Excessive accumulation of asbestos fibre in the bronchoalveolar space may be a marker of individual susceptibility to developing asbestosis: experimental evidence

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ABSTRACT Earlier studies have shown that only 60% of sheep exposed to a given chrysotile exposure developed asbestosis. Analyses of lung lavage (BAL) fibre content early in the disease showed that, despite identical injected doses, the subset of sheep with interstitial lung disease had significantly more fibre retention. To determine if the fibre retention preceded or followed early disease, 15 were exposed at 10 day intervals to 100 mg chrysotile by intratracheal injection. Animals were studied at three month intervals by chest radiograph (CR) and BAL. At month 15, 10 sheep had definitely abnormal CR (group B) and five had normal CR (group A). Fibre analyses of BAL reproduced earlier finding of a higher level of fibre retention early in the disease, month 15: 92 ± 2 f/ μ l in group B v 35 ± 19 in group A. Moreover, at month 3, when no disease was detectable, group B already had a significantly higher fibre retention level: 84 ± 2 in group B v 52 ± 3 in group A ($p < 0.05$). These data clearly imply that high alveolar dust retention precedes the disease process and that alveolar dust clearance capacity may be a major determinant of asbestosis.

Subjects exposed to asbestos in the workplace are at risk of developing asbestosis. For a given estimated inhalation exposure level, however, only a fraction of the men will develop the disease.¹ This fact has been largely attributed to the so called individual susceptibility. We have reproduced in a sheep model the selective development of the disease in some 60% of sheep exposed to the same intratracheal injection dose of the fibres and have found previously that at time of early asbestosis, the animals with the disease had significantly more fibres retained in their bronchoalveolar space.²

The present study was designed to test the hypothesis that the retention of asbestos fibres in the animals who develop asbestosis should precede the development of disease if it is to be a determinant of individual susceptibility.

Materials and methods

EXPERIMENTAL DESIGN

Twenty five sheep weighing 25-40 kg were used in this

study. They were prepared and accustomed to the pulmonary techniques as previously reported.²

The flock was divided into a group of 10 exposed to phosphate buffered saline only and a group of 15 exposed to 100 mg UICC Canadian chrysotile asbestos fibres in 100 ml phosphate buffered saline every 10 days.

These fibres are relatively uniform and well characterised, 92% being less than $0.25 \mu\text{M}$ in diameter and $20 \mu\text{M}$ in length. Exposures were carried out after nasotracheal intubation via repeated slow infusion of the suspension in the trachea at 10 day intervals until disease was detected clearly in more than 50% of the exposed animals by chest radiography.

CHEST RADIOGRAPH

Each sheep was positioned on a mobile cart with a wooden board and a grid cassette under the thorax. The x ray source was placed at a 30° caudal angle two feet from the cassette. The intubated animal was held at total lung capacity using a giant syringe and radiographs were taken at exposure factors 80 kV, 20 mAs, and 0.02 s. Each radiograph was scored according to the International Labour Organisation classi-

fication of radiographic profusion of parenchymal opacities.³ This classification recognises the existence of a continuum of change, from no opacity to the most advanced category. The scores were converted to a linear scale of 0 to 10 (12 categories) as follows: ILO grade 0/- (clearly normal) and grade 0/0 (normal after close examination) = 0 on the linear scale, 0/1 = 1, 1/0 = 2, 1/1 = 3, 1/2 = 4, 2/1 = 5, 2/2 = 6, 2/3 = 7, 3/2 = 8, 3/3 = 9, 3/4 = 10. In the ILO classification four categories are defined on the basis of these same profusion scores: category 0 = profusion scores 0/-, 0/0, and 0/1; category 1 = profusion scores 1/0, 1/1, and 1/2; category 2 = profusion scores 2/1, 2/2, and 2/3; category 3 = profusion scores 3/2, 3/3, and 3/4. The radiographs in category 0 are generally considered normal whereas those in category 1 or above are definitely abnormal. This was the criterion of the disease detection selected for this study as it is also commonly used in the detection of human asbestosis.¹

