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Smoking Inhibits Asbestos Clearance[1-3]

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SUMMARY To determine whether smoking affects the clearance of asbestos fibers, guinea pigs were given amosite asbestos by intratracheal instillation and divided into 3 groups: (1) nonsmokers, (2) animals exposed to smoke only after asbestos instillation, and (3) animals exposed to smoke both before and after asbestos instillation. Asbestos fiber concentrations and sizes were measured at 1 wk and 1 month after exposure. Between 1 wk and 1 month the asbestos burden in nonsmokers decreased on average 6-fold, whereas both smoking groups failed to show a significant decrease. Over this time period, the mean length of retained fibers increased in the nonsmokers but decreased in both smoking groups. This phenomenon was seen in tissue samples and lavage samples, although lavaged fibers were consistently shorter than tissue fibers. We conclude that in this model, cigarette smoking impedes asbestos clearance, largely by increasing retention of short fibers. This increased pulmonary fiber burden may be important in the increased disease rate seen in asbestos workers who smoke.

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Introduction

There is a substantial amount of epidemiologic and clinical evidence that asbestos workers who smoke cigarettes have more asbestos-related disease than those who do not smoke. Cigarette smoking workers have a risk of lung cancer, which in some studies is as much as 80 to 90 times that of the general population (1-3), and the effects of smoking and asbestos exposure in the genesis of lung cancer appear to be synergistic (3). There is also epidemiologic and roentgenographic evidence that workers who smoke have higher attack rates or progression rates for asbestosis (4).

Asbestos-related diseases are in general dose-related, and it has been suggested that smoking may exert its effects in part by interfering with the clearance of asbestos fibers from the lung, thereby increasing the effective dose (4). Because smoking has been shown to generally reduce particle clearance in humans (5, 6), this is a reasonable suggestion, but one which has never been tested.

Methods

Thirty female Hartley strain guinea pigs (mean weight, 400 g) were given 1 mg UICC amosite asbestos in 0.5 ml saline by intratracheal instillation using a procedure similar to that previously published (7). The animals were then divided into the following groups: nonsmokers (n = 10, referred to as "nonsmokers"), smoking commencing on the day after asbestos exposure (n = 10, referred to as "post-smoked"), and smoking commencing 2 wk before asbestos exposure and continuing for the duration of the experiment (n = 10, referred to as "presmoked").

The smoking apparatus is described and illustrated in reference 8. The machine operates by drawing 20 ml of air through a single cigarette and then expelling the 20 ml of smoke into a head-only exposure chamber. Each guinea pig is exposed in a separate chamber, breathing spontaneously in an awake state, and each cigarette feeds smoke to only 1 chamber. In this study, smoking animals were exposed to the whole smoke of 10 nonfilter standard cigarettes (200 puffs total, 20 ml smoke/puff) 5 days/wk. The cigarettes were obtained from the Tobacco Manufacturers Council of Canada; this type of cigarette has been previously used in studies on permeability of the airway epithelium (8-10) and is equivalent to a commercial nonfilter brand currently on the market. Animals were not exposed to smoke on the day of asbestos instillation.

Half of each group was killed by barbiturate overdose at 1 wk after instillation and half were killed at 1 month. The lungs were excised, and the right lung was fixed in glutaraldehyde by bronchial perfusion at a pressure of 100 cm of water for 30 min; this pressure is required to overcome the intense bronchospasm that occurs in guinea pigs immediately after death (11).

To determine asbestos fiber concentrations and sizes, we used the procedure that we have previously reported (12). Weighed pieces of lung constituting approximately half of the upper (cranial) and half of the lower (caudal) lobe were dissolved in filtered commercial laundry bleach for 24 h. The preparation was then centrifuged, the supernatant was discarded, and 30% hydrogen peroxide was added. The solution was allowed to stand for 4 h at 60° C. These procedures destroy the organic matter in the lung; our experience has shown that, using laboratory guinea pig lungs, virtually no organic matter remains and no other mineral particles are found after these steps. The solution was then filtered through an 0.45-μ pore size Millipore filter. Randomly selected portions of the filter were cut out and placed mineral side down on carbon/formvar-coated electron microscope grids, the grids were placed on pieces of urethane foam impregnated with acetone, and the filter was dissolved as capillary action drew the acetone onto the filter. This procedure transfers the particulates to the electron microscope grid. At the time of original tissue sampling, an adjacent piece of lung was dried to constant weight to allow expression of results as fibers/g dry lung.

