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Comparison of clastogenic effects of inorganic selenium salts in mice *in vivo* as related to concentrations and duration of exposure

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Abstract

Inorganic selenium compounds in the diet have been known to protect against cancer in laboratory animals, but were harmful in high concentrations. In the present work, the relative effects of two salts, sodium selenite and sodium selenate, administered to mice *in vivo*, in different concentrations and durations of exposure, were compared. Aqueous solutions of each salt (7, 14, 21 and 28 mg Kg^{-1} bw) were fed by gavaging to mice matched in age and sex. The animals were sacrificed at intervals of 6, 12, 18 and 24 h and chromosome preparations were made following the usual schedule of colchicine-hypotonic-fixative-airdrying-Giemsa staining. The endpoints screened were chromosomal aberrations (CA) and damaged cells (DC). Both salts affected chromosome structure and spindle formation, sodium selenite being more cytotoxic than sodium selenate. The frequencies of aberrations induced were directly proportional to the concentrations used and duration of exposure.

Introduction

Selenium is an essential trace element in both man and animals (WHO 1987; Fishbein 1991; Levander and Burk 1994). It is known to be a cofactor of the enzyme glutathione peroxidase (Rostruck *et al.* 1973) and protects cells from oxidative disintegration (Snyder 1986; Schrauzer 1992). Deficiency of selenium has been reported to increase the incidence of human cancers (Griffin 1979; Shamberger 1983). In view of its anticarcinogenic effects, diets are often supplemented with selenium in amounts greater than the recommended daily requirements (Shamberger 1985). Such higher doses of selenium containing compounds have been reported to be toxic to various organs in both man and animals (Olson 1986; Danielsson *et al.* 1990). Earlier reports on chromosome damage also have been made using micronuclei as endpoints (Itoh and Shimada, 1996). Since detailed investigations on exposure to ranges of dose and duration of the cell cycle had not been carried out, the present investigation was undertaken to compare the effects of four concentrations of two inorganic selenium salts at various time points of the cell cycle in mouse bone marrow cells using cytogenetic endpoints for monitoring.

Materials and methods

Salts tested

Sodium selenite (Na_2SeO_3, mol wt. 172.94, BDH, England, CAS No. 10102–18–8) and sodium selenate (Na_2SeO_4, mol wt. 188.94, Kotch Light, U.K., CAS No. 13410–01–0).

Animals

6–8 weeks old, laboratory bred male Swiss albino mice (*Mus musculus* L., 2n = 40) weighing 25–30 gm

were used as test system. These mice were housed in stainless steel wire mesh cages in air conditioned rooms under alternate light-dark cycles (light 06:00–18:00 h; dark 18:00–06:00 h). They were kept six per cage with husk bedding and were maintained on *ad libitum* access to commercial pellets (Lipton, India) and water.

Exposure to selenium salts

In vivo studies were performed according to the standard guidelines for genotoxicity assessment (Hsu 1982; Naismith 1987; Preston *et al.* 1981, 1987; Tice *et al.* 1994). The salts were dissolved in distilled water and single doses were administered by oral gavage to six male mice/dose/time point. The concentrations used were 7, 14, 21 and 28 mg kg^{-1} bw corresponding to $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{3}$ and $\frac{1}{2}$ of LD_{50} in case of sodium selenite and $\frac{1}{10}$, $\frac{1}{5}$, $\frac{1}{3}$ and $\frac{2}{5}$ of LD_{50} in case of sodium selenate respectively. Six mice received distilled water by oral gavage as negative control set (Table 1). Observations were made at 6, 12, 18 and 24 h after exposure for all sets.

Bone marrow chromosome preparation

Mice, from both exposed and control sets, were killed by cervical dislocation at 6, 12, 18 and 24 h after exposure to the chemicals. 1.5 h prior to sacrifice all animals were injected with 4 mg colchicine kg^{-1} bw. After sacrifice both femurs were removed. Bone marrow cells were flushed out in 75 mM KCl - hypotonic solution, kept for 20 min at 37 °C and fixed in 3:1 methanol-glacial acetic acid. Chromosome preparations were made following the standard procedure of air drying and then stained in 7% Giemsa solution (Preston *et al.* 1981, 1987; Sharma and Sharma 1994).

