Effects of selenomethionine on cell growth and on *S*-adenosylmethionine metabolism in cultured malignant cells

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The effects of selenomethionine (SeMet) on the growth of 17 cultured cell lines were studied. SeMet in the culture medium of three hepatoma cell lines promoted cell growth at subcytotoxic levels (1–20 μ M), but the growth of malignant lymphoid and myeloid cells was not stimulated. L-SeMet was cytotoxic to all 17 cell lines when assayed after culture for 3–10 days. A 50% growth inhibition was observed by 30–160 μ M-SeMet in a culture medium containing 100 μ M-methionine. SeMet cytotoxicity to normal (fibroblasts) and malignant cells was rather similar, excluding specific antineoplastic cytotoxicity. Cytotoxicity was increased by decreasing concentrations of methionine. The DL form of SeMet was less cytotoxic than the L form. L-SeMet was metabolized to a selenium analogue of *S*-adenosylmethionine approximately as effectively as the natural sulphur analogue methionine in malignant R1.1 lymphoblasts. Concomitantly, *S*-adenosylmethionine pools were decreased. This occurred early and at cytotoxic SeMet levels. Methionine adenosyltransferase activity was not altered by SeMet treatment. ATP pools were not affected early, and decreases in the synthesis of DNA and protein took place late and were apparently related to cell death. RNA synthesis was slightly stimulated at low cytotoxic SeMet levels by 24 h, but was markedly inhibited after 48 h. The SeMet analogue of *S*-adenosylmethionine could be effectively utilized in a specific enzymic transmethylation. Neither *S*-adenosylhomocysteine nor its selenium analogue accumulated in the treated cells. These findings together suggest a direct or indirect involvement of *S*-adenosylmethionine metabolism in SeMet cytotoxicity, but exclude a gross blockage of transmethylations.

INTRODUCTION

Selenium is an essential dietary component for mammals, including humans. Its only well-understood function is its presence in the active centre of glutathione peroxidase [1] in the form of selenocysteine [2]. Glutathione peroxidase catalyses the oxidation of glutathione and reduction of organic hydroperoxides or H_2O_2 , thereby protecting membrane lipids and macromolecules from oxidative damage. It has been shown experimentally that selenium supplementation in the diet protects against the development of tumours induced by various chemical carcinogens as well as by a virus [3]. Selenium has also been shown to inhibit mutagenicity of several carcinogens in the Ames *Salmonella* microsomal mutagen test [4]. Epidemiological findings together with accumulating evidence that selenium is effective in prevention of chemical carcinogenesis have created much interest in the possible use of selenium compounds as cancer chemoprevention agents [5–8]. Selenium may also be useful in protection of normal cells from oxidative damage caused by cancer chemotherapy. Furthermore, some reports suggest that selenium compounds may themselves have antineoplastic activity [9–12].

Several chemical forms of selenium, e.g. sodium selenate, sodium selenite, selenocystine and selenomethionine (SeMet), have been utilized in supplement therapy and in studies on prevention or on treatment of cancer. Dietary supplementation has been carried out commonly by using selenium-enriched nutritional yeast preparations. SeMet has been identified as the major selenium-containing compound in the yeast [13]. Similarly, SeMet may be a major form of selenium in plants and thus in food [14].

All selenium compounds are very toxic [15], and neither their metabolism nor the mechanisms of toxicity are fully understood. SeMet, a close analogue of methionine, is capable of forming a selenium analogue of *S*-adenosylmethionine [16–18]. *S*-Adenosylmethionine (AdoMet) is the methyl donor in numerous biological methylation reactions involved in cellular regulation at the level of DNA, RNA, protein and cell membrane. Tracer studies have indicated that, besides being incorporated into protein, SeMet is metabolized, possibly initially via the methionine metabolic pathways, resulting finally in the formation of dimethylselenide and trimethylselenonium [19], the methylation being carried out with AdoMet. All forms of selenium administered finally yield the same methylated end products, which are exhaled or excreted in urine. Impaired synthesis of AdoMet [20] and inhibition of its utilization in DNA methylation [21] have been previously tentatively implicated in selenite toxicity. We have now studied the growth-promoting and cytotoxic effects of SeMet in 17 cultured malignant and normal cell lines with special reference to the effects of SeMet on AdoMet metabolism.

A part of these findings has already been published in abstract form [22].

