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In vitro cytogenetic testing of an organoselenium compound and its sulfur analogue in cultured rat bone marrow cells

Jacob H Jacob*, Ahmad M Khalil and Ahmed O Maslat

Address: Department of Biological Sciences, Yarmouk University, Irbid – Jordan

Email: Jacob H Jacob* - jacob_gabi@yahoo.com; Ahmad M Khalil - ahmad200121163@yahoo.com; Ahmed O Maslat - mass7000@yahoo.com

* Corresponding author

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Abstract

Background: Selenium (Se) is a non-metal element, occurring in varying degrees in the environment and it has been found to be a component of several enzymes. Different selenium compounds have been associated with carcinogenicity, toxicity, modification of metal toxicity and prevention of cancer. Organoselenium compounds had substantially greater bioavailability and less toxicity than that of inorganic selenium. From a chemical point of view, Se resembles sulfur (S) in many of its properties, thus, Se and S may be considered to be isosteric. The ability of a synthetic organoselenium compound; cyclopenta-dienyldicarbonyl ironselenoterephthalic acid (CSe) and its sulfur analogue (CS) in the range of 10^{-8} to 10^{-5} M, to induce sister-chormatid exchanges (SCE) and alter cell division expressed as mitotic index (MI) as well as cell survival has been investigated.

Methods: Rat bone marrow cells were cultured in the presence of CSe and CS in the range of 10^{-8} to 10^{-5} M with a total exposure time of 4, 16 or 28 h at 37°C. Fluorescence-plus-Giemsa (FPG) technique was used to visualize chromosomes for SCE analysis and MI determination. Trypan blue exclusion technique was used to determine cell viability.

Results: At the three exposure times, cell survival progressively decreased with increasing concentration, but the effect of either chemical was not significant (ANOVA; $P < 0.05$) as compared to the negative control. Significant reductions in MI were calculated at the highest concentration (10^{-5} M) when either chemical was applied for 16 or 28 h. Furthermore, the mean SCE increased with longer exposure times and, in general, CSe had slightly greater effect on cell survival and caused higher frequencies of SCE than CS. The exception was the 10^{-8} M treatment. However, both CSe and CS failed to induce 2-fold SCE as that of the negative control and therefore they are not considered as mutagens.

Conclusion: Both CSe and CS in the range of 10^{-8} to 10^{-5} M could not double the SCE rate of the negative control and therefore not considered as mutagens at these experimental conditions.

Background

Selenium (Se) is a non-metal element [1], occurring in varying degrees in soil, water, plants and grains. Se has been found to be a component of glutathione peroxidase (GSH-Px) as selenocysteine (SeCys). In addition of many other enzymes, SeCys is also found in the active site of two mammalian enzymes, phospholipids hydroxide GSH-Px, and 5-deiodinase [2].

From a chemical point of view, Se resembles sulfur (S) in many of its properties [3]. Se and S may be considered to be isosteric, as originally defined by Langmuir,1919 [4].

The biological and pharmaceutical activities of different selenium compounds are of special interest because it has been associated with carcinogenicity [2], toxicity [5], modification of metal toxicity [6] and with prevention of cancer [7]. In the same field of research, the results of many studies [8-11] have related Se deficient bioavailability and intake to the human cancer mortality.

Organoselenium compounds had substantially greater bioavailability than that of inorganic selenium [12]. More importantly, organic selenium is usually found to be less toxic than inorganic forms of the element [13-16]. New synthetic selenium compounds may provide a way to minimize toxicity associated with higher selenium intake.

Recently, several forms of organoselenium have been studied for their cancer preventive activities. The dietary P-methoxybenzeneselenol, a synthetic organoselenium compound was found to inhibit azoxymethane-induced hepatocarcinogenesis in female F 344 rats without clinical signs of toxicity [17]. Furthermore, Jibril *et al.* [18] cited that El-Bayoumy in 1997 found that two newly synthesized selenium compounds; P-methoxybenzyl selenocyanate and 1, 4-phenylenebis (methylene) selenocyanate prevented both precancerous cell growth and tumor growth in animals after being treated with a colorectal cancer-inducing agent with no side effects. Moreover, the latter authors also cited that Mag *et al*. (1997) confirmed antihypertensive properties of some patented phenylaminoalkyl selenides.

