EXPERIMENTAL STUDIES IN RATS ON THE EFFECTS OF ASBESTOS INHALATION COUPLED WITH THE INHALATION OF TITANIUM DIOXIDE

J.M.G. DAVIS, MA., ScD. • A.D. JONES • IMELDA PARKER

Institute of Occupational Medicine, 8 Roxburgh Place
Edinburgh EH8 9SU

INTRODUCTION

Many inhalation studies in experimental animals have been undertaken to examine the pathogenicity of mineral fibres.[7,2,5] So far, however, work has concentrated on the effects of pure dust clouds in spite of the fact that, in the industrial environment, fibres are inhaled at the same time as isometric dust particles of many types. To examine the effects of other dusts inhaled with mineral fibres, we commenced a study in which amosite (a long fibre preparation) and chrysotile (UICC 'A') asbestos were administered to rats over the same time period as titanium dioxide, an innocuous particulate dust, or quartz, a highly toxic material.

MATERIAL AND METHODS

Groups of 48 rats of the AF/HAN strain were treated with one of the asbestos varieties at a dose level of 10 mg/m^3 of respirable dust with either titanium dioxide at 10 mg/m^3 or quartz at 2 mg/m^3. The inhalation period was one year and subsequently most of the animals were allowed to live out their full life span. Groups of four rats were killed at the end of the dusting period and similar groups six months later. From these animals the left lung was ashed to determine the content of retained dust, while the right was processed for histology. All lung tissue was serially sectioned with sections examined at multiple levels throughout the organ. Sections were also examined routinely from all major organs and all areas of pathological change detected macroscopically at autopsy. Sections were stained with haematoxylin and eosin, Van Gieson's method for collagen or Gordon and Sweet's method for reticulin. The area of lung tissue occupied by pulmonary interstitial fibrosis was measured using an automatic image analyser (Graphic Information Systems Ltd., GDS1). For the determination of retained asbestos, half of the left lung was ashed at low temperature in nascent oxygen and infrared analysis undertaken of a potassium bromide disc containing the dust residue. For titanium dioxide the rest of the lung tissue was analysed by atomic absorption following muffle ashing. Comparisons of levels of interstitial fibrosis and the retained asbestos content of lung tissue were undertaken using conventional analysis of variance techniques. Differences in the number of pulmonary tumours found in the experimental groups were examined using the Pearson chisquare statistic. The studies involving titanium dioxide and asbestos and quartz and asbestos were not undertaken synchronously. At present only the data from studies with titanium dioxide are complete and these are presented in this paper.

RESULTS

At the end of the dusting period, histological examination of lung tissue revealed large amounts of both asbestos and titanium dioxide intermingled within pulmonary macrophages and in deposits of fibrosing granulation tissue in the region of the terminal and respiratory bronchioles which are the characteristic early signs of lung pathology in rats exposed to asbestos (Figure 1). These lesions consisted mainly of macrophages, giant cells and fibroblasts with reticulin and collagen fibres found in increasing density as the study progressed. Giant cells were particularly noticeable in animals treated with asbestos and titanium dioxide with the phagocytosed titanium dioxide particles packed in the peripheral regions of cytoplasm along with the multiple nuclei. Short asbestos fibres were also found in this peripheral area but longer fibres transfixed the clear central regions of cytoplasm. As the rats aged, pulmonary fibrosis extended in some lung areas to involve the alveolar walls in the parenchyma between the terminal bronchioles. The first sign of this alveolar interstitial fibrosis is a rounding up of Type II epithelial cells which then progressively increase in number as the interstitial space is thickened with fibrous deposits until the airspaces may become lined completely by cuboidal epithelium. In some areas the fibrotic thickening of septa predominates but, in others, epithelial change is more pronounced leading to a pattern of adenomatosis. In the most advanced stages of this condition, some remodeling of the lung architecture occurs with thick walled airspaces no longer corresponding to the original alveoli. The process is probably equivalent to the development of honeycombing in human lungs. The area of lung tissue involved in this type of advanced 'fibrosis' in those animals surviving to within two months of the end of the study (age 34 months or more) is illustrated in Table I with comparable figures from recent studies using the same chrysotile or amosite samples on their own.[2,4] The inhalation of titanium dioxide as well as asbestos did not increase the amount of fibrosis produced.

