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Comparison of the clastogenic effects of antimony trioxide on mice *in vivo* following acute and chronic exposure

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Antimony trioxide (Sb_2O_3), in aqueous suspension, was administered by gavaging to mice and monitored for chromosomal aberrations in bone marrow and sperm head abnormalities in germ cells. Acute exposure to the doses followed by observations after 6, 12, 18 and 24 h did not show any clastogenic effects. Chronic exposure daily to different doses for periods up to 21 days induced chromosomal aberrations in bone marrow. The frequencies were dose-dependent to a significant extent but no relationship could be seen with the sex of the animal. The findings indicate the harmful effects of cumulative exposure for prolonged periods to Sb_2O_3, which is being increasingly used in various industries.

Keywords: antimony trioxide toxicity, chromosomal aberration

Introduction

Antimony and its compounds are known to be toxic to higher organisms at high doses. Antimony trioxide (Sb_2O_3), a trivalent compound, is used extensively in various industries and exposure to this compound is increasing. Trivalent compounds of antimony are 10 times more toxic than pentavalent forms. Toxic effects include high local irritation of the gastric mucosa, violent vomiting, diarrhoea, decreased body temperature followed by lowered respiratory rate and death, jaundice, and damage to heart (Venugopal & Luckey 1978), liver and kidney (Veiga *et al.* 1985).

Information on the mutagenic and clastogenic effects of antimony is relatively meagre. Antimony potassium tartrate was positively mutagenic in human cells *in vitro* and chloride was positive in modified *rec* assays (Kanematsu *et al.* 1980). Carcinogenic activity of Sb_2O_3 was recorded in rats (Groth *et al.* 1986; Vonk & Piver 1983). The present investigation was undertaken to study the effects of Sb_2O_3 on mice *in vivo* following chronic exposure over prolonged periods in both bone marrow and germ cells.

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Materials and methods

Aqueous suspensions of Sb_2O_3 (E. Merck, India) were administered orally. Concurrent controls received only distilled water. The doses used were calculated as a proportion of the oral LD_{50} (>20 000 mg kg^{-1} body mass according to Merck Index, 1983, Venugopal & Luckey 1978).

Swiss albino mice of both sexes were raised at the Departmental Animal House. The animals, with an average mass of 25–30 g, were treated at 8 weeks of age. They were housed in polycarbonate cages at temperatures of 22 ± 3 °C with a relative humidity of 50 ± 15% and a 12 h photo-period. They were maintained on standard pellet diet (Gold Mohur feed manufactured by Lipton India Limited) and given unlimited water.

Three doses of Sb_2O_3 (400, 666.67 and 1000 mg kg^{-1} body mass) were fed as aqueous suspensions by gavaging to mice. Mice of both sexes were used for acute exposure. Only male mice were subjected to chronic exposure to study the effect on germ cells. Following acute exposure, the observations were made after 6, 12, 18 and 24 h according to the schedule of Preston *et al.* (1987). For chronic exposure, the doses were administered daily to mice for 21 days and observations were made on days 7, 14 and 21.

In all cases, 1.5 h before sacrifice, the animal was injected intraperitoneally with 4 mg kg^{-1} colchicine and killed by cervical dislocation. Bone marrow of femurs was removed by flushing with 1% sodium citrate solution (prewarmed to 37 °C), incubated at 37 °C for 20 min,

centrifuged, fixed in cold ethanol/glacial acetic acid (3:1, by volume) and processed according to the standard schedule (see Preston *et al.* 1987). The epididymis content of male mice, after chronic exposure, was squeezed out in distilled water and flushed for studies on sperm head morphology (Sakamoto & Hashimoto 1988, Wyrobeck *et al.* (1984).

Bone marrow samples were washed twice in fixative and slides were prepared by flame drying, coded and stained in diluted Giemsa (Preston *et al.* 1987, Sharma & Sharma 1980). A total of 100 metaphase plates from each animal were scored, making 500 cells for each experimental set. Different types of chromosomal aberrations, chromatid gaps, chromatid breaks, centric fusions and polyploidy were recorded separately (Tice *et al.* 1987).

Epididymis content was smeared on clean slides which were air-dried, coded and stained in diluted Giemsa; 500 sperm heads from each animal were scored, making a total of 2500 sperm heads for each experimental set. Different types of sperm head abnormalities were recorded.

Data from short-term acute exposure were analyzed by the *t*-test (Fisher & Yates 1963) to find out the differences in frequencies of chromosomal aberrations between the sexes and between the doses used. In order to compare the effects of the duration after exposure and sex, if any, on the action of Sb_2O_3, a two-way analysis of variance test (ANOVA; see Sokal & Rohlf 1981) was used. The results of chronic exposure were analyzed by one-tailed trend test (Margolin *et al.* 1986). Two-way ANOVA, followed by Duncan's multiple range test (see Duncan 1955, Harter 1960, Sokal & Rohlf 1981), was used to analyze any significant differences between the different doses and sampling times on the effect of the compound. The sperm head abnormalities induced by the chemical were compared by the *t*-test with the control.

Results and discussions

Sb_2O_3 did not induce chromosomal aberrations following single acute exposures. Three doses of antimony trioxide were fed to mice. No statistically significant difference could be recorded between the treated and the normal control mice, of either sex, with respect to the frequency of chromosomal aberrations or mitotic index at 6, 12, 18 and 24 h after exposure. Therefore the compound is not clastogenic at single exposure. This may be explained by the observation that Sb(III) is very quickly removed from the plasma (Stolman & Stewart 1960).