These compounds lowered the blood pressure and increased blood velocity without increasing heart rate in experimental animals. More recently, Jibril and his group [18-20] have succeeded in preparing and characterizing a new class of organotransition metal S-and Se- bonded thiocarboxylate complexes. From a pharmaceutical point of view it seemed interesting to examine and compare the cytogenetic effects of cyclopentadienlydicarbonly ironselenoterephthalic acid (CSe) and its sulfur analogue (CS) [Figure 1] in cultured rat bone marrow cells. The cytogenetic endpoints investigated were SCE, mitotic index (MI) and cell survival. It should be indicated that these two compounds have been found to have antibacterial and antifungal activities. In addition, they were proved to be nonmutagenic in *Salmonella* Ames test [20]. However, in the literature, there are no data of chemopreventive effects with the two experimental compounds.

Methods

Chemicals

Cyclopentadienyldicarbonyl ironselenoterephthalic acid and cyclopentadienylicarbonyl ironthioterephthalic acid were synthesized and characterized previously [20]. Mitomycin C (MMC) was purchased from Janssen Chemica (Geel; Belgium).

Animals

Male and female Sprague-Dawely rats (10–12 week-old) were obtained from the animal house unit at Yarmouk University, Irbid – Jordan.

Preparation of bone marrow cells

Bone marrow cells were obtained according to Krishna *et al* [21] with slight modifications. Briefly, rats were lightly etherized to keep the cells in healthy conditions, then tibia and femora were isolated and washed in sterile Hanks Balanced Salt Solution (HBSS) followed by 70% ethanol. The dissected animals were completely etherized and properly discarded. Soft tissues and the ends of the bones were cut away and the cells were flushed using a syringe with HBSS. After centrifugation at 1000 × g for 10 min, cells were resuspended in an appropriate volume of fetal calf serum (FCS, PAA Laboratory, GmbH; Austria).

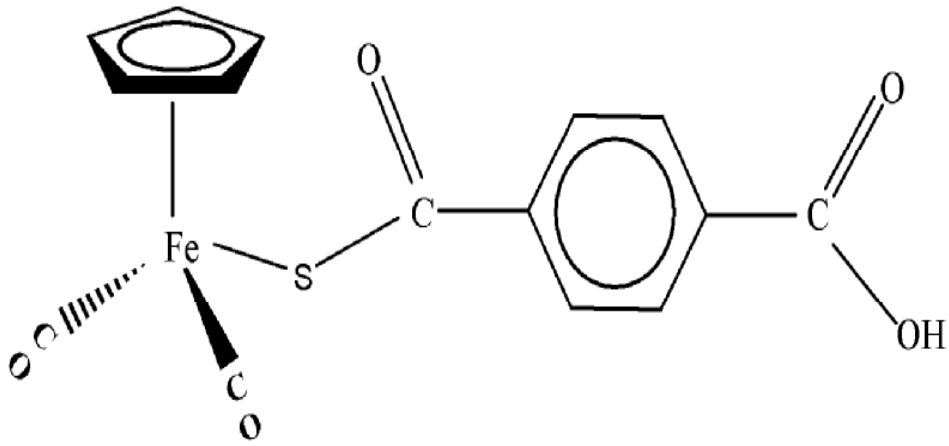
Cell cultures

Prior to establishing the cell cultures, the cells were washed and mixed evenly in fresh medium and counted in a hemocytometer to calculate the cell viability as described below.

