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CYTOTOXIC AND ANTIMUTAGENIC EFFECTS OF SODIUM SELENITE

Fikrat ABDULLAEV[1], Leticia RIVERÓN-NEGRETE[1], Israel PÉREZ-LÓPEZ[1], Rafael CAMACHO-CARRANZA[2] and J.J. ESPINOSA-AGUIRRE[2]

[1] Torre de Investigación "Dr. Joaquín Cravioto", Instituto Nacional de Pediatría Insurgentes Sur, 3700-C, 04530 México, D.F., México
[2] Instituto de Investigaciones Biomédicas, UNAM, Apartado Postal 70228, 04510 México, D.F., México. E-mail: jjea@servidor.unam.mx

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ABSTRACT

In the last decade, there is an increasing interest in selenium compounds because of their environmental, biological, and toxicological importance, and in particular, because of their anticancer activities. We have examined the effect of sodium selenite on colony formation of different human cultured malignant cells. In addition, the antimutagenic activities of this chemical was examined against the indirect-acting mutagen benzo[a]pyrene (BP) by using the Ames/*Salmonella* test assay TA98 strain. Results of these experiments demonstrated that sodium selenite caused a dose-dependent inhibitory effect on colony formation of tested malignant cells. It was also shown that sodium selenite exhibited dose-dependent antimutagenic effect on BP. These results suggest the potential use of selenium compounds as an anticancer and antimutagenic agent.

Palabras clave: selenito de sodio; citotoxicidad; mutagenicidad; antimutagenicidad; quimioprevención

RESUMEN

En la última década, se ha incrementado el interés en estudiar los compuestos que contienen selenio debido a su importancia toxicológica, ambiental, biológica y en particular por su actividad antitumoral. En este trabajo estudiamos el efecto del selenito de sodio sobre la formación de colonias de diferentes líneas celulares de origen humano. Adicionalmente, exploramos la actividad antimutagénica de este compuesto con el promutágeno benzo(a)pireno (BP) utilizando la prueba de Ames con la cepa TA98 de *Salmonella typhimurium*. Los resultados de estos experimentos mostraron que el selenito de sodio causó un efecto inhibitorio dosis-dependiente en la formación de colonias de células malignas humanas. Además, mostró un efecto antimutagénico dosis dependiente con el BP. Estos resultados sugieren que el selenio puede ser considerado un potencial agente antimutagénico y anticancerígeno.

INTRODUCTION

In the last decade, there is an increasing interest in selenium compounds because of their environmental, biological, and toxicological importance, and in particular, because of their anticancer activities (Combs 2001, Kim and Milner 2001). Selenium compounds has been shown to possess anticancer activity in both *in vivo* and *in vitro* experimental systems (Combs and Gray 1998). Literature data have indicated that the complementary supplementation of selenium is useful in the clinical treatment of different types of cancer (Bantzel 1999). The dose-dependent antimutagenic effect of sodium selenite on acridine orange

and 7,12-dimethylbenzo(a)anthracene (DMBA) was demonstrated in the Ames/*Salmonella* test system (Martin *et al.* 1981). Additionally, it was shown that sodium selenate and sodium selenite had a weak mutagenic activity in Kada´s rec-assay and Ames´s Salmonella test (Noda *et al.* 1979). By means of the wing spot test of *Drosophila melanogaster*, three selenium compounds: sodium selenite, sodium selenate, and selenious acid, were examined and neither compound proved to be genotoxic. Nevertheless, the antigenotoxic effects of sodium selenite by a complete suppression of mutant clones induced by potassium dichromate were demonstrated (Riski *et al.* 2001). It was reported that selenium could effectively protect Chinese hamster ovary cells from AFB1 cytotoxicity *in vitro* but not from AFB1-DNA adduct formation or mutagenesis (Shi *et al.* 1995). Another study indicated that two selenium compound were inconclusive in the Ames/*Salmonella/*microsome assay for antimutagenesis since the reduction in the number of revertants declined below the background level and might only reflect the toxic effects of these compounds (Romert *et al.* 1994). More recently, it was found that sodium selenite and ebselen (2-phenly-1, 2-benzisoselenazol-3(2H)-one) reduced the genotoxic effect of azoxymethane, methylmethansulphonate and hydrogen peroxide in Chinese V79 hamster cells. Sodium selenite is also involved in the detoxification of free radicals by increased the activities of glutathion peroxidase and catalase (Bronzetti *et al.* 2003). On the other hand, it was reported that selenium is able to prevent erythrocyte membrane damage induced by T-2 toxin (Keshavarz *et al.* 2001).

