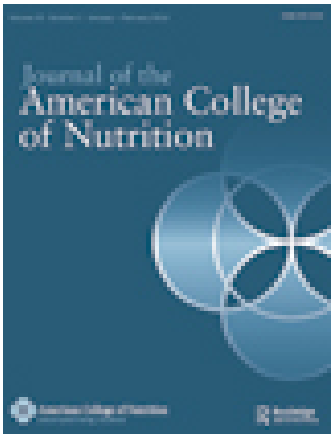

Journal of the American College of Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uacn20

D-Alpha-Tocopheryl Succinate (Vitamin E) Enhances Radiation-Induced Chromosomal Damage Levels in Human Cancer Cells, but Reduces it in Normal Cells

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Published online: 26 Jun 2013.

To cite this article: Bipin Kumar MD, Mitra N. Jha, William C. Cole PhD, Joel S. Bedford DPhil & Kedar N. Prasad PhD, FACN (2002) D-Alpha-Tocopheryl Succinate (Vitamin E) Enhances Radiation-Induced Chromosomal Damage Levels in Human Cancer Cells, but Reduces it in Normal Cells, Journal of the American College of Nutrition, 21:4, 339-343, DOI: 10.1080/07315724.2002.10719232

To link to this article: http://dx.doi.org/10.1080/07315724.2002.10719232

Original Research

D-Alpha-Tocopheryl Succinate (Vitamin E) Enhances Radiation-Induced Chromosomal Damage Levels in Human Cancer Cells, but Reduces it in Normal Cells

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Key words: irradiation, radiation therapy, carcinoma, chromatid damage

Objective: The purpose of this study was to measure and compare the effect of d-α-tocopheryl succinate (α-TS) in modifying radiation-induced chromosomal damage in human normal cells and cancer cells in culture.

Methods: Three human normal fibroblast cell lines (GM2149, AG1522 and HF19) and three human cancer cell lines, cervical cancer (HeLa) and ovarian carcinoma cells (OVG1 and SKOV3) were treated with α-TS (37.6 μM) 20 hours before 100 cGy γ-irradiation. After 30 minutes of irradiation, colcemid was added and cells were fixed. One hundred randomly selected metaphase cells were scored for the presence of chromatid gaps and breaks. To study the cellular accumulation of α-TS, cells were incubated in the presence of α-TS (18.8 and 37.6 μM) for 24 hours, and α-TS was extracted with hexane using α-tocopheryl acetate as an internal standard. The levels of α-TS were determined by HPLC.

Results: Results showed that α-TS induced chromosomal damage in both human cervical cancer cells and ovarian cancer cells, but not in human normal fibroblasts in culture. In addition, α-TS enhanced the level of radiation-induced chromosomal damage in cancer cells, but it protected normal cells against such damage. Both cancer cells and normal cells accumulated similar levels of α-TS, suggesting that increased sensitivity of cancer cells to α-TS is acquired during transformation.

Conclusion: The use of α-TS during radiation therapy may improve the efficacy of radiation therapy by enhancing tumor response and decreasing some of the toxicities on normal cells.

INTRODUCTION

Several studies on the radioprotective effects of vitamin E on normal cells in animals have been published [1–9]. We have reported that α-tocopheryl succinate (α-TS), the most effective form of vitamin E [10,11], enhances the growth-inhibitory effect of x-irradiation on neuroblastoma cells in culture [12]. Recently, we have shown that α-TS increases the levels of radiation-induced decrease in mitotic accumulation in human cervical carcinoma cells and ovarian carcinoma cells in culture, but it does not modify this effect of irradiation in normal fibroblasts in culture [13]. Ionizing radiation is well known to induce chromosomal damage in both normal and cancer cells; however, it is unknown whether α-TS can modify this effect of irradiation in cancer cells and normal cells. It is also unknown whether α-TS-induced damage in cancer cells is due to increased accumulation of α-TS in these cells.

We now report that α-TS by itself induces chromosomal damage in cancer cells, but not in normal cells in culture. In addition, it enhances the levels of γ-irradiation-induced chromosomal damage in cancer cells, whereas it protects normal cells against such damage. Both normal and cancer cells accumulate similar levels of α-TS, suggesting that increased sensitivity of cancer cells to α-TS is acquired during transformation.