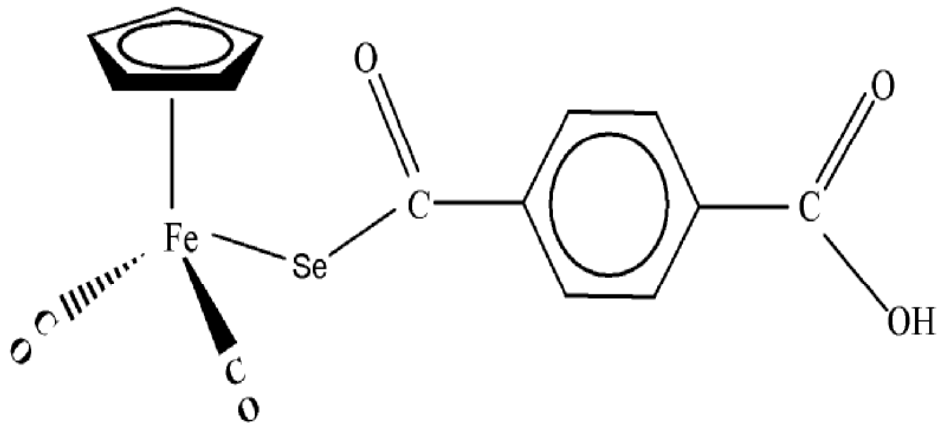
A standard bone marrow cell culture was employed through this study. About 2.0×10^6 cells / ml were placed in a final volume of 10 ml culture medium in 25 Cm^2 flasks. The medium consisted of RPMI 1640 buffered with 25 mM HEPES and supplemented with 15% FCS, 100 IU penicillin and 150 μg/ml streptomycin. Bromodeoxyuridine (ACROS Organics; USA) was added at the initiation time at a final concentration of 10 μg/ml. The test chemicals, dissolved in dimethyl sulfoxide (DMSO), were individually added at zero, 12 or 24 h of the incubation period (28 h) at 37°C at the following concentrations: 1.0×10^{-5}, 1.0×10^{-6}, 1.0×10^{-7} and 1.0×10^{-8} M. These doses were selected on the basis of solubility of the test chemicals and the toxicity of the solvent. This protocol gave a total exposure time of 28, 16 or 4 h, respectively. Mitomycin C (5.0×10^{-8} M) and DMSO (maximum volume 1%) were included as positive and negative controls, respectively. Four cultures were made for each treatment. The used DMSO concentration was based on previous experiments in our laboratory [22] and work by others [23,24].

Cell harvesting

Cells were harvested as reported previously [25].



Cyclopentadienyldicarbonyl ironthioterephthalic acid (CS).



Cyclopentadienyldicarbonyl ironselenoterephthalic acid (CSe).

Figure-1.

Figure 1
Cyclopentadienyldicarbonyl ironthioterephthalic acid (CS) and Cyclopentadienyldicarbonyl ironselenoterephthalic acid (CSe).

Table 1: The cell survival (%), in cultured rat bone marrow cells treated with either CS or CSe for 4, 16 and 28 hours.

	Concentration	Cell Survival (%)		
	(Molar)	4 hr.	16 hr.	28 hr.
Treatment		mean ± S.D.	mean ± S.D.	mean ± S.D.
MMC[a]	5.0×10^{-8}	81.73 ± 4.58	79.17 ± 2.49	70.24 ± 3.51
	1.0×10^{-5}	92.84 ± 1.95	92.84 ± 2.06	87.49 ± 5.12
	1.0×10^{-6}	94.34 ± 1.95	92.22 ± 2.1	88.44 ± 2.2
	1.0×10^{-7}	96.33 ± 3.09	92.97 ± 1.74	91.75 ± 3.16
CS	1.0×10^{-8}	98.19 ± 1.65	96.86 ± 0.35	94.86 ± 1.51
	1.0×10^{-5}	91.88 ± 3.38	88.6 ± 3.33	85.34 ± 3.87
	1.0×10^{-6}	93.08 ± 3.09	90.75 ± 2.05	87.19 ± 1.74
	1.0×10^{-7}	98.01 ± 0.80	92.27 ± 2.78	90.97 ± 2.85
Cse	1.0×10^{-8}	98.99 ± 1.10	96.41 ± 1.54	95.72 ± 0.38
DMSO[b]	10 μl	98.09 ± 1.29	94.76 ± 1.98	96.12 ± 1.47

[a] positive control. [b] negative control.

Cell survival

The harvested cells in each culture flask were mixed evenly as before and the cell viability was determined by the Trypan blue exclusion technique. The results were expressed as percentage (Table 1).

SCE frequencies

For every treatment, chromosomes were prepared, stained and analyzed for SCE as reported before [25]. The number of SCE/Cell was determined in 20–30 well differentiated second metaphase cells.

