Genotoxicity studies of sodium selenite and its effect on methyl-methanesulfonate-induced chromosome damage

G. Slapšytė[1],
A. Sukackaitė[2]

[1]*Department of Botany and Genetics, Vilnius University, M. K. Ciurlionio 21, LT-2600 Vilnius, Lithuania*
[2]*Lithuanian Oncology Center, Polocko 6, LT-2600 Vilnius, Lithuania*

The genotoxic activity of sodium selenite and its effect in combination with methyl methanesulfonate (MMS) were studied using chromosome aberration (CA) and sister chromatid exchange (SCE) tests in human peripheral blood lymphocytes *in vitro.* Only high doses of sodium selenite were found to be genotoxically active. A dose-related response in the SCE induction was determined. A decrease of MMS-induced chromosome aberrations was found in cultures treated concurrently with MMS and sodium selenite. However, the combined effect of MMS and sodium selenite resulted in a higher SCE rate as compared to that induced by MMS alone.

Key words: sodium selenite, chromosome aberrations, sister chromatid exchanges, human lymphocytes

INTRODUCTION

Selenium is an essential trace element. It was found in enzymes (glutathione peroxidase, thioredoxin reductase), tRNA, muscle proteins. It occurs naturally in water, soil, air, plants, food. The important physiological and nutritional roles of selenium have been recognised since 1970s. Epidemiological studies have shown that the deficiency of selenium may cause serious health complications. Increased incidence of a variety of cancers, including lung, stomach, bladder, ovaries, pancreas, thyroid, head and neck, cerebral cancers, melanoma were reported in human populations having a low selenium intake and consequently low plasma or serum selenium levels [1, 2]. On the other hand, dietary supplementation with selenium has been reported to reduce the incidence of cancer in animals [3] and in clinical and epidemiological studies [4].

However, data on the genotoxic and antigenotoxic effects of selenium compounds are rather scarce and contradictory. The mutagenic potential of sodium selenite has been shown in bacteria [5] and in yeast [6], it induced sister chromatid exchanges in whole blood cultures and in the Chinese hamster V-79 cell lines [7]. In addition, selenium selenite was found to increase the incidence of chromosome aberrations and sister chromatid exchanges in Chinese hamster bone marrow cells *in vivo* [8, 9]. But it must be indicated that in all these studies only very high (toxic) doses of selenite were found to be genotoxically active. In contrast, sodium selenite and other selenium compounds were reported to induce no chromosome aberrations in mouse bone marrow cells and primary spermatocytes [10] and somatic mutations and recombination in *Drosophila melanogaster* wing cells [11]. Besides, long-term low-level selenium supplementation in the treatment of human neuronal ceroid lipofuscinosis (a heritable lipopigment disorder with low activity of glutathione peroxidase) had no detectable effects on chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes [12].

As concerns the antigenotoxic properties of sodium selenite and other selenium compounds, there are several studies showing that they significantly reduce the frequencies of sister chromatid exchanges and micronuclei induced by carbon tetrachloride in ovine peripheral blood lymphocytes [13], prevent the cytotoxic effects of arsenic in mouse bone marrow cells [14], indicate protective effects against cadmium-induced chromosomal aberrations and micronuclei formation in root cells of *Hordeum vulgare* [15].

The present study was undertaken to evaluate the genotoxic effects of sodium selenite in chromosome aberration (CA) and sister chromatid exchange (SCE) tests in human lymphocytes *in vitro.* In addition, the possible effects of sodium selenite on methyl methanesulfonate (MMS) induced chromosome damage were also evaluated.

MATERIALS AND METHODS

Whole peripheral blood obtained from healthy donors was grown in RPMI 1640 medium containing 12% newborn calf serum, 2 mM α-glutamine, 7.8 μg/ml phytohemagglutinin P, 50 μg/ml gentamycin and 10 μg/ml 5-bromo-2'-deoxyuridine. All reagents for cell culture were purchased from Sigma (St. Louis, MO, USA). The cultures were incubated at 37 °C for 72 h treated with 0.5 μg/ml colchicine for the last 3 h of incubation, exposed to 0.075 M KCl for 20 min and fixed in 3:1 methanol : acetic acid. Treatment with sodium selenite (Na_2SeO_3; Sigma, St. Louis, MO, USA) or/and methyl methanesulfonate (MMS; Fluka Chemie, Switzerland) was carried out 48 h after culture initiation and lasted for a period of 24 h. Working solutions of the chemicals were made just before treatment. The chemicals, dissolved and diluted in RPMI 1640 medium, were applied by adding 50 μl solution to the culture flasks. Duplicate whole blood cultures were used for each treatment group.

