EVALUATION OF SCEs IN BONE MARROW CELLS OF MICE AFTER IN VIVO EXPOSURE TO ASBESTOS

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Abstract. Three samples of antigorite of different structure and a sample of anthophyllite were tested for induction of sister chromatid exchanges (SCEs) in bone marrow cells of male Balb C mice exposed to bromodeoxyuridine. The test substances were administered in the doses of 0, 1, 5, 10 and 20 mg per mouse. The SCE frequencies were analyzed in bone marrow cells in metaphase.

The results revealed that all studied kinds of asbestos elevated significantly the frequency of SCEs. The dose-effect relationships for all substances were similar.

The data suggest that the magnitude of effect was not related to the length and diameter or to the number of mineral fibres administered.

INTRODUCTION

It is known from numerous studies that asbestos fibres display mutagenic activity (8, 9) and are carcinogenic in man and animals (1, 6, 12, 16, 17). Several reports have suggested that asbestos fibres induce chromosomal changes when tested using some in vitro cell systems. The end points used to assess induced at the chromatin level included frequency of chromosomal aberrations and sister chromatid exchanges (3, 5, 8, 10, 11, 13, 14, 15). However, whereas chromosomal aberration frequency was generally elevated in the asbestos-treated cells, the SCEs were either slightly or not significantly increased when related to the control levels.

In this paper we report the effects of antigorite and anthophyllite of various structures on the mouse bone marrow cells in vivo.

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MATERIAL AND METHOD

a. Test agents

Four different kinds of asbestos were evaluated:

I. crushed antigorite, with destroyed fibrous structure, obtained by crumbling antigorite (II) in agate grinder and sifted through a sieve of 75 μm hole size.

II. a fibrous antigorite of the length and diameter of fibres of 4.8 μm and 0.3 μm, respectively.

III. another fibrous antigorite, characterized by the length and diameter of fibres of 12.0 μm and 0.13 μm, respectively.

IV. anthophyllite; here the length and diameter of fibres amounted to 16.0 μm and 0.6 μm, respectively.

TABLE 1. Sister Chromatid Exchange Frequency in Bone Marrow Cells of Mice Following In Vivo Exposure to Various Kinds of Asbestos

Test asbestos (mg/animal)	Number of animals	Number of metaphases scored	SCEs/metaphase /x±S.E.
Crushed antigorite (I)			
0	4	200	3.95±0.13
1	4	200	3.94±0.14
5	4	200	4.50±0.33
10	4	200	5.83±0.34 *
20	6	300	5.42±0.31 *
Fibrous antigorite (II)			
0	4	200	4.27±0.27
1	4	200	6.65±0.92 *
5	4	200	5.91±0.42 *
10	4	200	7.23±0.06 *
20	6	300	7.39±0.36 *
Fibrous antigorite (III)			
0	4	200	4.66±0.55
1	4	200	5.45±0.21
5	4	200	8.55±1.38 *
10	4	200	8.05±1.25 *
20	4	200	8.49±0.49 *
Anthophyllite (IV)			
0	4	200	4.66±0.55
1	4	200	5.72±0.63
5	4	200	7.55±1.09
10	4	200	9.92±0.37 *
20	4	200	8.88±0.82 *

* — significant at least at $p < 0.01$ vs. controls

All specimens of asbestos were obtained from the Department of Aerosols of the Institute of Occupational Medicine in Lodz.

b. Animals and administration of the test substances

Male Balb C mice, 8 — 10 weeks old and weighing 25—30 g were utilized. Into anesthetized mice tablets of 50 mg bromodeoxyuridine (BrdU) were implanted subcutaneously. One hr after implantation the mice received i.p. injection of one of the test substances (autoclaved before use), in the volume of 0.5 ml at doses of 0, 1, 5, 10 and 20 mg per mouse. All the groups consisted of 4 animals, except for the last 2 groups given crushed antigorite and fibrous antigorite (II), which consisted of 6 animals each. 20 hours after administration of asbestos and two hours prior to the sacrifice of animals Colcemid was injected i.p. at the amount of 0.03 mg per mouse. The animals were killed by cervical dislocation. The femurs were removed, muscle and connective tissues stripped away, epiphyses removed and bone marrow cells were flushed into Hank's solution. The cells were later centrifuged and the material was treated with hypotonic KCl (0.07 M) and afterwards with a fixative (methanol: acetic acid, 3 : 1). Flame-dried slides were prepared and differentially stained according to the method described by Antoshina and Porjadkova (2).

To determine the mean number of SCE per cell the 50 second-generation metaphase cells, as judged from the Harlequine stain of their chromosomes, were examined in each animal. Statistical significance of differences between means for groups of animals was determined by one way analysis of variance with multiple comparison tests. The differences were taken as statistically significant for which the chance that they were identical was less than 0.01.

RESULTS

The data on the induction of SCE in bone marrow cells, following exposure of the mice to various kinds of asbestos, are presented in Table 1. The one way analysis of variance with multiple comparison tests showed that all tested asbestos, independently of length and diameter of the fibres, and also the crushed antigorite (I) caused notable increase of SCE frequency in mouse bone marrow cells. The mean SCE frequency in the control group varied between 3.95 and 4.66/cell.