Mitotic index

In the same preparation used for SCE determination, the MI was calculated for each treatment by screening of 2000 cells from each culture and finding the proportion of mitotic nuclei.

Results

Cell survival in the CSe- and CS-treated cultures progressively decreased with increasing concentration of both chemicals, at the three exposure times (Table 1). Statistical analysis using ANOVA ($P < 0.05$) and Dunkan's multiple range test revealed no significant differences between means of cell survival and those of the negative control. The general trend was that, with the exception of the 10^{-8} M treatment, CSe had slightly greater, but still not significant, effects on cell survival (Table 1).

Comparison of the CSe and its sulfur analogue shows an overall phenomenon that is CS delayed cell division to greater extent than its selenium counterpart. However, the differences in the MI values were not statistically significant (Figures 2, 3). Interestingly, treatment of cultures with either of the test chemical for 16 h resulted in lower, but not significant, MI than those calculated in the shorter (4 h) and the longer (28 h) exposure times.

Unfortunately, the incubation period was not long enough for the cells to undergo three division cycles. Therefore, we have not encountered adequate number of cells that had divided three or more times (M3+) in culture to allow calculation of the replication index without bias.

In respect to SCE, The analysis of the data showed that the SCE frequencies were normally distributed. Thus, there was no need for either square rootor log transformations of the original data. Furthermore, the inter-culture variations within each treatment were not significant. Both chemicals under study applied for 4 h failed to produce significant differences over the negative control. But, at every concentration used, the longer was the period during which cells were incubated in the presence of either CS or CSe, the higher were the SCE levels. About 2-fold increases were seen, in case of CS, when the longest and shortest exposure durations were compared (Figure 4). Only the highest dose of CSe (10^{-5} M) for 16 or 28 h nearly doubled the SCE values recorded in the negative control (10.5 SCE / Cell versus 6.00 to 6.75 SCE/Cell), respectively. Finally, with exception of the 10^{-8} M treatment), CSe caused higher rates of SCE (Figures 4, 5).

Discussion

Reinforced by the recent discoveries on biochemical and pharmacological properties of some organoselenium compounds we have carried out the present research. To our knowledge, no work has been published on the

