Sister chromatid exchanges in rat pleural mesothelial cells treated with crocidolite, attapulgit, or benzo 3-4 pyrene

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Sister chromatid exchange (SCE) quantitation is a sensitive test with which to detect the effects of mutagens and has been used to determine the clastogenic potency of materials such as mineral fibres.¹⁻⁴ Attapulgit fibres are used for many different industrial purposes and their carcinogenic potency needs to be evaluated but until now few data have been available. Pott *et al* found that palykorskite fibres were carcinogenic in rats⁵ and Wagner reported that attapulgit from Spain induced mesotheliomas when injected into the pleural cavity of Fischer 344 rats⁶; short attapulgit fibres did not increase the incidence of tumours.⁷

The aim of the present work was to test the *in vitro* effect of attapulgit on SCE induction. Since pleural mesothelial cells are target cells in fibre related diseases, the test was performed on rat cell cultures and the effects were compared with those obtained with crocidolite fibres. In addition, benzo 3-4 pyrene (BP) was used to assess the reactivity of mesothelial cells to a known mutagen.

Material and methods

PARTICLES AND CHEMICALS

A sample of attapulgit was obtained from Senegal bed. Electron microscopic studies showed that the particles had a fibrous shape with a length less than 2 µm. Crocidolite was obtained from the Union Internationale contre le Cancer, BP was supplied by Sigma, and dimethyl sulphoxide (DMSO) by Merck. The particles were suspended in culture medium (200 µg/ml) and dispersed by sonication for 10 minutes (20 kHz, 20 w). BP was solubilised in DMSO or in acetone.

CELLS

Rat pleural mesothelial cells (RPMC) were cultured

as described elsewhere.⁸ Briefly, RPMC was grown at 37°C in Ham F10 supplemented with 10% fetal calf serum, cysteine 200 µg/ml, vitamin C50 µg/ml, glutamine 233 µg/ml, penicillin 100 units/ml, and streptomycin 50 µg/ml. The pH was adjusted with 1% HEPES. Secondary cultures were used; the cells were between the 12th and 15th passage (about 20-25 doublings).

RPMC were routinely passaged using a trypsinisation procedure. Twenty four hours after trypsinisation RPMC were treated with different concentrations of fibres (10, 15, and 20 µg/ml) for 48 hours or with 0.5 µg/ml BP solubilised either in DMSO or in acetone, for different periods; the final DMSO and acetone concentration were 0.1%. RPMC were then treated with 10 µg/ml of 5-bromodeoxyuridine (BrdUrd) for 70 hours corresponding to about two cell cycles.

Chromosome preparation and staining

Colcemid (final concentration 0.33 µg/ml) was added during the final 90 minutes of incubation. Mitotic cells were collected by mild trypsinisation, centrifuged (15 min, 300 × g), and treated at 37°C with 0.075 M hypotonic KCl for 30 minutes. Cells were fixed twice in methanol: glacial acetic acid (3:1) at +4°C. Spreading and staining were performed according to the method of Dutrillaux and Couturier.⁹ Slides were stained with Hoechst 33258 (5 µg/ml), exposed to a germicide lamp in 0.1 M phosphate buffer, pH 6.8 for two hours, and incubated in 2 × super concentrated solution (0.3 M sodium chloride 0.03 M trisodium citrate) at 60°C for 30 minutes. Cells were stained with 3% Giemsa.

Scoring and statistical analyses

Thirty metaphases having 38-42 chromosomes per metaphase were scored. The results were expressed as number of SCEs per metaphase. The Mann-Whitney test was used to compare the data obtained with untreated and treated cultures and the effect of dose or time of exposure to the particles or chemicals.

Table 1 Percentage of metaphases in a given cell cycle according to period of incubation

	Time of incubation with BrdU (hours)			
	50	64	67	72
1st cell cycle	90	26	12	2
2nd cell cycle	10	68	78	85
3rd cell cycle	0	4	10	13

100 metaphases were analysed for each incubation time.

Results

To obtain a maximal number of metaphases from cells having performed two cell cycles the frequency of these metaphases were determined according to the period of incubation with BrdU. Table 1 shows that the maximum number was obtained at 72 hours. A period of 70 hours was retained for the present experiments.

Attapulgit did not induce SCEs. Table 2 shows that no significant increase in the number of SCEs per metaphase was obtained with 10 or 20 µg/ml. Crocidolite fibres were potent in increasing the number of SCEs per metaphase, which was significantly enhanced at all experimental doses.

