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A Comparison Between Selenium Dioxide and Selenium Methionine Induced Cytotoxicity in Estrogen Receptor Negative and Positive Breast Cancer Cell Lines

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Abstract: Selenium is an essential trace element which has been shown to inhibit the growth of various cancers in numerous studies. Different forms of selenium have been reported to exert variable potencies against the cancer growth. In this study, the effect of selenium dioxide (SeO_2) and organic selenium (seleno-L-methionine) on the growth of the human breast cancer cell lines namely MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative) were compared. The standard MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to quantitate the viable cancer cells. Selenium dioxide inhibited cell growth at high concentrations (10^{-4} M to 10^{-3} M) but showed mild (not significant) stimulation of cell proliferation at lower concentration (10^{-12} to 10^{-5} M). However, selenomethionine exhibited biphasic effects on both cell lines. It inhibited cell growth at high concentrations (10^{-4} M to 10^{-3} M in MCF-7; 10^{-3}M in MDA-MB-231) but stimulated cell proliferation at lower concentrations. The findings in this study indicated that selenium dioxide was more potent than selenomethionine in inhibiting both cancer cell lines. Besides, both forms of selenium were also found to be less effective against MDA-MB-231.

Key words: Selenium, breast cancer

INTRODUCTION

Breast cancer is a common malignancy that affects women around the world, including Malaysia. The incidence of breast cancer has increased (30-35% of all female cancers)[1]. Women with estrogen receptor positive (ER+) breast tumors are known to respond better towards chemotherapy and survive with longer remission period compared to those with estrogen negative tumors. The ER+ breast cancer cell lines are characterized by a dependence on estrogen for growth *in vitro* or *in vivo* and by its sensitivity to the growth-inhibitory effects of anti-estrogenic and progestational drugs[2]. These cell lines tend to reflect the nature of ER+ tumors in breast cancer patients. MCF-7 is an ER+ cell line that is widely used and best characterized of all the human breast cancer cell lines[3]. The estrogen receptor negative (ER-) cell lines exhibit characteristics similar to those of ER- breast tumors. The ER- breast cancer is Estrogen-Receptor negative (ER-) and is usually associated with a poor prognosis and shorter survival of patients[4]. They tend to produce rapidly growing tumors that are highly invasive and some produce distant metastases[5]. The MDA-MB-231 cell line is one of the widely used ER- human breast cancer cell lines[6]. These cell lines can serve as an *in vitro* cellular model to study the potential anticancer drugs or compounds.

Selenium is an essential trace element. It is incorporated into a number of functionally active selenoproteins, including glutathione peroxidase, phospholipids hydroperoxide glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinases [7]. The enzyme glutathione peroxidase acts as a cellular protector against free radical oxidative damage[8]. There are several naturally occurring inorganic and organic forms of selenium. The forms of inorganic selenium include sodium selenite, selenate and selenium dioxide whereas examples of organic forms of selenium are selenomethionine and selenocysteine. Selenium enters the food chain through incorporation into plant proteins, mostly as selenocysteine and selenomethionine (SeMet) at normal selenium levels. However, with elevated selenium levels, Se-Methylselenocysteine (SeMCYS) can be the predominant selenocompound[9]. Unlike plants, animals cannot synthesize SeMet from inorganic selenium. Hence these selenoaminoacids are incorporated as part of the diet. Dietary selenium consists mainly of selenoaminoacids and analogs such as L-selenomethionine from cereal grains and animal proteins or L-selenocysteine from animal meats, poultry, fish and dairy products, with trace amounts of the selenium compounds such as L-Se-methylselenocysteine[9]. Inorganic selenium such as selenate and selenite are found as trace elements in water.

Many selenium compounds have been shown to inhibit the induction of cancer most notably in

thedimethylbenz [a] anthracene, DMBA-induced mammary tumor animal models[10,11] as well as in various tumor cell cultures *in vitro*. The aim of the present study was to compare the effects of inorganic (selenium dioxide) and organic (selenomethionine) forms of selenium on the growth of MCF-7 and MDA-MB-231 human breast cancer cell lines.

