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CONTROVERSY

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Pros and cons of antioxidant use during radiation therapy

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Radiation therapy is one of the major treatment modalities in the management of human cancer. While impressive progress like more accurate dosimetry and more precise methods of radiation targeting to tumor tissue has been made, the value of radiation therapy in tumor control may have reached a plateau. At present, two opposing hypotheses regarding the use of antioxidants during radiation therapy have been proposed. One hypothesis states that supplementation with high doses of multiple micronutrients including high dose dietary antioxidants (vitamins C and E, and carotenoids) may improve the efficacy of radiation therapy by increasing tumor response and decreasing some of its toxicity on normal cells. The other hypothesis suggests that antioxidants (dietary or endogenously made) should not be used during radiation therapy, because they would protect cancer cells against radiation damage. Each of these hypotheses is based on different conceptual frameworks that are derived from results obtained from specific experimental designs, and thus, each may be correct within its parameters. The question arises whether any of these concepts and experimental designs can be used during radiation therapy to improve the management of human cancer by this modality. This review has analyzed published data that are used in support of each hypothesis, and has revealed that the current controversies can be resolved, if the results obtained from one experimental design are not extrapolated to the other. This review has also discussed the scientific rationale for a micronutrient protocol that includes high doses of dietary antioxidants (vitamin C, vitamin E succinate and natural β-carotene) which can be used adjunctively with radiation therapy. © 2002 Published by Elsevier Science Ltd.

Key words: Radiation therapy; dietary antioxidants; endogenous made antioxidants; cancer.

INTRODUCTION

Currently in the United States, the incidence of new cancer is approximately 1.2 million cases per year with about 600 000 deaths due to cancer each year. The incidence of a second primary malignancy among cancer survivors is about 10–12% annually.

Radiation therapy is one of the major treatment modalities in the management of human cancer. x- or γ-irradiation is commonly used in fractionated doses; however, radio-immunotherapy and heavy particle radiation such as neutrons or protons are also occasionally used. The usual therapeutic radiation dose schedule includes 200 cGy/day, 1000 cGy/week, for a total of 3000–4000 cGy. x- or γ-irradiation causes damage to both normal and cancer cells primarily through free radicals (about 2/3rd of damage), and to a lesser extent through direct ionization. Neutron or proton irradiation produces damage mainly through ionization although free radicals are produced during irradiation.

Radiation therapy is often given in combination with surgery and/or chemotherapy, and this combination therapy has been useful in producing

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increased cure rates in certain tumors including Hodgkin's disease, childhood leukemia, and teratocarcinoma. However, the risk of a second malignancy and non-neoplastic diseases such as aplastic anemia, retardation of growth in some children and delayed necrosis in some organs such as brain, liver, bone and muscle exists. In addition, acute damage to normal tissues occurs during radiation therapy, and in some instances, such damage becomes the limiting factor for the continuation of therapy. At this time, the efficacy of radiation therapy has reached a plateau for most solid tumors in spite of impressive progress in physical parameters such as dosimetry and more efficient methods for delivery of radiation doses to tumors. Therefore, novel biological and chemical approaches to improve the efficacy of radiation therapy must be developed.

Radiation modifying agents which can either selectively protect normal cells, but not tumor cells, against radiation damage or which can selectively enhance the effect of irradiation on tumor cells, but not on normal cells, would improve the efficacy of radiation therapy. In spite of extensive radiobiological research and clinical evaluation of potentially useful chemical modifying agents, most of them have been ineffective in the management of human cancer because none of them were selective for tumor or normal cells and because most of them were found to be toxic in humans (1, 2). Although amifostine (WR-2731), an analog of cysteamine, protected normal cells without protecting most cancer cells (3–5) except glioma cells (4) against radiation damage, it, at protective doses, can cause nausea, vomiting, hypotension and marrow hypoxia (6, 7).

