Remote Effects in the Mutagenic Action of Chrysotile Asbestos and Zeolite Dust *in Vivo*

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UDC 612.6.05.06:613.633].084

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 5, pp. 484–486, May, 1993
Original article submitted December 17, 1992.

Key Words: *mutagenesis; chrysotile asbestos; zeolite*

It was shown earlier that the mutagenic action exerted by chrysotile asbestos (ChA) and zeolite (Z) dust in whole human blood cultures is mediated by active forms of oxygen species (AFO) arising as a result of dust particles contacting phagocytizing cells [1, 8]. AFO are highly reactive, and the rate at which the hydroxyl radical interacts with surrounding molecules is almost equal to its diffusion rate [10]. For this reason it would seem unlikely, on the face of it, that mineral dust is able to produce mutagenic effects outside the area of its direct contact with phagocytizing cells. However, AFO are capable of activating lipid peroxidation (LPO) processes [4, 6] whose intermediate and end products have been recently shown to possess mutagenic properties [11, 12]. The ability of particulate pollutants of various kinds to activate LPO has also been demonstrated by direct experiments [5, 7, 9]. Taken together, these findings strongly suggest that ChA and Z dust can cause damage *in vivo* not only to genetic structures of the cells that occur in immediate proximity to dust particles, but also to remote tissues, i.e., it can exert long-range mutagenic effects.

This investigation was undertaken to check this possibility experimentally by examining peritoneal exudate (PE) and bone marrow (BM) cells of mice for mutagenic effects of ChA and Z.

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MATERIALS AND METHODS

Dust samples of ChA (from the Bazhenovskoe deposit) and Z (from the Khonguruu, Pegasskoe, and other deposits) were used. The length of ChA fibers did not exceed 10 μ and the Z particles had a diameter of not more than 5 μ.

The mutagenic effects of these samples were assessed by recording chromosomal aberrations in PE and BM cells from male C57BL/6 mice 1.5-2 months of age and weighing 20-22 g obtained from the Svetlye Gory Nursery of the Russian Academy of Medical Sciences. ChA and Z dust was injected into the animals intraperitoneally on a single occasion at a rate of 50 mg/kg body weight and the results were recorded on days 1, 2, 3, 7, and 28 postinjection.

Cytogenetic preparations of BM cells were made in the usual way [3], while those of PE cells were made as described earlier [2].

RESULTS

The results of comparative studies of ChA and Z (clinoptilolite combined with heulandite) dust for its mutagenic effects on murine PE and BM cells in a subacute experiment are presented in Table 1.

0007-4888/93/0005-0524$12.50

Table 1. Results of a Cytogenetic Study of Peritoneal Exudate and Bone Marrow Cells of C57Bl/6 Mice Administered Chrysotile Asbestos (ChA) or Zeolite (Z) Dust

Group and days of exposure	№ of cells	№ (per 100 cells) of:		Total % of damaged metaphases	Significance of difference from control, p
		gaps	chromosomal aberrations		
Peritoneal exudate cells					
Control 1	300	2.0	9.7	8.3±1.6	
Control 2	200	1.3	10.7	8.3±1.6	
ChA dust, 50 mg/kg:					
1 day	200	–	2.5	2.5±1.1	<0.01
2 days	200	3.5	18.0	19.0±2.8	<0.001
7 days	200	4.5	27.0	22.0±2.9	<0.001
28 days	200	4.5	18.0	19.0±2.8	<0.001
Z dust, 50 mg/kg:					
1 day	400	0.8	1.0	1.8±0.7	<0.001
2 days	200	2.0	14.0	12.0±2.3	>0.05
7 days	200	1.0	20.0	16.5±2.6	<0.01
28 days	200	8.5	52.0	36.5±3.4	<0.001
Bone marrow cells					
ChA dust, 50 mg/kg:					
1 day	500	1.4	3.2	4.4±0.9	<0.01
2 days	500	0.8	3.8	4.0±0.9	<0.01
7 days	500	0.8	2.8	3.6±0.8	<0.05
28 days	500	0.6	2.6	3.2±0.8	<0.05
Z dust, 50 mg/kg:					
1 day	500	0.6	1.6	2.2±0.7	>0.05
2 days	500	1.2	1.6	2.8±0.7	>0.05
7 days	500	1.8	1.4	2.4±0.7	>0.05
28 days	700	1.4	2.0	3.6±0.7	<0.01

In the control (unexposed) animals spontaneous mutations were found to occur in 1.2±0.5% of BM cells (a figure that virtually does not differ from those reported by other authors [3]) and in 8.3±1.6% of PE cells from different groups of unexposed animals.

At 24 h (day 1) after intraperitoneal injection of ChA dust, the proportion of cells with abnormal metaphases fell sharply to 2.7±1.3% (p<0.01), followed by a large jump to 19.0±2.8% (p<0.001) at 48 h (day 2), to remain virtually the same throughout the observation period.

