Cigarette Smoke Increases the Penetration of Asbestos Fibers Into Airway Walls

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For study of the penetration of asbestos fibers into airway walls, guinea pigs were given amosite asbestos by intratracheal instillation. Half of the animals were also exposed to cigarette smoke. Animals were sacrificed at 1 week and 1 month, and numbers of fibers in airway walls were counted in histologic sections. In both smoke-exposed and nonexposed groups, numbers of fibers per square millimeter of airway wall increased from 1 week to 1 month in the respiratory bronchioles. At each time period, smoke-exposed animals had significantly higher numbers of fibers in the airway walls, compared with nonexposed animals. It is concluded that 1) continued transport of fibers into interstitial tissues may be the reason that asbestosis can progress after cessation of exposure; 2) cigarette smoke increases the penetration of fibers into airway walls. This effect may play a role in the increased incidence of disease seen in smoking, compared with nonsmoking, asbestos workers. (Am J Pathol 1986, 123: 95–99)

THERE IS considerable evidence that cigarette smoke potentiates the pathogenic effects of asbestos. Radiographically, cigarette smokers appear to have higher attack rates and/or faster progression rates for asbestosis compared to nonsmokers.[1] Smoking asbestos workers have a markedly higher incidence of lung cancer than nonsmoking workers, and the effects of the two agents are synergistic.[2]

The mechanisms by which asbestos causes fibrosis of the parenchyma and airways and lung cancer are poorly understood. It is clear that asbestos fibers, like many types of mineral particles, evoke an inflammatory response in the lung,[3] but it is not clear how this response evolves to parenchymal fibrosis. We have previously demonstrated[4] that asbestos fibers penetrate the walls of small airways, and there is some evidence that penetration of fibers into the pulmonary interstitium is important in the genesis of fibrosis. Pinkerton et al[5] have shown that, in an animal model, the amount of interstitial fibrosis is proportional to the burden of interstitial chrysotile fibers. We[6] have recently found that, in chrysotile asbestos miners, airways with marked fibrosis contain significantly larger numbers of mineral particles than those with little or no fibrosis, again implying that the numbers of particles reaching the interstitium is important in fibrogenesis.

In regard to lung cancer, it has been shown that administration of asbestos coated with benzo(a)pyrene increases the intracellular uptake and retention of the benzo(a)pyrene, compared with administration of the chemical alone.[7] Extrapolating to humans, it is possible that cigarette smoke carcinogens adsorbed to asbestos fibers act in a similar fashion.

These observations suggest that the amount of asbestos penetrating the airway wall through the airway epithelium may be an important factor in both carcinogenesis and fibrogenesis. In this article we examine the effects of cigarette smoke on penetration of fibers into airway walls.

Materials and Methods

Twenty 400-g Hartley strain female guinea pigs were divided into two groups: half of the animals were ex-

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Table 1—Respiratory Bronchioles: Asbestos Fibers per Square Millimeter of Airway Wall

	Fibers/sq mm				
	0	>0–10	11–100	>100	*P* less than
Smoke-exposed versus not exposed					
1 week: not smoke-exposed	165	2	15	8	0.01
1 week: smoke-exposed	133	4	26	48	
1 month: not smoke-exposed	142	0	19	43	0.01
1 month: smoke-exposed	99	1	15	80	
One week versus 1 month					
1 week: not smoke-exposed	165	2	15	8	0.01
1 month: not smoke-exposed	142	0	19	43	
1 week: smoke-exposed	133	4	26	48	0.01
1 month: smoke-exposed	99	1	15	80	

posed to cigarette smoke (see below) for 2 weeks; the other half were not. All animals were then given 1 mg UICC amosite asbestos in 0.5 ml saline by intratracheal instillation with the use of our previously published protocol.[4] Thereafter, the animals which had been exposed to smoke continued to be exposed for the duration of the experiment. Five smoke-exposed and 5 nonexposed animals were sacrificed 1 week after asbestos exposure, and the remaining 5 animals in each group were sacrificed 1 month after asbestos exposure.

