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Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines

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Abstract

Selenium supplementation has been shown for many years to work as an anticarcinogenic agent both in epidemiology and in in vitro studies. Selenium supplementation has recently been shown to decrease total cancer incidence. However, the mechanism of action of selenium as an anticarcinogenic agent has yet to be elucidated. Selenomethionine was the predominant form of selenium in the dietary supplement in the study by Clark et al. (Clark, L.C., Combs, G.F., Turnbull, W.B., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Glover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Lesher, J.L., Park, H.K., Sanders, B.B., Smith, C.L., Taylor, J.R. and The Nutritional Prevention of Cancer Study Group (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial. J. Am. Med. Assoc., 276 (24), 1957–1963) and therefore we evaluated the growth inhibitory effects of selenomethionine against human tumor cells. Selenomethionine was tested against each of three human tumor cell lines (MCF-7/S breast carcinoma, DU-145 prostate cancer cells and UACC-375 melanoma) and against normal human diploid fibroblasts. All cell lines demonstrated a dose-dependent manner of growth inhibition by selenomethionine. Selenomethionine inhibited the growth of all of the human tumor cell lines in the micromolar (μM) range (ranging from 45 to 130 μM) while growth inhibition of normal diploid fibroblasts required 1 mM selenomethionine, approximately 1000-fold higher than for the cancer cell lines. In short, normal diploid fibroblasts were less sensitive than the cancer cell lines to the growth inhibitory effects of selenomethionine. Furthermore, we show that selenomethionine administration to these cancer cell lines results in apoptotic cell death and aberrant mitoses. These results demonstrate the differential sensitivity of tumor cells and normal cells to selenomethionine. Published by Elsevier Science Ireland Ltd.

Keywords: Cancer chemoprevention; Selenomethionine; Growth inhibitory activity; Human tumor cell lines; Apoptosis

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1. Introduction

Since the 1960s, it has been suggested from geographical studies that there is an inverse relationship

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between environmental selenium (Se) levels and cancer incidence [1,2]. In 1991, Clark et al. [3] reported that cancer mortality rates were significantly lower in intermediate-selenium and high-selenium countries in comparison to low-selenium countries for total cancer and cancers of the lung, colon, rectum, bladder, esophagus, pancreas, breast, ovary and cervix.

A 10-year controlled clinical trial to test the efficacy of Se in preventing skin cancer, the Nutritional Prevention of Cancer with Selenium Trial, was recently completed. In addition to the primary endpoint of new non-melanoma skin cancers, the trial endpoints included the incidence of melanoma, lung, colorectal and prostate cancer and all-cause mortality. The study found Se supplementation to be associated with statistically significant reductions in several additional endpoints (total and lung cancer mortality, total cancer incidence and colorectal cancer and prostate cancer incidence), but not the primary endpoints of incidence of new basal and squamous cell carcinomas of the skin [4].

Since selenium (selenomethionine) had no effect on non-melanoma skin cancers, but did have an effect on carcinomas of the lung, colon and prostate, it is important to try to understand the mechanism of action of Se as a cancer prevention agent. Since the intervention agent, high Se Brewer's yeast, is predominately selenomethionine, the goal of this set of experiments was to gain insight into the mechanism of action of selenomethionine as an anticarcinogenic agent. As a first step toward elucidation of the mechanisms important for the anticancer activity of selenomethionine, we evaluated the growth inhibitory effects of selenomethionine against MCF-7/S breast carcinoma cells, UACC-375 melanoma cells, DU-145 prostate cancer cells and normal diploid fibroblasts. Our first objective was to determine if selenomethionine inhibited the growth of these aforementioned cell lines in a dose-dependent manner. Our second objective was to determine the inhibitory concentration at 50% growth inhibition (IC_{50}) for selenomethionine against each of the human tumor cell lines and normal diploid fibroblasts. The third objective of this study was to determine the effects of selenomethionine on cell morphology.

2. Materials and methods

2.1. Materials

The human prostate cancer DU-145 and human breast carcinoma MCF-7/S were obtained from ATCC (Rockville, MD). Normal diploid fibroblasts and the human melanoma cell line UACC-375 were obtained from the Arizona Cancer Tissue Culture Core. RPMI 1640 was purchased from ICN Biomedicals (Costal Mesa, CA). Fetal bovine serum was from Gemini Bioproducts (Calabasas, CA). L-Glutamine, penicillin, streptomycin and trypsin were purchased from Gibco (Grand Island, NY). Sulforhodamine B dye, D,L-selenomethionine, was from Sigma (St. Louis, MO).