Fibrous antigorite (II) induced conspicuous and significant increase of SCE frequency in the whole range of doses when compared with the control level. Mean SCE frequency in the animals treated with the two highest doses of fibrous asbestos (II) 10 and 20 mg (mice) was higher by

about 70 percent than the control level and significantly differed from the value observed after the dose of 5 mg/animal. Significant and similar number of SCE per cell (8.55; 8.05 and 8.49) was induced by three highest doses of fibrous antigorite (III) (70—80% higher than controls). Crushed antigorite (I) and anthophyllite (IV) induced significantly increased SCE frequencies in mouse bone marrow cells only at the two highest doses; they exceeded the control values to a similar degree for each substance, i.e. by 40—50 and 90—100%, respectively.

The dose-effect relationship observed for all tested kinds of asbestos were similar. The results indicate that the magnitude of effects was neither related to the length and/or diameter of the fibres nor to its number per unit mass of the substance.

DISCUSSION

As specified earlier in the text all tested kinds of asbestos differed from one another with regard to the length and the diameter of fibres. The number of fibres per 1 mg depended on their length and the diameter and therefore the number of fibres per 1 mg of fibrous antigorite (II), fibrous antigorite (III) and anthophyllite (IV) amounted to 1×10^7, 9×10^5 and 3×10^6, respectively. The fibrous structure of crushed antigorite (I) was destroyed by mechanical crushing.

The experiments showed that all tested specimens of fibrous asbestos (II, III, IV) as well as crushed antigorite (I) induced significant frequency of SCEs in mouse bone marrow cells in vivo at least at the two highest doses. The frequency of induced changes in four groups of animals treated with similar amounts of different asbestos was similar, and therefore independent both of the structure of the tested minerals and of the total number of fibres administered.

Summarizing, antigorite of different structure and anthophyllite are potent inducers of sister chromatid exchanges in mouse bone marrow cells in vivo.

The results presented above are not in agreement with observations made by other authors. High correlation between the length of fibres and their cytotoxic activity and with the yield of chromosomal aberrations was obtained by Brown et al. (4). These authors confirmed that together with the shortening of the fibres length, both the cytotoxic activity and ability to induce chromosomal aberrations were reduced. Likewise, Hesterberg and Barrett (7) while studying the Syrian hamster embryo cells, confirmed that asbestos as well as other fibres of the length above 10 μm and diameter from 0.1 to 0.2 μm displayed very high activity in inducing oncogenic transformation of cells. Thicker fibres (from

0.8 μm in diameter) showed weaker potency and shortening of the thin fibres below 1 μm eliminated their transformation potency.

REFERENCES

1. **Acheson E.** (et al): Mesothelioma in factory using amosite and chrysotile asbestos. Lancet 2, 1403—1406, 1981. — 2. **Antoshina M. and Porjadkova N.**: A technique for differential staining of sister chromatids without using fluorochrome. Citol. Genet. 4, 349—352, 1978. — 3. **Babu K.** (et al): In vitro cytological and cytogenetic effects of an indian variety of chrysotile asbestos. Environ. Res. 21, 416—422, 1980. — 4. **Brown R.** (et al): In vitro biological effects of glass fibres. J. Environ. Pathol. Toxicol. 2, 1369—1384, 1979. — 5. **Casey G.**: Sister chromatid exchange and cell kinetics in CHO-K1 cells, human fibroblasts and lymphoblastoid cells exposed in vitro to asbestos and glass fibre. Mutat. Res. 116, 369—377, 1983. — 6. **Harington J.**: Fiber carcinogenesis. Epidemiologic observations and the Stanton hypothesis. J. Natl. Cancer Inst. 67, 977—989, 1981. — 7. **Hesterberg T. and Barrett J.**: Dependence of asbestos and mineral dust-induced transformation of mammalian cells in culture on fibre dimension. Cancer Res. 44, 2170—2180, 1984. — 8. **Huang S.** (et al): Genetic effects of crocidolite asbestos in Chinese hamster lung cells. Mutat. Res. 57, 225—232, 1978. — 9. **Huang S.**: Amosite, chrysotile and crocidolite asbestos are mutagenic in Chinese hamster lung cells. Mutat. Res. 68, 265—274, 1979. — 10. **Lavappa K., Fu M. and Epstein S.**: Cytogenetic studies on chrysotile asbestos. Environ. Res. 10, 165—173, 1975.

11. **Livingston G., Rom W. and Morris M.**: Asbestos-induced sister chromatid exchanges in cultured Chinese hamster ovarian fibroblast cells. J. Environ. Pathol. Toxicol. 4—2, 373—382, 1980. — 12. **Mosseman B. and Craighead J.**: Mechanism of carcinogenesis, Environ. Res. 25, 269—280, 1981. — 13. **Oshimura M.** (et al): Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. Cancer Res. 44, 5017—5022, 1984. — 14. **Price-Jones M., Gubbings G. and Chamberlain M.**: The genetic effects of crocidolite asbestos; comparison of chromosome abnormalities and sister-chromatid exchanges. Mutat. Res. 79, 331—336, 1980. — 15. **Sincock A. and Seabright M.**: Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres. Nature (London) 257, 56—58, 1975. — 16. **Wagner J., Berry G. and Timbrell V.**: Mesotheliomata in rats after inoculation with asbestos and other materials. Br. J. Cancer 28, 173—195, 1973. — 17. **Wagner J.** (et al): A pathological and mineralogical study of asbestos-related deaths in the United Kingdom in 1977. Ann. Occup. Hyg. 26, 423—431, 1982.

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