JJBS

Jordan Journal of Biological Sciences

Volume 2, Number 1, March. 2009
ISSN 1995-6673
Pages 37 - 46

Inhibition of the *in Vitro* Growth of Human Mammary Carcinoma Cell Line (MCF-7) by Selenium and Vitamin E

Ahmad M. Khalil *

Department of Biological Sciences,Yarmouk University, Irbid, Jordan.

Abstract

The effects of the natural form of vitamin E; alpha - tocopherol and two forms of selenium; sodium selenite and selenomethionine on the *in vitro* growth of the human mammary cancer cell line; MCF-7 were investigated. Two experimental protocols were used. In the first, each of the test chemicals was included alone at various concentrations. In the second, one of the two selenium compounds was added at concentrations ranging from 1.0 x 10^{-8} M and 1.0 x 10^{-4} M in the presence of a fixed concentration of alpha - tocopherol (1.0 x 10^{-9} M, 1.0 x 10 M^{-8} M, or 1.0 x 10^{-7} M). In the individual treatment, high concentrations of all compounds caused statistically significant, and concentration – dependent decreases in cell viability. These depressions were in the following order: alpha - tochopherol › sodium selenite › selenomethionine. A combined dose of alpha – tocopherol and each selenium form maintained the same antiproliferative effects that were elicited by higher independent concentrations. In both protocols, a relatively higher inhibitory potency of sodium selenite over selenomethionine was obvious. Although the mechanisms of action are not well understood, several ones are discussed.

الملخص

هدفت هذه الدراسة الى البحث في تاثيرات اثنين من اشكال السيلينيوم (سيلينايت الصوديوم و السيلينو مثيونين) و الشكل الطبيعي للفيتامين (ه) و هو الفا توكوفيرول في نمو احد سلالات خلا يا الغدد اللبنية البشرية السرطانية . و لهذا الغرض، تم تنفيذ نوعين من التجارب؛ الاول زرعت فيه الخلايا لمدة ستة ايام بوجود كل مادة كيماوية بمفردها بتركيزات مختلفة. اما في الثاني، فقد احتوى الوسط الغذائي مستويات متفاوتة من احد شكلي السيلينيوم، اضافة الى تركيز محدد من التوكو فيرول. وبينت النتائج، ان التراكيز العالية من المواد الثلاث سببت تناقصا ذ ا دلالة احصائية قي نسبة الخلا يا التي بقيت حية. و يمكن ترتيب هذا الا ثر في التناقص من العالي الى المنخفض على النحو الآتي: الفا توكوفيرول، السيلينايت، السيلينو مثيونين. وعند اضافة احد مركبي السيمينيوم و الفيتا مين معا، ظهرت زيادة واضحة في مقدرة اي منهما في قتل الخلايا السرطانية. ما زالت هناك حاجة لاجراء مزيد من البحوث للكشف عن طبيعة هذه التا ثيرات و امكانية الافادة منها في مكافحة سرطان الثدي

Keywords: Mammary Carcinoma; MCF-7 Cell Line; Selenium; Vitamin E.

1. Introduction

Specific nutrients and dietary constituents are known to be important players in cancer prevention and treatment (Go et al., 2003; Kritchevsky, 2003; Bingham and Riboli, 2004; Menach et al., 2004). It is becoming increasingly clear that treatment of aggressive cancers that have metastasized to distant secondary sites is a daunting task, and expectations that a single agent will eliminate such cancers are not realistic. Furthermore, a chemopreventive agent should ideally be synthetic or natural component of the diet, and must be non-toxic to the host. Breast cancer has become the second cause of death in women, after lung cancer, and the leading cause of death for women between 35 and 54 (Kamangar et al., 2006). Worldwide, every year approximately one million women are newly diagnosed with breast cancer. Selenium (Se) and vitamin E (VE), both naturally occurring, are antioxidants. They are capable of neutralizing toxins known as free radicals that otherwise damage the genetic material of the cell and impair the immune system and possibly lead to cancer (Ambrosone, 1999; Thomson et al., 2007). Free radicals, specifically hydroxyl radicals, have been implicated in spread (metastasis) of breast cancer (Brown and Arthur, 2001). Researchers have determined that women with metastasized breast cancer exhibit twice as much as free radical damage to the breast tissue DNA than women with localized cancer do (Malins et al., 1996).

Accumulating evidence indicates that Se compounds possess anticancer properties (Beisel, 1982; Medina, 1986; Letavayova et al., 2006). Blood levels of selenium have been reported to be low in patients with prostate cancer (Willett et al., 1983). In preliminary reports, people with the lowest blood levels of Se had between 3.8 and 5.8 times the risk of dying from cancer compared with those who had the highest selenium levels (Salonen et al., 1985;

* Corresponding author: kahmad76@yahoo.com

Fex et al., 1987). In cultured tumor cells, supplementation with Se inhibited tumor growth and stimulated apoptosis (programmed cell death) (Ip and Dong, 2001). Selenite, an inorganic Se compound, was reported to induce DNA damage, particularly DNA strand breaks and base damage (Letavayova et al., 2006).

Relatively high blood levels of VE have been associated with relatively low levels of hormones linked to prostate cancer (Hartman et al., 1999). While a relationship between higher blood levels of VE and a reduced risk of prostate cancer has been reported only inconsistently (Hartman et al., 1998; Eicholzer et al., 1999), supplemental use of VE (Chan et al., 1999) has been associated with a reduced risk of prostate cancer in smokers. In a double-blind trial studying smokers, VE supplementation (50 IU of VE per day for an average of six years) led to a 32% decrease in prostate cancer incidence and a 41% decrease in prostate cancer deaths (Heinonen et al., 1998). Both findings were statistically significant (Heinonen et al., 1998). In the latter study, however, VE, *in vitro*, has been shown to enhance the cytotoxic effect of several anticancer drugs.

The goal of this study is to test the validity of the synergistic hypothesis of VE (in the form of natural source; alpha- tocopherol; α-TOH) with inorganic (selenite) and organic (selenomethionine; SeMet) selenium, on the inhibition of the *in vitro* growth of the MCF-7 human epithelial mammary cancer cell line. The remarkable capacity of mammary epithelium to undergo development and differentiation provides a research model in which the factors that influence growth, proliferation, morphologic patterning, and differentiation can readily be explored.