The left lungs were lavaged with 20 ml of saline in volumes of 10, 5, and 5 ml. The lavagate was filtered and prepared as above for fiber analysis. Only fiber sizes were determined on the lavage material.

Using the electron microscope, approximately 200 sequentially encountered fibers/animal were identified and measured from the tissue samples and 100 fibers/animal from the lavage samples. Fiber identity was confirmed by energy dispersive X-ray spectroscopy using a Kevex 7000 system attached to the electron microscope. Grid squares to be examined were selected by assigning every square a number and picking the actual squares using random numbers. Squares were selected and counted until the appropriate number of fibers had been found. For the tissue samples the fiber concentration was determined by an algorithm, relating weight of sample, and area of filter examined (12). Blank samples, which were run by going through the above steps but omitting tissue, failed to reveal any amosite fibers.

Six hundred ninety-seven fibers of the

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UICC amosite sample were sized as a reference standard. Because fiber size distribution is usually lognormal, fiber sizes and aspect ratios for both the standard and the test samples were expressed as geometric means and geometric standard deviations. The mean (SD) values for the standard were: for length, 5.1 (2.4) μ; for width, 0.31 (2.1) μ; for aspect (length to width) ratio, 20 (2.3) μ.

Geometric size data were compared statistically using t tests with a Bonferroni correction (13). Because of the small sizes of the groups, nonparametric statistical comparisons were used on the concentration data: fiber concentrations were ranked and were initially analyzed in a one-way layout with a Kruskal-Wallis test ($H = 15.8$, $p < 0.01$) followed by a nonparametric multiple comparison test based on ranks (14).

Results

The arithmetic mean and range of concentrations of amosite fibers in the various groups and time periods are shown in table 1. Comparing each group from 1 wk to 1 month, the nonsmokers had an approximately 6-fold decrease in mean fiber concentration ($p < 0.01$), whereas the 2 smoking groups each had an approximately 20% decrease in fiber concentration (p = NS). At 1 wk after exposure, the presmoked animals had significantly lower fiber concentrations than either of the other groups ($p < 0.01$). At 1 month, the nonsmokers had significantly lower concentrations than either of the other groups ($p < 0.01$), and the presmoked animals continued to have a lower concentration than the postsmoked animals ($p < 0.01$).

The tissue fiber sizes in the different groups at 1 wk and at 1 month are shown in table 2. In the nonsmokers, there was a significant increase in mean length between 1 wk and 1 month ($p < 0.001$). In both smoking groups, fiber lengths decreased significantly between 1 wk and 1 month (each, $p < 0.001$). Comparing animals at 1 month, both smoking groups retained fibers that were significantly shorter than those in the nonsmokers, (each comparison, $p < 0.001$), and the presmoked animals had fibers that were shorter than those in the postsmoked animals ($p < 0.001$). Fiber widths were not significantly different at 1 month. The aspect ratios at 1 month showed that the mean values for nonsmokers were greater than those for postsmoked animals, which in turn were greater than those for presmoked animals (each comparison, $p < 0.01$).

Similar data for the lavage samples are shown in table 3. Overall, the lavaged fibers were shorter, thinner, and of lower aspect ratio than the tissue samples. Again, the mean fiber length increased in the nonsmokers ($p < 0.001$), but in the smoking animals there was either no increase in fiber length with time or a decrease. The absolute numbers of fibers in the nonsmoking and postsmoked groups divided into those shorter and longer than 10 μ are shown in table 4.

TABLE 1

MEAN FIBER CONCENTRATIONS (RANGE) × 10^6/g DRY LUNG

Group	One Week	One Month
Nonsmokers	95 (18–167)	18 (6–27)
Postsmoked animals	100 (53–232)	78 (52–99)
Presmoked animals	50 (14–87)	40 (17–70)

TABLE 2

TISSUE FIBER SIZES IN MICRONS AS GEOMETRIC MEAN [GEOMETRIC SD] (NUMBER OF PARTICLES MEASURED IN PARENTHESES)

Group	One Week	One Month
Lengths		
Nonsmokers	5.6 [2.4] (1,020)	8.3 [2.3] (1,010)
Postsmoked animals	5.8 [2.5] (1,016)	5.1 [2.3] (1,046)
Presmoked animals	5.9 [2.5] (1,013)	4.6 [2.7] (1,030)
Widths		
Nonsmokers	0.28 [1.9] (1,020)	0.30 [2.0] (1,010)
Postsmoked animals	0.34 [2.0] (1,016)	0.28 [1.6] (1,046)
Presmoked animals	0.32 [2.0] (1,013)	0.31 [1.9] (1,030)
Aspect Ratios		
Nonsmokers	19 [2.4] (1,020)	21 [2.3] (1,010)
Postsmoked animals	17 [2.5] (1,016)	18 [2.3] (1,046)
Presmoked animals	19 [2.4] (1,013)	14 [2.4] (1,030)