MATERIALS AND METHODS

Chemicals

L-Selenomethionine (SeMet), DL-SeMet, DL-selenoethionine, selenocystamine, 6-selenopurine and selenodioxide (SeO_2) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Selenocystine was from Serva (Heidelberg, Germany), and sodium selenite

Abbreviations used: AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; MeSA, methylthioadenosine; AdoSeMet, adenosylselenomethionine; AdoSeHcy, adenosylselenohomocysteine; MeSeAdo, methylselenoadenosine; Met, methionine; SeMet, selenomethionine; PBS, phosphate-buffered saline; MEM, (Dulbecco's) minimal essential medium.

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(Na_2SeO_3) and sodium selenate (Na_2SeO_4) were from Aldrich (Steinheim, Germany). Other reagents of analytical grade were from Sigma or E. Merck (Darmstadt, Germany) if not otherwise stated.

Cell culture and cytotoxicity assays

HL-60, L1210, R1.1, K-562, Raji, Daudi, EL4, MOLT-4, Ramos (RA1), CCRF-CEM, WiL2-NS and CHO-K1 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and were maintained in suspension culture in RPMI 1640 (Gibco, Uxbridge, Middx., U.K.) supplemented with heat-inactivated 10% (v/v) fetal-bovine serum, penicillin (100 units/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$) in humidified atmosphere of air/ CO_2 (19:1) [23]. Anchorage-dependent cell lines were cultured in Eagle's minimal essential medium (MEM; Gibco) with the above-mentioned supplements. The origin of the hepatoma lines Hepa and HTC was described by Hankinson *et al.* [24] and Thompson *et al.* [25] respectively. HepG2 and BHK-21(C-13) were from the American Type Culture Collection. Fibroblasts cultured from skin biopsy of human forearm were kindly provided by Dr. Douglas Smith of the Department of Nutrition of this University. They were subjected to growth assays after five passages *in vitro*. All cultures were tested with Mycoplasma T.C. II (Gen-Probe, San Diego, CA, U.S.A.), and only cells not contaminated with mycoplasma were used.

Cytotoxicity was evaluated by growth-inhibition assay as follows: serial 2-fold dilutions of SeMet were added to cells at an initial density of $1 \times 10^5/\text{ml}$. At indicated times, usually after 72–120 h in tissue culture, living cells not stained with Erythrocine B were counted manually. Plating efficiency of cells was measured as follows: SeMet at various concentrations was added 16 h after seeding of 200 cells to each culture dish (10 cm diameter; Nunc, Roskilde, Denmark). After culture for 7 days, cell plaques (more than 30 cells) were stained with Crystal Violet and counted visually. The reported values are means of at least two independent determinations.

The methionine (Met) concentration in the medium was varied by using an RPMI-1640 Select-Amine kit (Gibco).

Selenium content of cell-culture media

Selenium content was measured by electrothermal atomic-absorption spectrometry [26]. The fetal-bovine sera and the RPMI 1640 medium used in these studies contained 58 ± 15 nm- and 23 ± 3 nm-selenium respectively ($n = 4$; means $\pm$ S.D.). Since fetal-bovine serum was added to the growth medium at a 10% concentration, the final selenium levels in the culture media were about 30 nm.

Macromolecule labelling

The rate of macromolecule synthesis was determined by isotopic labelling of cultures treated with 0–2.5 mM-SeMet for 24 h or 48 h (initial cell densities 0.3×10^6 and $0.2 \times 10^6/\text{ml}$ respectively). The assays were done in triplicate with serial dilutions of SeMet on 96-well plates. L-[4,5- ^3H]Leucine (1 $\mu\text{Ci}/\text{well}$), [6- ^3H]thymidine (1 $\mu\text{Ci}/\text{well}$) or [2- ^{14}C]uridine (0.5 $\mu\text{Ci}/\text{well}$) dissolved in a small volume of culture medium (20–50 μl) were added to the wells containing 200 μl of culture medium. After 3 h in culture, labelling was stopped by harvesting cells with a Multimash 2000 (Dynatech Laboratory, Chantilly, VA, U.S.A.). The cells were collected on glass-fibre filters by washing the plate with phosphate-buffered saline (PBS; 150 mM-NaCl/10 mM-phosphate buffer, pH 7.4). Thereafter, cells labelled with leucine were washed with 10% (w/v) trichloroacetic acid. Uridine-labelled cells were washed with PBS and thymidine-labelled ones with water. The glass-fibre filters were counted for

radioactivity in ACS scintillation fluid (Amersham International).

Cell preparations and assay of methionine adenosyltransferase

For measuring methionine adenosyltransferase activity, washed cells (4×10^7) were frozen and thawed three times in distilled water (400 μl) and centrifuged at 14000 g for 5 min in Eppendorf tubes. The supernatant was assayed after dialysis for enzymic activity as described previously [23].