2.2. Cell lines

The tumor cell lines were maintained in monolayer culture in RPMI 1640 media supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 50 IU/ml penicillin and 50 mg/ml streptomycin in a humidified incubator with 95% air and 5% CO_2 at 37°C. Subculturing was done at subconfluent densities. The cells were dispersed with a phosphate-buffered saline solution of 0.25% trypsin.

2.3. Sulforhodamine B (SRB) assay

Details on cell growth determination using the sulforhodamine B (SRB) colorimetric protein stain assay have been described previously [5]. The optimum cell number per well was determined for each cell line over a 7-day period. Ninety-six-well microtiter plates were used for plating the cell lines and the cells were subsequently incubated for 24 h prior to the addition of compounds to be tested. The D,L-selenomethionine was solubilized in distilled Millipore water and at higher concentrations in 0.0073 M HCl. The vehicle (0.0073 HCl) at this concentration was non-toxic and had no effects on proliferation in this series of experiments. Selenomethionine was tested over a concentration range of six- to eight-fold dilutions. The media controls and each of the compounds were tested in a minimum of six wells per experiment. After an additional 6 days of culture, viable cells were fixed to the bottom of each well with cold 50% trichloroacetic

acid (TCA) at a final concentration of 10%. The plates were kept at 4°C for 1 h, the supernatant was then aspirated and the plates were washed with deionized water. SRB solution was prepared to 0.4% (w/v) in 1% acetic acid. SRB (50 ml) was added to each well and the cells were stained for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid followed by air drying. Bound stain was solubilized with 50 mM unbuffered Tris and optical density (OD) was measured by an automated spectrophotometer (Biomek 1000, Beckman, Fullerton, CA) at a single wavelength of 540 nm.

2.4. Determination of IC_{50} values

For each compound, a minimum of three experiments measuring the cell growth inhibition of all the cell lines was completed. The percentage cell survival was determined by taking the optical density of each treated cell row and dividing it by the optical density of the media- or vehicle-treated cells in the same 96-well plate. The inhibitory effect of the compound at each concentration was expressed as a percentage, i.e. (mean OD of treated cells/mean OD of control or media-treated cells) $\times$ 100. Each concentration of compound was then plotted against the inhibitory effect as a percentage using an Excel 5.0 program. In this manner, a dose–response curve was generated and the IC_{50}, the compound concentration which caused a 50% reduction in the mean OD value relative to the control, was estimated by interpolation from the inhibitory effects measured at each concentration tested as previously described [5,6].

2.5. Microscopy

The tumor cells were treated with media alone or with selenomethionine. The cells were collected by centrifugation and resuspended in PBS and four drops containing approximately 1×10^6 cells were subjected to cytospin for 2 min as previously described [6]. The cells that were deposited on slides by this procedure were next fixed in methanol for 30 s, stained with Wright–Giemsa stain (Diff-Quick Kit, Baxter Scientific Products, McGraw Park, IL) and then rinsed and air-dried. Apoptotic cells were determined using bright field microscopy at 100× under oil immersion, using the criteria of condensed chromatin, dark staining cytoplasm, cell shrinkage, nuclear fragmentation, cytoplasmic vacuolization and apoptotic body formation as previously described [7].

2.6. Statistical methods

The common log of both percent survival and dose was calculated. The log of survival was plotted against that of dose. On the log–log scale, survival decreased linearly. A linear regression was done using the log-transformed data beginning with an observable decrease in survival. The slopes of these lines were compared between the four groups using a one-way analysis of variance. If there was a significant difference, Tukey's test was used to determine which pairs of groups differed. The estimated dose where survival begins to drop below 100% was calculated using the regression equation as a measure of the minimum dose at which growth inhibition occurs. This estimated dose was also calculated among the four groups using a one-way analysis of variance.

3. Results

3.1. The effects of selenomethionine administration on tumor cell growth

We first investigated the ability of selenomethionine to inhibit MCF-7/S breast carcinoma cell growth by the SRB assay. We found that selenomethionine inhibited tumor cell growth in a dose-dependent manner. In the MCF-7/S breast carcinoma cell line, selenomethionine at concentrations of 10^{-8}–10^{-5} M did not affect cell viability. However, selenomethionine administration at concentrations of 10^{-4}–10^{-3} M markedly inhibited cell growth (Fig. 1A).

