

## Review

# $\alpha$ -Tocopheryl Succinate, the Most Effective Form of Vitamin E for Adjuvant Cancer Treatment: A Review

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**Key words:**  $\alpha$ -tocopheryl succinate, cancer, gene expressions, differentiation, growth inhibition, apoptosis

In 1982, it was established that alpha-tocopheryl succinate ( $\alpha$ -TS) was the most effective form of vitamin E in comparison to  $\alpha$ -tocopherol,  $\alpha$ -tocopheryl acetate and  $\alpha$ -tocopheryl nicotinate in inducing differentiation, inhibition of proliferation and apoptosis in cancer cells, depending upon its concentration. During the last two decades, several studies have confirmed this observation in rodent and human cancer cells in culture and *in vivo* (animal model). The most exciting aspect of this  $\alpha$ -TS effect is that it does not affect the proliferation of most normal cells. In spite of several studies published on the anti-cancer properties of  $\alpha$ -TS, the value of this form of vitamin E has not drawn significant attention from researchers and clinicians. Therefore, a critical review on the potential role of  $\alpha$ -TS in the management of cancer is needed. In addition, such a review can also provide in-depth analysis of existing literature on this subject.  $\alpha$ -TS treatment causes extensive alterations in gene expression; however, only some can be attributed to differentiation, inhibition of proliferation and apoptosis.  $\alpha$ -TS also enhances the growth-inhibitory effect of ionizing radiation, hyperthermia, some chemotherapeutic agents and biological response modifiers on tumor cells, while protecting normal cells against some of their adverse effects. Thus,  $\alpha$ -TS alone or in combination with dietary micronutrients can be useful as an adjunct to standard cancer therapy by increasing tumor response and possibly decreasing some of the toxicities to normal cells.

### Key teaching points:

- $\alpha$ -TS is the most effective form of vitamin E in comparison to  $\alpha$ -tocopherol,  $\alpha$ -tocopheryl acetate and  $\alpha$ -tocopheryl nicotinate in inducing differentiation, inhibition of proliferation and apoptosis in cancer cells without affecting the proliferation of most normal cells.
- $\alpha$ -TS treatment causes extensive alterations in gene expression in tumor cells; however, only some can be attributed to differentiation, inhibition of proliferation and apoptosis. Not only is the effect of  $\alpha$ -TS on inhibition of proliferation in cancer cells independent of antioxidant action, but the well-established antioxidants,  $\alpha$ -tocopherol and butylated hydroxyanisole (having antioxidant activity similar to  $\alpha$ -tocopherol but without vitamin E action), also may inhibit proliferation of cancer cells by mechanisms that are independent of their antioxidation activity.
- $\alpha$ -TS enhances the growth-inhibitory effects of ionizing radiation, chemotherapeutic agents, hyperthermia and some biological response modifiers on cancer cells, but not on normal cells.
- $\alpha$ -TS, together with dietary micronutrients, can be used as an adjunct to standard and experimental cancer therapies in order to improve their efficacy.

## INTRODUCTION

Although vitamin E was first recognized in 1922 [1], its isolation was not achieved until 1936 [2]. Subsequently, the structural formula of vitamin E was found to be  $C_{29}H_{50}O_2$  [3], and soon afterwards, its chemical synthesis was accomplished.

The name tocopherol for vitamin E was derived from the Greek term meaning "to bear offspring", because it was first recognized to be essential for reproduction in rats. Among various forms of  $\alpha$ -tocopherol ( $\alpha$ -T),  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  forms are important.  $\alpha$ -tocopherol is considered the most biologically active form of vitamin E,  $\beta$ -tocopherol possesses 25% to 40% of the activity

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This study was supported by Shafroth Memorial Fund.

Journal of the American College of Nutrition, Vol. 22, No. 2, 108–117 (2003)  
Published by the American College of Nutrition

of  $\alpha$ -T, while  $\gamma$ -tocopherol has only 20% of the activity of  $\alpha$ -T. Esterified forms of vitamin E such as  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA),  $\alpha$ -tocopheryl nicotinate ( $\alpha$ -TN) and  $\alpha$ -tocopheryl succinate ( $\alpha$ -TS) are also commercially available.