For metabolite analysis, the cultured cells were collected by centrifugation at 700 g for 5 min at 4 °C. After removal of supernatants, the cells were washed once with ice-cold PBS. The pellets were extracted with ice-cold 0.4 M- HClO_4 for 15 min. After centrifugation, the supernatant fractions were neutralized with Alamine-Freon [27] and subjected to h.p.l.c.

Assay of adenosyl derivatives of SeMet and Met

Sulphur- and selenium-containing metabolites of Met and SeMet were determined by a modification [27] of the h.p.l.c. method of Wagner *et al.* [28]. ATP levels were determined by using the Nucleosil HPLC-column (Vydac, Hesperia, CA, U.S.A.) according to the instructions of the manufacturer. Adenosylselenomethionine (AdoSeMet), adenosylselenohomocysteine (AdoSeHcy), decarboxylated AdoSeMet and methylselenoadenosine (MeSeAdo) were prepared and purified as described by Kajander *et al.* [27].

Kinetics of histamine N-methyltransferase

The Michaelis–Menten kinetic parameters of AdoMet, AdoSeMet and histamine for histamine N-methyltransferase were estimated as follows. The incubation mixture (250 μl) contained histamine (2–20 μM), and AdoMet or AdoSeMet at 2–20 μM concentrations, in 50 mM-potassium phosphate buffer, pH 7.4, containing 5 mM-dithiothreitol and 1 mM-EDTA. Incubation was started by adding 12 μg of histamine N-methyltransferase preparation (containing 1.2 mg of protein/ml; specific activity 2.9 munits/mg; total added activity 34 μunits) purified by DEAE-cellulose chromatography [29]. After incubation at 37 °C for 30 min, the reaction was stopped by adding 80 μl of 2.5 M- HClO_4 containing 50 μM -3-methylhistamine (Calbiochem–Behring, La Jolla, CA, U.S.A.) as an internal standard. After centrifugation at 10000 g for 2 min, the supernatant was mixed with 75 μl of 1.5 M-KOH and centrifuged. A 200 μl sample of this latter supernatant was mixed with 190 μl of methanol/250 mM-sodium borate (pH 10.0; 4:1, v/v) solution and 10 μl of 0.25% (w/v) *o*-phthalaldehyde (in methanol), and 100 μl of this mixture was applied to the h.p.l.c. column for 1-methylhistamine assay. The h.p.l.c. apparatus consisted of a model 110A solvent pump (Beckman Instruments, Berkeley, CA, U.S.A.), a Rheodyne (Cotati, CA, U.S.A.) model 7125 sample injector and a Varian Fluorichrom detector (Varian Aerograph, Walnut Creek, CA, U.S.A.) with excitation and emission wavelengths of 330 nm and 360 nm respectively. The analytical column was an Altex Ultrasphere TM-ODS column (4.6 mm $\times$ 250 mm; particle size 5 μm ; Beckman). Methanol/70 mM-sodium phosphate, pH 7.5 (1:1, v/v) was used as the mobile phase at a flow rate of 0.7 ml/min at ambient temperature.

Protein assay

Protein was assayed by the method of Spector [30], using Coomassie Brilliant Blue G-250. Bovine serum albumin (Sigma) was used as standard. Cell protein in Fig. 1 (below) was analysed from a 96-well plate after dissolving the protein from PBS-washed wells in 0.1 M-NaOH and by the method of Lowry *et al.* [31].

RESULTS

Stimulation of cell growth by SeMet

The effects of SeMet on the growth of 17 different cultured leukaemia, lymphoma, hepatoma and fibroblast-like cell lines were analysed by growth assay under standard conditions. As shown in Fig. 1, the growth of human (HepG2), rat (HTC) and mouse (Hepa) hepatoma cells was stimulated by additions of low SeMet concentrations to the culture medium. About 50% increase was seen in the cell number of Hepa, and slightly less in HTC. The cell protein of HepG2 increased by more than 50%, although the cell number was not increased. Micromolar additions of SeMet to the culture medium slightly increased the plating efficiency of Hepa cells (Fig. 1b). Growth stimulation was not seen with lymphoma, leukaemia or fibroblast-like cells.

Inhibition of cell growth by SeMet

L-SeMet showed a marked cytotoxicity at concentrations above 40 μM in all the cell lines studied. A 50% growth inhibition in a 3-day culture was caused by 30–160 μM -SeMet in the normal culture medium. Total selenium level in the unsupplemented medium was only about 30 nM. Growth inhibition curves were very similar for all tested cell lines (see Fig. 2). From these curves, SeMet additions were calculated that caused 50% growth inhibition under conditions where control cultures without additions just reached confluence (3–10 days) (Table 1). Growth of the malignant cells was inhibited in a similar way to cultured human fibroblasts. Cytotoxicity tests were also carried out with DL-SeMet using R1.1, WiL2, CHO and HepG2 cells. DL-SeMet was less growth-inhibitory for all these cells and approx. two-times-higher DL-SeMet than L-SeMet additions were needed for 50% growth inhibition (results not shown).