2. Materials and Methods

2.1. Test Chemicals

α-TOH (from Sigma - Aldrech; Steinheim, Germany) was dissolved in ethanol (EtOH), and then taken through step-wise dilutions until the desired concentrations were reached. The final dilution using media brought the final concentration of EtOH to 0.1%, so that it does not affect the cell growth. Selenite and SeMet (both from Sigma – Aldrech) were dissolved step-wise in media until the desired concentrations were reached. Enough EtOH was added to the final Se solutions to bring the 0.1% EtOH.

2.2. Cells and Cell Culture

The human mammary epithelial cancer cell line, MCF-7, is derived from a Caucasian woman with metastatic breast cancer (no. 86012803, European Collection of Cell Culture, Salisbury, UK). This cell line is fully characterized, hormonally responsive and carries various steroid hormone receptors, including estrogen receptors (Marth et al., 1985). Cultures were made and maintained according to Maras et al. (2006). Briefly, the cells were cultured in standard growth medium (Dulbecco's minimum essential medium; DMEM, Gibco BRL, Life Technologies, Paisley, Scotland) supplied with 2 mM glutamine, 1 % nonessential amino acids, 15% heat-inactivated fetal bovine serum (FBS, Gibco BRL), phenol red as an indicator of pH, 1 ml of each antibiotic (penicillin and streptomycin, Gibco BRL) and 1 ml of antimycotics (fungizone, Squibb) per one liter of DMEM.

Untreated cells were grown in monolayer in T-75 plastic culture flasks. Treated cells were grown in monolayer in 6-well polystyrene plates (dia. 33 mm). All cultures were kept in an atmosphere of 5% CO_2 at 37 ºC. Untreated cells were fed with fresh supplemented DMEM medium on average of every three days.

2.3. Treatment

The treated cells were plated into 6-well plates with 2.5 ml of supplemented DMEM at initial density of 2.5 x 10^4 viable cells per well. One day after plating (to allow cells to attach to the surface of the well), solutions of α-TOH, or selenite or SeMet or various combinations, freshly prepared as mentioned above, were added to the wells in 2.5 ml aliquots of supplemented DMEM. α-TOH was administered alone at 1.0 x 10^{-5}, 1.0 x 10^{-6}, 1.0 x10^{7-}, 1.0 x 10^{8-}, and 1.0 x 10^{-9} M. Selenite and SeMet were administered individually at 1.0 x 10^{-4}, 1.0 x 10^{-5}, 1.0 x 10^{-6}, 1.0 x 10^{-7}, and 1.0 x 10^{-8} M. Combinations of α-TOH and Se were performed by keeping concentrations of α-TOH constant and varying those of the Se forms. α-TOH was administered to all cultures at 1.0 x $^{10-7}$ M and selenite or SeMet added in varying amounts of 1.0 x 10^{-5}, 1.0 x 10^{-6}, 1.0 x 10^{-7}, and 1.0 x 10^{-8} M. The same was repeated with α-TOH being kept at 1.0 x 10^{-8} M in all the wells and then again at 1.0 x 10^{-9} M, and the Se compounds were given at the various concentrations indicated above. The control wells were plated at the same time as the treated ones with 2.5 ml of supplemented DMEM and incubated for one day. An additional 2.5 ml of supplemented DMEM containing 0.1% EtOH were added to the control wells when the other wells were treated with 2.5 ml of treated supplemented DMEM.

2.4. Cell Harvesting

Harvesting was always done before the untreated cells become confluent or 6 days after treatment. The untreated cells were always fed the day before harvesting to reduce the trauma caused by the process. After the medium was removed, the cells were washed twice with cool Hank's-balanced salt solution (HBSS) to remove any residual medium. Then, the cells were washed once with 0.1% trypsin. After that, the cells were detached from the flasks or the wells with trypsin and EDTA (0.04%) in a 2: 1 ratio, while on a hot plate at 37 ºC. The detached cells were quickly washed into centrifuge tubes with ambient supplemented DMEM at quantities 10 times that of trypsin in the flask and centrifuged at 1000 rpm for 5 min. The cells were then re-suspended in fresh DMEM plus antibiotics. A small portion of cells was aliquoted for counting and the rest, if untreated, was re-plated at appropriate concentrations.

2.5. Cell Counting/ Viability

The cells were counted using a hemocytometer. The viability was checked by the trypan blue exclusion test.

2.6. Phase-contrast Light Microscopy

Black and white photographs of MCF-7 cells in culture were taken using a camera attached to a phase-contrast microscope.

2.7. Scanning Electron Microscopy

The scanning electron micrographs were taken while the cells were plated in Petri dishes containing circular cover slips with diameters of 12 mm. After that, the supplemented DMEM was removed very gently, and the cells were fixed by very slowly adding glutaraldehyde. After one day, the fixative was removed, and the cells were dehydrated in increasing concentrations of anhydrous EtOH (70%, 95% and 100%); two washings in each concentration for a minimum of 5 min., each. Once the cells were in 100% EtOH, EtOH was removed using CO2 Critical Point Drying. Then, the cover slips were mounted onto the scanning electron microscope (SEM) stubs with silver paints. After allowing the silver paints to dry, the cover slips were gold coated and placed into the SEM for observation and photography.

2.8. Statistics.

Results are expressed as means ± SD of three determinations. Using SPSS, values of control and treatment cells were compared by applying ANOVA on the whole treatments followed by two-sample Student's *t* test between control and each treatment group. The statistical significance of difference ($P \leq 0.05$) for the treatment groups was determined relative to the control group.

3. Results

The shape of the MCF-7 is density-dependent. When plated at low density (1.0×10^4 cells/ cm²), MCF-7 form islands of cells. The cells can migrate outward from a group in an amebiod fashion (Figure 1). Migrating cells appear thinner and more elongated producing a more three-dimensional cell than when stationary. When stationary, cells are provided with enough space and flatten out producing a clear distinction between the endoplasm and the ectoplasm (Figure 2). A degree of polyploidy is evident, and the nucleus usually contains one large dense nucleolus, but may contain as many as five and the presence of two is not uncommon. The exterior of the cells is covered with microvilli (Figure 3).