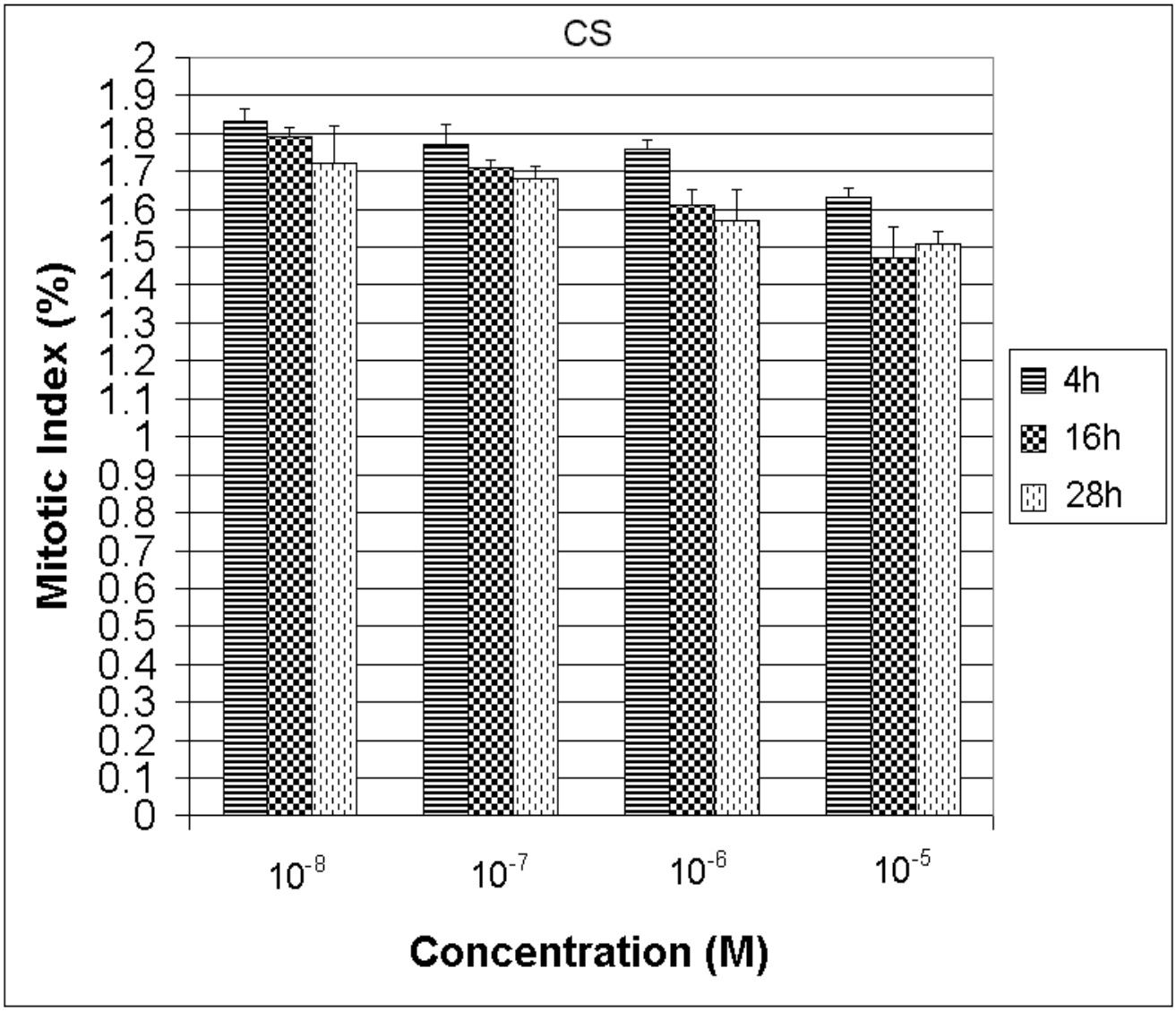


Figure 2
Mitotic indices (%) in cultured rat bone marrow cells exposed to different concentrations of CS for three different exposure times, 4 h, 16 h and 28 h.

biological activities of the two cyclopentadienydicarbonyl iron derivatives used in this study except one [20].

The culture of bone marrow cells is widely accepted as an *in vitro* system having an inherently dividing cell population without the need for mitogenic induction. It is also known that the arrangement of cells in the bone marrow is so disorderly and the cells intermingle with one another [26]. However, it can be assumed that most of the cells examined were the lymphoid and the myeloid progenitor cells which are characterized by their rounded or oval shapes.

As expected, the positive control (MMC, 5.0×10^{-8} M) induced 20.25 and 22.75 SCE/Cell when included in the culture medium for 4 and 28 h, respectively. These values are consistent with those reported previously [25] where under similar experimental conditions 18.4 to 26.7 SCE were observed. This confirms the reproducibility of the experimental protocol.