With the production of pulmonary tumours, however, a marked difference was found between those animals inhaling asbestos only and those inhaling asbestos and titanium dioxide (Table II). For this comparison, two studies using UICC chrysotile 'A' were available.[2,4] In the two studies with UICC chrysotile alone, the number of pulmonary

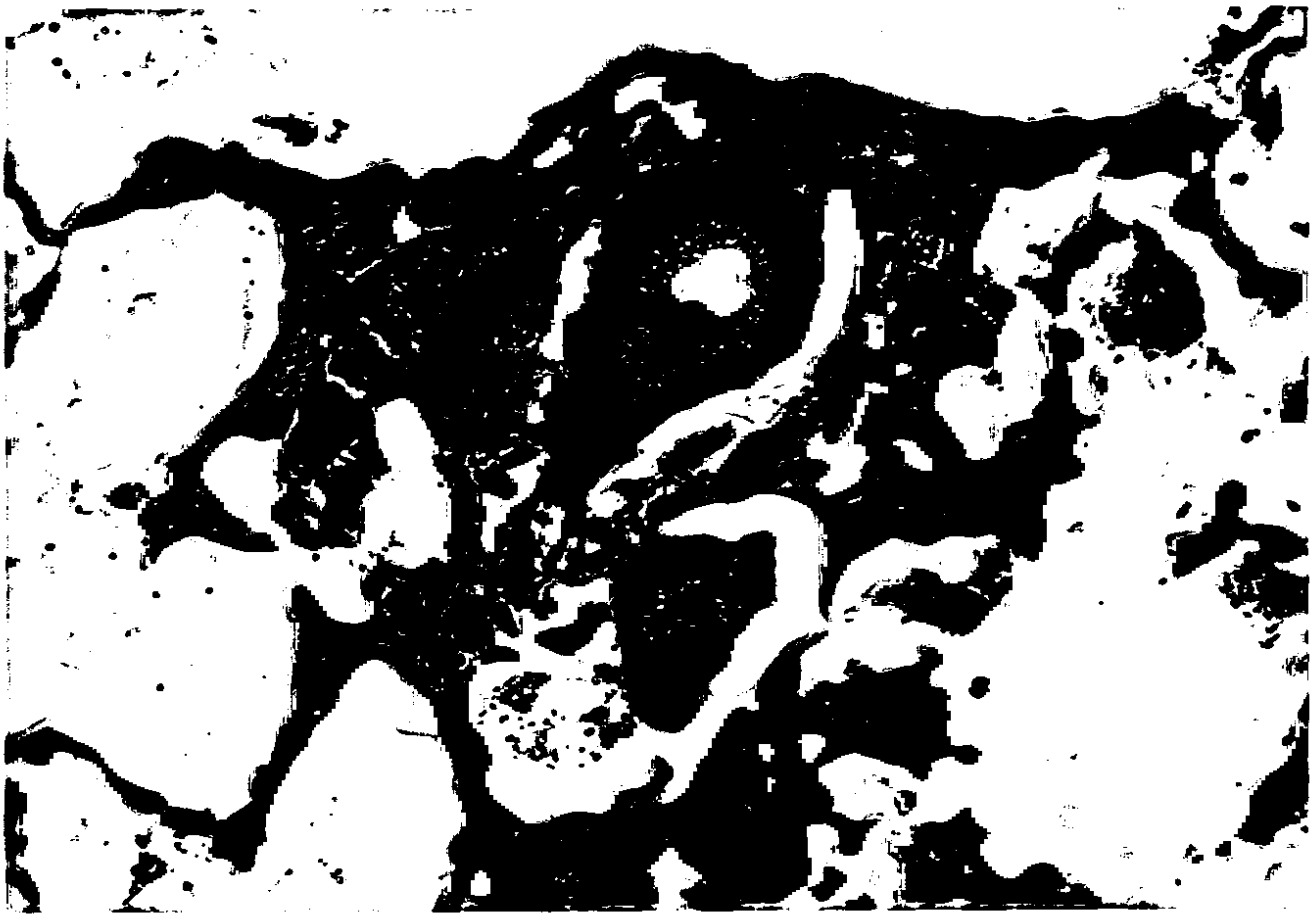

Figure 1. An area of fibrosing granulation time formed in the walls of respiratory bronchioles in a rat after 12 months inhalation of amosite and titanium dioxide. Fibres and particles of titanium dioxide (which appear black) are mingled together in phagocytic cells both free in the alveolar spaces and in the solid tissue of the lesion. Two foreign body giant cells are present with dust packed in the peripberal regions of the cytoplasm but with relatively clear centres. Magnification x 400.

Table I

The Percentage of Lung Parenchyma Occupied by Pulmonary Interstitial Fibrosis

CHRYSOTILE	CHRYSOTILE PLUS TITANIUM DIOXIDE	AMOSITE	AMOSITE PLUS TITANIUM DIOXIDE
12.2% (1.5-24.3)	12.9% (3.8-26.1)	11.0% (0.4-34.6)	9.5% (0.7-20.7)

Figures are means of all animals surviving until within two months of the end of the study. Group sizes varied from 12 - 18 months.

