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ANTICANCER EFFECTS OF SELENIUM COMPOUNDS ON HUMAN COLONIC CARCINOMA CELLS

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Studies performed so far on different human carcinoma cell lines, as well as numerous case-control and epidemiological studies have given proof to the protective effects of selenium against cancer. However, the anticancer properties of selenium are site-specific. The aim of this work was to evaluate the cytotoxic effect of selenium against CaCo2 human colon carcinoma cells, and SW620 lymph node metastasis of colon carcinoma cell line. Three selenium compounds, seleno-DL-cystine (SeC), seleno-L-methionine (SeM) and sodium selenite were used. Initial number of cells was 2×10^4 and the cells were incubated for 72 h with the aforementioned Se compounds at 10, 100 and 1000 μmol Se concentrations. Cytotoxicity was measured by the MTT cell survival assay. In the present study, decreased viabilities of both CaCo2 and SW620 cells were established following the treatment with selenite, SeC, and SeM. At 10 μmol Se levels all three chemical forms exerted a more or less anticipated cytotoxic effect with viability decreases ranging from 22 to 37%. However, the other two levels of 100 and 1000 μmol Se did not exhibit an expected proportional rise in cytotoxic effect compared to 10 μmol , which warrants further research on the reasons for increased resistance of these cells. Cell morphology also indicates that investigated Se forms induced apoptotic cell death in both cell lines. The results confirm the applicability of Se in the prevention and treatment of the investigated cancer sites.

Keywords: selenium, cytotoxicity, colonic carcinoma

A great body of evidence for the protective role of selenium against cancer has accumulated over the last three decades, ever since the first ecological studies have suggested an inverse relationship between selenium concentration in soil, food, and/or blood and cancer incidence (ALLAWAY et al., 1968; SHAMBERGER & FROST, 1969; SCHAMBERGER & WILLIS, 1971; SCHRAUZER et al., 1977). Selenium's anticancer properties have also been extensively investigated in numerous case-control and epidemiological studies, most of which support the hypothesis that this microelement is an indispensable risk factor for cancer (MCCONNELL et al., 1975; SALONEN et al., 1984;

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COMSTOCK et al., 1992; RUMI et al., 1992). Probably the most convincing epidemiological proof to the hypothesis is the intervention trial in which total cancer mortality in the selenium-supplemented group was 50% less than in the control group (CLARK et al., 1996). Various selenium compounds have also been demonstrated to inhibit chemically and virally-induced carcinogenesis, both in cell culture and in vivo (SCHRAUZER et al., 1978; MEDINA & SHEPHERD, 1980; THOMPSON & BECCI, 1980; MEDINA et al., 1983; MILKS et al., 1985; LIAO et al., 1988; APPEL & WOUTERSEN, 1996), as well as UVR-induced skin carcinogenesis (OVERVAD et al., 1985). The protective effects of selenium have been established in both the initiation and promotion phases of carcinogenesis (THOMPSON & BECCI, 1980; APPEL & WOUTERSEN, 1996). Additionally, selenium completely inhibits cancer propagation, according to GREEDER and MILNER (1980) who examined the effect of selenium on mice with Ehrlich ascites tumors. Many mechanisms for the anticarcinogenic and anticancer properties of selenium have been proposed, including its antioxidant activity through selenium containing glutathione peroxidase (GPX), involvement in carcinogen metabolism affecting cytochrome P-450, inhibition of certain enzymes, stimulation of apoptosis, inhibition of protein synthesis, modulation of endocrine and immune responses, etc. (MILNER, 1985; IP, 1986; EL-BAYOUMY, 1991). Cell culture experiments have provided most of the current knowledge on mechanisms of the element's anticancer activity. Some selenium compounds are genotoxic while others are not, but both groups inhibit growth of different cell lines (LU et al., 1995; STEWART et al., 1999). The extent of the determined cytotoxic and antiproliferative effects of selenium depends on both the selenium chemical form and the investigated cell line. It has been observed before that the strength of selenium's protective activity may be site-specific in humans, and the best evidence is in relation to cancers of colon, rectum and other gastro-intestinal carcinomas (CLARK, 1985; CLARK et al., 1996).