3.1. Independent Chemical Administration

The number of control MCF-7 cancer cells harvested at the end of culture period (2.66×10^5 cells /well) is used as a standard to 100 % proliferation of the cells. Figure (4) presents the degree of potency between individual treatments with α-TOH, selenite or SeMet. An increase in the concentration of α-TOH from 1.0×10^{-9} M to 1.0×10^{-5} M, as well as selenite and SeMet from 1.0×10^{-8} M to 1.0×10^{-4} M resulted in clear progressive increases in the inhibition of the proliferation of the MCF-7 cancer cells. In general, there were statistically significant differences ($p \leq 0.05$) between the higher concentrations used for each compound relative to the negative control. Also, at all concentrations used, except at 1.0×10^{-9} M (α-TOH) and the lowest two concentrations of selenium (1.0×10^{-8} M and 1.0×10^{-7} M), which showed viability values slightly above the control cell viabilities were far below those of the negative control. At a concentration of 1.0×10^{-6} M, α-TOH showed an inhibition of 37 %, selenite demonstrated an inhibition of 60 %, and SeMet produced an inhibition of 87 %. At this concentration, the viabilities of the independently treated cells (Table 1 and Figure 4) as well as in those treated in combination (data not shown in table 1) were all approximately 90 %.

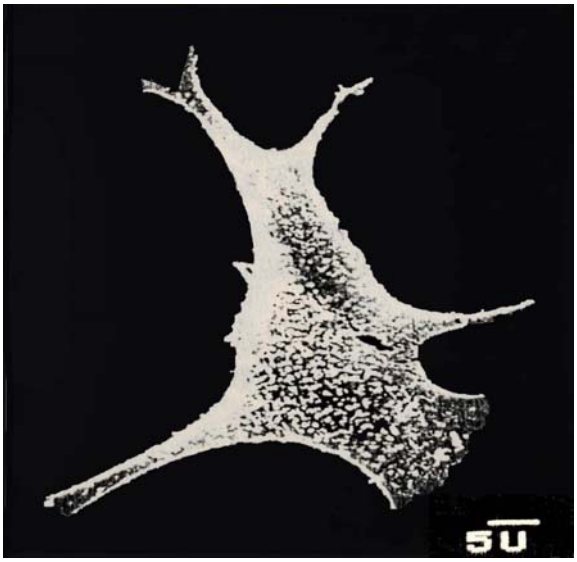

Figure1. Scanning electron micrograph of an MCF-7 human mammary epithelial cancer cell in supplemented DMEM (1000 X). The cell was in the process of extending and retracting pseudopodia to move itself across the cover slip on which it was growing. The white speckles are microvilli, which are less numerous as one goes towards the ends of pseudopodia. The dark patch and breaks in the pseudopodia are artifacts incurred during the drying process.

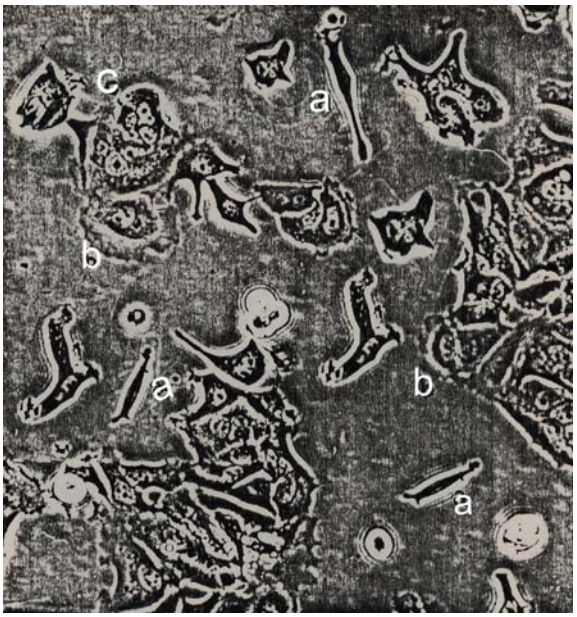

Figure2. A phase-contrast micrograph of live MCF-7 human mammary epithelial cancer cells plated in monolayer in supplemented DMEM (200 X). [a] cells moving across the flask surface. These cells appear dark due to reduced transmitted light through these thicker and more elongated cells than stationary cells [b]. The latter cells have outer, lighter and homogenous ectoplasm as opposed to the inner, darker and heterogeneous endoplasm.

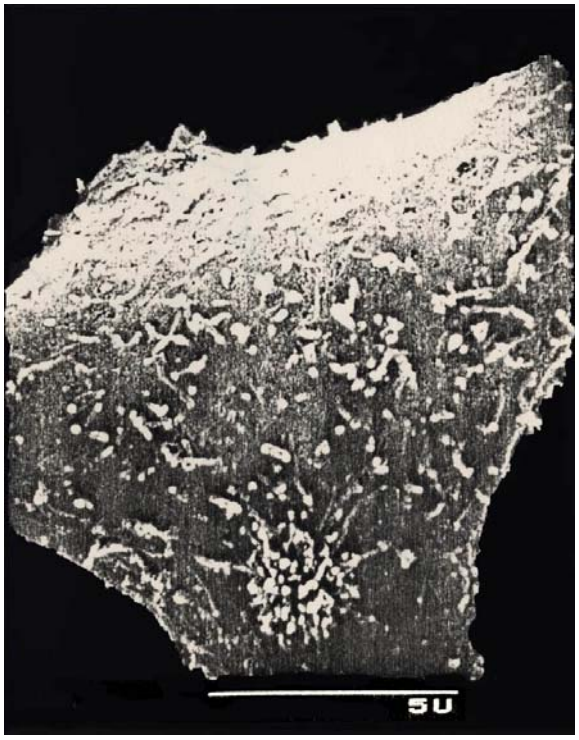

Figure3. Scanning electron micrograph of a portion of the surface of an MCF-7 human mammary epithelial cancer cell in supplemented DMEM (10000 X). Note the presence of microvilli displayed on the cell surface. The aggregation of microvilli near the bottom of the picture is obviously some form of arrested cellular-surface activity, the function of which is not clear. The small darker holes are artifacts incurred during the drying process.

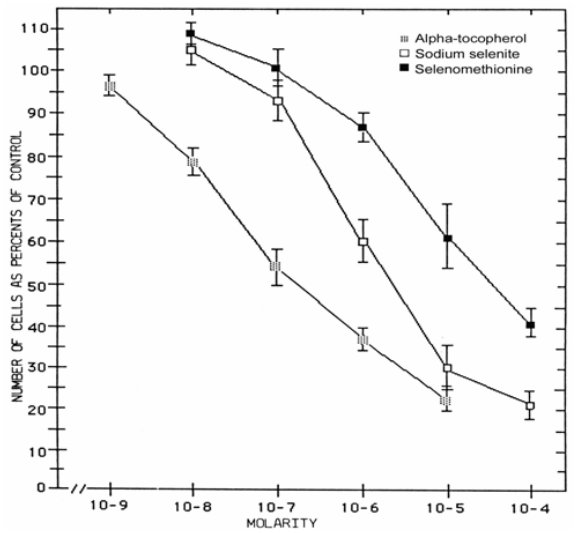

Figure 4. Dose - dependent effects on proliferation of MCF-7 cells by individual treatments. Each data point represents the mean of readings from three wells and the vertical lines are the standard deviations. The results are expressed as percents of the number of cells in the control group (100 % being the control which corresponds to 2.66 x 10^5 cells /33 mm. dia. Well). One day after plating, the cells were independently treated with the indicated concentrations for 6 days.