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Journal of the American College of Nutrition, Vol. 21, No. 4, 339–343 (2002)
Published by the American College of Nutrition

MATERIALS AND METHODS

Cell Culture

Human cervical carcinoma cells (HeLa cells from Dr. L.J Tolmach) ovarian carcinoma cells (OVG1 from Drs J.B. Mitchell and J.A. Cook, and SKOV3 from Dr. L. Smith) and human normal skin fibroblasts (GM 2149 from the NIGMS Human Genetic Mutant Cell Repository, AG1522 from the NIA Cell Repository and HF19 from Dr. D. Goodhead) were grown in culture as described earlier [13].

Treatment of Cells and Assay of Chromosomal Damage

The procedures for making solutions of d-α-tocopheryl succinate (Henkel, Chicago), colcemid (Sigma, St. Louise) and their storage have been published earlier [13]. In brief, α-TS was dissolved in ethanol at a concentration of 5 mg/mL. Cells were treated with α-TS (20 μg/ml or 37.6 μM) for 20 hours before γ-irradiation (100 cGy). The above concentration of α-TS and treatment period were selected because this concentration under the above experimental conditions inhibits the growth of cancer cells, but not of normal fibroblasts in culture [13]. It has been shown that a daily oral intake of 800 IU α-TS increases plasma levels of vitamin E (α-tocopherol, 60 μg/mL and α-TS, 6 μg/mL) [14]. The basal level of α-tocopherol in plasma is about 6–10 μg/mL. After 30 minutes of irradiation, colcemid (0.1 μg/ml) was added and one hour later the cells were fixed with 3:1 methanol and acetic acid for an hour and stained with Giemsa. The slides were randomized and coded to ensure that the observer was unaware of conditions of treatment at the time of scoring the chromosomal damage. For aberration frequency, 100 randomly selected metaphase-like cells were scored for the presence of chromosomal aberrations (chromatid gaps and breaks) according to the criterion suggested by Savage [15].

Intracellular Uptake of A-TS

To study the uptake of α-TS by cancer cells and normal cells, cells were incubated in the presence of α-TS (18.8 and 36.6 μM) for 24 hours and then removed and washed with PBS. Vitamin E was extracted with hexane using α-tocopheryl acetate (α-TA) as an internal standard. The levels of α-TS and α-TA were determined by HPLC using a C^{18} column (4.6 mm $\times$ 250 mm, 5 μm particle size) eluted with acetonitrile: MeOH (0.028% TFA) 60:40 (v/v) at a flow rate of 2.0 mL/minute. The level of each form of vitamin E was determined from a standard curve and expressed as μg of vitamin E/mg protein. Protein was determined by the method of Lowry *et al.* [16].

Statistical Analysis

Each experiment was repeated twice. The significant difference between control and experimental groups was determined by one-tail Student's t test at $p = 0.05$.

RESULTS

Results showed that treatment with α-TS (20 μg/mL or 37.6 μM) for a period of 20 hours increased the level of chromosomal damage (chromatid gaps and breaks) in HeLa cells and ovarian carcinoma cells, but not in three lines of normal human fibroblasts (Fig. 1). Solvent treatment did not alter chromosomal damage in cancer cells or normal fibroblasts in comparison to untreated control cells (data not shown). In cancer cell lines, the differences between untreated or solvent treated control and α-TS treated cells were significant at $p < 0.05$. In addition, α-TS treatment 20 hours before γ-irradiation enhanced the level of radiation-induced chromosomal damage in all tumor cell lines. On the other hand, a similar treatment with α-TS reduced radiation-induced chromosomal damage in all normal human fibroblast cell lines (Fig. 1). Solvent treatment did not alter radiation-induced chromosomal damage either in cancer cells or normal fibroblasts (data not shown). In both cancer cells and normal fibroblasts, the differences between irradiated cells and cells treated with α-TS and irradiation were significant at $p < 0.05$. It should be pointed out that all cancer cell lines are of epithelial origin; therefore, the observations made on fibroblasts may or may not be relevant to normal human epithelial cells.

In order to establish whether the higher sensitivity of cancer cells to α-TS is related to the increased accumulation of this vitamin E, the levels of α-TS in cancer cells and normal fibroblasts were determined by HPLC after incubation of cells in the presence of 18.8 or 37.6 μM of α-TS for 24 hours at 37°. Results showed that HeLa cells and normal cells after 24 hours of incubation accumulated similar levels of α-TS (Table 1). The level of α-TS was undetectable in both cancer cells and normal fibroblasts, suggesting that these cells do not convert α-TS to α-tocopherol in any significant amounts within 24 hours. These results indicate that increased sensitivity of cancer cells to α-TS is acquired during transformation and is not due to enhanced accumulation of this form of vitamin E.