Antioxidants could represent the most useful selective radiation modifying agents that are nontoxic to humans. However, the results on the effects of antioxidants in modifying radiation injuries on normal and cancer cells have led to opposing hypotheses. Our hypothesis states that supplementation with multiple micronutrients including high doses of dietary antioxidants (vitamins C and E, and β-carotene) may improve the efficacy of radiation therapy by increasing tumor response and decreasing some of its toxicity on normal cells (8–10). The other hypothesis suggests that antioxidants (dietary or endogenously made) should not be used during radiation therapy, because they would protect cancer cells against radiation damage (11, 12). At present, most radiation oncologists believe the second hypothesis and do not recommend antioxidants to their patients during radiation therapy, believing that they may protect both normal and cancer cells against radiation damage. Some of them may recommend a multiple vitamin preparation that contains low doses of antioxidants after completion of radiation therapy. In spite of the reservation of oncologists, over 70% of the patients are taking nutritional supplements including antioxidants, with or without the knowledge of their oncologist. These practices by patients and their oncologists may be harmful for two reasons; first, because certain antioxidants such as low doses of endogenously made antioxidants (SH-compounds) (1, 2) or dietary antioxidants (13, 14) that do not affect the growth of cancer cells may protect these cells against radiation damage, and second, because low doses of individual antioxidants taken alone such as vitamin C (15, 16) and polar carotenoids (17) may stimulate the growth of some cancer cells. Therefore, supplementation with low doses of dietary or endogenously made antioxidants may be counterproductive during and after radiation therapy.

Each of the proposed hypotheses is based on a different conceptual framework that is derived from specific experimental designs, and thus, each may be correct within its parameters. This review has discussed the biological basis of current controversies, and has revealed that the failure to recognize differences between two distinct conceptual frameworks, each of which is based on specific experimental designs, is responsible for this debate. This review has also discussed the scientific rationale for a micronutrient protocol that include high doses of dietary antioxidants which can be used as an adjunct to radiation therapy in a clinical trial.

Definition of types of antioxidants and their doses

It is important to distinguish between dietary (such as vitamins A, C and E and carotenoids) and endogenously made antioxidants (such as SH-compounds like glutathione, and antioxidant enzymes), because they modify the effects of irradiation on normal and cancer cells differently. It is equally important to define doses of these antioxidants, because they are often referred to as low, high and toxic without specific reference to any biological criteria. For this review, low doses are referred to as those that do not affect the growth of normal or cancer cells. In humans, antioxidant micronutrient supplements at about RDA doses can be defined as low-dose. In tissue culture, vitamin C doses of up to 50 μg/ml, vitamin E (α-tocopherol) doses of up to 5 μg/ml, α-TS doses of up to 2 μg/ml, retinoid doses of up to 5 μg/ml and β-carotene of up to 1 μg/ml can be defined as low-dose. High doses are referred to those that inhibit the growth of cancer cells without affecting the growth of normal cells. Based on human studies,

oral supplementation with vitamin C doses of up to 10 g/day, vitamin E of up to 1000 I.U./day, vitamin A doses of up to 10 000 IU/day and natural β-carotene doses of up to 60 mg/day can be defined as high-dose. In tissue culture, vitamin C doses of up to 200 μg/ml, vitamin E doses of up to 20 μg/ml, retinoid doses of up to 25 μg/ml and carotenoid doses of up to 15 μg/ml can be considered high-dose. Toxic doses are referred to those that can inhibit the growth of both normal and cancer cells; and therefore, they are not used in any experimental systems. Although oral retinoic acid doses of 300 000 I.U./day, vitamin E doses of 2000 mg/day, β- carotene doses of 150 mg/day and vitamin C doses of 20 grams or more per day have been used in cancer patients, their toxicities are limited to organs such as liver and skin toxicity with retinoids, defect in blood clotting with vitamin E, diarrhea with vitamin C, and bronzing of skin with β-carotene.

