particles of all types, and, at least experimentally, this applies to asbestos as well as non-asbestos particles.^{5,21,22} Lastly, it is possible that fibres actually enter the bronchial mucosa but are cleared much faster from the airways than they are from the parenchyma; there are no data that allow comment about this possibility, but, with exposures of up to 36 years, it seems unlikely that all fibres would be cleared from the mucosa, particularly given the well established finding that amphibole asbestos is generally cleared very slowly from lung tissue.

There is considerable controversy on the question of whether rates of lung cancer are increased with any amount of exposure to asbestos or only in those with asbestosis.²³⁻²⁹ This study was not designed to consider that issue; however, the fact that we cannot show any significant amount of asbestos in the mucosa of the large airways, the airways in which bronchogenic carcinomas are believed to arise, does raise doubts about the ability of asbestos to act as a direct airway carcinogen in humans.

This work was supported by grants from the National Cancer Institute of Canada and Medical Research Council of Canada.

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Accepted 6 July 1992