Table II
Pulmonary Tumours

TUMOUR TYPE	CHRYSOTILE		CHRYSOTILE PLUS TITANIUM DIOXIDE	AMOSITE	AMOSITE PLUS TITANIUM DIOXIDE
	1	2			
Adenoma	7	6	4	3	1
Adenocarcinoma	6	4	12	3	8
Squamous carcinoma	2	4	3	4	3
Mixed/ undifferentiated			5	1	5
Pleural mesothelioma			2	2	2
Peritoneal mesothelioma				1	
TOTAL	15	14	26	14	19
No. of animals	40	37	41	40	40

tumours produced was almost identical, indicating a good degree of reproducability in the animal model. The inhalation of titanium dioxide as well as chrysotile resulted in approximately twice the number of pulmonary tumours. With amosite and titanium dioxide, tumour production was approximately 50% higher than with amosite alone. For the experiments with chrysotile asbestos, the figures for all pulmonary tumours were significantly different ($P < 0.02$) and even more significant if only malignant tumours were considered ($P < 0.004$). For amosite the difference did not reach statistical significance with the group sizes used ($P > 0.10$).

Figures for the lung dust content of animals six months after the end of the dusting are illustrated in Table III. The presence of titanium dioxide, a particulate dust normally considered to be innocuous is associated with double the amount of chrysotile as normally retained at the same timepoint following this asbestos dose on its own. For animals treated with amosite and titanium dioxide, the retained amosite dose was 60% more than with amosite alone. Even with very small groups of only four rats, the differences in chrysotile retention were significant ($P < 0.05$). With the amosite experiments once again the differences were not large enough to reach significance ($P > 0.10$).

DISCUSSION

The results of studies with titanium dioxide and asbestos indicate that the inhalation of a particulate dust normally considered to be innocuous may increase the carcinogenicity of both amosite and chrysotile. Lung dust analysis suggests that this may result from increased retention of asbestos with the increase in pulmonary tumours very closely matching the increase in lung dust content. Whether or not this finding indicates an increased hazard for asbestos workers exposed to mixed dusts in the industrial environment needs careful consideration. Studies examining the buildup of amosite and titanium dioxide in the lungs of rats over a one year exposure period followed by a short clearance period of 38 days have been reported.[6] In this study no reduction of amosite clearance was found compared to similar studies with amosite alone. However, dose levels were different from those in the present study (2.5 mg/m^3 for amosite and 15 mg/m^3 of titanium dioxide) and the clearance period was short covering time when much dust is known to be in macrophages free in the alveoli. The six month period covered by the present paper is a time when dust is being incorporated into solid lesions in the lung parenchyma. The increased retention reported for amosite (as well as chrysotile) may reflect an increase in this process.

Table III

Lung Asbestos Burdens Six Months After the End of Dust Exposure

CHRYSOTILE	CHRYSOTILE PLUS TITANIUM DIOXIDE	AMOSITE	AMOSITE PLUS TITANIUM DIOXIDE
315 (49)	710 (71)	3080 (370)	4980 (499)

Figures are in microgrammes and are the means of groups of four animals. Standard deviations in brackets.

A continuing debate about asbestos-related pulmonary carcinomas concerns the question of whether or not these tumours occur in the absence of pulmonary fibrosis.[1] In the present study, levels of fibrosis were found not to increase with an increase in lung tumours when asbestos was administered with titanium dioxide. However, the principal does appear to apply since all the animals developing pulmonary tumours did have quite large amounts of pulmonary fibrosis as well. It may be that while fibrosis is an essential precursor of tumour development, the area of fibrosis is not the most important factor. The amount of dust retained in any area of fibrosis and the cellularity of the lesions may be much more important. In addition, the method of measuring advanced interstitial fibrosis that we have adopted involves ignoring, in animals with tumours, those areas of lung occupied by the tumour itself and estimating fibrosis as a percentage of the remainder. Thus a tumour may arise in a large area of fibrosis but overgrow this and eliminate it. The percentage of fibrosis in the remaining lung tissue may be relatively small.

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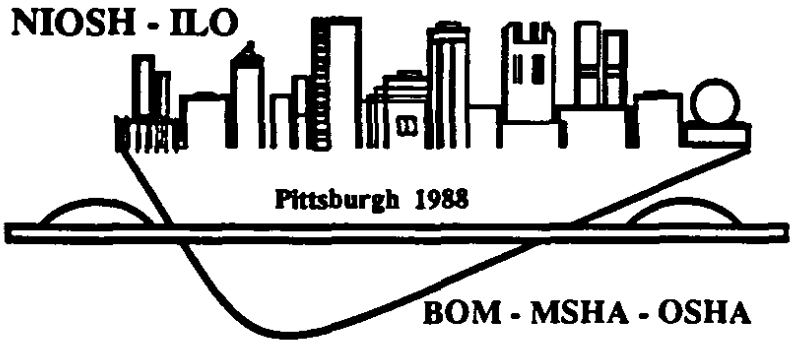

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