In the melanoma cell line UACC-375, selenomethionine also inhibited tumor cell growth in a dose-dependent manner. No effect on cell growth was seen at low doses (i.e. 10^{-8} and 10^{-6} M). However, beyond this concentration, selenomethionine inhibited melanoma cell growth (Fig. 1B).

In the prostate cancer cell line DU-145, selenomethionine at concentrations of 10^{-7}–10^{-5} M did not inhibit growth. Beyond this concentration, there was marked inhibition of growth with a rapid decline in cell numbers associated with an IC_{50} between 10^{-5} and

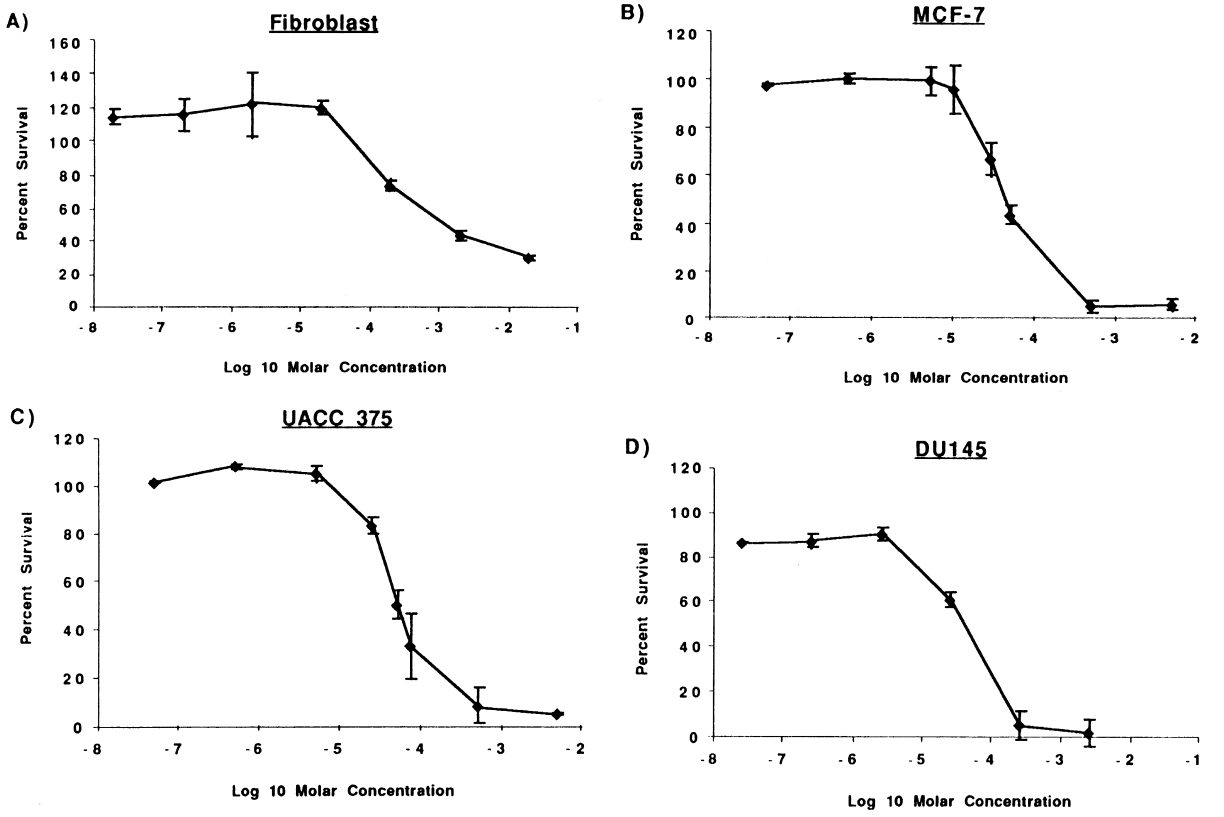

Fig. 1. The effects of D,L-selenomethionine administration on the growth of normal diploid fibroblasts (A), MCF-7 breast cancer cells (B), UACC-375 melanoma cells (C) and DU-145 prostate cells (D).

10^{-4} M. At doses greater than 10^{-4} M, growth was 4% of control and complete cell death occurs at the 10^{-2} M level. Similar effects on the lung cancer cell line A-549 and the colon cancer cell line HT-29 were also seen [6].