LUNG LAVAGE

Bronchoalveolar lavage (BAL) was carried out in a randomly selected subsegment of lung after wedging the distal tip of the bronchoscope in a distal airway by a slow infusion of four 50 ml 39°C aliquots of saline and by gentle aspiration of the effluent. The effluent of the fourth 50 ml infusion was used for fibre analyses as in our previous report.²

ELECTRON MICROSCOPY

The analytical transmission electron microscope was used to look for the presence of chrysotile fibres in lung lavages. All samples were prepared in a "clean room," using filtered chemicals and carefully cleaned glassware. Thirteen millilitres were isolated from the third effluent of lavage and allowed to react at room temperature with sodium hypochlorite in order to digest biological material. An aliquot of the suspension, corresponding to 5 ml of BAL, was filtered on a polycarbonate membrane filter 25 mm in diameter with a pore size of 0.2 µm pore size (Nucleopore Corp, Pleasanton, CA). Particles collected at the upper surface of the membrane were embedded in a carbon film and subsequently transferred on to 200 mesh grids.⁴ Several grid openings were observed in the transmission mode at a screen magnification × 10 000. Chrysotile fibres were identified by their morphological features and their elemental composition as determined by energy dispersive x ray spectrometry (PGT system IVR; Princeton Gamma Tech, Princeton, NJ). The length of each individual fibre encountered was measured directly on the screen to the nearest 0.2 µm; diameter was measured using an eyepiece graticule. Fibre count analyses were done at months 3 and 15 of the study.

STATISTICAL ANALYSIS

In the presentation of the results the data for each group of sheep are followed by the standard error as an index of dispersion. The data were evaluated by analysis of variance for experiments having repeated measurements on the same subjects. When a significant effect was detected, a Kruskal-Wallis test was used to determine which group means were significantly different.⁵ Differences with $p < 0.05$ were considered significant.

Results

The chest radiographs of the control sheep remained normal throughout the study. The first abnormal radiograph in the exposed sheep occurred during month 9 of the study and by month 15, 10 of the 15 asbestos exposed sheep had a definitely abnormal (ILO category 1) chest radiograph. The two subsets of sheep were then composed of 10 asbestos exposed sheep with early asbestosis (group B) and five similarly exposed sheep without asbestosis (group A).

Analyses of fibre content of BAL were done at the time of early disease (month 15) and at month 3, at which time all exposed sheep had normal radiographs. The results are presented in the table where it can be appreciated that fibre content of the bronchoalveolar space at the time of early disease was 2.6 times higher in group B compared with group A ($p < 0.05$) and that the higher BAL content of fibre in the sheep of group B was already present at month 3, before disease could be detected. Fibre length was comparable in all samples.

Discussion

This study extends our earlier investigation of the relation of exposure to asbestos dust, dust retention in the bronchoalveolar space, and the development of asbestosis.² Firstly, this work reproduces the early disease process in a subset of exposed sheep (group B) and documents again that the sheep with early asbestosis have significantly more fibres retained in their bronchoalveolar space than sheep similarly exposed to

Bronchoalveolar fibre content

	Group A Normal radiograph (n = 5)	Group B Abnormal radiograph (n = 10)
Month 3:		
Fibre	52 ± 3 fibre/µl	84 ± 2* fibre/µl
Length	4.3 ± 1.5 µM	3.7 ± 1.4 µM
Month 15:		
Fibre	35 ± 19 fibre/µl	92 ± 2* fibre/µl
Length	4.6 ± 1.7 µM	5.2 ± 1.4 µM

* $p < 0.05$ for group B v A.

Groups A and B are defined clearly in the "Result" section.

asbestos but without the disease. In this study also fibre length was comparable in both subsets of animals.

Secondly, the study reports new data on fibre retention. In this experiment we wished to ascertain that the higher fibre retention in the animal with early asbestosis was not related to the disease process itself. Therefore, we looked at fibre content of the bronchoalveolar space of our two subsets of sheep, before disease could be detected, at month 3 of this study. There, it is clearly seen that sheep which will develop asbestosis in the ensuing months of exposure to asbestos already have a significantly higher retention of fibres in their bronchoalveolar space.

Because the BAL fibre content relates to the lung tissue burden,⁶⁷ our data clearly strengthen our prior proposal that bronchoalveolar fibre dust clearance capacity may be a major determinant of individual susceptibility to develop asbestosis.

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