The aim of this study was to examine the cytotoxic effects of three different forms of selenium in two human cancer cell lines, CaCo2 human colon carcinoma and SW620 lymph node metastasis of colon carcinoma.

1. Materials and methods

1.1. Cell lines and culture

Human colon carcinoma cells (CaCo2) and poorly differentiated cells from lymph node metastasis of colon carcinoma (SW620) were kindly provided by Professor Krešimir Pavelić (Division of Molecular Medicine, Ruder Bošković Institute,

Zagreb, Croatia), and were grown in DME medium which was supplemented with 10% fetal bovine serum, 2 mmol L-glutamine, 100 IU ml⁻¹ penicillin and 100 mg ml⁻¹ streptomycin. The cultures were equilibrated with humidified 5% CO₂ in air at 37 °C.

The effects of the investigated forms of selenium on CaCo2 and SW620 cell morphology were also studied by light microscopy.

1.2. Cell viability assay

The quantity of cells was determined using the tetrazolium dye (MTT) test, as described previously by HORIUCHI and co-workers (1988). CaCo2 and SW620 cells (2×10⁴ cells/ml) were plated onto 96-microwell plates. The tested selenium compounds were sodium selenite, seleno-DL-cystine (SeC), and seleno-L-methionine (SeM). They were added to the cells at 10, 100 and 1000 μmol selenium concentrations, respectively, 24 h after plating. After 3 days of incubation, the cells were stained with 20 μl of 5 mg ml⁻¹ MTT in phosphate buffered saline for 4 h. The precipitate was solubilized with 50 μl DMSO and the plates were read with an Elisa reader at 570 nm. All experiments were performed at least 2 times with 6 wells for each.

1.3. Determination of DNA synthesis

DNA synthesis in CaCo2 cells was determined using ³H-thymidine incorporation assay. Six hours before end of 3-day culturing, ³H-thymidine (25 mCi ml⁻¹) was added to a final concentration of 1 μCi ml⁻¹. The cells were harvested onto glass wool filters using a cell harvester and the incorporated radioactivity was measured in a scintillation counter.

2. Results

2.1. Cytotoxicity

The results of cell viability assay are given in Table 1. The survival of both CaCo2 and SW620 cells decreased with the increasing concentration of selenite, SeC and SeM, respectively.

2.2. DNA synthesis

Incorporation of radioactive precursor ³H-thymidine was only monitored in CaCo2 cells at 10 and 100 μmol selenium concentrations, and the results are given in Table 2. A dose-dependent decrease of DNA synthesis was observed, consistent with the results of the MTT-test.

Table 1

Effects of selenite, selenocysteine and selenomethionine on the viability of SW620 and CaCo2 cells^a

Treatment	SW620	CaCo2
None	100	100
Selenite, 10 µmol	74.5 ± 3.5	63.1 ± 2.5
Selenite, 100 µmol	59.1 ± 2.7	58.4 ± 1.7
Selenite, 1000 µmol	54.8 ± 3.9	50.2 ± 3.2
Selenocysteine, 10 µmol	63.1 ± 1.9	66.9 ± 7.9
Selenocysteine, 100 µmol	61.2 ± 0.8	62.8 ± 1.7
Selenocysteine, 1000 µmol	56.2 ± 4.8	44.9 ± 16.5
Selenomethionine, 10 µmol	68.2 ± 2.1	77.9 ± 1.7
Selenomethionine, 100 µmol	60.4 ± 3.1	64.8 ± 2.6
Selenomethionine, 1000 µmol	60.2 ± 3.6	58.2 ± 3.7

^a data are mean percentages of live cells ± SD

Table 2

Effects of selenite, selenocysteine and selenomethionine on DNA synthesis in CaCo2 cells

Treatment	% Incorporation of ³ H-thymidine
None	100
Selenite, 10 µmol	63.6
Selenite, 100 µmol	57.9
Selenocysteine, 10 µmol	71.7
Selenocysteine, 100 µmol	53.9
Selenomethionine, 10 µmol	68.2
Selenomethionine, 100 µmol	61.4