Table 1 shows the relationship between the chromosomal aberrations induced by different doses following chronic exposure for long periods. The abnormalities induced were scored separately as total aberrations (with or without gaps) and as breaks per cell. In general the frequency of aberrations (with gaps) increased proportionately with dose of the chemical to a highly significant level ($P \leq 0.001$). The highest dose, 1000 mg kg^{-1} body mass, was lethal on day 20 of treatment. Frequency of aberrations (without gaps) increased proportionately with dose administered to a highly significant level ($P \leq 0.001$) for the first 14 days. Longer exposure (21 days) was lethal. A similar value was seen for the frequency of breaks induced.

Table 2 gives the analysis of variance (ANOVA) to compare the relative frequency of chromosomal aberrations induced by two doses (lower and middle) and three durations of exposure to the chemical. The highest dose was lethal. It is seen that the frequencies of chromosomal aberrations increased to a statistically significant level with increasing dosage but no such significant relationship could be seen with the period of exposure.

The Duncan's multiple range test for studying details of differences among sample means at different doses showed that the frequencies of chromosomal aberrations were significantly different between all the doses at the $P \leq 0.05$ level. The frequency of abnormal sperms did not differ significantly between treated and control mice at the $P = 0.05$ level, indicating that the compound did not exert a marked effect on testes even after prolonged treatment. The chronic action of the compound is also seen in its accumulation with consequent harmful effects like skin tumors (Gulati & Mangal 1985) and decreased maternal and pup mass in mice (Rossi *et al.* 1987).

Trivalent antimony salts cause *in vitro* transformation of cells and this may be related to transformed genotypes. Certain antimony compounds have been found to have DNA-damaging capacity and mutagenicity when tested in *Bacillus subtilis rec* assays (Kanematsu *et al.* 1980). Antimonial drugs like tartar emetic and bilharcid produced chromosomal aberrations in laboratory rats (El Nahas *et al.* 1982).

Occupational exposure to airborne dust containing up to 88% of Sb_2O_3 for at least a decade induced antimoniosis in workers of antimony smelting plants (Potkonjak & Pavlovich 1983). In this connection, the clastogenic activity of Sb_2O_3 observed following prolonged treatment, compared to its absence after acute treatment in mice *in vivo*, is interesting.

Antimony compounds show a pronounced concentration in the bone marrow of rats (Martindale & Turner 1980, Nagai & Yanagimoto 1980, Noronha & Sewatkar 1986), which may account for increased clastogenic activity following prolonged exposure.

However, there was no significant increase in the number of sperm head abnormalities. This observation may be related to the fact that earlier workers

Table 1. Data on bone marrow chromosomal aberrations in male mice

Duration (days)	Dose (mg kg^{-1} body mass)	log Dose	No. of animals taken	Total chromosomal aberrations						Frequency of aberrations (%)		Break/cell
				G′	G″	B′	B″	RR	Polyploids	Including gap	Without gap	
7	control	2.40	5	6	0	5	0	0	2	2.6 ± 0.894	1.4 ± 1.140	0.01
	400	2.60	5	8	1	9	0	0	2	4.2 ± 1.095	2.2 ± 0.447	0.018
	666.67	2.82	5	11	0	11	0	0	6	5.6 ± 0.547	3.4 ± 0.547	0.022
	1000	3	5	21	0	19	0	9	11	13.8 ± 0.447	9.6 ± 1.140	0.074
Trend test *P* value										***6.715	***4.88	***5.45
14	control	2.40	5	6	0	5	0	0	3	2.8 ± 0.447	1.6 ± 0.547	0.01
	400	2.60	5	9	0	11	0	0	5	5 ± 0.707	3.2 ± 0.447	0.022
	666.67	2.82	5	17	0	13	0	0	7	7.4 ± 0.547	4 ± 0	0.026
	1000	3	5	26	2	23	0	10	8	16.2 ± 0.447	10.2 ± 0.836	0.086
Trend test *P* value										***5.50	***4.72	***7.5
21	control	2.40	5	6	0	5	0	0	3	2.8 ± 0.447	1.6 ± 0.547	0.01
	400	2.60	5	8	0	13	0	0	10	6.2 ± 0.836	4.6 ± 0.547	0.026
	666.67	2.82	5	19	0	16	0	2	4	8.6 ± 0.894	4.8 ± 0.836	0.04
	1000	3	5	—	—	—	—	—	—	—	—	—
Trend test *P* value										−1.18	−2.82	−0.39

Abbreviations: G′ and G″, chromatid and isochromatid gap; B′ and B″, chromatid and isochromatid break. The frequency of aberrations was calculated as the percentage of total chromosomal aberrations ± standard deviation of the mean among five animals per set. The trend test *P* values were determined by a one-tailed trend test; *** indicates significantly different at $P \leq 0.001$

Table 2. Two-way ANOVA for chromosomal aberrations in male mice

Dose (mg kg^{-1} body mass) [factor B]	Frequency of aberrations (%) after exposure duration [factor A] of			
	7 days	14 days	21 days	Σ
0	2.6	2.8	2.8	8.2
400	4.2	5	6.2	15.4
666.67	5.6	7.4	8.6	21.6
Σ	12.4	15.2	17.6	45.2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F_s
Factor A: duration (column)	2	4.52	2.26	4.33
Factor B: dose (row)	2	29.99	14.99	28.72*
Error	4	2.09	0.52	

The test was only done for the lowest and middle doses as the highest dose was lethal; * indicates significant, level of significance $P = 0.05$

(Mangal & Sohal 1978) were unable to detect this metal in the testes.

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