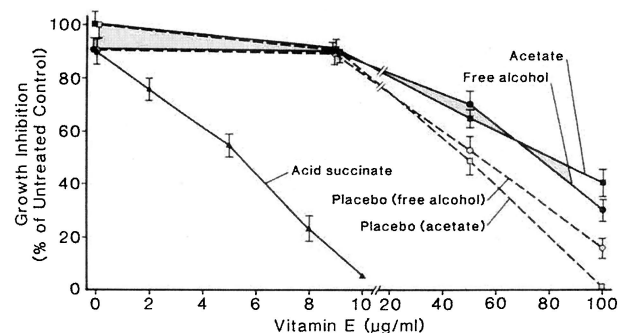
Although a few reviews have focused on vitamin E and cancer prevention [4–6], the role of vitamin E in cancer treatment has not received adequate attention. This may have been due to the fact that the most widely used forms of vitamin E ( $\alpha$ -T and  $\alpha$ -TA dissolved in ethanol) exhibited no anti-cancer activity on tumor cells in culture or *in vivo*. Since the discovery of  $\alpha$ -TS as the most effective form of vitamin E in comparison to the water soluble preparation of  $\alpha$ -T, and  $\alpha$ -TA or ethanol soluble  $\alpha$ -T,  $\alpha$ -TA and  $\alpha$ -TN for inducing differentiation, inhibition of cell proliferation and cell death in murine melanoma cells in culture [7], several publications have shown that  $\alpha$ -TS produces similar effects on a variety of human and rodent tumor cell lines without affecting the proliferation of most normal cells *in vitro* [8–37] and *in vivo* [10,19,20,38–41]. Results of several studies have revealed that  $\alpha$ -TS affects these biological processes by altering expression of those genes that are involved in differentiation, regulation of proliferation and apoptosis [13–15,17–19,23,31,34–37,42–48]. In addition, the role of  $\alpha$ -TS in modifying the effects of radiation [49], chemotherapeutic agents [11,21,22], hyperthermia [50,51] and biological response modifiers [17,20,52–54] on tumor and normal cells has also been investigated. Recently, Kline *et al.* [34] and Neuzil *et al.* [55] have reviewed anti-cancer activity of  $\alpha$ -TS and its potential mechanisms. It has been suggested that  $\alpha$ -TS exhibits dual functions: anti-cancer activity when present as  $\alpha$ -TS and anti-inflammatory effect when converted to  $\alpha$ -T [55]; however, Weber *et al.* [20] have reported that  $\alpha$ -T exhibits a weak anti-cancer activity *in vivo* in comparison to  $\alpha$ -TS. These reviews did not include studies on the interaction between  $\alpha$ -TS and standard and experimental cancer therapeutic agents. Furthermore, in view of the fact that  $\alpha$ -TS may be more useful in cancer treatment when employed in combination with current therapeutic modalities than by itself, a detailed critical review on  $\alpha$ -TS and cancer treatment would be helpful.

This review provides the following: (a) a brief description of the historical perspective of the discovery of  $\alpha$ -TS as an effective anti-cancer agent in inducing differentiation, inhibition of proliferation and apoptosis; (b) changes in expressions of genes and their products, and their translocation after  $\alpha$ -TS treatment, and (c) the interaction of  $\alpha$ -TS with commonly used tumor therapeutic agents and some biological response modifiers on cancer cells and normal cells, and their potential mechanisms of action.

## HISTORICAL PERSPECTIVE OF THE DISCOVERY OF $\alpha$ -TS AS AN ANTI-CANCER AGENT

It is essential that a review provides a brief description of the scientific processes leading to the discovery of a new

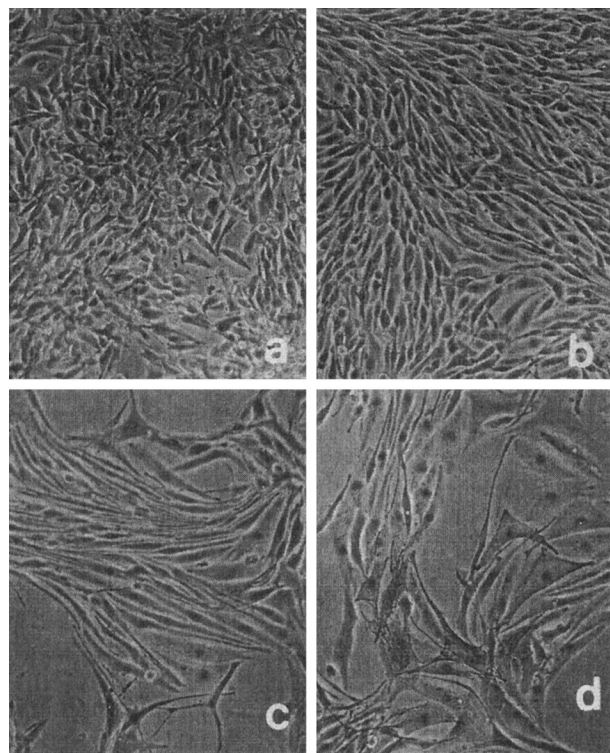
carcinogenic mechanism or a novel anti-cancer agent in order to acquaint researchers with the methods or events that led to such a discovery. In 1979, we demonstrated, for the first time, that aquasol vitamin E (dl- $\alpha$ -TA, USV laboratories, Tucoma, N.Y.) induced morphological differentiation and enhanced the effect of x-irradiation on murine neuroblastoma (NB) cells in culture [56] and certain chemotherapeutic agents on NB and rat glioma cells [57]. Subsequently, it was found that the solvent for aquasol vitamin E by itself was toxic to melanoma cells, suggesting that part of vitamin E's effect could have been due to its solvent [7]. The solvent was considered a trade secret; therefore, the solvent composition was never revealed. In order to avoid the inherent difficulty of using an aqueous form of vitamin E,  $\alpha$ -T and  $\alpha$ -TA obtained in oil were dissolved in ethanol. Both forms of vitamin E in this solvent were found to be inactive in melanoma cells in culture [7]. This led to the use of two other esters of vitamin E,  $\alpha$ -TN and  $\alpha$ -TS. These forms of the vitamin E were never tested in any experimental systems. In 1982, we reported that  $\alpha$ -TS induced morphological and biochemical differentiation, inhibition of proliferation and cell death in murine melanoma (B16) cells in culture, depending upon the concentration of  $\alpha$ -TS (Fig. 1). It is interesting to note that the dose range of  $\alpha$ -TS between growth inhibition and lethality is very narrow. A concentration of about 5  $\mu$ g/mL (9.4  $\mu$ M) of  $\alpha$ -TS caused about 50% growth inhibition without any significant cell death, whereas a concentration of 10  $\mu$ g/mL (18.8  $\mu$ M) caused almost 100% lethality. When the effect of aqueous preparations of vitamin E was compared with  $\alpha$ -TS, it