3.2. Combined Chemical Treatment

In the second protocol, addition of α-TOH with either selenite or SeMet produced a synergistic increase in the level of inhibition of the MCF-7 cells. This is obvious from the significant lines-shift to the left of the combined treatments towards lower concentrations for the same percent proliferation of the control. Table (1) shows the trends of the additive effects on inhibition of proliferation produced by combined treatment and the relative higher potency of selenite over SeMet.

When α-TOH was used alone at 1.0 x 10^{-7} M, the cells were inhibited to 55 % of the control. Selenite, alone, at 1.0 x 10^{-6} M inhibited the cell growth to 60 % of the control. Figure (5) shows that when α-TOH at 1.0 x 10^{-7} M and selenite at 1.0 x 10^{-6} M were used together, much lower percentages of viability relative to control were observed. Similarly, α-TOH, at the same level, in the presence of 1.0 x 10^{-6} M SeMet decreased the number of viable cells to 50 % of the control; which is again below the values recorded for either of the individual treatment; 55 % and 87 % for α-TOH and SeMet, respectively (Table 1 and Figure 6). A higher concentration of SeMet (1.0 x 10^{-5} M) was needed to lower cell viability to 35 % (close to the level [33%] produced with selenite at 1.0 x10^{-6} M). Similar differential inhibition patterns were exhibited when α-TOH was applied at 1.0 x 10^{-8} M (Table 1 and figures 7 and 8) and at 1.0 x 10^{-9} M (Table 1 and figures 9 and 10) with either forms of selenium.

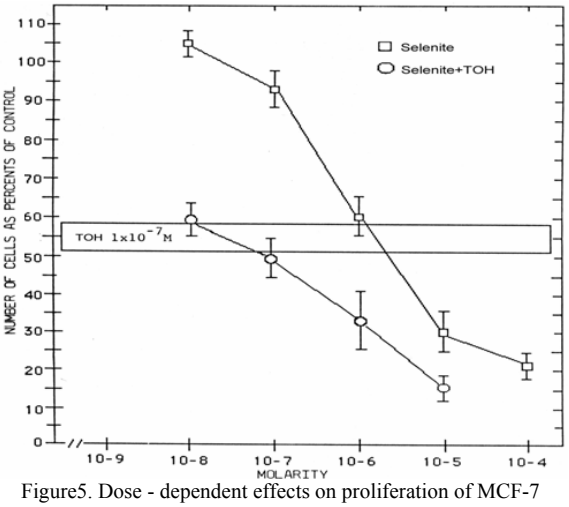

Figure5. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of alpa-tocopherol (α-TOH) at 1.0 x 10^{-7} M with varying concentrations of sodium selenite (selenite). The effects of individual treatment of selenite are plotted for comparison. The horizontal rectangle represents the effects on proliferation of the constant concentration of the α-TOH with the vertical component indicating standard deviation. Other experimental conditions are as in legend of figure. 4.

4. Discussion

This study has demonstrated that α-TOH, selenite, and SeMet caused concentration- dependent decreases in the number of cells harvested as evidenced by the reductions in the cell viability. These decreases may be due to the inhibition of cell proliferation rather than cytotoxicity of the compounds used. The antiproliferative efficacy of the three test chemicals was in the following decreasing order: α-TOH, selenite, SeMet. Furthermore, synergism between

α-TOH and both forms of selenium has been illustrated by treating MCF-7 cells with a combined regimen of α-TOH with a selenium compound. A combined dose of α-TOH and selenium maintained the same antiproliferative effects that were elicited from higher individual concentration. At a certain level of α-TOH administration, higher concentrations of SeMet were needed to produce equivalent inhibitions than those recorded under lower concentrations of selenite.

In reviewing the literature to compare our findings, very few published investigations were encountered. In the present

Table1. Inhibition of proliferation of the MCF-7 human epithelial mammary cancer cell line by α-tocopherol (TOH), sodium selenite (Selenite) and selenomethionine (SeMet) administrated individually or in combination.

Treatment	Concentration (Molar)	Percent of control in Independent Treatment*	Percent Cell Viability	Percent of Control in Combined Treatment (TOH and Selenium) ** TOH (Molar) 1.0×10^{-7}	1.0×10^{-8}	1.0×10^{-9}
TOH	1.0×10^{-5}	23	94	-----	------	-----
	1.0×10^{-6}	37	91	-----	-----	-----
	1.0×10^{-7}	55	95	-----	------	------
	1.0×10^{-8}	79	96	------	-------	------
	1.0×10^{-9}	96	95	-------	-------	-------
Selenite	1.0×10^{-4}	21	88	-------	--------	------
	1.0×10^{-5}	35	94	16	12	13
	1.0×10^{-6}	60	89	33	30	35
	1.0×10^{-7}	93	92	49	56	60
	1.0×10^{-8}	105	99	59	79	80
SeMet	1.0×10^{-4}	41	95	22	17	17
	1.0×10^{-5}	62	93	35	37	40
	1.0×10^{-6}	87	92	50	73	75
	1.0×10^{-7}	101	97	57	97	100
	1.0×10^{-8}	109	98	----	-----	------

- The percents in this column are the number of individually treated cells harvested as percentage of the number of control cells (cells untreated supplemented DMEM plus 0.1 % ethanol) harvested. The number of control cells harvested is equal to 100 %. The MCF-7 cells were either treated with α-TOH, selenite, or SeMet.
- The percents in these three columns are the number of cells harvested after combined treatments as a percentage of the number of control cells harvested. The number of harvested cells is normalized to 100 %. The MCF-7 cells were treated with various concentrations either selenite or SeMet in presence of a constant concentration of 1.0×10^{-7} M, 1.0×10^{-8} M, or 1.0×10^{-9} M.

experiments, consistent changes in the cell shape and appearance of MCF-7 cells were observed 6 days following treatment with α-TOH and/or selenium. Similar results were reported by Schwartz and Shklar (1992) who studied the cytotoxic effects of α-TOH on the in vitro growth of breast and other human tumor cell lines. Although not directly comparable, these findings may explain the observed cellular changes.