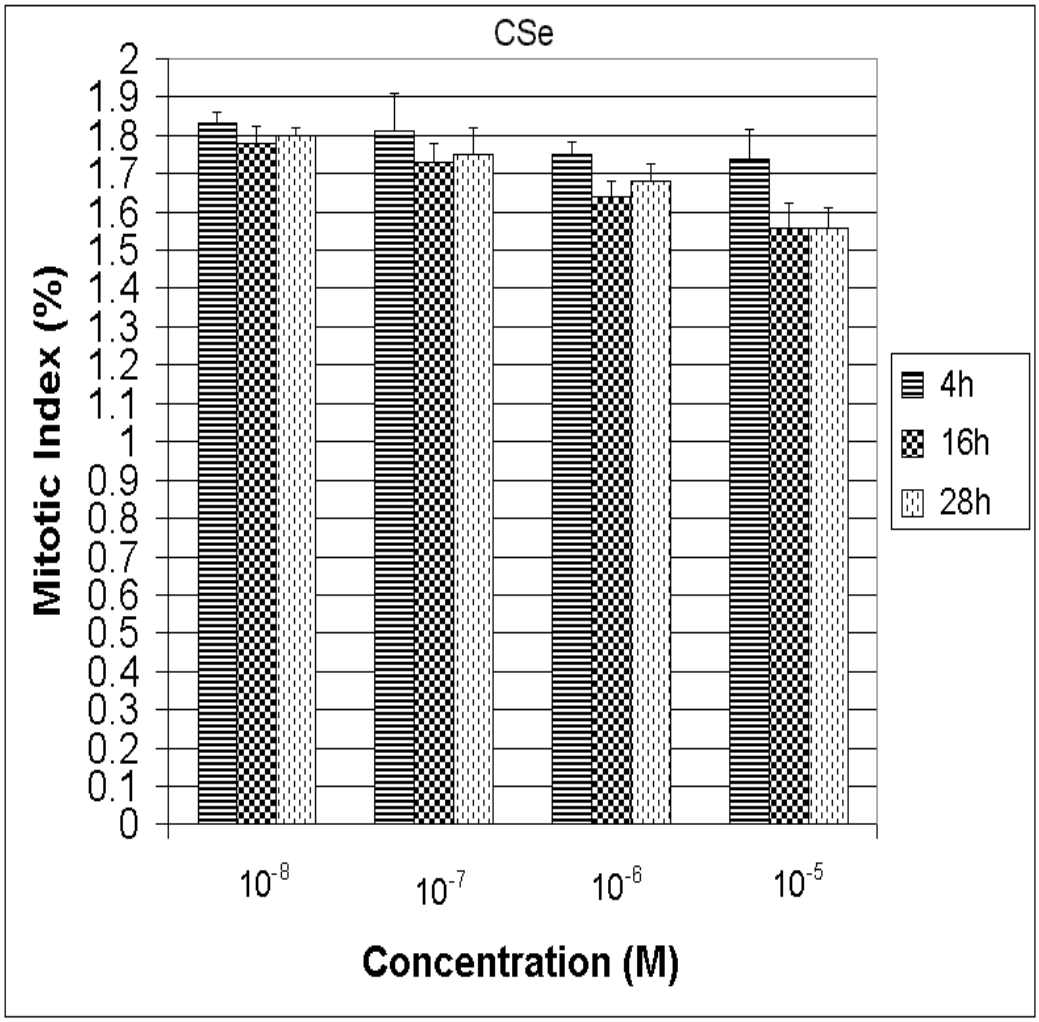


Figure 3
Mitotic indices (%) in cultured rat bone marrow cells exposed to different concentrations of CSe for three different exposure times, 4 h, 16 h and 28 h.

Our data reflect to certain extent the higher reactivity of organoselenium compounds over their sulfur counterparts reported by Odom [1]. Generally speaking, CSe had slightly greater effect on cell survival (Table 1) and with the exception of the 10^{-8} M treatment, it caused more SCE counts (Figures 4, 5) than its sulfur analogue (CS). In contrast, CS seems to have greater abilities to stop cell division as indicated by MI (Figures 2, 3). However, not all these differences were statistically significant. These findings are also in agreement with the results reported in literature [20] that selenium-containing derivatives were generally more potent as antimicrobial agents than their sulfur-containing ones.

The results presented here indicated an inverse relationship between reductions in mitotic activity, and cell viability on one hand and the elevation of SCE rates on the other. Such relations were reported in Chinese hamster ovary cells [27], human lymphocytes [22] and rat bone marrow cells [25].

One major conclusion from the present work is that both CSe and CS are either not mutagens or weakly mutagens. According to the GENE-TOX report [28] and the guidelines of UKEMS [29], an agent is judged as positive SCE-inducer when eitherit causes an SCE frequency at least 2-fold over the baseline level or demonstrates a 3-point