Table 2 SCE induction by minerals

Treatment (µg/ml)		Control		Attapulgit		Crocidolite		
				10	20	10	15	20
No of chromosomes per metaphase*	1	40.7 ± 1.2		41.1 ± 1.4	41.7 ± 1.4	41.6 ± 0.9	40.6 ± 1.4	41.5 ± 0.8
	2	40.2 ± 1.4		40.5 ± 1.4	41.7 ± 0.4	40.7 ± 1.3	40.4 ± 1.5	—
SCEs per metaphase*	1	9.9 ± 2.1		9.9 ± 3.4	11.2 ± 3.9	13.6 ± 3.8	14.3 ± 3.3	12.4 ± 4.0
	2	9.7 ± 2.2		9.8 ± 2.2	10.3 ± 2.6	12.9 ± 3.5	14.5 ± 3.6	—
Test, p ≤	1	—		NS	NS	0.001	0.001	0.05
	2	—		NS	NS	0.001	0.001	—

*Values represent mean ± standard deviation for 30 metaphases. 1 and 2 = different experiments. Test compares control at each particle concentration.

Table 3 Induction of SCEs by BP

Time (hours)			Treatment				
			A None	B DMSO	C BP in DMSO	D Acetone	E BP in acetone
SCEs per metaphase†	5	1	11.6 ± 2.9	11.6 ± 2.4†	23.0 ± 5.7**	12.0 ± 3.1†	26.1 ± 4.7**
		2	9.7 ± 2.2	11.3 ± 2.5*	22.2 ± 3.8**	11.4 ± 2.5*	24.1 ± 3.9**
	20	1	11.2 ± 3.5	12.9 ± 3.0†	28.6 ± 3.8**	12.8 ± 3.5†	28.6 ± 5.9**
		2	10.8 ± 2.8	12.6 ± 3.4†	29.9 ± 3.9**	11.7 ± 2.2†	29.7 ± 4.3**
	30	1	9.5 ± 2.2	10.5 ± 2.5†	30.3 ± 2.9**	10.7 ± 2.9†	30.8 ± 4.0**
		2	10.7 ± 2.1	11.1 ± 2.4†	31.6 ± 3.2**	11.4 ± 1.9†	30.3 ± 3.0**
	48	1	10.2 ± 3.6	11.3 ± 2.7†	31.4 ± 5.8**	15.5 ± 4.2**	39.4 ± 9.6**
		2	11.0 ± 2.9	11.4 ± 2.3†	30.5 ± 3.1**	13.8 ± 2.7†	38.3 ± 4.8**

Test: comparison between DMSO/BP in DMSO, acetone/BP in acetone, none/DMSO, or none/acetone.

*p ≤ 0.01; **p ≤ 0.001.

†Values represent mean ± standard error for 30 metaphases.

‡Not significant.

1 and 2 = Two different experiments.

After treatment with BP a significant increase in SCEs was obtained whatever the solvent, DMSO, or acetone (table 3). The SCE level increased with time; this effect was stronger at the beginning of the experiment and was more or less stationary after 20 hours.

It should be noted that 0.1% DMSO slightly increased the number of SCEs per metaphase, but the difference when compared with untreated controls was not significant, except after five hours in one experiment. Acetone 0.1% also increased SCEs and the difference was significant at 48 hours. The effect of BP solubilised in acetone was higher than when solubilised in DMSO.

Discussion

To our knowledge the cytogenetic effects of attapulgit have not been published. Recently Denizeau *et al* reported negative results when testing for unscheduled DNA synthesis by attapulgit fibres on hepatocytes.¹⁰ Our data indicate that attapulgit did not induce SCEs in cultured RPMC; this was not due to a lack of fibre ingestion because intracellular fibres were seen on sections observed with electron microscopy. According to our results, crocidolite contrasted with attapulgit; indeed 10 µg/ml of crocidolite fibres increased the level of SCEs by about

30% and by 40% in a concentration of 15 µg/ml. In previous experiments chrysotile fibres also enhanced the number of SCEs.¹¹ The lower effect with 20 µg/ml of crocidolite fibres could be related to a cytotoxicity. Indeed, the basis of the SCE method consisted in the observation of metaphase cells; it is possible that the cells having a high SCE level are killed or strongly injured cells and are then not detected. Similar results have been obtained when chromosomal aberrations were determined in chrysotile treated RPMC¹²; in these experiments low doses induced a dose dependent increase in abnormal metaphases whereas higher concentrations resulted in several abnormal metaphases similar to the number obtained with the control. Conflicting data have been obtained with crocidolite fibres since Casey³ and Price-Jones *et al*² did not find SCEs increased in V 79-4 or CHO-K1. By contrast, Livingston *et al* observed a significantly raised SCE rate in medium and larger chromosomes of CHO cells.¹ These discrepancies could be related to the different nature of the cells and also to the methodology. Price-Jones *et al* used an experimental procedure different from the one described in the present paper since the dusts were added while the cells were in suspension.² In our experiments RPMC were firstly plated and then dust treated before their exposure to BrdU and arrest in metaphase; this was done to assess the unrepaired remaining DNA lesions.