Screening for aberrations

Fifty clear metaphase plates with normal chromosome number $2n = 40$ were examined from each animal, giving a total of $50 \times 6 = 300$ plates for each set. The types of aberrations screened were chromatid, isochromatid and chromosome gaps, breaks, rearrangements and polyploidy according to standard WHO guidelines for evaluation of genetic toxicity (WHO 1985). All the aberrations were considered to be equal, regardless of the number of breakage involved (Tice *et al.* 1987). The results were expressed as percentages of total chromosomal aberrations without gap (Total% CA), number of chromosomal breaks per cell (CB/cell) and percentage of damaged cells (% DC), when a single cell contained more than one aberrations.

Statistical analysis

Comparison was made between the effects of the two salts at different concentrations and different durations of exposure. Data were analyzed by three way ANOVA (Sokal and Rohlf 1987) followed by Duncan's multiple range test in order to compare the effects of the two salts in relation to dosage and exposure (Table 2 and 3) (Duncan 1955; Harter 1960; Kotz and Johnson 1982). Student's t-test (Fisher and Yates 1963) was also performed to compare the results of treatment and control sets.

Results and discussion

Inorganic selenium in the diet has been observed to protect laboratory animals against several forms of cancer (El-Bayoumy 1991; Ip 1986). However chronic feeding at levels of > 5 ppm was reported to be toxic to rodents. Mutagenic effects were recorded in bacteria (Lofroth and Ames 1978; Noda *et al.* 1979; Kramer and Ames 1988) and yeast (Anjaria and Madhavnath 1988); clastogenic effects in *in vitro* human leucocyte cultures (Nakamuro *et al.* 1976; Khalil 1989; Khalil and Maslat 1990) and human fibroblast cultures (Lo *et al.* 1978)) and *in vivo* bone marrow cells of Chinese hamster and rats (Norppa *et al.* 1980a; Newton and Lilly 1986). Micronuclei were reported in fish erythrocyte cell (Al-Sabti 1994) and mouse bone marrow cells (Itoh and Shimada 1996). Unscheduled DNA synthesis in cultured human skin fibroblasts (Whiting *et al.* 1980) and sister chromatid exchanges in human lymphocyte cultures (Ray 1984; Ray and Altenberg 1978, 1980, 1982) were also reported.

The reason for the present investigation was to find out the range of concentrations of Se, the form and the period of exposure which would induce chromosome damage. Earlier studies have indicated either a very high near lethal dose (Norppa *et al.* 1980a) or a very low dose to be cytotoxic using micronuclei technique (Itoh and Shimada 1996). The usual amount of selenium prescribed for human consumption is 200 μg/day which is equivalent to 2.5–5 μg kg^{-1} for an 80 kg individual. We therefore tried to determine the lowest concentration which was genotoxic, using chromosomal abnormalities as endpoints (as per EPA guidelines).