The smoking apparatus has been previously described and illustrated by Simani et al.[8] The machine operates by drawing 20 cc of air through a single cigarette and then expelling the 20 cc of smoke (one "puff") into a head only-exposure chamber of 1 liter volume. One puff is delivered every minute. Each guinea pig is exposed in a separate chamber, and each cigarette sends smoke to only 1 chamber. The animals are awake and spontaneously breathing during exposure.

Cigarettes were obtained from the Tobacco Manufacturers' Council of Canada; they correspond to a commercially available brand of nonfilter cigarette. This type of cigarette has been previously used for studies on airway permeability.[8,9] For the present experiments, animals were exposed to the whole smoke of 10 cigarettes/day (200 puffs total) 5 days/wk.

Animals were sacrificed by intraperitoneal barbiturate overdose. The lungs were removed, and the right lung inflated via the trachea with 2% glutaraldehyde at a pressure of 100 cm water for 30 minutes. This pressure is necessary to overcome the intense bronchospasm which occurs in guinea pig airways after death.[10] The left lungs were used for lavage studies which will be reported separately.

For each lung, a midsagittal slice was taken of the upper lobe and of the lower lobe; these slices encompassed virtually the entire lobe and have been shown to be ideal slices for morphometric analysis.[11] Slices were photographed before processing to permit correction for shrinkage. Slices were embedded in paraffin and sections cut at 5 μ and stained with hematoxylin and eosin.

For each section, every membranous bronchiole (defined as noncartilaginous airways with continuous fibromuscular walls) and respiratory bronchiole (defined as noncartilagenous airways with partially alveolated

Table 2—Membranous Bronchioles: Asbestos Fibers per Square Millimeter of Airway Wall

	Fibers/sq mm				
	0	>0–10	11–100	>100	*P* less than
Smoke-exposed versus not exposed					
1 week: not smoke-exposed	26	0	2	0	NS
1 week: smoke-exposed	37	1	4	1	
1 month: not smoke-exposed	47	0	4	1	NS
1 month: smoke-exposed	40	0	1	3	
One week versus 1 month					
1 week: not smoke-exposed	26	0	2	0	NS
1 month: not smoke-exposed	47	0	4	1	
1 week: smoke-exposed	37	1	4	1	NS
1 month: smoke-exposed	40	0	1	3	

Table 3—Respiratory Bronchioles: Numbers of Fibers in Epithelium/Airway

	Fibers/airway				
	0	1–5	6–10	>10	*P* less than
Smoke-exposed versus not exposed					
1 week: not smoke-exposed	152	35	1	2	NS
1 week: smoke-exposed	162	44	4	1	
1 month: not smoke-exposed	159	40	3	2	NS
1 month: smoke-exposed	157	34	3	1	
One Week versus 1 Month:					
1 week: not smoke-exposed	152	35	1	2	NS
1 month: not smoke-exposed	159	40	3	2	
1 week: smoke-exposed	162	44	4	1	NS
1 month: smoke-exposed	157	34	3	1	

and partially fibromuscular walls) was identified. The wall area of each airway was determined by tracing inner and outer contours with a computer-assisted digitizer; the computer then calculated the actual area. The inner contour was defined as the epithelial basement membrane and the outer contour as the adventitia.

For each airway, the number of fibers greater than 5 μ in length (measured at 400× with an eyepiece graticule) in the airway wall was counted; a separate count was made for fibers in the epithelium itself. The 5-μ length was chosen so that fiber identification was unequivocal; it can be difficult to be sure that smaller particles are actually asbestos fibers. Values given in Results and Tables 1 and 2 as fibers per square millimeter indicate fibers in the wall only. Epithelial areas were not measured. Values given in Results and Tables 3 and 4 indicate fibers in the epithelium per airway (not per unit area). The reproducibility of the counting system was checked by recounting 50 airways; the correlation coefficient for the first and second counts was 0.99.