The exact molecular mechanisms underlying the development of breast cancer in general and estrogen-associated breast carcinogenesis, in particular, are not completely understood. It is generally believed that the initiation of breast cancer results from uncontrolled cell proliferation, as a consequence of cumulative genetic damages that lead to genetic alterations. In this regard, several molecular defects in the BRCA1 and BRCA2 have been associated with increased incidence in breast cancer (Bonadona et

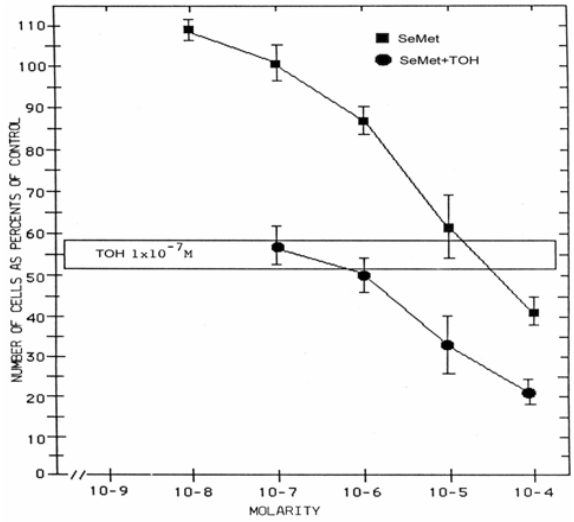

Figure 6. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of α-TOH at 1.0 x 10^{-7} M with varying concentrations of selenomethionine (SeMet). The effects of individual treatment of SeMet are plotted for comparison. Other experimental conditions are as in legends of figs. 4 and 5.

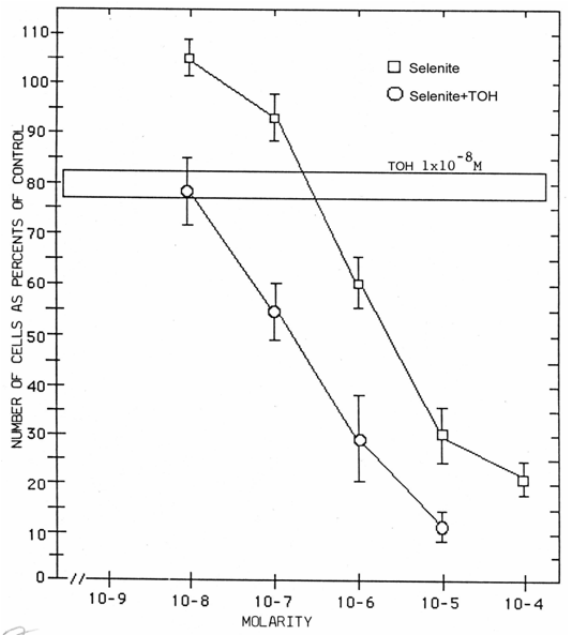

Figure 7. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of α-TOH at 1.0 x 10^{-8} M with varying concentrations of selenite. For details see legend of figure.5.

al., 2005; Vasickova et al., 2007; Krajc et al., 2008). The possibility that has been raised is that decreased lipid peroxidation may be a mechanism responsible, at least in part, for the increased risk associated with several hormonal and non-hormonal risk factors for breast cancer (Ambrosone *et al*., 1999; Gago-Dominguez *et al*., 2005). Selenium has several anti-carcinogenic properties, including protection against oxidation and enhancing nucleotide excision repair. In non-dividing cultured human skin fibroblasts, selenocystine induced significant levels of DNA repair (Whiting et al., 1980). In addition, when women with BRCA1 mutation were given Se for three months**,** the number of chromosome breaks (which can lead to breast cancer) was reduced to normal level (Kowalska et al., 2005). The effects of selenium

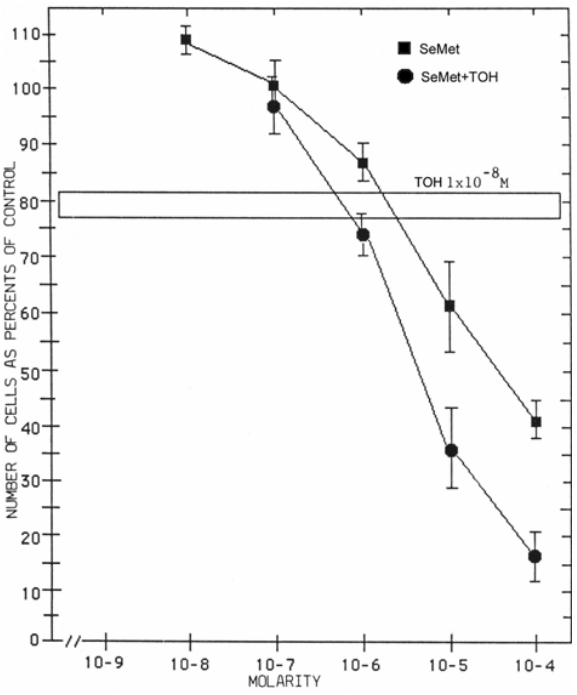

Figure 8. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of α-TOH at 1.0 x 10^{-8} M with varying concentrations of SeMet For details see legend of figure.6

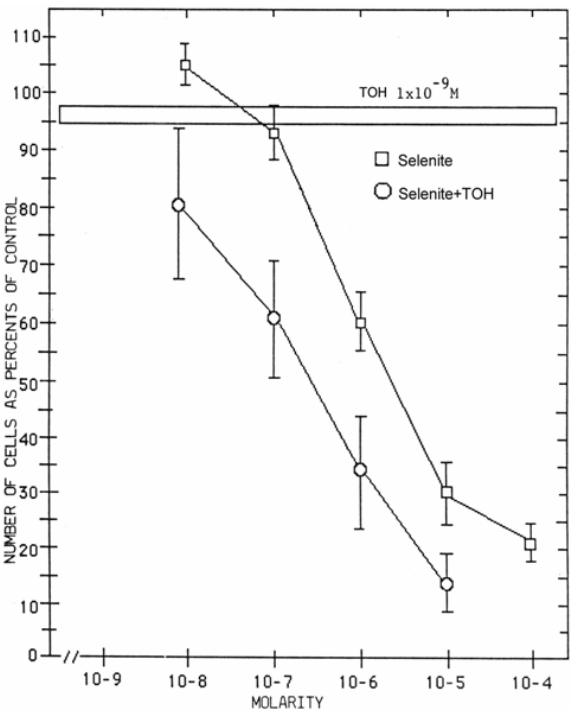

Figure 9. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of α-TOH at 1.0 x 10^{-9} M with varying concentrations of selenite. For details see legend of figure.5.

compounds on the expression levels of growth arrest and DNA damage-inducible (gadd) genes, and on selected cell death genes were examined in mouse mammary MOD cells (Kaeck et al., 1997). Selenium induced growth arrest and death of these cells. They also induced specific patterns of expression of gadd genes indicating that these genes may mediate some selenium-induced cellular responses. The findings further imply that selenium compounds may be effective chemopreventive agents for human breast carcinogenesis, in which p53 mutations are frequent.