DISCUSSION

The present study shows that α-TS treatment induces chromosomal damage in cancer cells, but not in normal cells. This selective effect of α-TS on human cancer cells is consistent with the results published earlier by us and others on other criteria of damage [10,11,14,17–20] and in animal tumor models [21,22]. To determine whether the increased sensitivity of

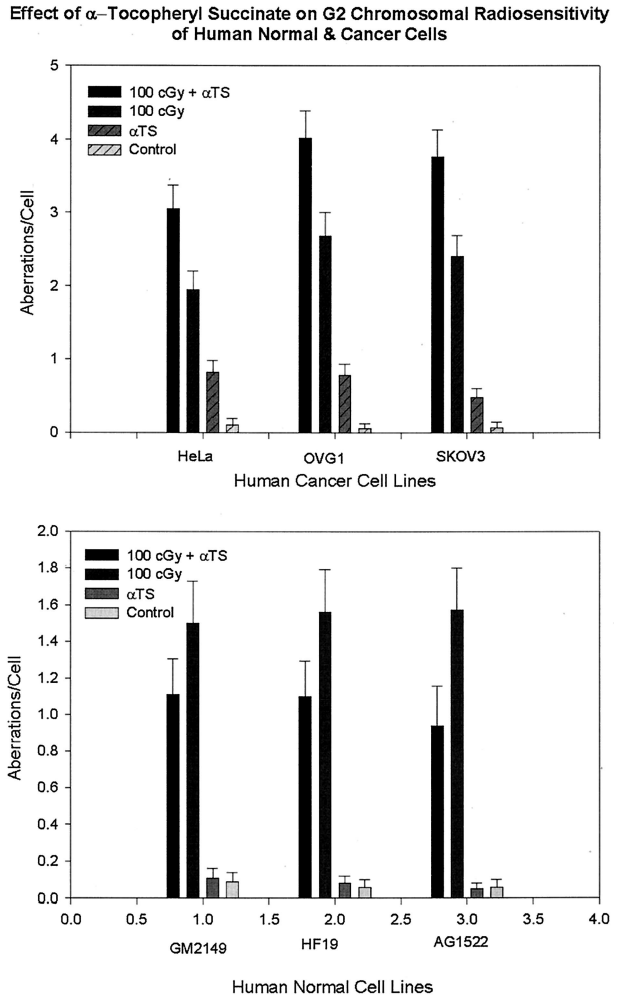

Fig. 1. Effect of d-α-tocopheryl succinate (α-TS) on the level of radiation-induced chromosomal damage in human cervical cancer (HeLa cells), ovarian carcinoma cell lines (OVG1 and SKOV3) and in human normal skin fibroblasts (GM2149, HF19 and AG1522). α-TS treatment alone increased chromosomal damage in all three cancer cell lines, but not in any normal cell lines. α-TS treatment also enhanced the levels of radiation-induced chromosomal damage in cancer cells but it protected normal cells against such damage. The bar is standard error of the mean; the difference between control and experimental groups in cancer cells, and between control (irradiation alone) and experimental groups (irradiation plus α-TS) is significant at $p < 0.05$.

cancer cells to α-TS is related to the enhanced accumulation of this form of vitamin E in these cells, cancer cells and normal fibroblasts were incubated in the presence of α-TS for 24 hours, and cellular levels of α-TS were determined by HPLC. Results showed that both cancer cells and normal cells accumulated similar levels of α-TS. This suggests that the increased sensitivity of cancer cells to α-TS in comparison to normal cells is acquired during transformation.

When α-TS is given before irradiation it increases the level of radiation-induced chromosomal damage in human cervical carcinoma and ovarian carcinoma, but protects normal human

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

Concentrations of α-TS	Accumulation of α-TS (μg/mg protein)	
	Fibroblasts	HeLa Cells
20 μg/mL (37.6 μM)		
Experiment 1	1.07	1.23
Experiment 2	0.89	1.02
10 μg/mL (18.8 μM)		
Experiment 1	1.38	1.87
Experiment 2	1.04	0.93

α-TS was extracted in hexane, and α-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 α-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.