**Fig. 1.** Effect of various forms of tocopherol (vitamin E) on the growth of mouse melanoma (B-16) cells in culture. Cells ( $10^5$ ) were plated in Lux tissue culture dishes (60 mm). Twenty-four hours after plating, D- $\alpha$ -tocopherol acid succinate (Sigma; soluble in ethanol), DL- $\alpha$ -tocopherol free alcohol (Hoffmann-La Roche; soluble in specialized solvent) and Aquasol DL- $\alpha$ -tocopherol acetate (USV Laboratories; soluble in specialized solvent) at various concentrations were added individually to separate dishes. Growth medium and drug were changed at two days after treatment, and the growth inhibition, based on the amount of protein per dish, in the treated culture was determined at three days after treatment. The average value of the untreated controls was considered 100%, and the growth inhibition of treated cultures was expressed as percentage of untreated controls. The amount of protein per dish in the untreated cultures was  $850 \pm 67 \mu$ g. Each point on the curve represents an average of nine samples; bars, S.D. [7].

was found that  $\alpha$ -TS was more effective than  $\alpha$ -T or  $\alpha$ -TA in reducing the growth of murine melanoma cells in culture (Fig. 2). Thus,  $\alpha$ -T and  $\alpha$ -TA in water soluble preparations exhibit a weak anti-cancer activity *in vitro*, whereas ethanol soluble  $\alpha$ -T and  $\alpha$ -TA were inactive.

During 1982–1990, we published additional papers [8,9,49–53,58] showing that  $\alpha$ -TS inhibited the growth of other tumor cell lines such as murine NB (NBP2) and rat glioma (C6) cells in culture, inhibited ligand-stimulated response of adenylate cyclase, enhanced the effect of x-irradiation hyperthermia, cAMP and sodium butyrate. We also showed that  $\alpha$ -TS increased PKA activity [59] and reduced the expression of c-myc and H-ras [42,43] in tumor cells in culture. During this period, only a few studies on  $\alpha$ -TS and cancer were published by others. It was demonstrated that  $\alpha$ -TS inhibited the growth of human NB cells in culture and in nude mice [10] and reduced the growth of oral cancer cells in hamster pouch [38]. Ripoll *et al.* demonstrated that  $\alpha$ -TS reduced the growth of human prostate carcinoma cells (DU-145) in culture



**Fig. 2.** Melanoma cells ( $10^5$ ) were plated in Lux tissue culture dishes (60 mm), and D- $\alpha$ -tocopherol (vitamin E) acid succinate (soluble in ethanol) and sodium succinate plus ethanol were added to separate cultures 24 hours after plating. Drugs and medium were changed at two and three days after treatment. Photomicrographs were taken four days after treatment. Control culture contained fibroblastic cells as well as round cells in clumps (a) Cultures treated with ethanol (1%) and sodium acid succinate (5 to 6  $\mu$ g/mL) also exhibited fibroblastic morphology with fewer round cells (b). Vitamin E acid succinate-treated cultures [5  $\mu$ g/mL (c); 6  $\mu$ g/mL (d)] showed a dramatic change in morphology.  $\times 450$  [7].

and enhanced the growth-inhibitory effect of adriamycin on these cells [11]. Two papers that were relevant to cancer prevention were published independently by Radner and Kennedy [60] and Borek *et al.* [61]. They showed that  $\alpha$ -TS treatment was most effective in reducing radiation-induced transformation of murine fibroblast cells (CH3/T101/2 clone).

During the 1990's, the Kline group published a series of articles [12–18,23,35] on the mechanisms of growth inhibitory action of  $\alpha$ -TS on tumor cells that generated increased interest in the anti-cancer potential of this form of vitamin E. In addition, two *in vivo* studies on the role of  $\alpha$ -TS in the prevention of chemical-induced cancer in hamster buccal pouches were reported [62,63].

In 2000–2002, the demonstration that IP administration of  $\alpha$ -TS inhibited the growth of several tumor types in athymic mice by Malafa *et al.* [39–41], Neuzil *et al.* [19] and Weber *et al.* [20] is going to have considerable impact on further evaluating the anti-cancer potential of  $\alpha$ -TS. Furthermore, Neuzil *et al.* have also shown that  $\alpha$ -TS treatment did not affect the proliferation of several human normal cell lines while inhibiting the growth of several human cancer cell lines [24]. This should further support the idea that  $\alpha$ -TS treatment exhibits high specificity for inhibiting the proliferation of cancer cells. Previous studies [60–63] together with a recent study published by Wu *et al.* [64] may stimulate research on the role of  $\alpha$ -TS in cancer prevention.