Comparison of the growth-inhibitory effects with other selenium-containing compounds

Concentrations of various selenium forms producing 50% growth inhibition is shown in Table 2. SeMet was the least toxic selenium compound tested. All selenium derivatives tested caused growth inhibition at micromolar concentrations and became cytotoxic at higher concentrations, killing all cells in culture.

Effect of Met on SeMet cytotoxicity

Normal cell culture media contain 100–200 μM (both RPMI 1640 and MEM contain 100 μM) Met. Cytotoxicity of SeMet might be affected by Met levels in the medium, because Met and SeMet probably share similar uptake and metabolic routes. Indeed, medium Met did affect SeMet cytotoxicity, as can be seen in Table 3. SeMet was found to be more cytotoxic when the Met concentration was decreased. When the medium concentration of Met was decreased from the normal 100 μM level by 10-fold, SeMet cytotoxicity increased by 1.5–4-fold in various cell lines. 10 μM -Met or SeMet supported cell growth as long as cell numbers were below $0.5 \times 10^6/\text{ml}$. At higher cell densities, Met was rapidly exhausted, resulting in cessation of cell growth and in cytotoxic effects. 10 μM -SeMet (in the absence of added Met) supported the growth of several cultured cell lines (Table 3). None of the cell lines grew if Met (in the absence of SeMet) was 2 μM .

SeMet and cellular AdoMet pools

Owing to the fact that SeMet cytotoxicity was very similar in cultured cells originating from different tissues, R1.1 T-lymphoma cell line was selected as a model. The time course of SeMet cytotoxicity was analysed by incubating R1.1 cells with SeMet and at various time points calculating manually cells not

Fig. 1. Effects of L-SeMet on the growth (a) and plating efficiency (b) of murine hepatomas, and on the growth of a human hepatoma cell line HepG2 (c)

(a), mouse (Hepa) and rat (HTC) hepatoma cells were incubated for 72 h in MEM medium on 96-well plates (2×10^4 cells/well initially), and the indicated SeMet was added either during seeding or after 4 h incubation. The ordinate indicates the percentage of control growth without added SeMet. $\square$, Hepa; $\blacktriangle$, Hepa (4 h); $\square$, HTC; $\blacklozenge$, HTC (4 h). In (b), Hepa and HTC cells were plated on dishes, incubated for 16 h and then subjected to the indicated SeMet levels. After 7 days, cell colonies were counted. The ordinate indicates the percentage of control colonies observed without SeMet addition. $\square$, Hepa; $\blacklozenge$, HTC. In (c), the growth of HepG2 cells was determined in the absence of SeMet after 5 days culture as described for (a) (left ordinate) and by measuring total cell protein (right ordinate). $\square$, Cell number; $\blacklozenge$, protein.

Fig. 2. Effects of SeMet on the growth of human and mouse lymphoma and leukaemia cell lines

The experimental conditions were similar to those of Fig. 1(a), except that the incubations were carried out in RPMI 1640 medium. □, HL-60; ◆, R1.1; ■, Daudi; ◇, EL-4; ●, K-562.

stained with Erythrocin B. When R1.1 cells were incubated with 100 μ M-SeMet, substantial cell death was observed after an initial lag period of 20–24 h. Thus early biochemical events leading to cytotoxicity were searched for at the time point of 16–24 h. SeMet has been reported to form the selenium analogue of AdoMet (see the Introduction). The effects of SeMet on cellular AdoMet and AdoHcy pools were thus examined and are shown in Table 4. Results at 16 and at 24 h (not shown) were similar. Cytotoxic levels of SeMet (over 40 μ M) resulted in decreased AdoMet pools. AdoHcy pools were very low in all experiments. AdoSeMet was present in the treated cells, and its levels increased with SeMet added to the medium. AdoSeHcy levels were very low. Addition of Met to the culture medium resulted in elevated AdoMet pools. The added pools of AdoMet plus AdoSeMet of SeMet-treated cultures practically equalled AdoMet pools of only Met-treated cultures.