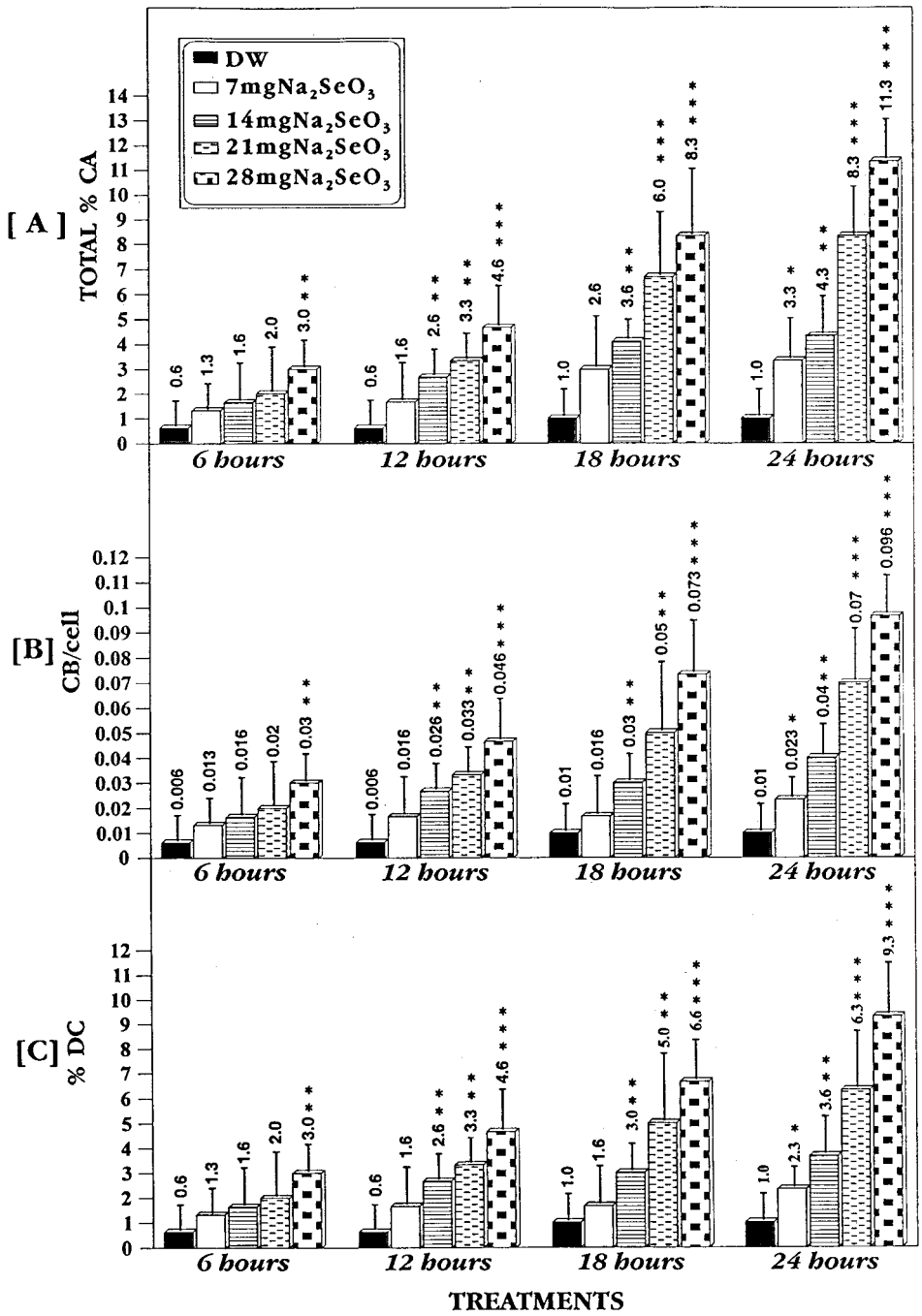

Figure 1. Frequency of [A] total % chromosomal aberrations [B] chromatid breaks per cell [C] percentages of damaged cells induced by sodium selenite at different durations after exposure in male mice.

Table 1. Schedule of exposure to selenium salts

Chemicals used	Concentrations (mg/kg bw)	Corresponding fractions of LD_{50}	Period of exposure (h)
Sodium selenite (Na_2SeO_3)	7, 14, 21, 28	$\frac{1}{8}, \frac{1}{4}, \frac{2}{5}, \frac{1}{2}$	6, 12, 18 and 24
Sodium selenate (Na_2SeO_4)	7, 14, 21, 28	$\frac{1}{10}, \frac{1}{5}, \frac{1}{3}, \frac{2}{5}$	