Interestingly, the growth of normal diploid fibroblast cells was not as markedly affected as tumor cells by selenomethionine. While micromolar concentrations of selenomethionine inhibited the growth of the tumor cell lines tested, selenomethionine in millimolar concentrations was necessary to inhibit normal fibroblast cell growth. For fibroblast growth to be inhibited by selenomethionine, concentrations of 10^{-4} M and higher were required (Fig. 1D).

A comparison of the IC_{50} values represents a means of determining the potency of a given agent in inhibiting cell growth. The lower the IC_{50} value, the more potent is the test agent as an inhibitor of tumor cell growth. Table 1 shows a summary of all the IC_{50} values for selenomethionine against the tumor cell lines and normal diploid fibroblast cells. The prostate tumor cell line and breast cancer cell line MCF-7/S appeared to be the most sensitive with IC_{50} values of 40 and 45 μM, respectively. The growth of UACC-

Table 1
Growth inhibition of human tumor cell lines and normal diploid fibroblasts by D,L-selenomethionine[a]

Cell line	Concentration (IC_{50})[b]
MCF-7/S (breast cancer)	45 μM
UACC-375 (melanoma)	50 μM
HT-29 (colon cancer)	130 μM[c]
DU-145 (prostate cancer)	40 μM
A-549 (lung cancer)	65 μM[c]
Fibroblasts (normal diploid)	1 mM

[a]7-day exposure measured with sulforhodamine B assay.
[b]IC_{50} values were derived from triplicate experiments.
[c]From Redman et al. [6].

Table 2

Comparison of the slopes of the dose–response curves for human tumor cell lines and normal diploid fibroblasts treated with D,L-selenomethionine[a]

Cell line	*N*	Mean (log)	SD (log)	Mean
Fibroblasts[b]	3	–0.20	0.032	0.63
UACC-375	3	–0.54	0.019	0.29
MCF-7/S	3	–0.55	0.096	0.28
DU-145	3	–0.61	0.092	0.25

[a]A linear regression was done using the log-transformed data beginning with an observable decrease in survival. The slopes of these lines were compared between the four groups using a one-way analysis of variance (ANOVA).
[b]Significantly different from the tumor cell lines using ANOVA and Tukey's test ($P = 0.0004$).

375 human melanoma and A-549 human lung tumor cells was reduced to 50% by concentrations of 50 and 65 μM, respectively. A higher concentration of 130 μM was required to cause 50% growth inhibition in the human colon cancer cell line HT-29. Finally, normal human fibroblasts were the least affected by selenomethionine. The inhibitory concentration of selenomethionine for 50% cells for normal diploid fibroblasts was 1.3 mM.

The slopes of the dose–response curves among the four different groups was compared using one-way analysis of variance (ANOVA). The slopes of the regression lines significantly differed among the groups ($P = 0.0004$). Tukey's test indicated that the slope for the normal fibroblast cells significantly differed from the tumor cell lines. The descriptive statistics for the slope for each of the cell lines are shown in Table 2. The mean slope for the fibroblast cells is less steep than for the other three cell lines, indicating that the growth inhibition was less severe in the fibroblast cells. There was no significant difference between the groups on the estimated dose where survival begins to drop below 100% (data not shown).

3.2. The effects of selenomethionine on tumor cell morphology

Next we evaluated the morphology of cells treated with a growth inhibitory concentration of selenomethionine. Morphological studies clearly showed that all three of the cancer cell lines treated with selenomethionine induced apoptosis at concentrations that inhibited 50% growth. The apoptotic characteristics of chromatin condensation, decreased nuclear to cytoplasmic ratio and vacuolization were obvious in selenomethionine-treated tumor cell lines (Fig. 2). TUNEL labeling followed by confocal microscopy confirmed the light microscopy findings (data not shown). Confocal microscopy clearly showed the characteristic apoptotic body formation and fluorescently-labeled DNA strand breaks in the apoptotic cell resulting from selenomethionine administration. In addition to apoptosis, selenomethionine treatment also caused aberrant metaphase cells. These cells were typically larger than control cells and the chromosomes were scattered within the cytoplasm (Fig. 1B).