Statistical analysis for fibers per unit wall area or fibers per airway epithelium was performed using a chi-square test with Bonferroni correction.

Results

A total of 167 membranous bronchioles and 800 respiratory bronchioles was examined. Table 1 shows the numbers of respiratory bronchioles with 0 fibers, greater than 0–10 fibers, 11–100 fibers, and greater than 100 fibers/sq mm. At both 1 week and 1 month smoke-exposed animals had greater numbers of fibers per square millimeter than animals not exposed to smoke. In both smoke-exposed and nonexposed animals, the number of fibers/sq mm increased from 1 week to 1 month. Similar results were obtained when the data was instead analyzed as fibers per airway (data not shown). An odds ratio test showed that the rate of increase from 1 week to 1 month was not statistically different between smoke-exposed and nonexposed animals.

Table 2 shows similar data for membranous bronchioles. There were no differences for this type of airway either between smoke-exposed and nonexposed groups at either time period or between time periods.

Tables 3 and 4 show the breakdown of fibers in epithelium per airway for membranous and respiratory bronchioles. No differences were seen for smoking status or time period.

Table 4—Membranous Bronchioles: Numbers of Fibers in Epithelium/Airway

	Fibers/airway				
	0	1–5	6–10	>10	*P* less than
Smoke-exposed versus not exposed					
1 week: not smoke-exposed	26	2	0	0	NS
1 week: smoke-exposed	36	5	2	0	
1 month: not smoke-exposed	49	3	0	0	NS
1 month: smoke-exposed	38	6	0	0	
One week versus 1 month					
1 week: not smoke-exposed	26	2	0	0	NS
1 month: not smoke-exposed	49	3	0	0	
1 week: smoke-exposed	36	5	2	0	NS
1 month: smoke-exposed	38	6	0	0	

Table 5—Percentage of Respiratory Bronchioles With Any Fibers in Walls

Not smoke-exposed: 1 week	13%
Not smoke-exposed: 1 month	30%
Smoke-exposed: 1 week	37%
Smoke-exposed: 1 month	49%

Discussion

In this paper we have begun to examine the effects of smoking on fiber penetration into airway walls. Our method of asbestos administration, namely, intratracheal instillation, is artificial and tends to result in a fairly inhomogeneous distribution of particles in the parenchyma in comparison with exposure by inhalation,[12,13] but does have the advantage of presenting high particle burdens to the airways and, most important, does reproduce lesions which are found in humans.[14] Because both smoke-exposed and nonexposed animals were given asbestos in the same fashion, and because we counted every airway in the tissue sections, inhomogeneity of distribution *per se* should not influence our results.

It is also possible that the reaction of the airway epithelium to masses of instilled fibers is different from that to inhaled fibers. This is a difficult problem to assess because little information is available on airway responses to asbestos in animals, and nothing is known about the acute human airway response. We did not perform a systematic evaluation of histologic changes in our animals, but at least to casual observation the epithelium was for the most part intact, and only occasional small focal ulcerations were seen. As shown in Tables 3 and 4, fibers were commonly found in the airway epithelium. We document below the abundant experimental evidence for transport of asbestos fibers and other mineral particles through epithelial cells into the interstitium, and we have also found fibers in the airway walls in chrysotile miners.[6] We believe, therefore, that our results reflect transepithelial penetration of asbestos fibers, and it is likely, but not proved, that transepithelial migration of fibers also occurs in man. However, the problems relating to method of administration of asbestos should be borne in mind when comparing our results to the human situation.