3. Discussion

The well-documented anticarcinogenic and antitumor action of Se cannot be fully explained by the antioxidant activity of GPX (DAVIS et al., 1999; STEWART et al., 1999). The link between thioredoxin reductase and anticancer activity of selenium has not been elucidated as yet. The selenium-containing enzyme reduces thioredoxin which stimulates cell growth and is overexpressed in cancer cells (POWIS et al., 1997; BERGGREN et al., 1999). The cytotoxic activity of Se has been the focus of the research

concerned with its antitumor properties. Selenium compounds exert this activity differently and with varying efficacy. In the present study the cytotoxic effects of selenite, SeC and SeM were established against CaCo2 and SW620 cells. At 10 μmol Se levels all three chemical forms exerted a more or less expected cytotoxic effect with viability decreases ranging from 22 to 37% (Table 1). However, the other two levels of 100 and 1000 μmol Se caused a minor increase in the cytotoxic effect compared to 10 μmol which is especially evident in SW620 cells. None of the forms precipitated a 50% decrease in viability after 72 h of exposure of this cell line to Se (Table 1). The percentage of CaCo2 cell viability decrease reached 50% at 1000 μmol selenite-Se, while the same concentration of SeC decreased the number of viable CaCo2 cells by 55%. The unexpectedly low cytotoxic effects of selenite, SeC and SeM at 100 and 1000 μmol Se in both investigated cell lines could be an indication of their adaptation to high Se concentrations, as observed before in a relatively Se-insensitive cell line (FICO et al., 1986). It has also been established (FEARON & VOGELSTEIN, 1990) that p53 gene is usually mutated in colonic carcinoma cells, which disables the p53-dependent apoptosis or programmed cell death. The absence of p53 expression produces a dramatic increase in resistance of such cells to anticancer agents (LOWE et al., 1993).

Most other authors who investigated selenite-Se cytotoxicity on different cell lines with a dye exclusion test reported lower concentrations of selenite required to induce a 50% decrease in cell viability (i.e. IC_{50} value). This can, in part, be explained by study design to assess selenium cytotoxicity, implying different duration of treatment, plating density, etc. In HL-60 human leukemia cells the viability fell to 50% of control at 34 μmol selenite level (BATIST et al., 1986). FICO and co-workers (1986) determined 97, 52, 40, and 18% decreased viabilities in different canine mammary tumor cell lines after 72 h of incubation with 10 μmol of Se as selenite. Selenite was also shown to be very cytotoxic to mouse keratinocyte BALB/c MK2 cell line after 24-h incubation at 317 μM (STEWART et al., 1999). No viable cells were detected at that and higher levels. Cell viabilities were decreased 12 and 40% in B16 murine melanoma cells at 10 and 100 μmol selenite-Se concentrations, respectively (SIWEK et al., 1994). In pB16 cells the corresponding viability decreases were 39 and 54%.

The cytotoxicity of selenite is based on its ability to generate superoxide and oxidative free radicals which have been shown as capable of inducing cell death (GARBERG et al., 1988; HOCKENBERY et al., 1993; SPALLHOLZ, 1994). Selenite reacts with glutathione (GSH), as well as other thiol-containing proteins, to give selenodiglutathione (SDG) (i.e. corresponding selenotrisulfides; RSSeSR) (GANTHER, 1968), which is an even more potent inhibitor of cell growth in vitro than selenite itself (FICO et al., 1986; CAFFREY & FRENKEL, 1991; LANFEAR et al., 1994). SDG is then reduced to a selenopersulfide anion (GS-Se^-) which in turn redox cycles producing free radicals. Several groups of authors observed a genotoxic effect as a result of selenite-

induced redox cycling in different cell lines (GARBERG et al., 1988; WILSON et al., 1992; SINHA et al., 1996; STEWART et al., 1999). It has been reported that concentrations of 10 μmol selenite and higher induce apoptotic cell death (ZHU et al., 1996; STEWART et al., 1997). The induction of apoptosis is attributed to the free radical oxidative stress by the oxidation of cellular GSH (DAVIS & SPALLHOLZ, 1996), and to the endonuclease activation (LU et al., 1994). However, some authors suggested the involvement of a Se-binding protein in growth inhibition by selenium (WU et al., 1995), while others propose conversion of selenite to methylated compounds which indiscriminately bind to proteins and nucleic acids (IP & GANTHER, 1990; IP et al., 1991; WILSON et al., 1992; IP & GANTHER, 1993).