The aim of the present study was to examine the cytotoxic and antimutagenic activities of sodium selenite using the *Salmonella assay* and different human cell cultures.

MATERIALS AND METHODS

Chemicals

Sodium selenite was purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco´s modified Eagle's medium (D-MEM) was purchased from Gibco/BRL Products (Grand Island, NY). Fetal bovine serum (FBS) was obtained from HyClone (Road Logan, UT). Culture dishes and flasks were obtained from Costar (Corning, NY). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium selenite (Na_2SeO_3), was dissolved in distilled water, adjusted to pH=7.4 and added directly to the culture medium. The final concentration of Na_2SeO_3 in the cultures was ranged from 173 to 672 μg/plate for *Salmonella typhimurium,* and from 10 to 200μM for cultured tumor cells, as indicated in **tables**.

Cell culture

For our experiments we chose normal and human malignant cells with different origin: CCD-18Lu (human lung), HeLa (human cervix epitheloid carcinoma), A-204 (human rhabdomyosarcoma) and HepG2 (human hepatocellular carcinoma) cells which were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in D-MEM with 10% FBS in a CO_2 water-jacketed incubator (Nuaire, Plymouth, MN) at 37ºC in humidified atmosphere of 5% CO_2 and 95% air.

Colony formation assay

Cells (2 x 10^5) were seeded into 100-mm Petri dishes with 10 ml D-MEM medium. After 2 days, different concentrations of tested agent was added and incubation continued 1 h. The cells were then trypsinized (0.05M trypsin), counted and 200 cells were seeded in 60-mm Petri dishes for determination of colony formation. At least 3 replicate colony determinations were carried out for each culture. After 10 days of incubation, the resulting colonies were rinsed with 50 mM phosphate buffer, pH 7.6, containing 150 mM NaCl, fixed with methanol, stained with Giemsa (Sigma) and the number of colonies (with diameter > 0.05 mm) per dish were determined as described previously [Abdullaev and Frenkel, 1994].

Mutagenicity and antimutagenicity assays

Dr. B.N. Ames, University of California at Berkeley kindly provided *Salmonela typhimurium* TA98 tester strain. The mutagenic and the antimutagenic activities of sodium selenite were determined using the plate incorporation test (Maron and Ames 1983) and indirect-acting mutagen: benzo(a)pyrene (BP). BP (10 μg/plate) was chosen as positive control for the antimutagenicity studies, since this dose is not toxic for TA98 strain. 0.5 ml of S9 mixture (10% rat liver S9 fraction, 8 mM $MgCl_2$, 33 mM KCl, 4 mM NADP, 5 mM glucose-6-phosphate and 100 mM sodium phosphate buffer (PBS), pH 7.4 were added to the molten top agar and plated on minimal agar. Toxicity of the tested agent was assessed by observation of the background bacterial growth due to histidine traces in the medium. Revertant colonies were counted after a 72-h incubation period at 37º C using a MiniCount colony counter (Biotran II, New Brunswick Scientific, Edison NJ). All determinations were done in triplicate.

Antimutation index was calculated as AMI= x_1/x_0 where x_1 is the number of revertant colonies found in BP plates and x_0 is the number of revertants at each

concentration of the tested agent.

A test compound is considered antimutagenic if the number of His^+ colonies decreases in at least three of the concentrations tested, all of them resulting in AMI values> 1 and a reproducible dose response curve can be obtained.

Statistical analysis

Data were analyzed using Statistical Analysis System software (SAS Institute Inc., Cary, NC 27511, U.S.A. Release 6.02). Values were considered significant when $p< 0.05$.

RESULTS

Cytotoxic effect of sodium selenite on tumor cells

The effect of different concentrations (from 10 to 200 μM) of sodium selenite on colony formation of normal human cells (CCD-18Lu) and human malignant (HeLa, A-204 and HepG2) cells *in vitro* was tested. Results (**Table I**) indicated that sodium selenite had no effect on normal cells but inhibited colony formation of malignant cells in a dose-dependent manner. No significant difference in sensitivity to sodium selenite between tested malignant cells was found.

Antimutagenic activity of sodium selenite

Data reveal (**Table I**) that increasing the amount of sodium selenite per plate up to 672 μg was dose-dependently effective in reducing the mutagenicity of BP. In these experiments 10 μg BP per plate gave the greatest mutagenicity, resulting in 405 revertants per plate. Higher concentration of sodium selenite (672 μg/plate, approx. 44 ppm) induced about 37 % reduction in BP mutagenicity. Previously, it was shown that sodium selenite had no mutagenic effect in the Ames/*Salmonella* test system (Noda *et al.* 1979; Romert *et al.* 1994).