### ***In vitro* Data on $\alpha$ -TS-Induced Differentiation, Inhibition of Proliferation and Apoptosis in Cancer Cells**

$\alpha$ -TS induced differentiation, inhibition of cell proliferation and cell death in murine B-16 melanoma cells in culture, depending upon the concentration [7].  $\alpha$ -TS also inhibited growth in human melanoma cells [21], murine NB cells [8], rat glioma cells [8], human NB cells [10], human prostate cancer cells [11,18,31,36] human parotid carcinoma cells [22], human breast cancer cells [14,15,17,32,35,45], virally transformed rodent cancer cells [12], several cell lines of human hematopoietic cancer [13,16,24,25], adenocarcinoma cells of lung (A549), bronchiocarcinoma (BEAS-2B), several cell lines of colon carcinoma [24], human gastric carcinoma (SgC-7901) [26,27], human pancreatic cancer cells [28], human cervical and ovary cells [29] and human oral squamous cell carcinoma [30]. These data show that  $\alpha$ -TS can reduce the proliferation of tumor cells of different cellular origin. Concentrations used in these studies varied from 10–50  $\mu$ M, and treatment time varied from a few hours to a few days. The types of effect (differentiation, inhibition of proliferation and apoptosis) depended upon the concentration of  $\alpha$ -TS, period of treatment, form of tumor cells and culture conditions. Lower concentrations can cause cell differentiation, inhibition of proliferation, whereas higher concentrations can induce apoptosis in tumor cells. Longer treatment times are needed for a maximal effect of  $\alpha$ -TS on cancer cells.

In contrast to the growth-inhibitory effect of  $\alpha$ -TS on cancer cells, this form of vitamin E failed to affect cell proliferation, mitotic accumulation and chromosomal damage on three different cell lines of normal human fibroblasts [29,65], rodent fibroblasts [9], prostate epithelial cells [31] and other normal cells (human peripheral monocyte cells, monocyte derived macrophages, fibroblasts and umbilical vein endothelial cells, murine peritoneal macrophages, rat intestinal epithelial cells, neonatal cardiomyocytes, neonatal hepatocytes and smooth muscle cells [24]). However, it has been reported that  $\alpha$ -TS can cause apoptosis in human umbilical vein endothelial cells in culture [66]. It is interesting to note that even  $\alpha$ -T at 50  $\mu$ M concentration inhibited the growth of vascular smooth cell proliferation in culture via inhibition of PKC [67]. This effect was considered independent of antioxidant activity. These data suggest that  $\alpha$ -TS is the most effective form of vitamin E for reducing the proliferation of cancer cells and that at similar concentrations it produces no significant effect on the proliferation of most normal cells. The selectivity of  $\alpha$ -TS effect on cancer cells has not yet been fully recognized by oncologists.

#### ***In vivo* Data on $\alpha$ -TS-Induced Growth Inhibition**

Helson *et al.* demonstrated for the first time that I. P. administration of  $\alpha$ -TS dissolved in ethanol (50 mg/kg of body weight) for a five-day period markedly reduced the growth of human NB cells in athymic mice [10]. Another study reported that a direct injection of  $\alpha$ -TS dissolved in sesame oil (250  $\mu$ g/injection twice a week) for a period of four weeks caused regression of chemical-induced tumor in the oral cavity of hamster [38]. Since then, no *in vivo* studies were initiated until in 2000, when Malafa *et al.* showed that I.P. administration of  $\alpha$ -TS dissolved in sesame oil (150 mg/Kg of body weight) reduced the growth of human breast cancer cells [39], murine melanoma cells [40] and murine colon carcinoma cells [41] in athymic nude mice. Weber *et al.* [20] reported that I.P. administration of  $\alpha$ -TS dissolved in dimethylsulfoxide (DMSO) (100 mg/Kg of body weight, every third day) for a 12-day period reduced the growth of human colon cancer in athymic mice by 80% and  $\alpha$ -T by only 35%. Similar observations were made by Neuzil *et al.* [19]. In addition to exhibiting anti-tumor activity *in vivo*,  $\alpha$ -TS also reduces colon cancer liver metastasis [41]. Oral [68] or sub-cutaneous [39,40] administration in rodents was ineffective, suggesting that most of  $\alpha$ -TS under these conditions may be hydrolyzed before entering into the blood stream. It has been observed that daily oral doses of  $\alpha$ -TS in humans are not fully hydrolyzed in the intestinal tract [69]. This was evidenced by the fact that  $\alpha$ -TS at a concentration of about 6  $\mu$ g/mL in comparison to a concentration of  $\alpha$ -T of about 60  $\mu$ g/mL was detectable in the blood of these patients. It is unknown whether this blood level of  $\alpha$ -TS can be considered sufficient to inhibit the growth of tumor cells *in vivo*. It has been reported that  $\alpha$ -TS can readily bind with lipoprotein, and this complex continues to maintain potent anti-cancer activity

in human breast cancer cells *in vitro* [32]. In order to avoid the possibility of hydrolysis in the gut, the nonhydrolyzable ether form of  $\alpha$ -TS (TSE) was synthesized by Farris *et al.* [33]. This analog as well as  $\alpha$ -TS caused growth inhibition in murine leukemia cell lines (myeloid and lymphocytic), but they did not affect the growth of normal bone marrow cells. In fact, the growth of these normal cells was stimulated by  $\alpha$ -TS and its analog, TSE [33].