4. Discussion

This study demonstrates that selenium (selenomethionine) exerts antiproliferative activity against several different solid tumor cells in vitro. In the present study, the IC_{50} for selenomethionine ranged from 40 to 130 μM. Other researchers have found similar concentrations of selenomethionine to inhibit growth of other cell lines. Porter et al. [8] in 1984 found that 130 μM selenomethionine inhibited 50% of the growth of murine L1210 leukemia cells using a 48 h viability assay (different from the SRB assay we utilized in these studies). Batist et al. [9] also studied selenomethionine-induced cytotoxicity in the human promyelocytic leukemia cell line HL-60. Of all the selenium compounds they studied in this cell line, selenomethionine was found to be the least cytotoxic. However, doses of selenomethionine up to 100 μM did not inhibit the growth of the HL-60 cells significantly. This can most likely be explained by the short 24-h cytotoxicity assay and possibly the type of viability tests used. Lastly, Kajander et al. [10] tested D,L-selenomethionine in cytotoxicity assays using R1.1, WiL2, CHO and HePG2 cells. They found that in these aforementioned cell lines, selenomethionine was cytotoxic to the cells in the 60–320 μM range for 50% growth inhibition.

The doses used in the present study are slightly higher than the blood levels noted in individuals receiving dietary selenium supplements. However, it