Six male mice were used per treatment set

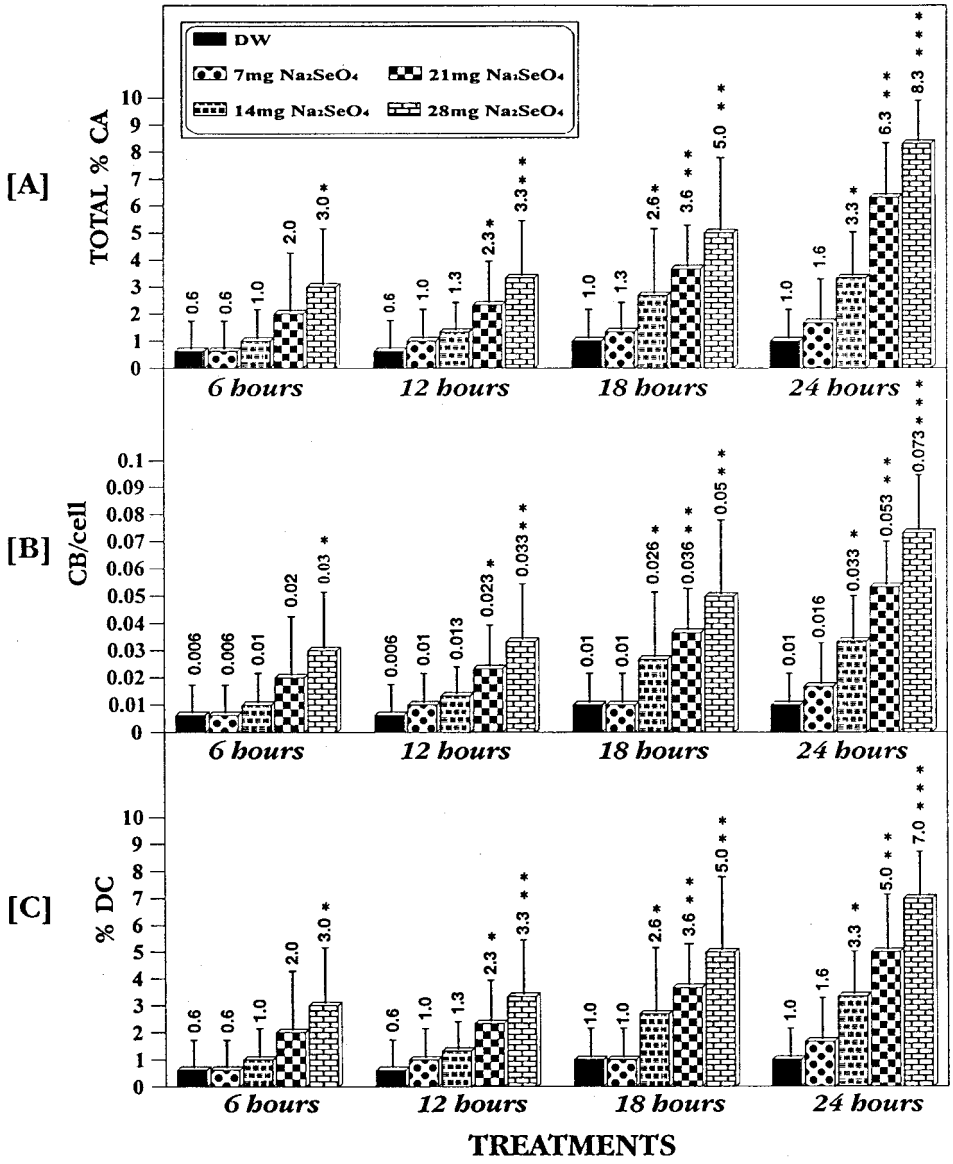

Figure 2. Frequency of [A] total % chromosomal aberrations [B] chromatid breaks per cell [C] percentages of damaged cells induced by sodium selenate at different durations after exposure in male mice.

In the present study, sodium selenite and sodium selenate were screened separately for their ability to induce chromosomal aberration (CA) in *mice* bone marrow cells *in vivo*. The effects of four concentra-

Table 2. Three way ANOVA (without replication)