For BM cells from ChA-exposed animals, a statistically significant increase to 4.4±0.9% in the proportion of cells with chromosomal aberrations was observed on day 1 and at all times subsequently.

In tests with Z dust samples, the proportion of PE cells with chromosomal aberrations was found, as in tests with ChA dust samples, to have significantly decreased (to 1.8±0.7%) on day 1 postinjection; on day 2, 12.0±2.3% of PE cells were damaged, which did not differ significantly from the proportion of such cells in intact animals, and it was only on days 7 and 28 of exposure that the number of cells with abnormal metaphases rose significantly to reach 16.5±2.6% and 36.5±3.4%, respectively.

For BM cells of Z-exposed mice, a significant rise in the proportion of aberrant metaphases was recorded on day 28 only.

These results indicate that the two minerals studied both display mutagenic properties *in vivo*, but differ in the time course of their cytogenetic action. In the case of ChA, the proportion of damaged cells increased shortly (on days 1-2) after the start of exposure and remained almost unchanged throughout the observation period, whereas the mutagenic effect of Z was manifested later and the proportion of damaged cells rose along with the duration of exposure.

This study thus shows that ChA and Z can damage the genetic structures not only of nearby cells but also those of cells in remote tissues, and it is important to note that BM cells appear to be at least as sensitive to the mutagenic activity of these minerals as are the PE cells in direct contact with the latter. Indeed, statistically significant increases in the number of BM cells with chromosomal aberrations after the injection of Z were recorded at the same times as those in the number of PE cells with such aberrations, while ChA exerted a mutagenic effect on BM cells as early as on day 1, i.e., earlier than it did on PE cells (day 2).

In order to confirm the ability of particulate pollutants to produce mutagenic effects at a distance, further experiments were carried out with three zeolite varieties - clinoptilolite, chabazite, and mordenite; the results are shown in Table 2.

Table 2. Cytogenetic Damage Caused by Various Zeolites to Bone Marrow Cells of C57Bl/6 Mice

Group and days of exposure	№ of cells	№ (per 100 cells) of:					Total % of damaged metaphases	Significance of difference from control, p
		gaps	single fragments	paired fragments	exchanges	chromosomal aberrations		
Control		500	0.2	1.0	0	0	1.0	1.2±0.
Clinoptilolite, 50 mg/kg:								
1 day	500	1.2	1.2	0	0	1.2	2.4±0.7	>0.05
3 days	500	1.2	1.0	0	0	1.0	2.2±0.7	>0.05
7 days	500	0.2	1.4	0	0	1.4	1.6±0.6	>0.05
28 days	500	1.4	2.6	0	0	2.6	3.8±0.9	<0.01
Chabazite, 50 mg/kg:								
1 day	500	1.0	1.4	0	0	1.4	2.4±0.7	>0.05
3 days	500	0.6	1.2	0	0.2	1.4	1.8±0.6	>0.05
7 days	500	1.6	1.4	0.2	0	1.6	3.0±0.8	<0.05
28 days	500	1.6	2.4	0	0	2.4	3.8±0.9	<0.01
Mordenite, 50 mg/kg:								
1 day	500	0.8	1.0	0	0	1.0	1.8±0.6	>0.05
3 days	500	0	0.4	0	0.2	0.6	0.6±0.3	>0.05
7 days	500	0	0.6	0	0	0.6	0.6±0.3	>0.05
28 days	500	1.4	3.2	0	0	3.2	4.2±0.9	<0.01

A cytogenetic analysis of BM cells isolated from the femoral bones of mice 24 hours (day 1) after the injection of these zeolites at a rate of 50 mg/kg body weight did not reveal any statistically significant rise in the proportion of cells with chromosomal abnormalities, which agrees with the results of the preceding experiments (Table 1). On day 7 of exposure, however, a significant increase to 3.0±0.8% in the number of cells with chromosomal aberrations was recorded for cells from chabazite-exposed mice, followed by a further increase to 3.8±0.9 on day 28.

For clinoptilolite and mordenite dust, a statistically significant rise in the proportion of abnormal metaphases was observed only on day 28 of exposure, with clinoptilolite inducing chromosomal aberrations in 3.8± ±0.9% and mordenite in 4.2±0.9% of the tested BM cells.

The results obtained with these three zeolite varieties confirm the ability of this mineral to exert its mutagenic effect at a distance.

In general, the findings of this investigation warrant detailed studies of the mechanisms by which such a remote effect is brought about and indicate a need for revising the maximum permissible concentrations currently in force for ChA and Z to take into account their genetic hazards, as well as for examining the mutagenic properties of other particulate pollutants; they also point to the desirability of undertaking a search for compounds capable of preventing particulate pollutants from causing damage to genetic structures.

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