TABLE I. ANTIMUTAGENICITY OF SODIUM SELENITE, ASSAYED IN THE *Salmonella typhimurium* PLATE INCORPORATION TEST, WITH METABOLIC ACTIVATION (S9MIX) AND 10 μG PER PLATE OF BP

Sodium selenite (μg/plate) (mean ± SD)[a]	TA98 his^+ revertants/plate	AMI
Untreated control	45 ± 5.6	-
0	405 ± 16.2	-
173	350 ± 8.3 *	1.1
346	289 ± 22.4*	1.4
519	269 ± 11.7 *	1.5
672	255 ± 9.7 *	1.6

[a] Each value represents the mean ± standard deviation (SD) of triplicate plates
*$p<0.05$

DISCUSSION

The cytotoxic and antimutagenic effects of sodium selenite on human tumor and bacterial cells *in vitro* have been described. First, sodium selenite had a dose-dependent inhibitory effect over colony formation in all the tumor cells tested *in vitro* (**Table II**). Our previous results and data from other laboratories have indicated that selenium compounds differ in their capacity to inhibit cell growth (Abdullaev *et al.* 1992, 1994, 1995, Fico *et al.* 1986, Frenkel *et al.* 1987, 1991). These experiments comparing normal and malignant cells revealed differences in the cellular sensitivity to selenium and suggested that the origin of those differences lies in the intracellular levels of SH-compounds. High intracellular levels of sylfhydryl (SH) compounds in tumor cells can be an important factor contributing to the relative sensitivity of malignant cells to sodium selenite, since SH compounds play an important role in the cytotoxicity of sodium

TABLE II. INHIBITORY EFFECT OF SODIUM SELENITE ON COLONY FORMATION OF TESTED CELLS

Sodium selenite (μM)	HeLa cells(%)	A-204 cells(%)	HepG-2 cells(%)	CCD-18Lu(%)
0	0	0	0	0
10	26 ± 2.6*	17 ± 0.9	9 ± 1.5	0
50	34 ± 1.7*	31 ± 3.7*	34 ± 1.8*	8 ± 3.8
100	42 ± 5.1*	38 ± 4.5*	40 ± 4.7*	12 ± 1.5
200	52 ± 7.2*	53 ± 6.4*	56 ± 3.4*	15 ± 2.9

Cells were exposed to the indicated concentrations of sodium selenite for 1 h.
The number of cells that were able to form colonies was determined as described in Materials and Methods. Results are as percentage of inhibition in treated cells normalized to untreated controls. Each value represents the mean ± S.D. of triplicate plates.
*$p<0.05$

selenite (Abdullaev and Frenkel 1994, Abdullaev and Gonzalez 1995, Shen *et al.* 2002). It was also shown that sodium selenite increased glutathione peroxidase and catalase activities, involved in the detoxification of free radicals (Bronzetti *et al.* 2003).

Second, our results demonstrated that sodium selenite itself reduces the mutagenicity of BP (**Table I**) and that this antimutagenic effect is dependent on the concentration of selenium. BP is a polycyclic aromatic hydrocarbon found in tobacco smoke and various environmental contaminants and has been shown to be carcinogenic to animals and mutagenic to cells in culture (Olson *et al.* 1995).

Since the plate-incorporation reversion test used in our studies does not directly measure toxicity, a decreased reversion frequency could be attributable to a toxic effect of the agents. To examine this possibility, the background growth on the plates was observed under a microscope when the revertant colonies were counted. No obvious toxic effect by sodium selenite at the concentrations tested was noted. Thus, our results reveal that sodium selenite possesses selective cytotoxic as well as antimutagenic properties.

It has been proposed that selenium exerts its anticarcinogenic actions by multiple mechanisms. Besides the selective killing of transformed cells mentioned above, selenium compounds appear to function as antimutagenic agents, preventing the initiation and progression steps in the malignant transformation of normal cells. These preventive effects of selenium may be primarily associated with its presence in glutathione peroxidases, which are known to protect DNA and other cellular components from damage by free radicals (Schauzer 2000). Experimental and clinical findings indicate that selenium-containing drugs should be recommended for potential use in cancer patients (Lush *et al.* 1999, Medina *et al.* 2001).

In conclusion, the present study supports the increasing evidence that selenium compounds may have an important role in cancer treatment as well as in chemoprevention. Additional studies are needed to determine the most effective approach for treatment and doses of these synthetic agents, as well as the molecular mechanism(s) of their anticarcinogenic activities.

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