Conceptual framework of our hypothesis

This hypothesis is based on the following concepts: (a) dietary antioxidants such as vitamins A, C and E and β-carotene can produce biological effects on cancer cells by mechanisms that are not related to their antioxidant action, (b) dietary antioxidants at high doses inhibit the growth of cancer cells in culture, in animal and human tumor models without affecting the growth of normal cells; (c) dietary antioxidants at high doses enhance the effect of x-irradiation on cancer cells, but protect normal cells against some of its damage; and (d) prolonged treatment time before and after irradiation is necessary for selectively enhancing the effect of irradiation on tumor cells.

Conceptual framework of other hypothesis

This hypothesis is based on the following concepts: (a) the only function of antioxidants is to destroy free radicals; (b) antioxidants do not affect the growth of cancer cells; (c) antioxidants protect cancer cells and normal cells against radiation damage; and (d) no considerations are given to doses and types of antioxidants and treatment period.

Thus, there are major differences in the conceptual frameworks of the two proposed hypotheses. These differences appear to be primarily due to differences in experimental designs, doses and types of antioxidants and treatment period before and after irradiation.

Experimental designs of our hypothesis

This hypothesis is based on the results obtained on the following experimental conditions: (a) dietary antioxidants are given several hrs to days before and after x-irradiation in more than one dose for the entire experimental period; (b) high doses of dietary antioxidants are generally used in combination with radiation; and (d) one or more dietary antioxidants are used in combination with radiation.

Experimental designs of other hypothesis

This hypothesis is based on the results obtained on the following experimental conditions: (a) antioxidants (dietary or endogenously made) are given shortly before x-irradiation one time in a single dose, and generally removed immediately after irradiation; (b) low doses of antioxidants are used in combination with radiation; (c) only one antioxidant is used in combination with radiation.

Types of antioxidants

Our hypothesis is based on the effect of dietary antioxidants in modifying radiation damage on normal and cancer cells. The other hypothesis does not distinguish between dietary and endogenously made antioxidants in modifying radiation injury.

Thus, if the conceptual framework and its respective experimental designs of one hypothesis are not extrapolated to the other, the current debates regarding the use of antioxidants during radiation therapy can easily be reconciled without questioning the validity of the conceptual framework of each hypothesis.

In order to understand better the biological basis of our proposed hypothesis, it is essential that the effects of individual dietary and endogenously made antioxidants and their mechanisms of action are briefly described. All studies described below have been performed with high doses of dietary antioxidants and under experimental conditions in which the agents are present throughout the experimental period.

Effect of high doses of individual dietary antioxidants

Several studies have now established that high-doses of individual antioxidant micronutrients such as

vitamin A (including retinoids) (16–19), vitamin C (16, 17, 20), vitamin E (21–26), and carotenoids including β-carotene (27–30) inhibit the growth and cause differentiation and apoptosis in cancer cells in culture. They also reduce the growth of tumors in animal models (27, 31–34) and certain human tumors (35–41) without affecting the growth of normal cells. More recently, we have shown that d-α-tocopheryl succinate (α-TS) inhibits the growth and reduces the levels of mitotic accumulation in human cervical cancer cells and human ovarian carcinoma cells, but it has no such effect on three lines of human normal fibroblasts (42). α-TS also increases the level of chromosomal damage in cancer cells without producing such effects on normal cells (43). The growth-inhibitory doses of these antioxidant micronutrients vary from one species to another for the same tumor type. They also vary from one tumor type to another within the same species.

The extent and type of effect on tumor cells depends upon the type and form of micronutrients. For example, α-TS induces cell differentiation (Fig. 1), growth inhibition and apoptosis in murine melanoma cells in culture, but α-tocopherol, α-tocopheryl acetate and α-tocopheryl nicotinate at similar concentrations were ineffective (21). α-TS induces only growth inhibition in human melanoma cells (17), Certain cancer cells such as rat glioma cells (C6) are more sensitive to natural (d)-α-TS than to synthetic (dl)-α-TS on the criterium of growth inhibition whereas other tumors are equally sensitive to both natural and synthetic forms of α-TS (10).