Recently, evidence showing an association between Se, reduction of DNA damage, and oxidative stress together with data showing an effect of selenoprotein genotype on cancer risk implies that selenoproteins are indeed implicated (Rayman, 2005).
The observations of this study, as well as those of others, are in accord with previous reports indicating that Se may lead to cell death, and hence delay in cell proliferation as a result of decreased protein (Vernie, 1984) or RNA (Billard and Peets, 1974) synthesis. Another possibility is that selenium, as an important component of the antioxidant enzyme glutathione peroxidase (GPX), inhibits cell proliferation; and in animal studies protects against a variety of cancers (Ip, 1986) including rat mammary tumors (Chidambaram and Baraclarajon, 1996). In another study (Al-Jassabi and Khalil, 2007), selenium was able to protect mouse liver from microcystin-induced oxidative damage. This was evidenced from the favorable changes in the biochemical markers; alanine transaminaes (ALT), liver glycogen content, thiobarbituric acid (TBA), GPX and glutathione-s-transferase (GST).

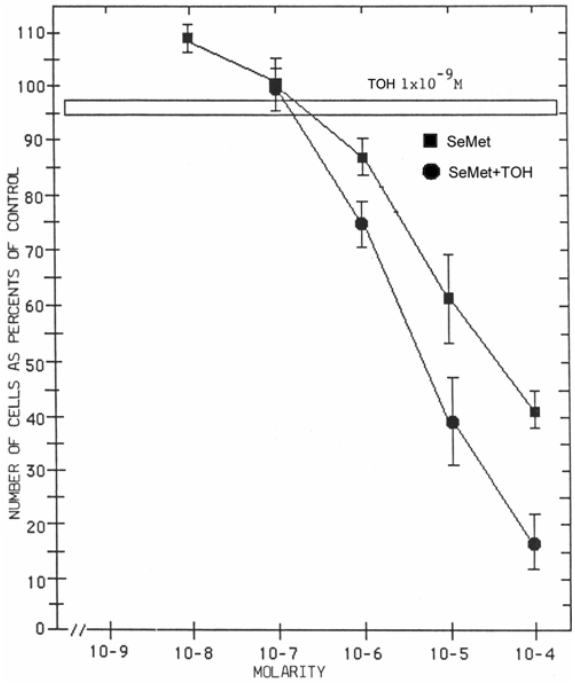

Figure 10. Dose - dependent effects on proliferation of MCF-7 cells by combined treatment of α-TOH at 1.0 x 10 $^{-9}$ M with varying concentrations of SeMet For details see legend of figure.6.

With respect to VE, it was reported that women who did not take this vitamin had a 3.8 times higher risk than did women who were supplemented with VE (Ambrosone *et al*., 1999). A form of VE called VE succinate (VES) has been shown to inhibit the proliferation of estrogen receptor- negative human breast cancer cell lines (Turley et al., 1997). This action, at least in part, is due to induction of apoptosis (or cell death to cancer cells) by VES (Zhao and Yu, 1997). In some experiments (King and McCay, 1983), VE also inhibited mammary tumors in rodents. Not only do the tocopherols quench several of the oxygen free radicals, such as peroxyl, singlet oxygen, and superoxide, but they appear to neutralize some of the nitrogen species as well (Cooney et al., 1993). Nitrogen dioxide, in biological systems, has been recognized as a possible carcinogen that can deaminate DNA bases, resulting in mutations (Christen et al., 1997). In a review of seven case-control and three prospective studies, an inverse association was found between vitamin E intake and breast cancer incidence (Kimmick et al., 1997). Significant inverse relationship between VE intake and pre-menopausal women with a family history of breast cancer was also reported (Ambrosone et al., 1999).

In vitro, VES has been shown to be a potent inhibitor of murine (Slack and Proulx, 1989) and human neuroblastoma (Helson and Parasad, 1983), rat glioma cells (Rama and Prasad, 1983) murine B-16 melanoma cells (Prasad and Edwards-Prasad, 1982), human prostate carcinoma cells (Ripoll et al., 1986), avian lymphoid cells (Kline et al., 1990a) and human promyelocytic cells (Turley et al., 1997). In a more recent study, Al-Jassabi and Khalil (2006) have shown that VE is capable of reducing microcystin-induced damage if administered prior to toxin dose. This effect was explained by VE radical scavenging potentials through inhibition of 8-hydroxydeoxyguanosine, a biomarker for oxidative damage, and generation in DNA. Vitamin E, including the tocotrienols, possesses important cellular functions outside its antioxidant activity, especially in the case of the malignant cell. It was shown that RRR-alpha-tocopherol succinate demonstrated a powerful ability to induce apoptosis in MDA-MB-435 human breast cancer cells in culture (Yu et al., 1997a). At four days following exposure, 74% of the cells were apoptotic. Utilizing antibodies to block Tumor Growth Factor (TGF)-beta, it was shown that the cytotoxic effect of VES could be completely blocked, indicating that the apoptosis was induced by stimulating TGF-beta production. In a further study, Yu and colleagues exposed murine EL-4 T-lymphocytes to VES and found a 95% apoptosis rate within 48 hours (Yu et al., 1997b). Analysis demonstrated that the cells treated with VES were locked in G_1 cell cycle phase, with decreased c-myc and increased bcl-2, c-fos, and c-jun mRNAs. There was also an increase in the transcriptional factor (activation protein1, AP-1) binding. The exact cause of the induced apoptosis remains unknown and is not entirely related to TGF-beta since VES can induce cell arrest in non-TGF-beta-responsive human prostate cells. It is important to note that cell arrest and cell growth inhibition affect only cancer cells and not normal cells which makes VES a valuable adjunct in the treatment of cancer. Another way VE affects cancer growth is by stimulating the immune system. Vitamin E has been shown to enhance both cellular and humoral immunity and to induce macrophages to produce elevated levels of interleukin-1 (IL-1) and/or down-regulate prostaglandin E2 (PGE2) synthesis (Tengerdy and Brown, 1977; Kline et al., 1990b; Meydani et al., 1990). Elevated PGE2 is known to suppress immunity. Vitamin E has been shown to inhibit the activation of phospholipase A2 and hence the initiation of the eicosanoid cascade (Douglas et al., 1986).