## **MECHANISMS OF ACTION OF $\alpha$ -TS**

### **Relation Between $\alpha$ -TS, Antioxidants, Antioxidation Activity and Inhibition of Proliferation**

It has been reported that  $\alpha$ -TS-induced inhibition of cell proliferation *in vitro* requires that this molecule remains intact [33]. Since  $\alpha$ -TS is not hydrolyzed by cancer cells *in vitro* [65], the above suggestion appears rational. Since ethanol solubilized  $\alpha$ -T did not exhibit anti-cancer activity *in vitro* [7,34,55], it has been presumed that  $\alpha$ -TS-induced inhibition of cell proliferation and apoptosis does not involve antioxidation activity. Here, we propose that all antioxidants that inhibit the proliferation of cancer cells at concentrations higher than those produced by  $\alpha$ -TS may also not involve antioxidation mechanism and that  $\alpha$ -TS induced growth inhibition *in vivo* in part may be mediated via  $\alpha$ -T. This is supported by the following observations: (a) water soluble  $\alpha$ -T inhibited the proliferation of cancer cells at high concentrations *in vitro* [8,56,57]; (b) butylated hydroxyanisole (which exhibits antioxidant activity similar to that of vitamin E, but without vitamin E activity) inhibited the proliferation of melanoma cells and NB cells at concentrations similar to that of  $\alpha$ -TS, but to a lesser degree than that produced by  $\alpha$ -TS [8]; (c) trolox was less effective than  $\alpha$ -TS in both NB cells and melanoma cells in culture [8], but at higher concentrations can cause apoptosis in human colon cancer cells in culture [70], and (d) treatment with  $\alpha$ -T *in vivo* reduced the growth of tumor less than that produced by  $\alpha$ -TS treatment [20]. These studies suggest that  $\alpha$ -TS in its intact form can exhibit most potent anti-cancer activity *in vitro*; however, *in vivo*, part of the activity of  $\alpha$ -TS may be mediated via  $\alpha$ -T.

### **Studies on Cellular Uptake of $\alpha$ -TS**

In order to explain the mechanisms of the growth inhibitory effect of  $\alpha$ -TS *in vitro*, it was postulated that cancer cells may accumulate more  $\alpha$ -TS than normal cells [69]. To test this hypothesis, human cervical carcinoma cells (HeLa cells) and normal fibroblasts were incubated in the presence of  $\alpha$ -TS (10  $\mu$ g/mL) for 4 and 24 hours, and then  $\alpha$ -TS was extracted, using  $\alpha$ -TS as an internal standard. Results showed that both cancer cells and normal cells accumulated similar levels of  $\alpha$ -TS at 24 hours after treatment (Table 1), suggesting that tumor cells have acquired an increased degree of sensitivity to  $\alpha$ -TS [65]. It was interesting to note that  $\alpha$ -T was not detectable in the cell,

**Table 1.** Accumulation of D- $\alpha$ -Tocopheryl Succinate ( $\alpha$ -TS) in Human Cervical Cancer (HeLa cells) and Normal Human Fibroblasts after 24 Hours of Treatment with  $\alpha$ -TS

Concentrations of $\alpha$ -TS	Accumulation of $\alpha$ -TS ( $\mu$ g/mg protein)	
	Fibroblasts	HeLa Cells
20 $\mu$ g/mL (37.6 $\mu$ M)		
Experiment 1	1.07	1.23
Experiment 2	0.89	1.02
10 $\mu$ g/mL (18.8 $\mu$ M)		
Experiment 1	1.38	1.87
Experiment 2	1.04	0.93

$\alpha$ -TS was extracted in hexane, and  $\alpha$ -tocopheryl acetate was used as an internal standard to determine the efficiency of extraction procedure. The recovery efficiency was 55% to 75%. The levels of accumulation after treatment of cells with  $\alpha$ -TS for 24 hours were similar in both HeLa cells and normal fibroblasts. Each measurement was repeated twice, and they were reproducible within the same experiment [65].

implying that there was no significant conversion of  $\alpha$ -TS to  $\alpha$ -T within the cell. A cellular concentration of about 0.5  $\mu$ g/10<sup>6</sup> cells, which was about 40 times lower than that added to the growth medium, was sufficient to inhibit the proliferation of Hela cells.