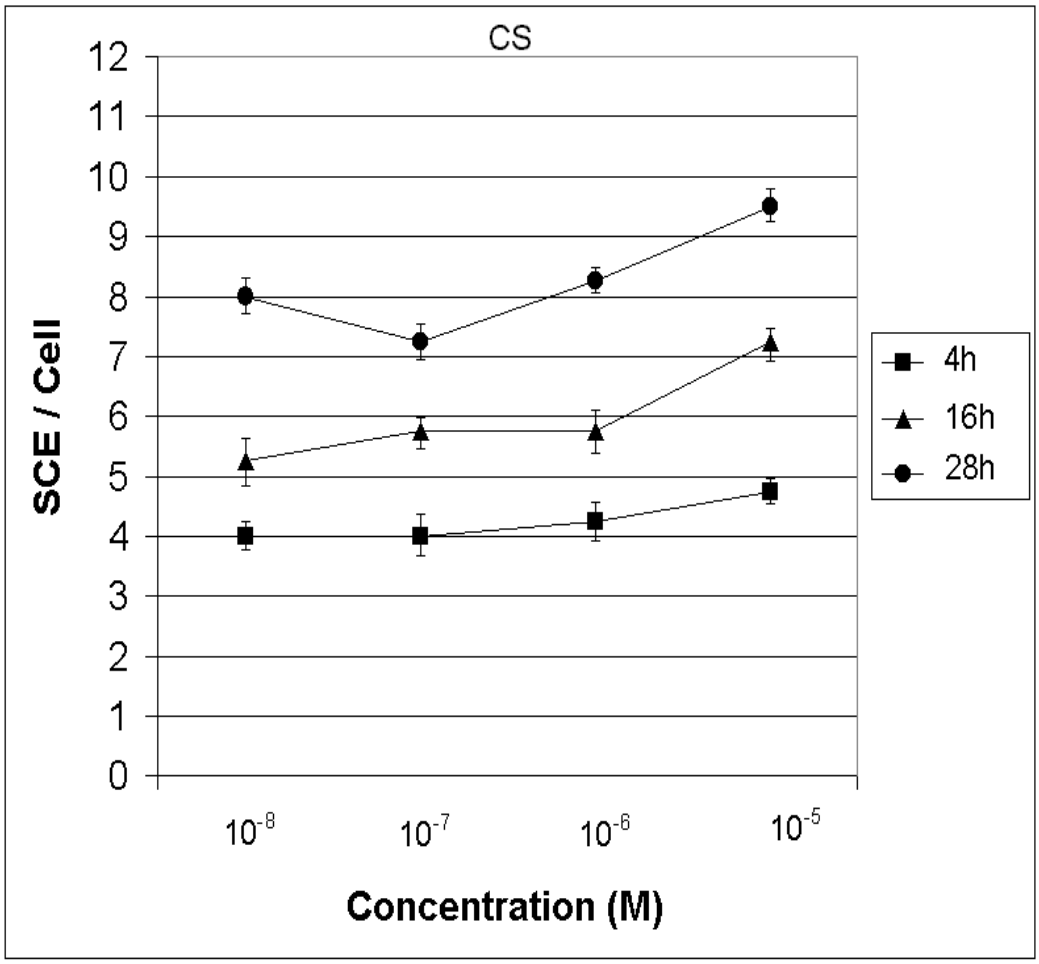


Figure 4
Frequency of SCE/cell in cultured rat bone marrow cells treated different concentrations of CS for three different exposure times, 4 h, 16 h and 28 h.

dose-response curve showing a progressive increase over baseline SCE frequency.

The two compounds under investigation were shown to be non-mutagenic in *Salmonella typhimurium* [20]. In view of this, CSe looks safer than already tested organoselenium compounds [22,30] as well asselenopuridine and selenocystine [15] which had obvious cytogenetic effects oncultured human lymphocytes.

It has been reported [22] that *in vitro* exposure of human lymphocytes to a dimer of Pmethoxybenzeneselenol and benzylselenocyanate caused 3-fold increase in sister-chromatid exchanges and 2 to 3-fold increase in the incidence of chromosome aberrations (CA). The two compounds led to inhibition of cell proliferation. In *Salmonella typhimurium*, the above two compounds were founds to be base-pair substitution mutagens using TA100 strain [30].

Conclusions

CSe has safer cytogenetic effects than already tested organoselenium compounds. Thus, the outcomes of the present work are not merely of theoretical interest, they also have practical significance because by the use of suitable substituents in organoselenium and organouslfur compounds it might be possible to obtain drugs with certain desired features. Clearly, further studies on the two compounds and other novel ones, used separately or in combination in different systems, are warranted.