In conclusion, selenium at both low and high concentrations induced growth arrest and death of human breast cancer MCF-7 cells and enhanced rather than antagonized the anticancer effect of α-TOH. The mechanism(s) of this enhanced interactive effect is not clear yet, but may be related to their complementary action as antioxidants. Although these results have been generated with an *in vitro* system using a single cell line, they are encouraging and provide some scientific justification for further research using other mammary carcinoma cell lines as well as for clinical testing in both pre- and post-menopausal breast cancer patients.

References

Al-Jassabi S and Khalil A. 2007.The role of selenium and lycopene in the protection of mice against microcystin-induced toxicity. Abhath Al-Yarmouk Basic Sci. Eng. 16, 49-57.

Al-Jassabi S and Khalil A. 2006. Microcystin-induced 8-hydroxydeoxyguanosine in DNA and its reduction by melatonin, vitamin C and vitamin E in mice. Biochemistry (Moscow). 71, 1115-1119.

Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T and Shields PG. 1999. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer Res. 59, 602-606.

Beisel WR. 1982. Single nutrients and immunity. Am. J. Clin. Nutr. 35, 417-68.

Billard W and Peets E. 1974. Sulfhydryl reactivity: mechanism of action several antiviral compounds selenocyctine, 4-(-α-propinyloxy)-β-nitrostyrene and acetylaranotin. Antimicrob. Agents Chemoth. 5, 19-24.

Bingham S and Riboli E. 2004. Diet and cancer-the European prospective investigation into cancer and nutrition. Nat. Rev. Cancer. 4, 206-215.

Bonadona V, Sinilnikova OM, Chopin S, Antoniou AC, Mignotte H, Mathevet P, Brémond A, Martin A, Bobin JY, Romestaing P, Raudrant D, Rudigoz RC, Léoné M, Chauvin F, Easton DF, Lenoir GM and Lasset C. 2005. Contribution of BRCA1 and BRCA2 germ-line mutations to the incidence of breast cancer in young women: results from a prospective population-based study in France. Genes Chromosomes Cancer. 43, 404-413.

Brown KM and Arthur JR. 2001 Selenium, selenoproteins and human health: a review. Public Health Nutr. 4(2B) :593-599.

Chan JM, Stampfer MJ, Ma J, Rimm EB, Willett WC and Giovannucci EL. 1999. Supplemental vitamin E intake and prostate cancer risk in a large cohort of men in the United States. Cancer Epidemiol. Biomarkers Prev. 8, 893-899.

Chidambaram N and Baraclarajon A. 1996. Influence of selenium on glutathione and some associated enzymes in rats with mammary tumor induced by 7, 2-dimethyl6enz (o)onthracene. Mal. Cell Biochem.156, 101-07.

Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW and Ames BN. 1997. Gamma- tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. Proc. Natl. Acad. Sci. USA. 94, 3217-3222.

Cooney RV, Franke AA, Harwood PJ, Hatch-Pigott V, Custer LJ, Mordan LJ. 1993. Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha- tocopherol. Proc. Natl. Acad. Sci. USA. 90, 1771-1775.

Douglas CE, Chan A and Choy PC. 1986. Vitamin E inhibits platelet phospholipase A2. Biochem. Biophys. Acta. 876, 639-645.

Eicholzer M, Stäelin H, Lüdin E and Bernasconi F. 1999. Smoking, plasma vitamins C, E, retinol, and carotene, and fatal prostate cancer: seventeen-year follow-up of the Prospective Basel Study. Prostate. 38, 189-198.

Fex G, Pettersson B and Akesson B. 1987. Low plasma selenium as a risk factor for cancer death in middle-aged men. *Nutr Cancer* 10, 221-229.

Gago-Dominguez M, Castelao JE, Pike MC, Alex Sevanian A and Haile RW. 2005. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. Cancer Epidemiol. Biomark. Prevent. 14, 2829-2839.

Go V.L.W., Butrum, R. R. and Wong, D. A. 2003. Diet, nutrition, and cancer prevention: The postgenomic era. J. Nutr. 133, 3830S-3836S.

Hartman TJ, Albanes D, Pietinen P, Hartman AM, Rautalahti M, Tangrea JA and Taylor PR. 1998.The association between baseline vitamin E, selenium, and prostate cancer in the alpha-tocopherol, beta-carotene cancer prevention study. Cancer Epidemiol. Biomarkers Prev. 7, 335–340.

Hartman TJ, Dorgan JF, Virtamo J, Tangrea JA, Philip R. Taylor PR, Albanes D. 1999. Association between serum α-tocopherol and serum androgens and estrogens in older men. Nut. Cancer. 35, 10–15.

Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, Haapakoski J, Malila N, Rautalahti M, Ripatti S, Maenpaa H, Teerenhovi L, Koss L, Virolainen M and Edwards BK.1998. Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J. Nat. Cancer Inst. 90, 440–446.

Helson L, Verma M and Helson C. 1983. Vitamin E and human neuroblastoma. In: Meyskens FL and Prasad KN, (editors) Modulation and Mediation of Cancer by Vitamins. Basel: Karger, p. 258-265.

Ip C 1986. The chemopreventive role of selenium in carcinogenesis. Adv. Exp. Med. Biol. 206, 431-447.

Ip C and Dong Y. 2001 Methylselenocysteine modulates proliferation and apoptosis biomarkers in premalignant lesions of the rat mammary gland. Anticancer Res. 21(2A):863-867.

Kaeck M, Lu J, Strange R, Ip C, Ganther HE and Thompson HJ. 1997. Differential induction of growth arrest inducible genes by selenium compounds. Biochem. Pharmacol. (USA), 53, 921-926.

Kamangar F, Dores GM and Anderson WF. 2006. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J. Clin. Oncol. 24, 2137 – 2150.

Kimmick GG, Bell RA and Bostick RM. 1997. Vitamin E and breast cancer: a review. Nutr Cancer. 27, 109-117.

