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A STUDY OF SELENIUM GENOTOXICITY IN THE MICRONUCLEUS TEST ON MICE

Č. RUSOV*, RADMILA ŽIVKOVIĆ*, B. SOLDATOVIĆ**, LJILJANA JOJIĆ-MALIČEVIĆ* and Z. STANIMIROVIĆ**

*Department of Hematology, Scientific Institute for Veterinary Medicine of Serbia, Auto-put, 3, 11070 Belgrade, Yugoslavia **Department of Biology, Faculty of Veterinary Sciences, University of Belgrade, Bul. JNA 18 11000 Belgrade, Yugoslavia

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Genotoxicological characterization of sodium selenite in the micronucleus test on BALB/c mice showed that a very small dose (0.2 mg/kg body weight), as well as higher doses (2.0 and 10.0 mg/kg body weight), applied intramuscularly, induced higher micronuclei counts (11.20 ± 1.92; 23.20 ± 3.42; 40.00 ± 4.00) in polychromatic erythrocytes of the bone marrow. The count of induced micronuclei is statistically significant ($P<0.001$) compared to that of spontaneous micronuclei (2.25 ± 1.49) found in mice inoculated with physiological saline solution. Therefore, much care should be taken when selenium preparations are used alone or in combination with vitamin E in the prevention or therapy of different pathological states due to potential genotoixc effects.

Key words: selenium genotoxicity, micronucleus test, bone marrow cells, polychromatic erythrocytes, BALB/c mice.

INTRODUCTION

Selenium is an essential but a very toxic element. In veterinary medicine its salts are widely used alone or in conjuction with vitamin E to prevent muscular dystrophy and exudative diathesis in chicks, white muscle disease in sheep and infertility in ewes. They have been reported to prevent pneumonia in premature lambs and calves and to control hepatitis in swine (Elkin and Margrave 1968). Also it was shown that selenium decreased the frequency of some reproductive disorders (Julien et al., 1976; Harrison et al., 1984), reduced the incidence and alleviated the course of mastitis (Smith, 1984). Moreover, selenium activated cells of the reticuloendothelial system (RES) showed antiinflammatory and stimulating effects on humoral and cell mediated immunity, and acted anticarcinostatically (Spallholz et al., 1981).

However, selenium can be toxic for experimental and domestic animals. The toxic effects of selenium are dependent on the species and age of the

animals, dose levels and inoculation routes, as well as other factors. Toxic dose levels for domestic and experimental animals are well known toay (Stecher, 1968; Pletnikova, 1970; Underwood, 1971; McDonald et al., 1981, Goehring et al., 1984; Blodgett, 1984; Ivandija, 1988; Choy et al., 1993).

The previous investigations of mutagenic and clastogenic effects of selenium, in vitro and in vivo (Magos, 1991; Balansky, 1991; Shelby et al., 1993; Berces et al., 1993), show that high doses of selenium act mutagenically/carcinogenically.

Due to the present popularity of selenium in the therapy or prevention of many disorders in domestic animals it has become necessary to determine its genotoxic potential. The aim of this work was to estimate the genotoxicity of different doses of sodium selenite in the micronucleus assay.

MATERIALS AND METHODS

Before the micronucleus assay was started the LD_{50} of solium selenite was determined over 7 days. In groups of 5 BALB/c female mice with a mean body weight (b.w.) of 20.8± 2.1 g, the toxic effects of selenium at dose levels of 0.2, 10.0 and 20.0 mg/kg b.w. were investigeted.

The results showed that the selenium $LD_{50}/7$, given intramuscularly (i.m.), was 20 mg/kg b.w.

For the micronucleus assay, the animals were divided into 5 groups with 5 BALB/c females in each group. Their mean b.w. was 21.20 ± 3.42 g. Physiological Saline was administered twice at the dose of 0.1 ml per ouse at 24-hour intervals. The animals in the positive control group were treated with cyclophosphamide (Endoxan) at a single dose of 50 mg/kg b. w. In the three experimental groups, animals were treated twice with sodium selenite at the dose levels of 0.2, 2.0 and 10,0 mg/kg b. w. respectively at 24-hour intervals. Applied i.m. (All test compounds were applied i.m.) The mice were sacrificed 48 hours after the first treatment with the test compound. Their femurs were prepared for bone marrow sampling. Bone marrow was flushed from their femurs into fetal calf serum. The method according to Schmid (1976) was used for the preparation of micronuclei. Two slides of sedimented bone marrow cells stained with May-Grünvald Giemsa were made for each animal.

The frequency of micronuclei, their morphological and tinctorial characteristics were established for each animal by counting a total of 1000 polychromatic erythrocytes (PCEs), as was described in our previous paper (Rusov et al., 1988).

