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Original Article

The Inhibitory Effects of Ascorbic Acid, α-Tocopherol, and Sodium Selenite on Proliferation of Breast Cancer Cell Lines

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Abstract

The role of antioxidants in prevention and treatment of cancers have been reported by several studies. In our investigation we studied the effects of ascorbic acid, α-tocopherol, and sodium selenite on proliferation of two breast cancer cell lines: T47D (estrogen-receptor positive) and MDA-MB-231 (estrogen-receptor negative). We also used 17-β-estradiol as positive control for proliferation of T47D cells. The viability of cells after 7 days of exposure to different concentrations of test compounds was determined by resazurine based method. Ascorbic acid and α-tocopherol significantly inhibited cell growth at a concentration of 10^{-4} M in both cell lines and antagonized the cell proliferation induced by 17-β-estradiol in T47D cells. Sodium selenite at concentrations above 10^{-6} M strongly inhibited the cell growth in both cell lines and suppressed the stimulated growth of T47D cells by 17-β-estradiol. Our results with different strengths of activity of test compounds, further confirmed the findings of previous studies that showed the inhibitory effects of these antioxidants on other malignant cell lines.

Keywords: Antioxidants; Breast cancer; T47D cells; Cell proliferation; α-Tocopherol; ascorbic Acid; Sodium selenite.

Introduction

Breast cancer is one of the most common types of cancers and cause of cancer death in women worldwide (1, 2). The cause of breast cancer is not completely understood, but based on epidemiological studies, most common areas for etiology are endocrine, environmental, and genetic factors (3). The importance of environmental factors was demonstrated by the study on immigrant women from low-incidence to high-incidence areas. This study showed relative equality of incidence between immigrant women and natives (4). Diet, as an obvious environmental factor, is one the most important etiological basis of breast cancer. Animal and epidemiological studies have demonstrated the relationship between dietary fat and incidence of mammary carcinomas (5, 6).

A number of studies have shown that diets containing vegetables and fruits can reduce the incidence of various cancers. These diets provide a wide range of phytochemicals acting as chemopreventive substances including antioxidants, vitamins and minerals (7, 8). These natural and dietary antioxidants like vitamin E, C, α-carotene and selenium have been identified as chemopreventive agents in carcinogenesis by several mechanisms including: a) inhibition or decrease of oxidative stress, b) acting as a co-factor or part of antioxidant enzymes, c) scavenging of free radicals, d) induction of apoptosis, and e) cell cycle arrest (9, 10, 11, 12, 13).

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The aim of this study was to determine the effects of Ascorbic Acid (Vit. C), α-tocopherol (Vit. E), and inorganic salt of selenium (sodium selenite), as known antioxidants which are effective in cancer prevention and treatment, in proliferation of T47D and MDA-MB-231 breast cancer cell lines.

Experimental

Materials

Sodium selenite (S1382), ascorbic acid (A5960), 17-α-estradiol (E-8875) and resazurine (R6892) were purchased from Sigma (Steinheim, Germany) and α-tocopherol (89550) from Fluka (Steinheim, Germany). The stock solutions of estradiol and α-tocopherol were prepared in absolute ethanol and for sodium selenite and ascorbic acid in deionized distilled water. The final concentration of solvent was 0.5% (v/v). The stock solutions were stored in 2-8^{o}C. Subsequent dilutions for all compounds were made in complete culture medium. For culture of cells, RPMI 1640 (21870-076) and without phenol red (11853-030), Dulbecco's Modified Eagle Medium or DMEM (11960-044) and without phenol red (31053-028), FBS (16000-044), L-Glutamine (25030-081), Trypsin-EDTA (25200-71) and Penicillin-Streptomycin were purchased from Gibco BRL (Grand Island, New York), and Charcoal-Dextran Treated FBS or CDT-FBS (SH30068.03) from Hyclone (Logan, Utah).

Cell lines and culture

Human breast cancer cell lines, T47D (estrogen receptor-positive), passage number 18-25, and MDA-MB-231 (estrogen receptor-negative), passage number 44-51 were purchased from American Type Culture Collection (Manassas, Virginia). T47D cells were maintained in RPMI 1640 with added 5% FBS, 1% L-Glutamine and Penicillin-Streptomycin at 37°C in a 5% CO_2 incubator. MDA-MB-231 cells were maintained in DMEM with added 10% FBS, 1% L-glutamine and penicillin-streptomycin at 37°C in a 5% CO_2 incubator. Two days before experiment, the media of cells were removed and phenol red-free medium with CDT-FBS were added to cells.

Methods

Cells were harvested by trypsin-EDTA and resuspended in fresh medium and seeded in 96-well culture plates at a density of 4000 cells/well. After one day of incubation for attaching the cells, the phenol red-free media with CDT-FBS were added to the cells for two days. On day 3, the medium was removed and fresh medium containing sodium selenite, α-tocopherol and ascorbic acid at different concentrations were added to the cells and after 7 days of exposure with only one change of media in between, the plates were prepared for viability assay. Experiments were performed in quadruplicate each time and repeated three to five times.