Effect of SeMet on methionine adenosyltransferase activity

Low AdoMet pools in SeMet-treated cells were not caused by a change in methionine adenosyltransferase activity, because identical activities were measured from cells cultured in the presence of the same additions (10–100 μ M) of either Met or SeMet (Table 5). The apparent 'inhibition' by both L-SeMet and

Table 1. Cytotoxicity of L-SeMet for various human and murine cell lines

Cell growth was measured as a percentage of that of control viable cells without added SeMet, and values for 50% growth inhibition are given. Each value is a mean for duplicate experiments performed twice.

Cell line	Origin	Test time (days)	[SeMet] causing 50% growth inhibition (μ M)
HL-60	Human, promyelocytic leukaemia	3	45
K-562	Human, chronic myelogenous leukaemia	3	93
Daudi	Human, Burkitt lymphoma	3	75
Raji	Human, Burkitt lymphoma	3	68
Ramos	Human, Burkitt lymphoma	5	30
WiL2	Human, B-lymphocyte	3	78
MOLT-4	Human, acute lymphoblastic leukaemia	3	58
CCRF-CEM	Human, T-lymphoblastoid cell	3	93
L1210	Mouse, lymphocytic leukaemia	3	62
R1.1	Mouse, T-cell lymphoma	3	83
EL4	Mouse, T-cell lymphoma	3	44
HepG2	Human, hepatoma	5	135
HTC	Rat, hepatoma	3	85
Hepa	Mouse, hepatoma	3	135
CHO	Chinese hamster, ovary	3	150
BHK	Syrian or golden hamster, kidney	3	156
Fibroblast	Human, normal skin	10	160

Table 2. Concentrations of various selenium compounds decreasing the growth of human and murine cell lines by 50% in 3-day cultures

Values are expressed as the concentration of selenium compounds causing 50% growth inhibition (measured as percentage of that for control viable cells). Each value is the mean of duplicate measurements carried out twice.

Compound	Cell line...	Concn. causing 50% growth inhibition (μ M)			
		HL-60	L1210	R1.1	HepG2
Selenomethionine		45	62	83	80
Sodium selenite		4	2	1	3
Sodium selenate		7	34	29	19
Selenodioxide		15	3	2	2
Selenocystine		18	2	3	2

Table 3. SeMet concentrations causing 50 % growth inhibition in low-methionine media

Cell growth was measured as a percentage of that for control viable cells and the values for 50 % growth inhibition are given. Each value is the mean of duplicate measurements carried out twice. The culture time was 3 days. Control cultures contained the indicated methionine concentration without SeMet addition, except that the 0 μM -methionine culture contained 10 μM -SeMet and no added methionine (less than 2 μM -methionine was present, originating from serum).

Cell line	Added methionine concn. in the culture medium (μM)...	Added SeMet concn. causing 50 % growth inhibition (μM)		
		100	10	0
K-562		93	70	40
Raji		68	40	18
Daudi		75	37	12
EL4		44	14	10

Table 4. Effects of SeMet and Met additions on cellular AdoMet and AdoHcy pools

R1.1 cells were incubated in RPMI-1640 ([Met] = 100 μM) medium with the indicated additions of either SeMet or Met. The initial cell density was $3 \times 10^5/\text{ml}$. After 16 h, the cells were counted and extracted with 0.4 M-HClO₄. Values are means of three determinations ($\pm$ S.D.) and are expressed as pmol/10⁶ cells.

Addition to culture medium	Pool size (pmol/10 ⁶ cells)			
	AdoHcy	AdoSeHcy	AdoMet	AdoSeMet
Control	1.6 $\pm$ 0.6	0	35.8 $\pm$ 5.0	0
+ SeMet				
10 μM	1.7 $\pm$ 0.1	< 1	33.8 $\pm$ 4.3	< 3
40 μM	1.7 $\pm$ 0.1	< 1	32.7 $\pm$ 3.1	6.9 $\pm$ 0.9
100 μM	1.3 $\pm$ 0.2	< 1	25.6 $\pm$ 6.4	25.0 $\pm$ 5.2
150 μM	1.3 $\pm$ 0.2	< 1	11.3 $\pm$ 3.6	37.4 $\pm$ 7.9
+ Met				
10 μM	1.6 $\pm$ 0.4	0	39.9 $\pm$ 2.8	0
40 μM	1.4 $\pm$ 0.3	0	35.8 $\pm$ 3.3	0
100 μM	1.3 $\pm$ 0.1	0	41.2 $\pm$ 4.8	0
150 μM	1.3 $\pm$ 0.2	0	49.6 $\pm$ 4.2	0

Table 5. Methionine adenosyltransferase activity in R1.1 cell extracts prepared after incubating the cells for 16 h with the indicated SeMet or Met addition to the culture medium (contains 100 μM -Met)

The cells were thoroughly washed, frozen and thawed three times and centrifuged in Eppendorf tubes (14000 g, 5 min). Dialysed supernatant fractions were used for enzyme-activity analysis. Values are means $\pm$ S.D. for four experiments.