TABLE 3

LAVAGE FIBER SIZES IN MICRONS AS GEOMETRIC MEAN [GEOMETRIC SD] (NUMBER OF PARTICLES MEASURED IN PARENTHESES)

Group	One Week	One Month
Lengths		
Nonsmokers	2.3 [2.6] (498)	3.0 [2.3] (501)
Postsmoked animals	2.9 [2.5] (499)	2.5 [2.1] (500)
Presmoked animals	2.7 [2.6] (500)	2.8 [2.3] (502)
Widths		
Nonsmokers	0.19 [2.3] (498)	0.22 [1.8] (501)
Postsmoked animals	0.22 [1.8] (499)	0.21 [2.0] (500)
Presmoked animals	0.20 [1.9] (500)	0.20 [2.0] (502)
Aspect Ratios		
Nonsmokers	12 [2.1] (498)	13 [2.1] (501)
Postsmoked animals	13 [2.2] (499)	13 [2.1] (500)
Presmoked animals	13 [2.2] (500)	12 [2.0] (502)

TABLE 4

ABSOLUTE FIBER NUMBERS BY SIZE (MEAN CONCENTRATIONS × 10^6/g DRY LUNG)

		< 10 μ	> 10 μ
Nonsmokers	1 week	74	21
	1 month	13	3
Postsmoked animals	1 week	75	25
	1 month	66	12

Discussion

In this series of experiments, we were interested in examining the question of whether smoking affects asbestos clearance. The intratracheal instillation model that we have chosen is, like all intratracheal instillation models, unphysiologic: the doses given are high, and there is nonuniform fiber distribution within the parenchyma (15). Because all animals were treated in the same fashion, we do not believe that the method of asbestos administration, per se, should affect our results. However, it should be appreciated that different results might be obtained with an inhalation model, and that changes in the amount of asbestos administered and amount of smoking might also change the results. Thus, our comments on relevance to humans are meant to be interpreted with caution.

Bearing these caveats in mind, it is evident that, in our model, smoking decreased asbestos fiber clearance. This phenomenon was seen in both smoking groups; neither showed any statistical evidence of fiber clearance between 1 wk and 1 month. By contrast, in the nonsmokers only approximately one sixth of the fibers that were found at 1 wk were still present at 1 month.

The 2 different smoking protocols were used because we wished to determine whether exposure to cigarettes commencing before exposure to asbestos, i.e., the usual situation in humans, would have the same effect as acute exposure commencing only after exposure. Although the effects of smoking were parallel in both groups (no clearance), the presmoked group initially had a lower fiber burden than the group that was smoked only after asbestos instillation. A possible explanation for this difference is tha

smoking sensitizes the guinea pig airways so that bronchospasm is rapidly induced by asbestos administration, and more of the administered dose is deposited very proximally, from whence it is rapidly cleared, and less of the administered dose reaches distal airways and parenchyma, hence the apparent lower retained dose at 1 wk in the presmoked group. This sequence of events has been shown experimentally in humans inhaling bronchoconstrictors and particles (16). These problems do not, however, affect our conclusion that clearance is impaired in the presmoked animals.

A second conclusion from our data is that the increased fiber burden in the smokers compared to that in the nonsmokers at 1 month is composed in large part, although not entirely, of short fibers. This phenomenon is best seen in table 4, which we have restricted to the nonsmokers and the postsmoked animals, because these groups have almost identical mean concentrations of fibers at 1 wk. In the smokers, approximately half of the fibers longer than 10 μ were cleared by 1 month, whereas virtually no fibers shorter than 10 μ were cleared. However, the clearance failure was not entirely confined to short fibers since the smoking animals still retained 4 times the number of long fibers than did the nonsmoking animals.

It has been shown (17, 18) that, ordinarily, short fibers are cleared more effectively from the lung than are long fibers, and hence retained fiber length appears to increase with time. This phenomenon was seen in our nonsmoking animals for both tissue fibers and lavaged fibers. In both groups of smoking animals, on the other hand, the length and aspect ratio of retained tissue fibers decreased from 1 wk to 1 month, and a similar process was seen in the lavaged fibers. Presumably, the differences in fiber sizes between smoked and nonsmoked animals reflect smoke-induced interference with mucociliary transport and/or macrophage clearance of fibers; phagocytosis by macrophages is thought to be the first step in clearance of short asbestos fibers from the lung parenchyma (17, 18). It is interesting that the presmoked group had the shortest mean tissue fiber lengths and lowest aspect ratios at 1 month, an observation which may imply that the interference with clearance mechanisms is related to cigarette dose. There is some data in humans that clearance times are directly proportional to dose as measured by pack-years of smoking (6).