King MM and McCay PB. 1983. Modulation of tumor incidence and possible mechanisms of inhibition of mammary carcinogenesis by dietary antioxidants. Cancer Res. Suupl. 2485S-2490S.

Kline K, Cochran GS and Sanders BG. 1990a. Growth-inhibitory effects of vitamin E succinate on retrovirus-transformed tumor cells in vitro. Nutr. Cancer. 14, 27-41.

Kline K, Rao A, Romach EH, Kidao S, Morgan TJ, Sanders BG. 1990b. Vitamin E effects on retrovirus-induced immune dysfunctions. Ann. NY Acad. Sci. 587, 294-296.

Kowalska E, Narod SA, Huzarski T, Zajaczek S, Huzarska J, Gorski B, Lubinski J. 2005. Increased rates of chromosomes breakage in BRCA1
carriers are normalized by oral selenium supplementation, Cancer Epidemiol.
Biomark. Prevent. 14, 1302-306.

Krajc M, Teugels E, Zgajnar J, Goelen G, Besic N, Novakovic S, Hocevar M and De Grève J. 2008. Five recurrent BRCA1/2 mutations are responsible for cancer predisposition in the majority of Slovenian breast cancer families. BMC Med. Genet. 9, 83 [on line].

Kritchevsky D. 2003. Diet and cancer: What's next? J. Nutr. 133, 3827S-3829S.

Letavayova L, Vlckova V and Brozmanova J. 2006. Selenium: From cancer prevention to DNA damage. Toxicology. 227, 1-14.

Malins, DC, Polissar, NL and Gunselman SJ. 1996. Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. Proc. Natl. Acad. Sci. USA, 2557-63

Maras M, Vanparys C, Muylle F, Robbens J, Berger U, Barber J L, Blust R, and De Coen W. 2006. Estrogen-like properties of fluorotelomer alcohols as revealed by MCF-7 breast cancer cell proliferation. Environ.Health Perspect.114, 100-105.

Marth C., Bock G., Daxenbichler G. 1985. Effect of 4-hydroxyphenylretinamide and retinoic acid on proliferation and cell cycle of cultured human breast cancer cells. J. Natl. Cancer Inst., 75: 871-875.

Menach C, Scalbert A, Morand C, Remesy C. and Jimenez L. 2004. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 79, 727-747.

Medina D. 1986. Mechanisms of selenium inhibition of tumorigenesis. Adv. Exp. Med. Biol. 206, 465–72.

Meydani SN, Barklund MP, Liu S, Meydani M, Miller RA, Cannon JG, Morrow FD, Rocklin R and Blumberg JB. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. Am. J. Clin. Nutr. 52, 557-563.

Prasad KN and Edwards-Prasad J. 1982. Effects of tocopherol (vitamin E) acid succinate on morphological alterations and growth inhibition in melanoma cells in culture. *Cancer Res.* 42, 550-554.

Rama BN and Prasad KN. 1983. Study on the specificity of alpha tocopherol (vitamin E) acid succinate effects on melanoma, glioma and neuroblastoma cells in culture. Proc. Soc. Exp. Biol Med. 174, 302-307.

Rayman MP. 2005. Selenium in cancer prevention: a review of the evidence and mechanism of action. Proc. Nutr. Soc. 2005, 64:527-42.

Ripoll EAP, Rama BN, and Webber M. 1986. Vitamin E enhances the chemotherapeutic effects of adriamycin on human prostate carcinoma cells in vitro. J. Urol.136, 529-531.

Salonen J, Salonen R, Lappetelainen R, Maenpaa P H, Alfthan C., and Puska P. 1985. Risk of cancer in relation to serum concentrations of selenium and vitamins A and E; matched case-control analysis of prospective data. BMJ 290, 417–420.

Schwartz J and Shklar G. 1992. The selective cytotoxic effect of carotenoids and alpha-tocopherol on human cancer cell lines in vitro. J. Oral Maxillofac. Surg. 50:367-373.

Slack R and Proulx P. 1989. Studies in the effects of vitamin E on neuroblastoma NIE 115. Nutr. Cancer. 12, 75-82.

Tengerdy RP and Brown JC. 1977. Effect of vitamin E and A on humoral immunity and phagocytosis in E.coli infected chickens. Poultry Sci. 56, 957-963.

Thomson CA, Stendell-Hollis NR, Rock CL, Cussler EC, Flatt SW and Pierce JP. 2007. Plasma and dietary carotenoids are associated with reduced oxidative stress in women previously treated for breast cancer. Cancer Epidemiol. Biomark. Prevent. 16, 2008-2015.

Turley JM, Ruscetti FW, Kim SJ, Fu T, Gou FV, and Birchenall-Roberts MC. 1997. Vitamin E succinate inhibits proliferation of BT-20 human breast cancer cells: increased binding of cyclin A negatively regulates E2F transoctivation octivity. Cancer Res. 57, 2668- 2675.

Vasickova P, Machackova E, Lukesova M, Damborsky J, Horky O, Pavlu H, Kuklova J, Kosinova V, Navratilova M, and Foretova L. 2007. High occurrence of BRCA1 intragenic rearrangements in hereditary breast and ovarian cancer syndrome in the Czech Republic. BMC Med. Genet. 8, 32. [On Line].

Vernie LN. 1984. Selenium carcinogenesis. Biochem. Biophys. Acta. 738, 203-217.

Whiting RF, Wei l. and Stich HF. 1980. Unscheduled DNA synthesis and chromosome aberrations induced by inorganic and organic selenium compounds in the presence of glutathione. Mutat. Res. 78, 159-169.

Willett WC, Polk BF, Morris JS, Stampfer MJ, Pressel S, Rosner B, Taylor JO, Schneider K, and Hames CG. 1983. Prediagnostic serum selenium and risk of cancer. Lancet. 42, 130–134.

Yu W, Sanders BG, and Kline K. 1997 a. RRR-alpha-tocopherol succinate inhibits EL4 thymic lymphoma cell growth by inducing apoptosis and DNA synthesis arrest. Nutr. Cancer. 27, 92-101.

Yu W, Heim K, Qian M, Simmons-Menchaca M, Sanders BG, and Kline K. 1997 b. Evidence for role of transforming growth factor-beta in RRR-alpha-tocopherol succinate-induced apoptosis of human MDA-MB-435 breast cancer cells. Nutr Cancer. 27, 267-278.

Zhao B and Yu W. 1997. Involvement of activator protein- 1 (AP-1) in induction of opoptosis by vitamin E succinote in human breast cancer cells. Mal. Carcinog. 3, 180-190.