MATERIALS AND METHODS

MCF-7 and MDA-MB-231 adenocarcinoma cell lines were obtained from American Type Culture Collection, USA. Seleno-L-Methionine was obtained from Sigma Chemicals, USA. Selenium dioxide sublimed and 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were from MERCK chemicals, Germany. L-glutamine, fetal bovine serum, RPMI 1640 medium without L-glutamine and tripsin were from FLOWLAB, Australia. All other chemicals used were of pure analytical grade.

The two human breast cancer cell lines, MCF-7 and MDA-MB-231 were routinely grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 5% fetal bovine serum (FBS) maintained at 37°C in humidified air containing 5% CO_2.The number of cells in 1 mL of growth medium was estimated by using a Naebauer Haemocytometer. The cell suspension was diluted with growth medium to establish 3000 cells per 100 µl in each well of a 96-well culture plate. The cultures were then incubated at 37°C in 5% CO_2 for 24 hours prior to the treatment with the two types of selenium.

The selenium solutions (10 µL) of various concentrations were added into each of the 100 µl cell suspension after filter-sterilization using 0.22 µm pore-size syringe filters (Millipore). The cultures were then incubated at 37°C in humidified air containing 5% CO_2 for 48 hours. The control contained 10 µL of sterile distilled water instead of the selenium solution. The cell growth was quantitated by using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) according to the method outlined by Mosmann[12]. Forty-eight hours after introducing the test samples into the cells, MTT solution (10 µl of 5 mg/mL in PBS stock) was added to each well and was incubated for 4 hours. At the end of the incubation period, the medium was removed and isopropanol was added to solubilize the formazan formed as a result of tetrazolium ring cleavage by the dehydrogenase enzymes in the cell. The absorbance was measured at 560 nm. The absorbance measured was directly proportional to the number of viable cells.

Statistical Analysis: The Student's t-test was used to determine the significance of the results.

RESULTS AND DISCUSSIONS

This study demonstrated that selenium dioxide inhibited cell growth at high concentrations (10^{-4} M to 10^{-4} M) but showed mild (not significant) stimulation of cell proliferation at lower concentration (10^{-12} to 10^{-5} M) (Fig. 1 and 2). However, selenomethionine exhibited biphasic effects on both cell lines. It inhibited cell growth at high concentrations (10^{-4} M to 10^{-4} M in MCF-7; 10^{-3}M in MDA-MB-231) but stimulated cell proliferation at lower concentrations (Fig. 1 and 2). This is consistent with an earlier study by Medina and Oborn[13] that reported a biphasic effect of sodium selenite on YN-4 mouse mammary epithelial cells. However, the possible mechanisms in which selenium stimulate cancer cell growth at low concentrations are not fully understood and was not investigated in this study.

Selenium dioxide, which is an inorganic form of selenium, was found to be more potent against both breast cancer cell lines when compared with selenomethionine (Fig. 1 and 2). At higher concentrations (10^{-4} and 10^{-3} M), the presence of selenium dioxide inhibited MCF-7 cell proliferation almost completely. Comparison of the IC_{50} values of selenium dioxide and selenomethionine in MCF-7 cells showed that selenomethionine has a higher IC_{50} (80 µM) value than selenium dioxide (35 µM) and was less potent against MCF-7 cell growth. Besides, the IC_{50} (Concentration of selenium that caused 50% inhibition) value of selenomethionine against MCF-7 (80 µM) estimated in this study was higher than the value reported by Redman *et al.*[14] who demonstrated that 45 µM of selenomethionine was needed to inhibit 50 % of MCF-7 cell growth.

Selenium dioxide has also been shown to inhibit the cell proliferation, viability and prompted apoptosis of both immortal human hepatic cell line (HL-7702) and human hepatome cell line (SMMC-7721) markedly after 48 hours treatment[14]. Selenium dioxide could also down-regulate the Bcl-level greatly in HL-7702 but regulate wild type p 53 level significantly in SMMCA- 7721 cells[14]. In the present study, selenomethionine was found to have a lower inhibitory effect against both breast cancer cell lines especially MDA-MB-231cells. This may be because selenomethionine is relatively non-toxic as shown by many studies[15-17] also postulated that the low inhibitory effects of selenomethionine might be caused by the absence of α-lyase enzyme that is required to form the cytotoxic metabolite, i.e. methylselenol. They suggested that selenomethionine is very inactive in arresting cell growth in culture at low concentration but are highly active in chemoprevention *in vivo* by likely

