主論文の要旨

RAT MODEL DEMONSTRATES A HIGH RISK OF TREMOLITE BUT A LOW RISK OF ANTHOPHYLLITE FOR MESOTHELIAL CARCINOGENESIS

（ラットモデルにおける中皮腫発がんリスクは
トレモライトが高くアンソフィライトは低い）

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【Introduction】

Chrysotile (white asbestos), crocidolite (blue asbestos) and amosite (brown asbestos) are all categorized as major asbestos. Substantial data are available on major asbestos fibers, including studies published by the authors of this study. Crocidolite has been used in most of the asbestos cell culture experiments. However, recent studies on minor asbestos, actinolite, tremolite and anthophyllite, are lacking.

Here we performed a carcinogenesis experiment with Japanese tremolite ($[Ca_2Mg_5Si_8O_{22}(OH)_2]_n$) and Afghan anthophyllite ($[(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2]_n$). The mesothelial carcinogenicity of tremolite with short fibers was potent, whereas Afghan anthophyllite did not induce MM within a period of 550 days.

【Methods】

The animal experiment committee of the Nagoya University Graduate School of Medicine approved the experiments performed in this study. Six-week-old 150 female and 120 male F1 Fischer-344 x Brown-Norway hybrid rats (Charles River, Japan) were used, which is consistent with our previous experiments. No spontaneous MM has been reported in these rats. A total of 270 animals were used, and they were divided into the following three groups: untreated control (NTA or saline), tremolite and anthophyllite. We used two injection doses of each asbestos fiber (4 groups of 54 animals each, consisting of 24 males and 30 females). The animals received two intraperitoneal injections of either 0.5 or 5 mg asbestos fibers in 1 ml saline with a week-interval. The total asbestos injected was either 1 or 10 mg per animal. Half of each group received intraperitoneal injections of 80 mg/kg NTA once a week for 12 weeks to promote an iron-catalyzed Fenton reaction, as previously described. Either NTA alone of the same dose or physiological saline solution was administered to the remaining rats as two control groups. Animals were maintained in a specific pathogen-free environment at 24^{o}C with a 12-h dark/light cycle. When the animals showed massive ascites or were dying, they were euthanized, and an autopsy examination was performed. The experiments were terminated 550 days after the first injection of asbestos. Excised organs were fixed in phosphate-buffered 10% formalin and underwent paraffin embedding and routine hematoxylin and eosin staining processes or immunohistochemistry. Additionally, Perls' iron staining and Masson trichrome staining were performed.

【Results】

Fiber morphology

The diameter of more than 50% of tremolite fibers was < 500 nm, and the diameter was

uniform throughout the entire fiber length **(Fig. 1A and B)**. In contrast, the diameter of most anthophyllite fibers was > 1000 nm, and they sometimes had irregular ends, though the samples also contained thinner needle-like fibers. Some of the thin anthophyllite fibers revealed either one-sided bluntness or slight bulginess in the center **(Fig. 1C-F)**. Approximately 15% of the tremolite fibers and 100% of the anthophyllite fibers were longer than 20 μm **(Fig. 1G)**.

Tremolite induced malignant mesothelioma (MM) but anthophyllite did not

Tremolite induced peritoneal MM in a dose-dependent manner **(Fig. 2).** Most rats (96.1%; male NTA, 10/10; male saline, 11/11; female NTA, 13/15; female saline, 15/15; 3 males died during injection period) administered with 10 mg tremolite developed MM within 550 days of the initial asbestos administration, and 51.9% of rats administered with 1 mg tremolite developed MM within 550 days (male NTA 11/12; male saline 9/12; female NTA 5/15; female saline 3/15). Males were more susceptible than females . Animals with MM (tremolite groups only) often suffered from massive bloody ascites. Macroscopically, the tumors were scattered in the peritoneal cavity, and the largest nodules were most commonly longer than 5 mm in diameter. These tumors sometimes proliferated on the liver surface **(Fig. 3A and B).** The severity of dissemination was different in each case; therefore, this was the factor used to determine the macroscopic grading of MM **(Fig. 3B).** The sarcomatoid subtype of MM was the most common histological finding **(Fig. 3C)**. Pathologic diagnosis was confirmed using immunohistochemistry, which revealed positivity for cytokeratin (AE1/3), calretinin, podoplanin, mesothelin and WT1 in the epithelioid subtype and positivity for cytokeratin, mesothelin and WT1 in the sarcomatoid subtype. Desmin (a myogenic marker) and S-100 (a neural marker) were negative or only partially positive **(Fig. 4A and 4B).** We applied the same criteria used for human MM for the pathologic diagnosis. We observed heavy and scattered iron deposits in the mesothelial cells and near the MM in most rats injected with tremolite **(Fig. 5A-D).** However, iron deposition was much less in rats injected with anthophyllite **(Fig. 5E and F)**.

【Discussion】

Fibrous minerals have been unexpected causes of human cancer. Few carcinogenesis studies have been performed on minor asbestos. We studied the carcinogenicity of two minor asbestos types, tremolite and anthophyllite, after characterization.

Tremolite from Yamaga City, Kumamoto, Japan, was potently carcinogenic after intraperitoneal injection in rats and generated MM that was confirmed using immunohistochemistry. The carcinogenic potential of tremolite, tested within the period

required for mesothelial carcinogenesis, was stronger than that of the chrysotile, crocidolite and amosite asbestos types and was as potent as multiwalled carbon nanotubes with a 50 nm-diameter with experiments using the same F1 rats. We assume that it is not coincidence that of the rats with MM induced by tremolite or multiwalled carbon nanotubes, the majority developed the more malignant sarcomatoid subtype. It is still unknown at present which factors are responsible for differentiating between the epithelioid and sarcomatoid MM subtypes. There are two hypotheses for this: 1) the initiated cells are different in the two subtypes and 2) the epithelioid subtype progresses into the sarcomatoid subtype. These hypotheses are being extensively investigated in our laboratory.

In the tremolite group, we found that males were more susceptible to MM than females. This suggests the involvement of sex hormones and, presumably, the resulting difference in iron metabolism. These findings are consistent with the findings of a previous study related to chrysotile-induced mesothelial carcinogenesis, particularly in the group that received additional NTA injections. Unexpectedly, NTA did not promote tremolite-induced mesothelial carcinogenesis. We observed iron deposits in the spleen and in the tissue surrounding asbestos deposits. This is most probably due to the ferroportin block via foreign body-associated inflammation. Therefore, involvement of local iron overload is plausible in tremolite-induced carcinogenesis as well. We suspect that calcium within the chemical formula for tremolite ($[Ca_2Mg_5Si_8O_{22}(OH)_2]_n$) might have blocked the access of NTA to iron.

In conclusion, short fiber preparation tremolite was potently carcinogenic to mesothelial cells. In contrast, Afghan anthophyllite in the current preparation was not carcinogenic. Thus, the fiber diameter appears to be more important than the fiber length for mesothelial carcinogenesis, although the fiber length appears more important for clearance from the lung when exposure is via the respiratory tract. Anthophyllite is also contaminated with talc deposits and chrysotile A. Our results suggest these contaminants are less responsible for MM, especially for chrysotile asbestos. Genomic alteration of tremolite-induced rat MM is under investigation in our laboratory.

【Conclusion】

The results suggest that the carcinogenicity of anthophyllite is weaker than formerly reported, whereas that of tremolite is as potent as major asbestos.