	Source of variation	Degress of freedom (df)	Sum of squares (SS)	Mean sum of squares (MSS)	F
CB/cell[a]	A (Salt)	1	0.000612306	0.000612306	38.269125***
	B (Dose)	4	0.011037263	0.002759315	172.4571875***
	C (Duration)	3	0.004096933	0.001365644	85.35275***
	A × B (Salt × Dose)	4	0.000237501	0.000059375	3.710953125*
	A × C (Salt × Duration)	3	0.000094987	0.000031662	1.978875ns
	B × C (Dose × Duration)	12	0.002235579	0.000186298	11.64364063***
	A × B × C (Salt × Dose × Duration)	12	0.000192001	0.000016	
% DC[b]	A (Salt)	1	5.1337191	5.1337191	36.7473683***
	B (Dose)	4	101.8087289	25.45218223	182.1877466***
	C (Duration)	3	34.9410339	11.6470113	83.36977647***
	A × B (Salt × Dose)	4	1.8771431	0.469285775	3.359166498*
	A × C (Salt × Duration)	3	0.6974813	0.232493766	1.664199751ns
	B × C (Dose × Duration)	12	18.4898283	1.540819025	11.02922447***
	A × B × C (Salt × Dose × Duration)	12	1.6764365	0.139703041	
Total% CA[c]	A (Salt)	1	11.3763556	11.3763556	48.87468356***
	B (Dose)	4	130.7989346	32.69973365	140.4834018***
	C (Duration)	3	65.3849115	21.7949705	93.63475649***
	A × B (Salt × Dose)	4	3.8718114	0.96795285	4.158483692
	A × C (Salt × Duration)	3	2.9576452	0.985881733	4.235509105*
	B × C (Dose × Duration)	12	35.138645	2.928220417	12.58011364***
	A × B × C (Salt × Dose × Duration)	12	2.7931898	0.232765816	

CB/cell[a] = Mean chromosomal breaks/cell (not including gap);%DC[b] = Mean percentage of damaged cells. Total% CA[c] = Total percentage of chromosomal aberrations (not including gap); $*p \leq 0.05$; $***p \leq 0.001$ significantly different followed by three way ANOVA; ^{ns} = Nonsignificant.

Table 3. Duncan's multiple range test

	Salt		Sodium selenite		Sodium selenate	
	Sample mean		0.6362		0.4797	
CB/cell	Dose (mg/kg bw)	Control	7	14	21	28
	Sample mean	0.0668	0.1131	0.1964	0.3065	0.4331
	Salt		Sodium selenite		Sodium selenate	
	Sample mean		61.662		47.332	
% DC	Dose (mg/kg bw)	Control	7	14	21	28
	Sample mean	6.668	11.332	19.331	29.665	41.998
	Salt		Sodium selenite		Sodium selenate	
	Sample mean		71.663		50.331	
	Dose (mg/kg bw)	Control	7	14	21	28
Total % CA						
	Sample mean	6.668	13.666	20.664	33.993	46.998
	Duration (h)	6	12	18	24	
	Sample mean	16	21.664	35.332	48.998	

Duncan's multiple range test denotes that there is significant differences at $p \leq 0.05$ level.

tions of each salt, administered to mice by gavaging, were recorded at 6, 12, 18 and 24 h after exposure. In general, the frequencies of chromosomal breaks per cell (CB/cell), percentages of damaged cells (% DC) and total percentages of chromosomal aberrations (Total% CA) induced following exposure to the salts, increased significantly as compared with controls as shown by the *t*-test (see Figures 1 and 2).

With sodium selenite at 6 h, only the highest concentration (28 mg kg^{-1} bw) induced significantly high percentages of chromosomal aberrations. At 12 and 18 h, three highest concentrations i.e 14, 21 and 28 mg kg^{-1} bw were clastogenic and the frequency of chromosomal aberrations was significantly high. After exposure for 24 h, all concentrations showed significantly high frequency of chromosomal alterations (Figure 1).

Following exposure to sodium selenate for 6 h, only the highest concentration showed statistically significant number of chromosomal aberration. After 12 h, the two highest concentrations, (21 and 28 mg kg^{-1} bw) induced aberrations in frequencies significantly higher than control. Exposure for 18 and 24 h to the three highest concentrations i.e., 14, 21 and 28 mg kg^{-1} bw showed statistically significant increase in chromosomal aberrations (Figure 2).