Our basic observations are two. First, the burden of fibers in the walls of respiratory bronchioles increases with time for both smoke-exposed and nonexposed animals, despite the fact that only a single dose of asbestos was administered at a single time point. Because both airway wall fibrosis and parenchymal fibrosis appear to reflect numbers of fibers present in the interstitium (see Introduction), we suggest that continuing transport of fibers from the air spaces to the interstitium after cessation of exposure may produce continuing fibrosis. This phenomenon could play a role in progression of asbestosis after exposure has ceased, an event which has been documented in both animals[5] and humans.[15,16]

Second, smoking increases the number of fibers which penterate into the walls of the respiratory bronchioles. This is conveniently seen in Table 5, which shows the percentage of airways containing any fibers visible by light microscopy. Statistically the difference between smoke-exposed and nonexposed animals is highly significant (Table 1). Again, if one accepts the idea that fibrosis in airway walls or alveolar parenchyma is proportional to the burden of fibers in these sites, then smoke-induced increases in transport of fibers into the interstitium could be the mechanism which produces the increased severity and/or progression of asbestosis seen radiographically in cigarette smokers.[1] Similarly, increased transport of asbestos fibers bearing carcinogens adsorbed from cigarette smoke could be a mechanism for the increased lung cancer rate seen in smoking asbestos workers.

We failed to find a significant increase in fiber penetration into the more proximal membranous bronchioles with either increasing time or exposure to smoke. This observation may reflect the fact that we counted only fibers easily visible in the light microscope, a relatively crude technique which only detects a minority of fibers. It is possible that electron-microscopic study might reveal increased penetration for smaller fibers as well. Certainly, examination of membranous bronchioles in asbestos-exposed humans and animals by either visual grading techniques to evaluate fibrosis[14] or actual measurements[4] indicates that these airways become thick-walled and fibrotic; and asbestos fibers can be found in the walls by electron microscopy,[6] which implies that the membranous bronchioles are subject to much the same lesions as the respiratory bronchioles.

Although fibers were readily found in the epithelium in both types of airway, we were unable to show a difference between smoke-exposed and nonexposed groups or an increase with time. This problem may again reflect the use of light-microscopic counting. Alternately, it may indicate that fibers move through the epithelium so rapidly (as they do through the alveolar epithelium; see below) that increased penetration is not visible at any one time point, and the amount of migration can only be seen in the wall itself, where the fibers "pile up." In either case, one implication of observing increased numbers of fibers in airway walls is that increased numbers of fibers must have passed through the epithelial cells in smoked-exposed animals.

How asbestos fibers in general penetrate the epithelium and reach the interstitium is uncertain. Brody and colleagues have shown that, in alveolar ducts, fibers are phagocytized by epithelial cells and rapidly transported

through the cell, reaching the interstitium in as little as 5 hours after exposure.[17,18] In explants of tracheal epithelium, Mossman and associates have found that fibers can move between cells and are also phagocytized by tracheal (and presumably bronchial and bronchiolar) epithelial cells; some (all?) fibers finally reach the submucosa.[19] This phenomenon is not specific for asbestos, because quartz,[20] iron oxide,[21] carbon black,[22] volcanic ash,[23] uranium oxide, and barium sulfate[24] are also translocated into the alveolar and/or airway epithelium and interstitium.

Brain[25] suggests that particle transport through the epithelium does not appear to be mediated by macrophages, and that in fact macrophages normally protect the epithelium by phagocytizing exogenous particles. Some evidence supporting this notion has been provided by Adamson and Bowden,[22] who showed that, with increasing particle doses, local macrophage response was overwhelmed and more particles were translocated into the interstitium. Thus, it is possible that the increased fiber penetration we have observed reflects smoke-induced interference with macrophage function, resulting in persistence of fibers in the airway lumens, which would normally be cleared by macrophages. However, cigarette smoke has been shown to increase the permeability of airway epithelium to horseradish peroxidase, at least in part by increasing junctional permeability, and asbestos has the same effect on alveolar tissue.[9,26] It is also possible, therefore, that cigarette smoke affects penetration by directly affecting epithelial cells.

Both parenchymal fibrosis and carcinoma of the lung have been found epidemiologically to show a dose-response effect, with increased exposure producing a higher incidence of disease.[27] Increased penetration of fibers into the airway epithelium and eventually into the interstitium, resulting in functionally increased doses of fibers to the tissues, may be one of the mechanisms whereby smoking potentiates asbestos disease.

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