### Studies on Adenylate Cyclase Response to Ligands After $\alpha$ -TS Treatment

Adenylate cyclase (AC) mediates the action of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) in inducing differentiation and inhibition of proliferation in NB cells and of melanocyte-stimulating hormone (MSH) in regulating proliferation and differentiation in melanocytes. Growth inhibitory concentrations of vitamin E succinate inhibited basal, PGE<sub>1</sub>-stimulated AC in NB cells [9], MSH-stimulated AC in melanoma cells [58] and sodium fluoride (NaF)- and forskolin-stimulated AC in both NB cells and melanoma cells in culture. These data suggest that  $\alpha$ -TS inhibited the activity of GTP-binding protein (Gs) and the catalytic subunit of AC. These effects of  $\alpha$ -TS are not related to its growth-inhibitory effect, because PGA<sub>2</sub>, which inhibited the proliferation of melanoma cells, did not inhibit MSH-stimulated AC activity. The fact that butylated hydroxyanisole, which inhibited the proliferation of NB cells, did not affect basal or PGE<sub>1</sub>-stimulated AC activity in NB cells, suggests that inhibition of PGE<sub>1</sub>-stimulated AC by  $\alpha$ -TS is not due to its antioxidant action.  $\alpha$ -TS did not affect the proliferation of normal murine fibroblasts in culture, but it inhibited PGE<sub>1</sub>-stimulated AC activity [9]. These data suggest that  $\alpha$ -TS-induced inhibition of AC activity *in vitro* is not related to inhibition of proliferation of cancer cells or to proliferation of normal fibroblasts. The significance of  $\alpha$ -TS-induced inhibition of AC activity is unknown. Since PGs for most cells and MSH for melanocyte can act as tumor promoters, the above effect of  $\alpha$ -TS may be more relevant to cancer prevention than to cancer treatment.

### Studies on Expression of Genes and their Products and their Translocation after Treatment with $\alpha$ -TS

In order to understand the mechanisms of action of  $\alpha$ -TS on cancer cells, it was necessary to investigate the effect of  $\alpha$ -TS on expression of those genes that have been implicated in regulation of proliferation and apoptosis. Indeed, the results of several studies revealed that the expression of certain genes and their products are up- and down-regulated after treatment of tumor cells with growth inhibitory concentrations of  $\alpha$ -TS. The genes which were up-regulated included TGF- $\beta$ s and their products, and TGF- $\beta$  type II receptor in human breast cancer cells [15,35,44,46], mitogen-activate protein kinase (MAPK), Erk1m MEK1 and JNK1, but not p38, and phosphorylation of transcriptional factors (c-jun, AFT-2 and Elk-1) [45]. Similar observations were made on human gastric cancer cells [46]. The genes which were down-regulated include CD178 on the cell surface of ovarian carcinoma [48], prostate-specific antigen (PSA) in human prostate cancer cells at both the transcriptional and translational levels [31], vascular endothelial growth factor (VEGF) [36,39] and c-myc and H-ras in murine NB cells and melanoma cells [42,43].

In addition to changes in gene expression, the activities and levels of some gene products are increased, decreased or translocated after treatment with growth inhibitory concentrations of  $\alpha$ -TS. For example, the activities of caspase-3 in Jurkat lymphoma cells [37], caspase-3, 6, 8, and 9, but not caspase-1 in promyelocytic leukemia [25], PKA (cAMP-dependent kinase) in the cytosolic fraction of B-16 melanoma cells [59] increased, whereas, the activities of PKC *in vitro* [71], PKC- $\alpha$  subtype in human Jurkat lymphoma cells by increasing protein phosphatase 2A (PP2A) activity [19], basal and PGE<sub>1</sub>-stimulated AC in NB cells [9] and basal and MSH-stimulated AC activity in B-16 melanoma [58] cells were decreased.  $\alpha$ -TS causes translocation of Fas protein from the cytoplasm to plasma membrane [14]. It also inhibits activation of NF- $\kappa$ B and DNA-binding activity of activated NF- $\kappa$ B [72,73].  $\alpha$ -TS also reduces transcriptional activation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [47]. The Kline group has proposed that  $\alpha$ -TS-induced apoptosis in human breast cancer cells is mediated via Fas signaling pathway [14]. The most remarkable observation was that  $\alpha$ -TS treatment induced Fas-sensitivity into Fas-resistant human breast cancer cells. Fas, also referred to as Apo-1 or CD 95, can induce apoptosis upon trimerization with Fas ligand (CD95L) or upon cross-linking with Fas specific antibodies [17]. Weber *et al.* have proposed that  $\alpha$ -TS-induced apoptosis in cancer cells is independent of p53 and p21 [20]. This is in contrast to trolox and 5-FU which induces apoptosis via p53 and p21 [70]. Others have reported that  $\alpha$ -TS increases the expression of wild type p53, but decreases the expression of mutated p53 in chemical-induced tumor in hamster buccal pouches [63].

The levels of TGF- $\beta$  in human breast cancer cells increased [44], whereas the levels of VEGF in human prostate cancer cells *in vitro* [36] and breast cancer cells *in vivo* [39] and PSA

in human prostate cancer cells [31] decreased after treatment with growth inhibitory concentrations of  $\alpha$ -TS.