Analysis carried out by trend test showed a positive trend at durations of 6, 12, 18 and 24 h after exposure for both salts (Figures 1 and 2). Three way ANOVA followed by Duncan's multiple range test indicates that the two salts induce significantly high clastogenic effects, as compared with control, at all concentrations and all durations after exposure (Tables 2 and 3). The clastogenic effects depend directly on the concentration of the salt, increasing with increase in the concentration administered and also on the duration, increasing directly with the period of exposure.

The results indicate that the effects of inorganic selenium salts are principally at late S and G^2 since only chromatid breaks were observed, which increased with duration of exposure. At short durations after exposure (6 and 12 h), the action is entirely on chromosome structure. Spindle disturbances leading to polyploidy were observed at 18 hours onwards. Rearrangements were noted in a very low frequency only after 24 h following exposure to the highest concentrations of both salts. The highest numbers of chromosomal aberration (CA) and damaged cell (DC) were observed with highest concentrations (21 and 28 mg kg^{-1} bw) after exposure for prolonged periods (18 and 24 h). Thus the clastogenic activity is directly proportional to the concentrations used and durations of exposure. Norppa *et al.* (1980a) and Newton and Lilly (1986)

had earlier observed chromosomal aberrations in Chinese hamster and rat bone marrow cells at near lethal doses. However, another observation by Norppa *et al.* (1980b) found 0.8 mg kg^{-1} bw to be nonclastogenic, but the dose used was much lower than our lowest concentration (7 mg kg^{-1} bw). The duration of exposure was not studied. Our observations indicate that Se is cytotoxic at a range and concentration from $\frac{1}{10}$ to $\frac{2}{5}$ for sodium selenate or $\frac{1}{8}$ to $\frac{1}{2}$ for sodium selenite. These effects are related to period of exposure. Since Se is anticarcinogenic at lower concentrations as given by many authors it is suggested that the margin between clastogenic and anticlastogenic concentrations should be carefully monitored before prescribing selenium as an anticarcinogenic drug.

The mechanism of selenium toxicity remains ambiguous due to differences in the mode of action of various selenium compounds (WHO 1987). Selenate is slowly reduced to selenite by glutathione and other sulfhydryl compounds. Selenite interferes with glutathione enzyme activity and is converted into reactive compounds, selenide (HSe) and selenopersulphide (GS-Se). The latter two may be the ultimate mutagenic forms. Both these compounds can be easily oxidized and may produce free radicals which could react with and damage DNA, resulting in breakage of chromosome (Shamberger 1985). Our observations here and earlier (Biswas *et al.* 1997) indicate that sodium selenite is much more clastogenic than sodium selenate. This may be due to the fact the selenite-glutathione reaction generates superoxide anion-the oxygen free radical formation while selenate fails to do so (Yan and Spallholz 1993). Such selective involvement of oxygen radicals in selenium toxicity has been shown by increase in lipid peroxidation caused by selenite and not selenate *in vivo* (Dougherty and Hoekstra 1982). The high level of clastogenic effects, exhibited by sodium selenite as compared to sodium selenate may be related to their respective effects on cell metabolism. The lower response of sodium selenate may be attributed to kinetic differences in chromosomal aberration formation.

In conclusion, our observations of direct relationship between degree of clastogenicity and concentrations of the two salts indicate that given in low doses for short periods selenium is nontoxic. The four concentrations used by us ranged from $\frac{1}{8}$ th to $\frac{1}{2}$ of LD_{50} with sodium selenite and $\frac{1}{10}$ th to $\frac{2}{5}$ th of LD_{50} with sodium selenate. Even following exposure through oral gavaging to such high concentrations for shorter periods of 6 and 12 h the salts are relatively non toxic since chromosomal aberrations are minimal. These observations support the use of relatively low doses of selenium for its beneficial action (Khalil and Maslat 1990). It is nontoxic and thus nonclastogenic at the lower doses and for short periods of exposure. Since, clastogenic effects increase proportionately with concentration and period of exposure used, it might explain the cases when higher concentrations of selenium itself have been reported to stimulate tumorigenesis in rodent models (Levander and Burk 1994; Birt *et al.* 1989). Thus the use of selenium in cancer chemoprevention, should be under strictly controlled conditions with respect to concentration and duration of exposure.

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