fibroblasts against such damage. Solvent treatment does not modify radiation damage either in cancer cells or normal fibroblasts in culture. The radiosensitizing effect of α-TS is consistent with the results published earlier, in which treatment of tumor cells with α-TS before x-irradiation enhances the level of radiation-induced growth inhibition [12] and radiation-induced decrease in mitotic accumulation [13]. The radioprotective effect of α-TS on normal cells is also consistent with several studies reported earlier with other forms of vitamin E [1–9]. Most of these studies have focused on investigating the effect of vitamin E in modifying radiation response either in normal cells or in cancer cells. The present study is unique in the sense that it has compared the effect of α-TS in modifying radiation response on cancer cells and normal cells under similar experimental conditions. It should be pointed out that tumor cells used in this study are of epithelial origin, whereas normal cells are fibroblasts. Nevertheless, fibroblasts are also damaged during radiation therapy; therefore, if similar observations are made *in vivo*, the use of α-TS may improve the efficacy of radiation therapy by increasing tumor response and decreasing some of the toxicities.

Other antioxidant micronutrients such as vitamin C [23,24], all-trans-retinoic acid [25,26], retinyl palmitate (vitamin A) and β-carotene [27] when given before irradiation also enhanced radiation-induced growth inhibition in tumor cells in culture and *in vivo* without causing toxicity to normal cells *in vivo*. A recent study has reported that administration of multiple antioxidants (vitamin A, E and C) before and after radio-immunotherapy reduced the magnitude of myelosuppression without interfering with its efficacy on tumor cells [28]. Another study has shown that vitamin C, vitamin E acetate and β-carotene in combination with mitomycin and irradiation were more effective in reducing the growth human of leukemic cells (HL-60) in culture than the individual agents [29]. Limited clinical trials in humans have reported enhancement of the efficacy of radiation

therapy and chemotherapy with antioxidant micronutrients in patients with small-cell lung carcinoma [30,31] and breast cancer [32].

The exact mechanisms of differential modification of the effect of irradiation by α-TS on chromosomal damage in normal and cancer cells are unknown. We propose that an administration of α-TS at high doses before irradiation initiates damage in cancer cells as evidenced by the alterations in expression of those genes and cell signaling systems that are involved in regulation of growth and differentiation [11,17–21,33–37]. They include decreased expression of c-myc, N-myc, H-ras, mutated p53, tumor necrosis factor, transcriptional factor E2F and Fas, and reduced activity of protein kinase C. They also include increased expression of wild type p53, p21, transforming growth factor β (TGF-β), and the connexin gene, and activation of caspase. Since retinoic acid has been shown to reduce the repair of potential lethal damage [25], it is possible that α-TS may have similar effects on the repair of radiation damage in cancer cells. In addition, cancer cells, in general, have lower intracellular pH than normal cells; therefore, α-TS may not be able to perform its antioxidant function. This would then allow free radicals generated during irradiation to increase damage in cancer cells initiated by α-TS. In contrast to cancer cells, α-TS does not cause any chromosomal damage in normal cells, and at normal intracellular pH, α-TS continues to perform its antioxidant action. It is also possible that α-TS may enhance the rate of repair of potential radiation damage in normal cells. Therefore, it is not surprising that α-TS protects normal cells against radiation-induced chromosomal damage.

CONCLUSION

The role of antioxidant micronutrients in modifying radiation damage of cancer cells has generated extensive debates. At present, most radiation oncologists do not recommend such micronutrient supplements to their patients during radiation therapy, believing that antioxidants may protect both normal and cancer cells against radiation damage, since about two-third of radiation damage is mediated via free radicals. This belief has emerged from extrapolation of the results obtained on endogenously made antioxidants such as SH-compounds and their derivatives [1,2,38], and overexpression of certain antioxidant enzymes such as mitochondrial superoxide dismutase [39,40] which protect cancer cells against radiation damage to dietary antioxidant micronutrients such as vitamins A, C and E, and carotenoids which enhance the effect of irradiation on cancer cells [10–14,17–27]. The above belief is also maintained due to extrapolation of the results obtained on the radioprotective effect of vitamins A, C and E, and carotenoids on normal cells [1–9] to cancer cells which exhibit increased radiation damage in the presence of these micronutrients. In fact many *in vitro* studies, some animal studies and a few human studies show that supplementation with these micronutrients before radiation therapy may increase the efficacy of this treatment by increasing tumor response and decreasing some of the toxicities on normal cells [14,41]. Well-designed clinical trials with multiple micronutrients, which include high dose antioxidants such as vitamins A, C and E, and carotenoids, are needed to substantiate the above hypothesis.

ACKNOWLEDGMENT

This study was supported by Shafroth Memorial Fund.

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Received July 19, 2001; revision accepted September 28, 2001.