More recently, analysis of our data using gene array technique has allowed us to propose a novel concept with respect to the relationship between changes in gene expressions and growth inhibition after treatment of NB cells with  $\alpha$ -TS (our unpublished observation). For example, alterations in the expression of different sets of genes occurred that were unique either to a non-growth inhibitory or a growth inhibitory concentration of  $\alpha$ -TS. In addition, changes in expression of different sets of genes that were common to both non-growth inhibitory and growth inhibitory concentrations of  $\alpha$ -TS were found. These data suggest that profound changes in gene expression can occur without any significant alterations in growth or morphology of NB cells after treatment with a non-growth inhibitory concentration of  $\alpha$ -TS. They also suggest that not all changes in gene expression that are observed after treatment with growth-inhibitory concentrations of  $\alpha$ -TS are related to growth inhibition.

#### **$\alpha$ -TS in Combination with Dietary Micronutrients on Cell Proliferation**

$\alpha$ -TS in combination with dietary micronutrients such as retinoic acid, vitamin C and polar carotenoids is more effective in reducing the proliferation of human melanoma cells [21] and human parotid acinar carcinoma cell and immortalized acinar cells [22] in culture than the individual agents. This suggests that a combination of above agents rather than a single agent should be considered as an adjunct to standard cancer treatment for any clinical study.

#### **Modification of Radiation Damage on Cancer and Normal Cells by $\alpha$ -TS**

Ionizing radiation is commonly used as one of the standard cancer treatment modalities; therefore, we have investigated the effect of  $\alpha$ -TS in combination with radiation on normal and tumor cells. A growth inhibitory concentration of  $\alpha$ -TS in combination with  $x$ - or  $\gamma$ -irradiation reduced the growth of NB cells more than that produced by individual agents alone [49]. It also has been reported that a growth inhibitory concentration of  $\alpha$ -TS enhanced radiation-induced chromosomal damage in human cervical cancer cells, but protected normal human fibroblasts against such damage [65]. This form of vitamin E also increased radiation-induced delay in mitotic accumulation in human cervical cancer and ovary carcinoma cells without causing similar changes in human normal fibroblasts [29]. These data suggest that  $\alpha$ -TS can enhance the effect of irradiation on cancer cells, but it can protect normal cells against some of the toxicities.

#### **$\alpha$ -TS Enhances the Effect of Hyperthermia on Tumor Cells**

Hyperthermia is used as an experimental cancer therapy in the treatment of primarily local solid tumors. It has been reported  $\alpha$ -TS at a growth inhibitory concentration in combination with hyperthermia at 43°C enhanced the growth-inhibitory effect of hyperthermia on NB cells in a synergistic manner [50,51]. Even at a lower temperature of 41°C, the combination of  $\alpha$ -TS and heat was more effective than the individual agents. Butylated hydroxyanisole, an antioxidant without vitamin E properties, also enhanced the effect of hyperthermia but to a lesser degree that produced by  $\alpha$ -TS. These data suggest that in presence of  $\alpha$ -TS, hyperthermic temperature can be reduced from currently used temperatures of 42.5–43 to 41°C without sacrificing its efficacy on cancer cells. The currently used temperatures of about 43°C produced burns to normal tissue and such high temperatures cannot be given whole-body because of unacceptable side effects.

#### **$\alpha$ -TS Enhances the Effect of Chemotherapeutic Agents on Cancer Cells without Affecting the Proliferation of Most Normal Cells**

$\alpha$ -TS enhanced the growth-inhibitory effect of several chemotherapeutic agents on cancer cells in culture. For example,  $\alpha$ -TS enhanced the effect of adriamycin on human prostate carcinoma cells [11], human glioma and Hela cells (unpublished observations); the effect of *cis*-platin, tamoxifen and decarbazine (DTIC) on human melanoma cells [21] and human parotid acinar carcinoma cells [22], the effect of doxorubicin on murine leukemia cell lines [33]. However,  $\alpha$ -TS did not modify the effect of adriamycin on the growth of human normal fibroblasts (unpublished observations), but it protected bone marrow cells against the lethal effect of doxorubicin [33]. Thus,  $\alpha$ -TS can enhance the efficacy of some chemotherapeutic agents selectively on cancer cells. More recently, we have demonstrated that statins with a closed-ring structure such as mevastatin inhibited the growth of NB cells by inhibiting proteasome activity, and this effect of mevastatin was enhanced by  $\alpha$ -TS [74]. A recent study has shown that a mixture of micronutrients (vitamin C,  $\alpha$ -TS and beta-carotene) potentiates the growth-inhibitory effects of paclitaxel and carboplatin in human lung cancer cells in culture [75].