Viability Assay

For measurement of the cells viability, the resazurine method was applied. This method is designed for spectrophotometric or fluorometric determination of cell numbers as a function of metabolic activity using the dye resazurine. Bioreduction of the dye by viable cells reduces the amount of its oxidized form (blue) and eventually increases the amount of its fluorescent intermediate (red), indicating the degree of cells' viability following exposure to the test compounds, compared to the control. After 7 days of exposure to test compounds, the media of plates were removed and 10% (v/v) resazurine in phenol red-free medium with CDT-FBS was added to cells in plates and placed in the incubator. After 2-4 h of exposure to dye, plates were removed from the incubator and viability was measured fluorometrically through monitoring the increase in fluorescence at a wavelength of 590 nm, using a excitation wavelength of 560 nm, by plate reader. The plate reader was a dual-scanning spectrofluorometer, SPECTRAmax GEMINI XS made by Molecular Devices Co. (Sunnyvale, California).

Statistics

Data are presented as the mean±SD. The significance of results was determined using ANOVA and Dunnett's comparison of test versus control, in which $P<0.05$ was considered statistically significant.

Results

Effects of antioxidants on proliferation of MDA-MB-231 cells

Viability of the cells after 7 days of exposure to test compounds was determined by resazurine method and results are shown in figure 1. Effects of sodium selenite at concentrations more than 10^{-6} M were significantly (P<0.001) inhibitory on cell growth. Ascorbic acid and α-tocopherol at concentrations of 10^{-4} M significantly (P<0.05) reduced the growth of cells. The latter two compounds showed no effect on cell growth at concentrations less than 10^{-5} M.

Effect of antioxidants on proliferation of T47D cells

The response of T47D cells to test antioxidants was similar to MDA-MB-231 cells. Figure 2 shows that sodium selenite had a significant suppressing effect on cell growth at concentrations between 10^{-6} to 10^{-4} M (the highest tested concentration) and below that, showed no effect. Ascorbic acid and α-tocopherol at their highest concentration (10^{-4} M) had a significant inhibitory effect on cell growth. Concentrations < 10^{-5} M had no effect on cell growth.

Effect of antioxidants on proliferation induced by estradiol

17-α-estradiol at a concentration of 10^{-9} M, as a potent ligand for estrogenic receptors, stimulated growth of T47D cells by more than 2-fold (Figure 3). Sodium selenite at concentrations greater than 10^{-6} M, significantly (P<0.001) inhibited the cell proliferation induced by Estradiol. Also α-tocopherol and ascorbic acid at their highest concentration (10^{-4} M) significantly (P<0.01) reduced the proliferation of cells induced by estradiol.

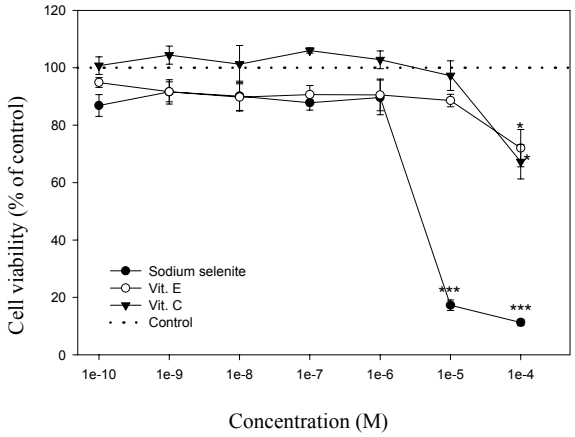

Figure 1. Effects of antioxidants on growth of MDA-MB-231 cells.

Cells were seeded in 96-wells ($4x10^3$ /well) and after 7 days of exposure to different concentrations of test compounds, cell viability was determined by resazurine based method. Data are presented as mean+/-SD (n=4). * P<0.05, *** P<0.001

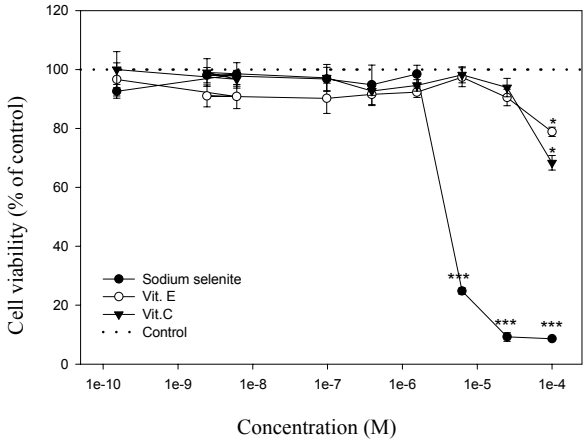

Figure 2. Effects of antioxidants on growth of T47D cells**.**

Cells were seeded in 96-wells ($4x10^3$ /well) and after 7 days of exposure to different concentrations of test compounds, cell viability was determined by resazurine based method. Data are presented as mean+/-SD (n=4). * P<0.05, *** P<0.001