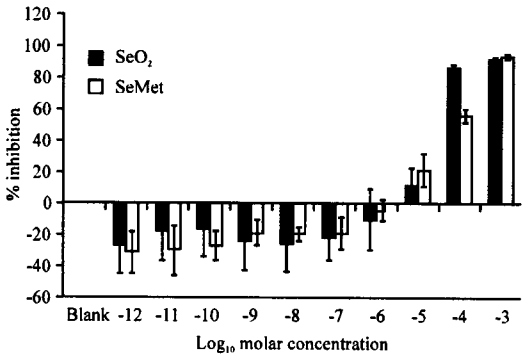

Fig. 1: Comparative effect of selenium dioxide (SeO_2) and selenomethionine (SeMet) on MCF-7 cell line

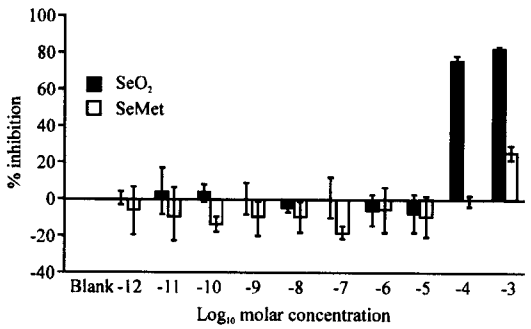

Fig. 2: Comparative effects of selenium dioxide (SeO_2) selenomethionine and (SeMet) on MDA-MB-231

forming methylselenols. Although selenomethionine was shown to be less effective in inhibiting both types of cancer cell growth, it may play an important role as a potent chemopreventive agent by inducing DNA repair response in normal cells *in vitro* and protecting cells from DNA damage as reported by Young *et al.*[18].

Selenium dioxide and selenomethionine might have different mechanisms in inhibiting the cancer cell growth. As reported by several studies, different selenium compounds were found to have different mechanisms in arresting cell growth *in vitro*[11,15]. Sodium selenite has been found to have greater effects on cell cycle arrest in S-phase than selenomethionine but less effect in G2-M phase[19]. Although selenomethionine has a lower potency than selenium dioxide, selenomethionine has been shown to inhibit tumor cells selectively[13,15,19] suggested that selenite, an inorganic selenium might have non-specific effects on cell growth *in vitro* in contrast to the organic selenium. They showed that selenite induced single strand DNA breaks rapidly when compared to organic selenium such as selenomethionine.

The anticancer effect and toxicity of selenium depend upon the concentration and the chemical form of selenium. Administration of high level of selenium compounds can lead to selenium toxicity in human. For example, it has been reported that a 10 g oral dose of selenium dioxide can cause death in human[20] selenite has been shown to be toxic at just 5 mg/kg^{-1} of dietary supplementation[21].

The first double blind, placebo controlled human selenium supplementation intervention trial was carried out by[22] which included 1312 individuals with non-melanoma skin cancer history. This trial showed that selenium supplementation of humans with 200 μg per day as selenium yeast, containing mostly L-selenomethionine and a small amount of se-methylselenocysteine, had no effect on the primary endpoint of non-melanoma skin cancer but reduced the incidence of lung, prostate and colorectal cancers.

Since selenomethionine is a major natural food form of selenium and the selenomethionine-containing yeast was used in the selenium supplementation clinical trial of [22] selenomethionine has been suggested to be the most appropriate supplemental form of selenium for chemoprevention in human.

Selenium has also been suggested to play an important role as an adjuvant therapy in cancer treatment. It has been suggested to reduce the adverse effect of chemotherapy or radiotherapy[23] enhance the chemotherapeutic effect of Taxol and Doxorubicin [24] and prevent the drug-resistance in cancer patients[25]. Inorganic forms of selenium especially sodium selenite has been shown in many studies as adjuvant therapy of cancer and no adverse effects were observed [23,25].

Selenium dioxide that was shown to have a good potency in inhibiting the proliferation of both breast cancer cell lines in our study might play a role as adjuvant therapy in cancer treatment especially for effective ER-tumor treatment. However, further studies are required to verify this speculation.

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