RESULTS AND DISCUSSION

The results concerning the genotoxicity of sodium selenite and other test compounds in the micronucleus assay are shown in Table 1.

Table 1. Frequency of micronuclei in polychromatic erythrocytes in BALB/c mause bone marrow 48 hours after the first treatment of the animals with the test compounds.

Test compounds	Dose (mg/kg b.w.)	Number of micronuclei per 1000 cells	p
Negative control (Physiological saline)	2 x 0.1 ml per mice	2.25 + 1.49	
Positive control (Cyclophosphamide)	50	21.20 + 3.42	< 0.001
Sodium selenite	0.2	11.20 1.92	< 0.001
Sodium selenite	2.0	23.20 + 3.70	< 0.001
Sodium selenite	10.0	40.00 + 4.00	< 0.001

Data presented in Table 1. show that cyclophosphamide induced a rise of micronuclei counts in PCEs of bone marrow (21.20 ± 3.12). This increase was statistically significant ($P<0.001$) when compared to the frequency of spontaneous micronuclei in mice inoculated with physiological saline (2.25 ± 1.49).

All the administered test doses of sodium selenite (0.2, 2.0 and 10.0 mg/kg b.w.) also caused large increases in micronuclei counts in PCEs of the treated animals. The counts of induced micronuclei (11.20 ± 1.92, 23.20 ± 3.70 and 40.00 ± 4.00) were also statistically significant ($P<0.001$) when compared to the frequency of spontaneous micronuclei in mice inoculated with physiological solution only.

Balansky (1991) observed two-three times higher micronuclei counts and increased levels of chromosome aberrations in the bone marrow of mice treated with sodium selenite at 10 ppm in the drinking water. The animals were previously treated intraperitonelly (i.per.) with Uretan at doses of 0.5 - 1.0 g/kg b. w. Selenium was found to increase microunuclei counts by 43.8% in PCEs of mice given Mitomycin C i. per. at a dose of 1.5 mg/kg b.w. When selenium was applied alone, however, micronuclei counts were not increased. These results confirmed the comutagenic and coclastogenic activity of selenium in prokaryotes and eukaryotes during in vivo and in vitro experiments.

The genotoxic effect of low concentrations of selenium alone and in combination with mercury, was tested in the micronucleus test on binucleated erythrocytes of Prussian carp. It was found that the rise in micronuclei counts was dependent on the dose levels of selenium and was higher than the spontaneous increase in corresponding controls (al Sabti, 1994).

Stanimirović et al. (1995) tested the genotoxic effect of sodium selenite in bone marrow cells of BALB/c mice and established that the dose level of 0.2 mg/kg b.w. inoculated i. m. neither affected mitotoxic activity nor induced chromosome aberration. The dose level of 2.0 mg/kg b.w., however, induced a rise of the mitotic index (MI) to 6.92 compared to 6.20 in the control group. This increase was not statistically significant. However, a statistically significant increase of chromosome gap frequency was found ($P<0.05$). At the dose level of 10.0 mg/kg b.w. the mitotic index was increased (8.45), the difference being statistically significant ($P < 0.01$). Robertson's translocations in heterozygote

states (16,60%), and the presence of aneuploidies at levels of 4,2% compared to 0,92% in control mice were also noticed.

The results of these investigations, together with the findings of Stanimirović et al. (1995) showed that parenteral inoculation of selenium at higher dose levels induced high micronuclei counts and chromosome aberrations in the bone marrow of mice.

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IZUČAVANJE GENOTOKSIČNOSTI SELENA MIKRONUKLEUS TESTOM NA MIŠEVIMA

RUSOV Č., ŽIVKOVIĆ RADMILA, SOLDATOVIĆ B., JOJIĆ-MALIČEVIĆ LJILJANA i STANIMIROVIĆ Z.

SADRŽAJ

Genotoksikološka karakterizacija natrijum selenita u mikronukleus testu na BALB/c miševima, pokazuje da i mala doza (0.2 mg/kg telesne mase), a naročito veće doze (2.0 i 10.0 mg/kg telesne mase), date intramuskularno, idukuju veliki broj mikronukleusa (11.20 ± 1.92; 23.20 ± 3.42; 40.0 ± 4,0) u polihromatofilnim eritrocitima kostne srži. Ove vrednosti su statistički visoko značajne ($P<0.001$) u odnosu na broj spontanih mikronukleusa (2.25 ± 1.49) u kontrolnih miševa tretiranih fiziološkim rastvorom.

S obzirom na genotoksični potencijal visokih doza selena, apliciranog parenteralno, pri primeni samo selena ili s vitaminom E za preveniranje i terapiju različitih patoloških stanja kod životinja, potrebna je velika obazrivost pri doziranju i dužini njegove primene.

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