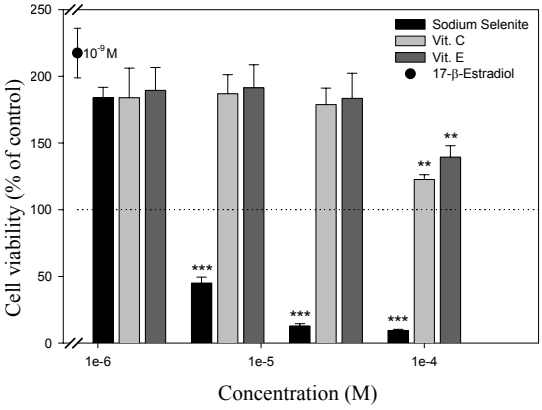

Figure 3. Effects of antioxidants on proliferation induced by 17-α-estradiol on T47D cells.

Cells were seeded in 96-wells ($4x10^3$ /well) and after 7 days of exposure to different concentrations of test compounds in the presence of 10^{-9}M concentration of 17-α-estradiol, cell viability was determined by resazurine based method. Data are presented as mean+/-SD (n=4). ** P<0.01, *** P<0.001

Discussion

In our study, we investigated effects of vitamins E and C as well as sodium selenite at different concentrations on the growth of two breast cancer cell lines, T47D (estrogen

receptor-positive) and MDA-MB-231 (estrogen receptor-negative). α-Tocopherol, one of the tested antioxidants, at its highest concentration (10^{-4} M) had a significant inhibitory effect on the growth of both cell lines. In a similar study, Sigounas and co-workers (14) tested four different concentrations of α-tocopherol on different hormone-responsive cancer cell lines such as MCF-7 (estrogen receptor-positive) and LNCaP (androgen receptor-positive). In their study, α-tocopherol at a concentration of 10^{-4} M, after 6 days of exposure inhibited MCF-7 cell growth by about 25%, whereas in our study the same concentration of α-tocopherol inhibited almost the same percentage in both tested cell lines at day 7, regardless of estrogen receptor status.

Ascorbic acid, another tested antioxidant, is known as an effective factor in prevention and treatment of various types of cancers. In vitro and in vivo studies have shown the effects of ascorbic acid on cell growth to be both inhibitory and stimulatory, depending on various cell types used (9, 13). In our study ascorbic acid showed an inhibitory effect on the cell growth (approximately 30%) in both our tested cell lines. A significant inhibition was observed at a concentration of 10^{-4} M. In a study conducted by Kurbacher and co-workers (15), ascorbic acid was found to have cytotoxic activity at high concentrations (10^{-3}-10^{-4} M), on MCF-7 and MDA-MB-231. They showed that concentrations of 10^{-4} M (moderately cytotoxic) could improve the cytotoxic action of chemotherapeutic agents. In another study, Ascorbic acid at aconcentration of 10^{-3} M, was able to remarkably decrease hepatoma cell growth (16).

In our study sodium selenite, an inorganic salt of selenium, strongly inhibited the growth of T47D and MDA-MB-231 cell lines; Concentrations more than 10^{-6} M had very significant inhibitory effects. In a similar investigation, Stewart and co-workers (17) studied the effect of sodium selenite (at concentrations of 10^{-4} to 10^{-6} M) on colon carcinoma cell lines. They found that concentrations greater than $5x10^{-6}$ M decreased cell growth and above 10^{-5} M induced cell differentiation and apoptosis. The study of Vadgama and co-workers using selenium compounds on various types of cancer cells such as MCF-7 and LNCaP, showed an increase in apoptosis measured by DNA fragmentation. The effect was dose-dependent and optimal inhibition was observed at concentrations between 4 and 40 ng/ml after 72 h of treatment (18).

Improvement in the effects of some anticancer drugs by these antioxidants has been demonstrated by previous studies (15, 18). We also studied the effects of these compounds on proliferation of T47D cells induced by 17-α-estradiol, the most potent ligand for estrogen receptors. As shown in figure 3, 17-α-estradiol stimulated the growth of T47D cells at a rate of more than two-fold, but ascorbic acid and α-tocopherol, at the highest concentration (10^{-4} M), significantly antagonized this effect. Concomitant exposure of T47D cells to 17-α-estradiol (10^{-9} M) and sodium selenite ($>10^{-6}$ M) showed a strong antagonism on cell growth.

Therefore, our results like many other studies on different types of cancer cell lines indicated the useful effects of these compounds and diets containing high amounts of antioxidants, not only in prevention but also in treatment of cancers including the breast cancer.

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