BP was more potent than crocidolite fibres in inducing SCEs. For example, after 30 hours of incubation SCEs were enhanced by 300% whereas SCEs enhancement did not exceed 48% after treatment with crocidolite. It should be noted that acetone induced a slight increase in SCEs after 48 hours of incubation. This was not observed with DMSO and suggests that the stronger effect of BP solubilised in acetone when compared with DMSO could be due to a synergism between acetone and BP. The results obtained with BP indicate that cultured RPMC synthesise BP metabolites which interact with DNA. This is in agreement with a previous study showing that RPMC are able to metabolise BP¹³; RPMC may be a useful model to study the chromosomal effects of chemicals.

Conclusion

This attapulgite sample did not induce SCE in cultured RPMC but crocidolite fibres produced SCEs. If the SCE technique is taken as an indicator of a carcinogenic potency these results do not disagree with animal data because the attapulgite sample used in this experiment contained short fibres by contrast with the paly-gorskite or Spanish attapulgite used by Pott *et al*⁵ and Wagner⁶ respectively in their animal experiments. According to Stanton's hypothesis these

short fibres would not be carcinogenic.¹⁴ Concerning crocidolite fibres, their positive effect is in agreement with their well known carcinogenicity in animals.^{15 16}

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References

- Livingston GK, Rom WN, Morris MV. Asbestos induced sister chromatid exchanges in cultured Chinese hamster ovarian fibroblast cells. *J Environ Pathol Toxicol* 1980;4:373-82.
- Price-Jones MJ, Gubbings G, Chamberlain M. The genetic effects of crocidolite asbestos; comparison of chromosome abnormalities and sister-chromatid exchanges. *Mutat Res* 1980;79:331-6.
- Casey G. Sister chromatid exchange and cell kinetics in CHO-K1 cells, human fibroblasts and lymphoblastoid cells exposed in vitro to asbestos and glass fibers. *Mutat Res* 1983;116:369-77.
- Tice RR, Lambert B, Morimoto K, Hollaender R. A review of the international symposium on sister chromatid exchanges: twenty five years of experimental research. *Environ Mutagen* 1984;6:737-52.
- Pott F, Dolgner R, Friedrichs KH, Huth F. Effect oncogène des poussières fibreuses; l'expérimentation animale et des relations avec la carcinogénèse humaine. *Annals of Anatomical Pathology* 1976;21:237-46.
- Wagner JC. *Compte rendu du symposium mondial sur l'amiante*. Montréal, Canada, 25-27 Mai, 1982. Montreal: Canadian Asbestos Information Center, 1982:263-86.
- Lipkin LE. Failure of attapulgite to produce tumors prediction of this result by in vitro cytotoxicity test. In: Beck EG, Bignon J, eds. *In vitro effects of mineral dusts*. Vol 3. (NATO ASI Series. Series G: ecological systems.) 1985:539-42.
- Jaurand MC, Bernaudin JF, Renier A, Kaplan H, Bignon J. Rat pleural mesothelial cells in culture. *In vitro* 1981;17:98-106.
- Dutrillaux B, Couturier J. *La pratique de l'analyse chromosomique*. Paris: Masson, 1981. (Technique de Laboratoire No 12.)
- Denizeau F, Marion M, Chevalier G, Cote MG. Absence of genotoxic effects of non asbestos fibres. *Cell Biology and Toxicology* 1985;1:23-32.
- Jaurand MC, Bastie-Sigeac I, Paterour MJ, Renier A, Bignon J. Possibility of using rat mesothelial cells in culture to test cytotoxicity, elastogenicity and carcinogenicity of asbestos fibers. *Ann NY Acad Sci* 1983;407:409-1.
- Jaurand MC, Kheuang L, Magne L, Bignon L. Chromosomal changes induced by chrysotile fibers or benzo 3-4 pyrene in rat pleural mesothelial cells. *Mutat Res* 1986;169:141-8.
- Bastie-Sigeac I, Hubert-Habart M, Jaurand MC, Bignon J. Metabolisme du Benzo(a)pyrène par des cellules mésothéliales pleurales de rat en culture. *Comptes Rendus de l'Académie des Sciences* 1984;298:423-6.
- Stanton MF, Layard M, Tegeris A, Miller E, May M, Kent E. Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. *J Natl Cancer Inst* 1977;58:587-603.
- Wagner JC, Berry G. Mesotheliomas in rats following inoculation with asbestos. *Br J Cancer* 1969;23:567-81.
- Monchaux G, Bignon J, Jaurand MC, *et al*. Mesothelioma in rats following inoculation with acid leached chrysotile asbestos and other mineral fibres. *Carcinogenesis* 1981;2:229-36.