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Asbestos fibers and human malignant mesothelioma

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Introduction

It is well known that human malignant mesothelioma is caused almost exclusively by exposure to asbestos. Currently, to prove exposure to asbestos, several approaches have been utilized such as occupational and environmental history, clinical and pathologic investigation and asbestos tissue burden study. In the last approach, asbestos fibers are typically identified and characterized in the lung tissue by analytical electron microscopy. Asbestos fibers have been generally sought in the lung tissue of mesothelioma patients. Such an approach has generally been carried out in studies of mesothelioma patients. However, we question the adequacy of this approach for assessing the carcinogenicity of asbestos in the pleural or peritoneal mesothelioma for the following reasons:

1) prior to development of the tumor, the mesothelial tissue must be directly exposed to asbestos;
2) both the parietal pleura and the peritoneum, which are known to be the primary site of mesothelioma, are far from the lung parenchyma;
3) intrapulmonary asbestos fibers translocate to these serosal tissues; and
4) there is a disproportion of both type and number of asbestos fibers between the lung and the serosal tissues in mesothelioma cases.

Our objective is to characterize asbestos fibers associated with the induction of human malignant mesothelioma by an asbestos tissue burden study on both the lung parenchyma and the mesothelial tissues, including pleural hyaline plaque and mesotheliomatous tissue.

Materials and Methods

To identify and characterize the asbestos fibers associated with the induction of malignant mesothelioma, asbestos fibers in lung parenchyma and mesothelial tissue representing mesotheliomatous tissue or hyaline plaque or both, obtained from human malignant mesothelioma cases were investigated. Ninety-two lung

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samples and 89 mesothelial tissue samples obtained from the 127 mesothelioma cases were used as materials. These samples comprised bulk tissues, 25 μm sections, or both. Twenty-seven lung samples and nine mesothelial tissue samples, obtained as bulk tissues from the general population were used as controls. These mesothelioma cases comprised various occupations, including asbestos insulation worker, pipe fitter, electrician, shipyard worker, sheet metal worker, power plant worker, seamen, brake lining, related worker and housewife. Diagnostic certainty of all of these mesothelioma cases was reconfirmed by Y.S. by systematic analysis including gross appearances. histology, histochemistry and immunocytochemistry. Ultrastructural diagnosis was also undertaken in a small number of these cases. For the asbestos tissue burden study, a digestion technique of the bulk tissues using bleach or KOH solution, and low temperature ashing technique of thick tissue sections, were both used. Details of these techniques have been reported elsewhere [1–3]. A high-resolution analytical electron microscope was used for the identification and characterization of asbestos fibers in these tissues; ultrastructure and energy dispersive X ray spectrometry were utilized, and selected area electron diffraction was used in a limited numbers of these cases.

Results

The results were as follows:

1. Asbestos fibers were present in all of the 92 lung tissues and in almost all (86/89) of the mesothelial tissues.
2. The most common asbestos types seen in the lung were an admixture of chrysotile with amphiboles (38/92; 41.3%); the majority of the admixed amphiboles was amosite. In 26 of the 92 cases (28.3%), the detected asbestos type in the lung was chrysotile only.
3. The asbestos type seen in the mesothelial tissues was chrysotile alone in the majority (68/86; 79.0%); although both chrysotile and amphiboles (mainly amosite, rarely crocidolite, tremolite or anthophyllite) were seen in the remaining 21 cases.
4. Quantitative analysis of asbestos fibers was done on the digested bulk lung tissues obtained from 34 mesothelioma cases. The number of asbestos fibers in the lung of these mesothelioma cases was 14.5×10^6/dry gram as geometric mean (GM), 456.4×10^6/dry gram in maximum, and 0.16×10^6/dry gram in minimum. Except for a single case, the number of fibers in the lungs of these 34 mesothelioma cases was larger than the maximum number (0.49×10^6/dry gram) found in the lungs of the general population.
5. The number of asbestos fibers in the mesothelial tissues was investigated in 17 of the 127 mesothelioma cases, all of which also had lung analyses. The number of fibers was 3.1×10^6/dry gram as GM, 240×10^6/dry gram maximum and 1.4×10^6/dry gram minimum. Except for a single case, the number of fibers in the mesothelial tissues was larger than the maximum number (2.24×10^6/dry gram) in the nonasbestos-related fibrous mesothelial tissue

obtained from the general population subjects having no known occupational exposure to asbestos.

6. A comparative study of the type and number of asbestos fibers between the lung and the mesothelial tissues was done in the above-mentioned 17 cases. It was noteworthy that the type and number of asbestos fibers were different between the lung and the mesothelial tissues. In the lung, chrysotile and amosite were major asbestos types although amosite fibers were generally larger in number. On the other hand, in the mesothelial tissues, chrysotile was the major asbestos type and the number of the chrysotile fibers was remarkably larger than that of other asbestos types.
7. Dimensions (length and diameter) of all of chrysotile and amosite fibers seen in the digested lung samples and the digested mesothelial tissue samples obtained from the above-mentioned 17 cases were measured. The chrysotile fibers were shorter in length and thinner in diameter than the amosite fibers. In the former, the length (GM) was 0.84 μm in the lung, and 0.6 μm in plaque and 0.68 μm in the mesothelial tissue, and the diameter (GM) was 0.04 μm in both the lung and the mesothelial tissue. In the latter, the length (GM) was 3.4 μm in the lung, 2.2 μm in the plaque and 3.0 μm in the mesotheliomatous tissue, and the diameter (GM) was 0.13 μm in the lung, 0.13 μm in the plaque and 0.15 μm in the mesotheliomatous tissue.
8. Long (>8 μm in length), thin (<0.25 μm in diameter) chrysotile fibers comprised of those found 0.7% in the lung, 1.6% in plaque and 0.4% in mesotheliomatous tissue. The amosite fibers of similar dimensions were seen in 8.9% in the lung, 4.4% in plaque and 7.9% in mesotheliomatous tissue.