Concn. (μM)		Methionine adenosyltransferase activity (pmol/min per mg)
SeMet	Met	
—	—	268.6 $\pm$ 35.4
—	10	319.7 $\pm$ 1.6
10	—	313.0 $\pm$ 18.7
—	40	291.8 $\pm$ 2.3
40	—	237.2 $\pm$ 19.7
—	100	273.4 $\pm$ 16.9
100	—	251.2 $\pm$ 24.9

L-Met of methionine adenosyltransferase in R1.1 extracts was practically identical (Table 6). The 'inhibition' was due to dilution of the radiolabelled methionine. Both DL-SeMet and DL-Met were less inhibitory, because the D form is not an effective

substrate. Thus it was apparent that SeMet and Met competed for the same enzymic reaction in the living cell (Table 4), as well as in the cell extract (Table 6).

Other selenium derivatives were also screened as inhibitors of methionine adenosyltransferase. Inorganic forms (Table 6) as well as selenocystamine, selenopurine and selenoethionine were poor inhibitors. AdoSeMet was a weaker inhibitor than AdoMet, but MeSeAdo was a much stronger inhibitor than MeSAdo.

Effect of Met and SeMet on cellular nucleotide pools

Adding Met and SeMet at concentrations of 10–150 μM to RPMI 1640 medium did not affect the cellular levels of ATP and GTP in cultured R1.1 cells. Control levels of these nucleotides were 2.1 and 0.5 nmol/10⁶ living cells respectively, and similar levels were found in treated cells at 16–24 h. Also, no changes in UTP levels were found (results not shown).

SeMet and transmethylation

AdoSeMet formed in cells treated with SeMet was tested as a substrate for enzymic transmethylation reaction catalysed by histamine N-methyltransferase. K_m and V_{max} values for substrate pairs AdoMet/histamine and AdoSeMet/histamine are shown in Table 7. The kinetic parameters were almost identical. Thus AdoSeMet could replace AdoMet in this transmethylation reaction.

Table 6. Inhibition of methionine adenosyltransferase by selenium compounds

Methionine adenosyltransferase activity was assayed in 76 mM-Tris/HCl, pH 7.4, containing 40 mM-KCl, 25 mM-MgCl₂, 10 mM-ATP, 2.5 mM- β -mercaptoethanol and 150 μ M-¹⁴C-methyl-labelled L-methionine (4.9 μ Ci/ μ mol). The dialysed supernatant fraction from a R1.1-cell extract was used as the enzyme preparation. Indicated compounds were added to the assay mixture 5 min before enzyme addition. Incubation was carried out at 37 °C for 45 min and stopped by adding 25 μ l of the reaction mixture to a P-81 phosphocellulose paper (diameter 2 cm). Phosphocellulose papers were then washed three times with 600 ml of distilled water, dried, and counted for radioactivity. The values given are means $\pm$ S.D. ($n = 3$) and indicate percentage inhibition as compared with the control activity (100% = 248 pmol/min per mg) determined in the absence of inhibitors.

Compound	Concn. in the incubation mixture (μ M)...	Percentage inhibition		
		750	150	30
Sodium selenate		13 $\pm$ 4	8 $\pm$ 2	5 $\pm$ 3
Sodium selenite		18 $\pm$ 7	0 $\pm$ 2	1 $\pm$ 4
Selenosulphide		20 $\pm$ 6	8 $\pm$ 8	6 $\pm$ 1
Selenodioxide		16 $\pm$ 7	3 $\pm$ 5	7 $\pm$ 1
Selenocystamine		21 $\pm$ 3	10 $\pm$ 8	7 $\pm$ 4
6-Selenopurine		4 $\pm$ 5	16 $\pm$ 5	20 $\pm$ 4
DL-Ethionine		7 $\pm$ 3	1 $\pm$ 3	1 $\pm$ 4
DL-Selenoethionine		22 $\pm$ 2	11 $\pm$ 4	6 $\pm$ 3
DL-SeMet		79 $\pm$ 2	27 $\pm$ 2	10 $\pm$ 5
DL-Met		75 $\pm$ 3	44 $\pm$ 5	29 $\pm$ 3
L-SeMet		85 $\pm$ 3	40 $\pm$ 2	14 $\pm$ 7
L-Met		85 $\pm$ 1	53 $\pm$ 2	33 $\pm$ 2
AdoSeMet		15 $\pm$ 11	8 $\pm$ 3	3 $\pm$ 2
AdoMet		90 $\pm$ 1	52 $\pm$ 1	25 $\pm$ 4
MeSeAdo		99 $\pm$ 4	81 $\pm$ 1	29 $\pm$ 3
MeSAdo		7 $\pm$ 5	4 $\pm$ 6	0 $\pm$ 5