Flame-dried chromosome preparations were made and differentially stained by fluorescence plus Giemsa technique [16]. Two hundred first-mitotic division cells for chromosome aberrations and 50 second-mitotic division cells for SCEs were analysed. The frequency of first, second and third mitotic division cells were scored from no less than 200 cells for each test condition to determine the replicative index (RI).

All statistical analyses were performed using InStat V2.02 (GraphPad Software, San Diego, CA, USA) statistical package.

RESULTS AND DISCUSSION

Table 1 shows that sodium selenite did not increase the frequency of chromosome aberrations at concentrations up to the top concentration of 0.75 μg/ml. At this concentration sodium selenite induced a borderline, but statistically significant increase of aberrations. Higher concentrations of sodium selenite (≥ 1 μg/ml) were cytotoxic. A dose-related response in the SCE induction was observed in cultures treated with sodium selenite (Table 2). The dose dependency was linear and might be described by equation $Y = 5.98 + 12.91\ X$ ($r^2 = 0.7565$, $P = 0.0243$). It should be mentioned that a significant increase of SCEs was evident only after treatment with the highest concentrations of sodium selenite (starting from 0.5 μg/ml). Thus, a relatively narrow range of concentrations leading to mutagenic effects before lethality was determined. Sodium selenite significantly decreased the RI values. However, no dose-related effect was observed ($r^2 = 0.03947$; $P = 0.7059$). The results of our study are in agreement with and support the previously established absence of genotoxic potential for sodium selenite at low concentrations in different experimental systems: whole blood cultures and in the Chinese hamster V-79 cell lines *in vitro* [7], Chinese hamster bone marrow cells *in vivo* [8, 9]. Only high doses of selenite were found to be genotoxically active. Superoxide and other oxy-radicals have been reported to be produced during selenium reductive metabolism [17]. Thus, the genotoxicity of selenite and its proximal metabolites is considered to be mediated by an oxy-radical-mediated mechanism [18].

Table 1. **Chromosome aberrations in human lymphocytes treated *in vitro* with sodium selenite and after cotreatment with sodium selenite (Se) in combination with methyl methanesulfonate (MMS)**

Compound concentration (μg/ml)	Number of cells analysed	Frequency of aberrant cells (% ± S. E. M.)	Aberrations[a]			
			csb	cse	ctb	cte
Control	200	1.0 ± 0.7	1	0	1	0
Sodium selenite						
0.05	200	3.0 ± 1.2	4	0	2	0
0.1	200	2.0 ± 1.0	2	0	2	0
0.25	200	2.0 ± 1.0	1	1	2	0
0.5	200	3.0 ± 1.2	2	0	4	0
0.75	200	4.0 ± 1.4[b]	3	0	5	0
MMS (0.02)	200	9.0 ± 2.0[b]	2	0	6	10
Cotreatment MMS + Se						
0.02 + 0.05	200	6.0 ± 1.7	1	0	9	2
0.02 + 0.1	200	3.0 ± 1.2[c]	0	0	6	0

[a] csb, chromosome breaks; cse, chromosome exchanges; ctb, chromatid breaks; cte, chromatid exchanges.
[b] $P < 0.05$ as compared to control.
[c] $P < 0.05$ as compared to MMS.