Discussion

In 1991, Kohyama and Suzuki reported an asbestos tissue burden study on seven cases of mesothelioma in asbestos insulators, together with three cases of the lung cancer and three asbestosis cases [1]. Quantitative comparison between chrysotile and amosite (the major amphibole fibers used by the insulators) was done between the lung and the mesothelial tissues (pleural hyaline plaque and mesotheliomatous tissue). In the insulators' lung tissues, both chrysotile and amphiboles (predominantly amosite) were present and amosite was larger in number compared with chrysotile. However, in the mesothelial tissues, the predominant asbestos type was chrysotile and its number was remarkably large. Such a disproportion of asbestos type and number of asbestos fibers had been reported by LéBouffant et al. [4] and Sébastien et al. [5]. Our present study on mesothelioma cases in which patients had been exposed to chrysotile and amphiboles, also showed the same trend as seen in our previous study.

We have strongly suggested that this disproportion was caused by chrysotile's remarkable capacity of translocation from the lung to the pleura and the peritoneum [1].

It is logical to consider that to develop malignant mesothelioma, pleural or

peritoneal tissues must be directly exposed to asbestos. If it is so, the type of asbestos in the mesothelial tissues is significant enough to be considered as the carcinogen of malignant mesothelioma.

It was interesting to find that in 28.3% (26 of 92) of our mesothelioma cases, the asbestos type detected in the lung was chrysotile only, indicating that these patients had been exposed to chrysotile almost exclusively. Similar results had been obtained by Morinaga et al. [2].

The role of chrysotile in the induction of human malignant mesothelioma has been disputed. However, based on our tissue burden study, it was concluded that chrysotile is an important carcinogen of human malignant mesothelioma, since the asbestos type detected in the mesothelial tissues was predominantly chrysotile and its number was much larger than other asbestos types. Presently, many researchers are still seeking asbestos fibers associated with the development of human malignant mesothelioma in the lung tissue only. However, we emphasize that asbestos fibers in the lung do not fully represent a total picture of asbestos exposure because translocated asbestos fibers are not retained in the lung. We also consider that the lung is not a wholly suitable organ to examine for the identification of asbestos fibers responsible for the induction of malignant mesothelioma, since a disproportion of both type and number of asbestos fibers is frequently seen between the lung and the mesothelial tissues.

There were obvious differences in dimensions between chrysotile and amosite (the major amphibole) in both the lung and the mesothelial tissues. Chrysotile was shorter and thinner than amosite in these tissues. Timbrell has reported that the smaller diameter is the essential factor for asbestos fiber's penetration into the deeper part of the lung [6]. The thin diameter of all types of asbestos fibers may be an important factor for the translocation of the fibers from the lung to other tissues.

Stanton hypothesized that long (8 μm and over in length), thin (0.25 μm and smaller in diameter) fibers were strongly carcinogenic [7]. Such long and thin asbestos fibers were quite small in numerical proportion in both the lung and the mesothelial tissues: the number of asbestos fibers corresponding to the dimension in Stanton's hypothesis were less than 2% in chrysotile and less than 9% in amosite in the lung and the mesothelial tissues. It was noteworthy that the majority of asbestos fibers in the pleural hyaline plaque and mesotheliomatous tissues were short, thin chrysotile fibers. It is reasonable to consider that such short, thin chrysotile fibers have played an important role in the development of both hyaline plaque and malignant mesothelioma.

Summary

To identify and characterize asbestos fibers contributing to the induction of human malignant mesothelioma, asbestos fibers in lung tissues (92 cases) and mesothelial tissues (hyaline plaque and mesotheliomatous tissues; 89 cases) obtained from 127 mesothelioma cases were investigated by a high-resolution

analytical electron microscope. Digestion of bulk tissues, or ashing of 25-μm tissue sections, or both were used for the tissue preparation:

1) A disproportion of types and number of asbestos fibers was seen between the lung and the mesothelial tissues in these mesothelioma cases and this disproportion was explained by chrysotile's strong capacity to translocate from the lung to the mesothelial tissues.
2) Chrysotile was considered to be an important carcinogen of human malignant mesothelioma, since the major asbestos type identified in the mesothelial tissues was chrysotile and asbestos type detected in the lung was chrysotile only in 28.3% of 92 consecutive cases of mesothelioma.
3) Asbestos fibers corresponding to Stanton's hypothesis (8 μm and longer in length and 0.25 μm and smaller in diameter) were a small proportion of asbestos fibers detected in the lung and the mesothelial tissues; less than 10% in amosite and less than 2% in chrysotile. A large majority of asbestos fibers were short and thin in dimension. We may not exclude fibrogenic and carcinogenic effects of such short, thin asbestos fibers, since they are predominant asbestos fibers seen in asbestos-induced fibrotic pleura (hyaline plaque) and mesotheliomatous tissue.

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