#### **$\alpha$ -Tocopheryl Succinate Enhances the Effect of Biological Response Modifiers on Cancer Cells**

It has been reported that  $\alpha$ -TS at a growth inhibitory concentration can enhance the levels of cAMP-induced differentiation of murine NB cells [52,54] and murine melanoma cells [53] in culture.  $\alpha$ -TS also enhanced the growth inhibitory effect of sodium butyrate, a 4-carbon fatty acid, on murine NB cells in culture [52].  $\alpha$ -TS in combination with other dietary micronutrients (vitamin C, carotenoids and retinoic acid) may be

more effective than the individual agents [21, 22]. This form of vitamin E also enhanced the effect of  $\gamma$ -interferon [21] on tumor cells in culture. This is a novel concept in the sense that it can modify the effect of some physiological substances in the body, because in the past, it has been presumed that vitamin E acts as an independent agent in modifying cellular functions. Turley *et al.* have reported that the combination of  $\alpha$ -TS plus anti-Fas increased apoptosis in Fas-resistant human breast cancer cells [17]. Weber *et al.* have shown that the combination of  $\alpha$ -TS and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was more effective than the individual agents in inducing inhibition of proliferation and apoptosis *in vitro* and *in vivo* without affecting the proliferation of normal tissues [20].

### **Proposed Mechanisms of Damage to Cancer Cells When $\alpha$ -TS is Combined with Radiation or Chemotherapeutic Agents**

Pre-treatment of cancer cells with high doses of  $\alpha$ -TS can cause damage by diverse mechanisms that include Fas-signal pathway [14,17], anti-angiogenesis [36,39], up-regulation of wild type p53 and down regulation of mutant p53 [63], activation of caspase activity [25,37], inhibition of PKC activity [25,37], inhibition of activation of NF- $\kappa$ B and DNA binding activity of activated NF- $\kappa$ B [72,73], down regulation of oncogenes, c-myc and H-ras [42,43]. High expression of c-myc and H-ras increases the radioresistance of cancer cells [76,77]. Since  $\alpha$ -TS down-regulates these genes in cancer cells [42,43], pre-treatment of these cells with  $\alpha$ -TS is expected to increase the sensitivity of cells to x-irradiation. 5-FU, a commonly used chemotherapeutic agent, induced apoptosis in cancer cells via p53 and p21 [70], whereas  $\alpha$ -TS produced such an effect by different mechanisms [14,17,36,39]. Therefore, the combination of  $\alpha$ -TS and 5-FU would be more effective in inducing apoptosis in cancer cells than the individual agents. It is unknown whether  $\alpha$ -TS when given after irradiation or chemotherapeutic agents would inhibit repair of damage; however, retinoic acid has been shown to inhibit the repair of radiation-induced potential lethal damage in cancer cells [78]. It has been reported that  $\alpha$ -TS can act as an anti-angiogenesis agent *in vivo* [36,39], whereas radiation or chemotherapeutic agents do not; therefore, the combination of two may be more effective in reducing the growth of cancer cells than the individual agents. These data suggest that a combination of  $\alpha$ -TS with radiation or chemotherapeutic agents is more effective than the individual agents, because their mechanism of action on tumor cells is different.

### **Clinical Studies with $\alpha$ -TS Alone or in Combination with Dietary Micronutrients as an Adjunct Standard Therapy**

No clinical studies have been performed with  $\alpha$ -TS alone or in combination with standard therapy. However,  $\alpha$ -TS in combination with dietary micronutrients has been used in randomized clinical trials. Kim's group has utilized micronutrients

including  $\alpha$ -TS in combination with radiation therapy for the treatment of breast cancer after surgical removal of a tumor [79], and Kochupillai's group has used a similar approach in combination with chemotherapy for the treatment of human non-small cell lung carcinoma [80]. Initial results were encouraging and showed that the combination treatment did not interfere with the efficacy of radiation or chemotherapy in cancer cells.

## **CONCLUSIONS AND FUTURE DIRECTION**

Results discussed above suggest that  $\alpha$ -TS inhibits the proliferation of rodent and human cancer cells without affecting the proliferation of most normal cells. In addition, they also show that  $\alpha$ -TS when used in combination with some standard and experimental cancer therapeutic agents may enhance their growth-inhibitory effect on cancer cells, while protecting normal cells against some of their toxicities. Since it has been demonstrated that  $\alpha$ -TS in combination with dietary micronutrients (retinoic acid, vitamin C and carotenoids) is more effective in reducing the proliferation of tumor cells in culture than the individual agents and in enhancing the effect of some chemotherapeutic agents on these cells, we have recommended the use  $\alpha$ -TS in combination with dietary micronutrients as an adjunct to standard therapy in the treatment of cancer [66]. Although preliminary clinical studies using  $\alpha$ -TS in combination with dietary micronutrients as an adjunct to standard chemotherapy appear encouraging, randomized double-blind-placebo control trials, using high doses of multiple micronutrients including  $\alpha$ -TS as an adjunct to standard cancer therapy as well as experimental therapy (hyperthermia and some biological response modifiers), should be initiated. In basic research, it is essential to identify additional genes that initiate damage in cancer cells as a function of time (a few minutes to a few days) after treatment with  $\alpha$ -TS. Although  $\alpha$ -TS does not affect the proliferation of most normal cells, analogous studies should be performed on normal counterpart cells in order to establish whether normal cells show any alterations in gene expression. This is particularly important, because our recent study (unpublished observation) has shown that a non-growth-inhibitory concentration of  $\alpha$ -TS markedly alters the expression of many genes in neuroblastoma cells, some of which exhibit changes similar to those produced by a growth-inhibitory concentration of  $\alpha$ -TS.

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*Received August 2, 2002; revision accepted December 10, 2002.*