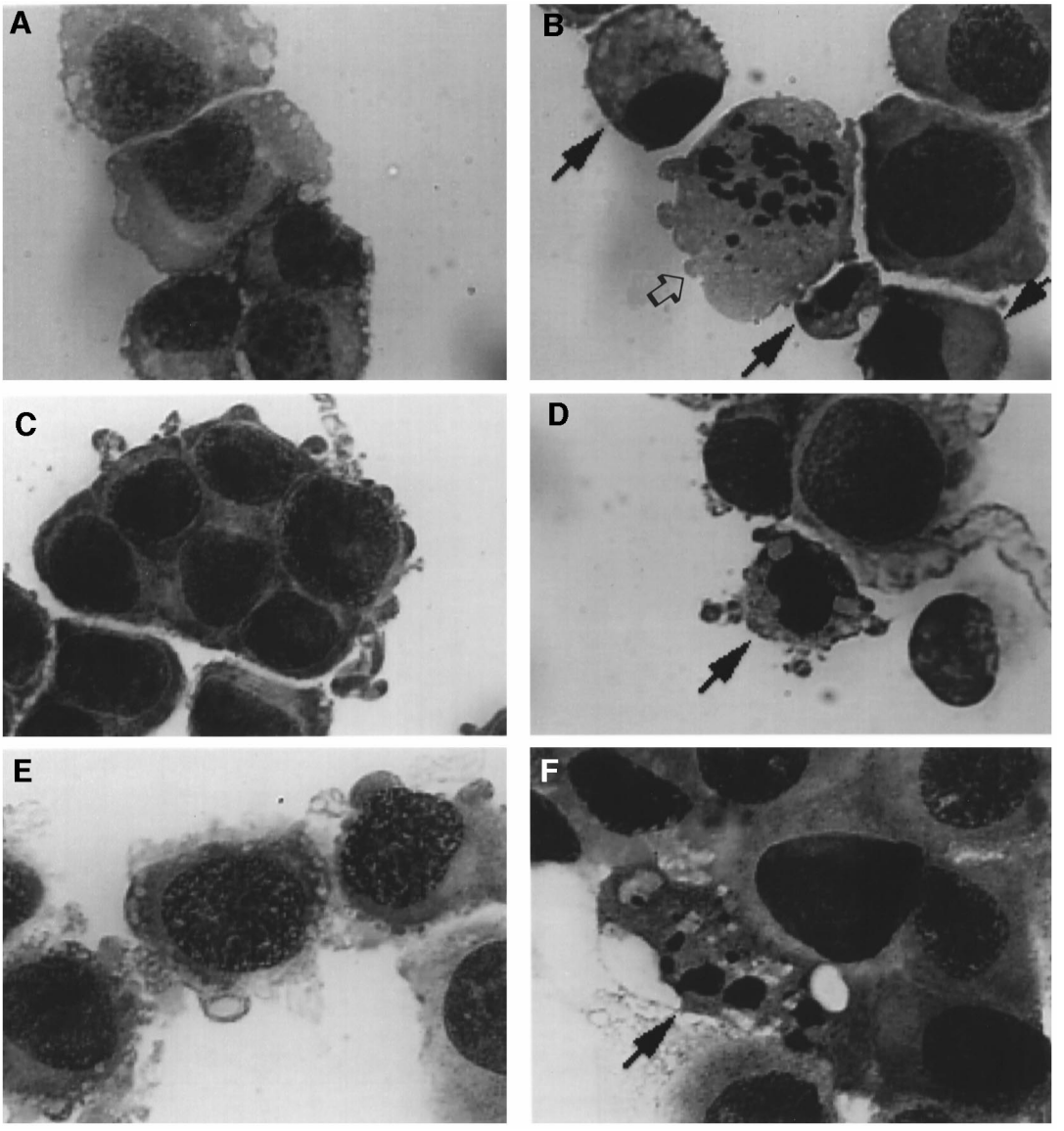

Fig. 2. The effects of D,L-selenomethionine administration on the morphology of tumor cell lines. Tumor cell lines were treated with selenomethionine (at the IC_{50} value for each cell line, see) and cell morphology was analyzed by light microscopy at 100 × . (A) Vehicle control DU-145 prostate cells. (B) Selenomethionine-treated DU-145 prostate cells. (C) Vehicle control MCF-7 breast cancer cells. (D) Selenomethionine-treated MCF-7 breast cancer cells. (E) Vehicle control UACC-375 melanoma cells. (F) Selenomethionine-treated UACC-375 melanoma cells. The solid arrow points to apoptotic cells induced by selenomethionine administration. The open arrow points to an aberrant metaphase cell induced by selenomethionine treatment.

is difficult to make comparisons between in vitro studies and human intervention (in vivo) studies for two reasons: (1) no human studies to date have measured tissue selenium levels in patients receiving selenium supplementation; and (2) it is important to understand that plasma selenium levels may not necessarily reflect tissue selenium levels. This is due to the fact that selenomethionine is incorporated into selenoproteins in tissues and therefore one may find higher levels of selenium in tissues than in plasma. Positive

findings from cancer chemoprevention trials reinforce the need to continue human Se kinetic and metabolism studies.

The mechanism by which selenomethionine exerts its antiproliferative effects against cancer cells is not totally clear. However, morphological data from the present study indicate that the induction of apoptosis would appear to be a plausible hypothesis. In addition, recent data from our laboratory group demonstrated an association between apoptosis and cell cycle changes and perturbations in polyamine metabolism [6]. Polyamines are mediators of numerous biological responses [11] and are important in neoplastic cell transformation [12].

As part of understanding how selenomethionine may exert its cancer chemopreventive effects, we need to know the effects of the drug on a normal cell type. Since normal melanocytes, normal prostate epithelial cells, normal colon epithelial cells, etc. are not easy to grow and maintain, we begin to examine this issue with normal diploid fibroblasts. However, it is important to note that the slopes of the dose–response curves of the tumor cell lines and the normal diploid fibroblasts were significantly different. The slope of the fibroblast dose–response curve was not as steep as the slope of the tumor cell line dose–response curve. This implies that the mechanism of growth inhibition by selenomethionine between normal cells and tumor cells might be different. The metabolism of selenomethionine, unlike that of selenite, does not involve interaction with glutathione. Selenomethionine is converted via the trans-sulfuration mechanism to selenocysteine, which in turn is hydrolyzed to hydrogen selenide by the enzyme selenocysteine lyase [13]. Thus, uptake and metabolism of selenomethionine to cancer preventive metabolites might be central to the difference in the sensitivity of normal fibroblast cells and tumor cells. Support for this hypothesis comes from the studies of Hwang and Milner [14], who showed that retention and distribution of Se are determinants of the degree of cellular growth inhibition caused by this trace element. The difference in the sensitivity between normal cells and tumor cells in the present study will require further investigation. In any case, further studies in normal cells from other tissues are in progress.

The mechanism of formation of the aberrant mitoses induced by selenomethionine treatment is not known at the present time. Since the scattering of chromosomes is suggestive of an anaphase arrest [15], interference with the spindle apparatus or inhibition of protein phosphatases 1/2A [15,16] by selenomethionine or its metabolites are possible.

In summary, this study provides information on the growth inhibitory effects of selenomethionine on human tumor cell lines. We have demonstrated that selenomethionine inhibits tumor cell line and normal diploid cell line growth in a dose-dependent manner. We have also demonstrated that a ‘normal’ cell type appears to be less sensitive than the human tumor cells to the toxic effects of selenomethionine. Finally, we have shown that selenomethionine administration to human tumor cells results in the formation of apoptotic cells and in cells with aberrant mitoses. These results have established a foundation which will facilitate the further elucidation of the anticarcinogenic mechanism of selenomethionine.

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