List of abbreviations

CA: Chromosome aberrations

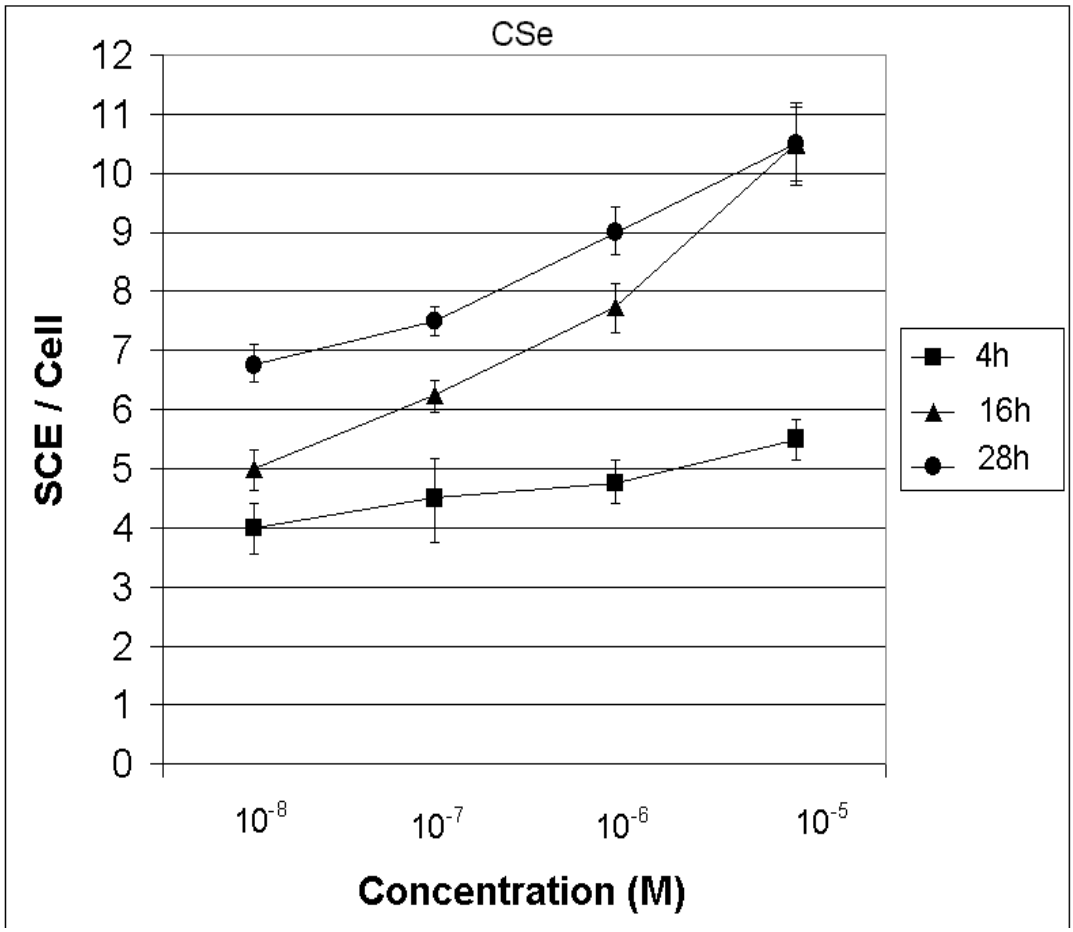


Figure 5
Frequency of SCE/cell in cultured rat bone marrow cells treated different concentrations of CSe for three different exposure times, 4 h, 16 h and 28 h.

CS: Cyclopentadienyldicarbonylironthioterephthalic acid

CSe: Cyclopentadienyldicarbonylironselenoterephthalic acid

DMSO: Dimethyl sulfoxide

FCS: Fetal calf serum

GSH-Px: Glutathione peroxidase

HBSS: Hanks balanced salt solution

MI: Mitotic index

MMC: Mitomycin C

SCE: Sister-chromatid exchanges

SeCys: Selenocysteine

UKEMES: United Kngdom Environmental Mutagen Society

Authors' contributions
The authors contributed equally to this work.

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