Table 2. Sister chromatid exchanges in human lymphocytes treated *in vitro* with sodium selenite (Se) and after cotreatment with sodium selenite in combination with methyl methanesulfonate (MMS)

Compound concentration (μg/ml)	Number of cells analysed	SCE / cell ± S. E. M.	Range	RI ± S. E. M.
Control	50	6.86 ± 0.43	2–15	2.51 ± 0.05
Sodium selenite				
0.05	50	7.84 ± 0.39	3–15	2.12 ± 0.05
0.1	50	7.34 ± 0.40	3–16	2.03 ± 0.06
0.25	50	7.82 ± 0.41	3–16	2.29 ± 0.05
0.5	50	8.98 ± 0.48[a]	4–17	2.17 ± 0.06
0.75	50	18.34 ± 1.79[a]	4–54	2.19 ± 0.06
MMS (0.02)	50	39.48 ± 1.25[a]	21–59	1.95 ± 0.05
Cotreatment MMS + Se				
0.02 + 0.05	50	46.43 ± 1.40[b]	28–68	2.25 ± 0.05
0.02 + 0.1	50	45.30 ± 2.0[b]	31–79	2.28 ± 0.05

[a] $P < 0.05$ as compared to control.
[b] $P < 0.05$ as compared to MMS.

The literature underlines the observation that selenium compounds at high concentrations can be toxic, mutagenic and carcinogenic, whereas at low concentrations they may have antimutagenic and anticarcinogenic effects. In a chromosome aberration assay we determined an increase and respectively a decrease in the frequency of chromosome aberrations in cultures treated with MMS alone and in cultures treated concurrently with MMS and 0.1 μg/ml sodium selenite (Table 1). MMS at a concentration of 0.02 μg/ml induced 9.00 ± 2.0 CA/100 cells. When the MMS treatment was concurrently followed by the treatment with 0.1 μg/ml sodium selenite, the frequency of aberrations decreased to 3.00 ± 1.21 CA/100 cells ($P < < 0.05$). For cells treated with MMS plus 0.05μg/ml sodium selenite, the decrease of the frequency of chromosome aberrations was less pronounced (6.00 ± ±1.68 CA/100 cells, $P > 0.05$). The decrease in aberration frequency was restricted to chromatid-type exchanges. However, the results involving induction of SCEs do not correspond to those obtained in the chromosome aberration assay. The combined effect of MMS and sodium selenite resulted in a significantly higher SCE rates (Table 2). At 0.02 μg/ml MMS induced less SCEs (39.48 ± 1.25 SCE/cell) than after treatment in combination with sodium selenite 0.05 μg/ml (46.43 ± 1.40 SCE/cell) and 0.1 μg/ml (45.30 ± 1.99 SCE/cell).

Our results concerning a combined effect of sodium selenite with MMS are contradictory, difficult to explain and need further investigations. There are several studies showing that selenite is effective in the reduction of the mutagenicity induced by a variety of mutagens: potassium dichromate [11], carbon tetrachloride [13], arsenic [14], cadmium [15] A to the possible mechanism of action of the antigenotoxic activity of selenium, it is ascribed to the ability of selenium to prevent the uncontrolled formation of free radicals and reactive oxygen species (for example, H_2O_2, $O_2^{\cdot -}$, $^{\cdot}OH$) and to inhibit their reactions with biological structures [19].

The results of the present study demonstrate that sodium selenite exerts both genotoxic (at high doses) and antigenotoxic (CA assay) effects. Bearing in mind the growing use of selenium compounds for dietary supplement, their genotoxicity testing may be an important field of future research.

Received
11 October 2002

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G. Slapšytė, A. Sukackaitė

NATRIO SELENITO GENOTOKSIŠKUMO TYRIMAI IR JO POVEIKIS METILMETANSULFONATU INDUKUOTŲ CHROMOSOMŲ PAŽAIDŲ DAŽNIUI

S a n t r a u k a

Tirtas natrio selenito (Na_2SeO_3) ir natrio selenito kartu su metilmetansulfonatu (MMS) veikimas, indukuojant chromosomu aberacijas (CA) ir seserinių chromatidžių mainus (SCM) žmogaus periferinio kraujo limfocitų kultūroje *in vitro.* Natrio selenitas indukavo CA ir SCM tik paveikus maksimaliomis tirtomis dozėmis (0,5 ir 0,75 μg/ml). Nustatytas patikimas MMS indukuotų CA kiekio sumažėjimas kultūrose, paveiktose MMS kartu su 0,01 μg/ml natrio selenito. Priešingai, SCM dažnis kulturose, paveiktose MMS kartu su natrio selenitu, buvo didesnis, nei paveikus tik MMS.