Table 7. Kinetic Michaelis-Menten parameters for histamine *N*-methyltransferase

Substrates	K_m (μ M)	V_{max} (pmol/min)
AdoMet	1.1 $\pm$ 0.2	14.8 $\pm$ 0.4
Histamine	1.7 $\pm$ 0.2	14.8 $\pm$ 0.3
AdoSeMet	0.9 $\pm$ 0.2	13.4 $\pm$ 0.3
Histamine	1.3 $\pm$ 0.3	15.0 $\pm$ 0.4

SeMet and synthesis of macromolecules

The rates of macromolecule synthesis by SeMet-treated R1.1 cells were carefully analysed by a pulse-labelling technique (Table 8). In cells cultured with the addition of 156 μ M-SeMet (a value relevant for cytotoxicity), thymidine incorporation (into DNA) was 80 and 60% of the control, that of leucine (to protein) 90% and 54% and that of uridine (to RNA) 108 and 35% at the time points of 24 and 48 h respectively (Table 8). The uridine-incorporation experiment (together with the others) was repeated

Table 8. Effect of L-SeMet on the rate of macromolecule synthesis by R1.1 cells

Experimental details are given in the Materials and methods section. Values (c.p.m.) given are means $\pm$ S.D. ($n = 3$).

Radiolabel	[SeMet] in culture medium (mM)	Culture time (h)...	Incorporation of radiolabel (c.p.m.)	
			24	48
Thymidine	0		935 $\pm$ 111	1035 $\pm$ 172
	0.039		898 $\pm$ 153	1365 $\pm$ 208
	0.078		839 $\pm$ 76	912 $\pm$ 120
	0.156		748 $\pm$ 132	626 $\pm$ 46
	0.31		534 $\pm$ 77	357 $\pm$ 37
	0.625		438 $\pm$ 70	126 $\pm$ 49
	1.25		344 $\pm$ 35	105 $\pm$ 29
	2.5		342 $\pm$ 42	94 $\pm$ 30
	0		853 $\pm$ 96	2070 $\pm$ 574
Leucine	0.039		1009 $\pm$ 150	2740 $\pm$ 433
	0.078		783 $\pm$ 160	2036 $\pm$ 444
	0.156		774 $\pm$ 149	1116 $\pm$ 228
	0.31		634 $\pm$ 91	555 $\pm$ 75
	0.625		495 $\pm$ 69	243 $\pm$ 8
	1.25		393 $\pm$ 60	167 $\pm$ 11
	2.5		390 $\pm$ 61	46 $\pm$ 26
	0		4205 $\pm$ 1075	16645 $\pm$ 5855
	0.039		4853 $\pm$ 1691	14150 $\pm$ 1259
Uridine	0.078		4901 $\pm$ 1298	10407 $\pm$ 3927
	0.156		4542 $\pm$ 488	5855 $\pm$ 1241
	0.31		3325 $\pm$ 511	3086 $\pm$ 901
	0.625		2090 $\pm$ 468	881 $\pm$ 61
	1.25		1591 $\pm$ 162	319 $\pm$ 44
	2.5		1328 $\pm$ 109	183 $\pm$ 24

three times (results not shown) and it always showed stimulation at a time point of 24 h at low SeMet levels. As a control, leucine incorporation was measured in microwell cultures identical with those in Table 8, except that cycloheximide was added at $10 \mu\text{g}/200 \mu\text{l}$ 30 min before addition of labelled leucine. Radiolabel was incorporated similarly in all wells (118 ± 26 c.p.m., $n = 39$). Because cycloheximide blocks protein synthesis, this value represents non-specific binding of the leucine label.