SeC was more effective than selenite in decreasing survival of B16 cells in an MTT-assay (SIWEK et al., 1994). At 10 and 100 μmol SeC concentrations the viabilities of B16 cells were decreased by 22 and 87%, respectively. The amount of living pB16 cells at these SeC levels dropped by 34 and 65%, respectively. Although 10 μmol SeC caused a stronger cytotoxic effect in both SW620 and CaCo2 cells (Table 1) than in both B16 or pB16 cells, a significant decrease in cell viability occurred in these cells at 100 μmol level which could not be seen in either SW620 or CaCo2 cells. A dose-dependent response exists but the lack of a more pronounced effect of both selenite and SeC as documented by most other researchers is difficult to explain. SeC decreased the number of viable L1210 cells by 29% after 3 h incubation at 6.33 μmol (MILNER & HSU, 1981), while the determined IC_{50} -value for HL-60 cells was 10.5 μmol (BATIST et al., 1986).

Similarly to selenite, SeC reacts with GSH to give selenocysteine which is then enzymatically converted to H_2Se by the enzyme selenocysteine lyase (DICKSON & TAPPEL, 1969; ESAKI et al., 1982). Involvement of selenide anions (Cy-Se^-) is implicated in the cytotoxicity of SeC (WHITING et al., 1980). Moreover, it has been suggested that the prooxidant catalytic action of Se is limited to the forms which can generate the selenide anion (R-Se^-) (YAN & SPALLHOLZ, 1993; SPALLHOLZ, 1994). The established lesser cytotoxicity and genotoxicity of SeC compared with selenite is attributed to the lower reactivity of the C-bonded selenide group of SeC (Cy-Se^-) than the S-bonded selenide anion of selenopersulfide (GS-Se^-) from inorganic Se compounds (SHAMBERGER, 1985). Both selenite and SeC can deplete cellular GSH levels (ANUNDI et al., 1984; YAN et al., 1991; STEWART et al., 1997). GSH is needed for the cytotoxic effect of selenite and SeC, since the reaction between these molecules is the first step in the metabolic conversion of selenite and SeC to Se species which redox cycle producing reactive free radicals and peroxides (CAFFREY & FRENKEL, 1991; YAN & SPALLHOLZ, 1993). GSH can also give significant protection against reactive oxygen species (ARRICK & NATHAN, 1984).

In SW620 cells, 10 μmol SeM-Se induced a 31.8% drop in cell viability which is better than its reported activity in UACC-375 melanoma or MCF-7/S breast cancer cells and similar to DU-145 prostate cancer cell line (REDMAN et al., 1998). The effect of SeM in SW620 cells did not increase significantly at 100 and 1000 μmol , not reaching 50% survival decrease (Table 1). Similar pattern was observed with SeM addition to CaCo2 cells. Most authors who investigated SeM cytotoxicity determined the IC_{50} of 40 to 320 μmol (PORTER et al., 1984; KAJANDER et al., 1990; REDMAN et al., 1998). In contrast, SeM induced only a small decrease of cell survival in B16 and pB16 cells at 10 and 100 μmol levels (around 10 and 5% for B16, and around 20% at both levels for pB16 cells) (SIWEK et al., 1994). SeM was neither toxic to HL-60 cells up to 100 μmol (BATIST et al., 1986) nor to BALB/c MK2 mouse keratinocyte cell line up to 3170 μmol (STEWART et al., 1999).