The actual mechanisms of carcinogenesis and fibrogenesis by asbestos are not clear, but from observations of disease incidences in laboratory animals, it is often claimed that long fibers are considerably more pathogenic than are short fibers in the genesis of asbestosis, carcinoma of the lung, and mesothelioma (19–21). However, the suggestion has also been made that a large number of short fibers might be just as dangerous as a few long fibers (22). Because our guinea pigs retain increased numbers of both long and short fibers, we cannot determine whether the increased disease rates seen in smoking asbestos workers should be imputed to long or short fibers; however, our data do suggest that short fibers should not be totally disregarded as possible agents in the pathogenesis of asbestos-related disease.

References

1. Selikoff IJ, Hammond EC, Seidman H. Mortality experience of insulation workers in the United States and Canada, 1943–1976. Ann NY Acad Sci 1979; 330:91–116.
2. Selikoff IJ, Seidman H, Hammond E, Culyer S. Mortality effects of cigarette smoking among amosite asbestos factory workers. JNCI 1980; 65:507–18.
3. Hammond EC, Selikoff IJ, Seidman H. Asbestos exposure, smoking, and death rates. Ann NY Acad Sci 1979; 330:473–92.
4. Weiss W. Cigarette smoke, asbestos, and small irregular opacities. Am Rev Respir Dis 1984; 130:293–301.
5. Cohen D, Arai SF, Brain JD. Smoking impairs long-term clearance from the lung. Science 1979; 204:514–6.
6. Bohning DE, Atkins HL, Cohn SH. Long-term particle clearance in man: normal and impaired. Ann Occup Hyg 1982; 26:259–71.
7. Filipenko D, Wright JL, Churg A. Pathologic changes in the small airways of the guinea pig after amosite asbestos exposure. Am J Pthol 1985; 119:273–8.
8. Simani AS, Inoue S, Hogg JC. Penetration of respiratory epithelium of guinea pigs following exposure to cigarette smoke. Lab Invest 1974; 31:75–87.
9. Hulbert WC, Walker DC, Jackson A, Hogg JC. Airway permeability to horseradish peroxidase in guinea pigs: The repair phase after injury by cigarette smoke. Am Rev Respir Dis 1981; 123:320–6.
10. Boucher RC, Johnson J, Inoue S, Hulbert W, Hogg JC. The effect of cigarette smoke on the permeability of guinea pig airways. Lab Invest 1980; 43:94–100.
11. Lai Y-L, Lamm WJE, Luchtel DL, Hildebrandt J. Massive postmortem bronchoconstriction in guinea pig lungs. J Appl Physiol 1984; 56:308–14.
12. Churg A. Asbestos fiber content of the lungs in patients with and without asbestos airways disease. Am Rev Respir Dis 1983; 127:470–3.
13. Miller RG. Simultaneous statistical inference. 2nd ed. New York: Springer-Verlag, 1981; 67–70.
14. Hollander M, Wolfe D. Nonparametric statistical methods. New York: John Wiley & Sons, 1973; 114–36.
15. Brain JD, Knudson DE, Sorokin SP, Davis MA. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ Res 1976; 11:13–33.
16. Svartengren M, Philipson K, Linnman L, Camner P. Airway resistance and deposition of particles in the lung. Exp Lung Res 1984; 7:257–69.
17. Holmes A, Morgan A. Clearance of anthophyllite fibers from the rat lung and formation of asbestos bodies. Environ Res 1980; 22:13–21.
18. Morgan A. Effect of length on the clearance of fibers from the lung and on body formation. In: Wagner JC, ed. Biological effects of mineral fibers. Lyon: IARC, 1980; 329–35.
19. Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press, 1978.
20. Davis JMG, Beckett ST, Bolton RE, Collings P, Middleton A. Mass and number of fibers in the pathogenesis of asbestos-related lung disease in rats. Br J Cancer 1978; 37:673–87.
21. Stanton MF, Layard M, Tegeris A, Miller E, May M, Morgan E, Smith A. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. JNCI 1981; 67:965–75.
22. Pott F. Animal experiments on biological effects of mineral fibers. In: Wagner JC, ed. Biological effects of mineral fibers. Lyon: IARC, 1980; 261–72.

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