DISCUSSION

The effects of SeMet on cell growth were screened in several established malignant cell lines. SeMet metabolism is known to be related to Met metabolism, which varies greatly from one cell type to another and is dependent on culture or growth conditions. Major steps in methionine metabolic pathways are transmethylation, trans-sulphuration, remethylation of homocysteine to methionine and polyamine synthesis. Their relative flows vary, e.g. in rat liver only about 1 % of AdoMet is used for polyamine synthesis [32], whereas rapidly growing cultured cells consume a major part for the synthesis of polyamines [33,34]. In cultured malignant cells, trans-sulphuration and remethylation of homocysteine to methionine consume only a few per cent of the L-homocysteine formed [33,34]. From this perspective it is remarkable that SeMet caused in the present study very similar cytotoxicity in different types of cultured cells. A 50 % growth inhibition was brought about at a narrow range of 30–160 μM -L-SeMet in the culture medium. That the cytotoxic compound is either SeMet itself or its metabolite is supported by dependency of SeMet cytotoxicity on methionine levels in the medium. The cytotoxicity increased with decreasing methionine concentrations. This situation favours increased uptake and metabolism of SeMet via methionine pathways. Intracellular targets involving enzymic steps are also implicated by the observation that DL-SeMet was less cytotoxic than the L form. Only the L form is a good substrate in AdoSeMet synthesis.

The growth effects of SeMet have previously been studied only in a few cell lines. Porter *et al.* [18] observed 50 % inhibition at 130 μM -SeMet in the growth of L1210 cells in an assay similar to ours. This is in excellent consistence with our data (62 μM), since they used the less cytotoxic DL-mixture. Batist *et al.* [9] studied cytotoxicity of several selenium compounds, including SeMet, on HL-60 cells. They also noticed that SeMet was the least cytotoxic form of selenium, in accordance with our data in Table 2. However, they did not observe cytotoxicity for SeMet at their highest dose of 100 μM . This can be explained by the use of DL-form of SeMet and by the short 1-day culture time.

Improved cell growth by selenium, including SeMet addition to the culture medium, has been reported previously by Beilstein *et al.* [35] in fibroblast and myoblast cultures. The improved growth of various hepatoma cell lines by SeMet observed in the present study may be related to high uptake rate of Met and SeMet [35] and a high utilization rate due to presence of two forms of methionine adenosyltransferase. The liver-specific enzyme form escapes feedback inhibition by AdoMet, whereas the enzyme form present in other tissues and cells is strongly inhibited by AdoMet.

SeMet addition to the culture medium did not specifically block the early synthesis of DNA, RNA or protein. However, RNA labelling was initially (24 h) stimulated by low cytotoxic levels of SeMet in R1.1 cells. By 48 h, RNA labelling had decreased more than DNA or protein labelling. RNA is normally highly modified by methylation, but also incorporation of selenium derivatives takes place, and the finding might relate to inefficient function or transport of hypo- or hyper-modified

RNA. This hypothesis implies that the modifying enzymes (e.g. methylases) would be affected by SeMet treatment. This initially could result in stimulation of RNA synthesis followed later by inhibition. In accordance with our findings, Porter *et al.* [18] reported surprisingly late inhibition of protein synthesis during SeMet toxicity in L1210 cells.

In SeMet-treated cells, no gross block was evident in transmethylation by R1.1 cells, as evidenced by lack of accumulation of AdoHcy or AdoSeHcy and by the excellent function of AdoSeMet in a specific transmethylation reaction by histamine N-methyltransferase. We have also shown that AdoSeMet can be decarboxylated and functions thereafter in polyamine synthesis of K-562 cells [36]. Here we showed that ATP levels were not affected by Met or SeMet. SeMet cytotoxicity might be due to selenocysteine formed via the trans-sulphuration pathway. This is supported by the finding that selenocysteine (selenocysteine yields two selenocysteine residues) was several times more cytotoxic than SeMet. Although this pathway has been described in rat liver [37], its function in SeMet catabolism has not been experimentally proved. It is noteworthy that selenium and sulphur metabolism differ in their end products, and labelled SeMet has been shown to produce dimethylselenide and trimethylselenonium as the final excreted products [19]. The activity of the trans-sulphuration pathway in cultured cells has been found to be low [33,34]. Further work is thus needed to elucidate the metabolism of SeHcy and its role in SeMet toxicity.

SeMet administration resulted in a profound decrease in AdoMet levels. This took place early (before a decrease in the number of viable cells occurred) and at cytotoxic SeMet levels. The fall in AdoMet was compensated for by the formation of AdoSeMet, which was found to function as a substrate for a methyltransferase. However, AdoMet is utilized in almost 100 reactions, some of which are of vital importance. It remains to be elucidated whether some of these reactions may not accept AdoSeMet as a substrate, or are inactivated or inappropriately regulated by high levels of AdoSeMet, resulting in cytotoxic effects. Finally, it must be emphasized that SeMet is the least cytotoxic form of selenium tested. Yet it is efficiently incorporated into tissues and shows biological activity, indicated by increased glutathione peroxidase activity [38], and thus is a useful source of this necessary trace element.

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