SeM is metabolized via the transsulfuration mechanism to selenocysteine, and then enzymatically to H_2Se , as already mentioned above. Considering very similar metabolic pathways, the consistently weaker cytotoxic effects of SeM compared to SeC are somewhat surprising (YAN et al., 1991; SIWEK et al., 1994). In part, this can be explained by the non-specific incorporation of SeM into proteins in place of methionine (IP & GANTHER, 1993). On the basis of their results, STEWART and co-workers (1999) consider SeM a relatively non-toxic and non-redoxing form of Se. REDMAN and co-workers (1997; 1998) have shown that SeM induces cell death by apoptosis which is independent of p53. They also suggested that depletion of polyamines by SeM leads to an apoptotic cell death and cell cycle changes.

There were no significant differences between the effects of selenite, SeC and SeM on cell morphology. After 24 h of treatment, the majority of cells became smaller, round shaped, and a proportion of cells spontaneously detached from the culture flask and floated in the medium. The apoptotic characteristics of cytoplasmic vacuolization and chromatin condensation were also observed in both cell lines after 24-h treatment with the three forms of Se.

The measurement of the rate of DNA synthesis in the presence of the three Se forms reveals their growth inhibitory effect on CaCo2 cells (Table 2). There is no significant difference between selenite, SeC and SeM at 10 μmol Se level. At 100 μmol there is a slightly stronger effect of selenite and SeC evident, in comparison with SeM (Table 2). ABDULLAEV and GONZALEZ DE MEJA (1996) reported similar inhibitory effect (70% of control) on DNA synthesis in HeLa cells after 1-h treatment with 10 μmol selenite. In MOD cells, however, after 24 h of Se treatment, 10 μmol selenite reduced DNA synthesis by more than 90% (LU et al., 1995).

In spite of the lack of a proportional drop of cell survival beyond 10 μmol Se, which warrants further research on these cell lines, the determined cytotoxic effect of Se supports the theory of its cancer chemopreventive activity. In addition, the levels of

most Se compounds that are 100 or 1000 higher than the normal blood concentration could find little use in cancer prevention or treatment because of the adverse effect on normal cells. Previous convincing findings of Se anticancer properties have led to the development of organoselenium compounds which have high chemopreventive activity and low toxicity, like xyleneselenocyanate (p-XSC) (EL-BAYOUMY et al., 1995). Co-administration of Se with other anticancer substances is another approach to bypass the toxicity of high concentrations of Se compounds to normal cells. Combinations of natural compounds with selenite or SeM were also found to be more effective than either of the substances alone (ABDULLAEV & GONZALEZ DE MEJIA, 1996). IP and LISK (1996) have focused their research on the attainment of Se cancer chemopreventive effect with Se-enriched garlic, which has been shown to be superior to regular garlic in the suppression of mammary cancers in an animal model (IP et al., 1992). It is obvious that cancer prevention through Se-enriched, i.e. functional foods is the best way to utilize its chemopreventive potency on larger populations. All three chemical forms of Se investigated in the present work occur naturally in human nutrition, although there are differences regarding their levels in foods and absorption. From the little data on Se species in different foods (MUTANEN, 1986), it can be concluded that SeMet is probably the most frequent form of Se in human nutrition. A body of work also indicates that SeMet is more readily absorbed from the gastrointestinal tract than the other two chemical forms (LEVANDER et al., 1983; MCADAM et al., 1985).

Studies aimed at establishing Se provision to domestic animals (GAVRILOVIĆ, 1981; GAVRILOVIĆ & MATEŠIĆ, 1987; VULENIC et al., 1987) and humans (MANDIĆ et al., 1995; KLAPEC et al., 1998) in eastern Croatia point to suboptimal intakes, and deficiency diseases in domestic animals have been reported. Age-adjusted colorectal cancer mortality for the city of Osijek in 1994 is 15.8, the corresponding data for US is 13.2 (source: www.cancermundial.com). If looked at from a perspective of Se intake, although one should always make such comparisons with caution because of numerous other factors affecting cancer prevalence, a higher incidence of cancer should be expected in eastern Croatia considering three to five times higher Se intakes in the USA (LONGNECKER et al., 1991; KLAPEC et al., 1998). Both our results and current knowledge on Se chemopreventive activity justify finding a proper way of raising Se provision to the population of eastern Croatia, hoping that immediate research will help clarify the role of this microelement in cancer prevalence in the area.

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