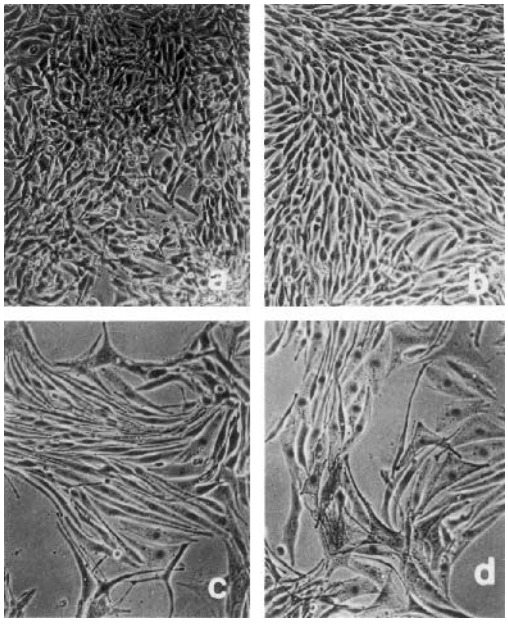

Figure 1 Melanoma cells (10^5) were plated in tissue culture dishes (60 mm), and *d*-α-tocopheryl succinate (α-TS) and sodium succinate plus ethanol were added to separate cultures 24 h after plating. Drugs and medium were changed at 2 and 3 days after treatment. Photomicrographs were taken 4 days after treatment. Control cultures showed fibroblastic cells as well as round cells in clumps (a); cultures treated with ethanol (1%) and sodium succinate (5–6 μg/ml) also exhibited fibroblastic morphology with fewer round cells (b); α-TS-treated cultures 5 μg/ml (c), and 6 μg/ml (d) showed a dramatic change in morphology. Magnification ×300 (21).

Mechanisms of action of high doses of dietary antioxidants on tumor cells

To study the mechanisms of differential effects of antioxidant nutrients in cancer cells, it is important to establish whether the greater sensitivity of cancer cells to dietary antioxidant micronutrients (vitamins A, C and E, and carotenoids) is due to increased accumulation of antioxidants in these cells in comparison to that found in normal cells or whether cancer cells and normal cells accumulate the same levels of these antioxidant micronutrients, with the cancer cells being more sensitive to these micronutrients than the normal cells.

Accumulation of dietary antioxidants in normal and cancer cells

Some studies have shown that tumor cells accumulate more vitamin C than normal tissue following the administration of radioactive labeled vitamin C into animals carrying transplanted tumor (44). A similar observation was made earlier in patients with leukemia (37). Thus, increased accumulation of vitamin C by tumor cells following high-dose supplementation may be responsible for its anti-cancer activity. Our results show that human cervical cancer cells (HeLa cells) and normal human fibroblasts in culture accumulate similar levels of α-TS within 24 h of treatment (Table 1). This suggests that the tumor cells acquired increased sensitivity to α-TS for growth-inhibition, differentiation and/or apoptosis during transformation. The relative uptake of other antioxidant micronutrients such as retinoids and carotenoids by normal and cancer cells in culture has not been studied.

The analysis of the basal levels of antioxidant micronutrients in human tumors and their adjacent normal tissues shows that the levels of individual antioxidant micronutrients in tumor tissue may be higher, lower or the same in comparison to those found in the adjacent normal tissues (45–48). The exact reasons for these variations are not known. Several factors may account for the above results. They include differences in the dietary intake,

TABLE 1 Accumulation of *d*-α-tocopheryl succinate (α-TS) in human cervical cancer (HeLa cells) and normal human fibroblasts after 24 h 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 55–75%. The levels of accumulation after treatment of cells with α-TS for 24 h were similar in both HeLa cells and normal fibroblasts. Each measurement was repeated twice and they were reproducible within the same experiment (43).

TABLE 2 Effects of retinoids, β-carotene and vitamin E on gene expression in tumor cells in culture

Reduced gene expression and/or activity	Increased gene expression and/or activity
p53 mutant	p53 wild-type
c-myc	p21
H-ras	c-fos
Bcl_2	c-jun
c-neu	HSP70
c-erbβ_2	HSP90
VEGF	connexin
Phosphotyrosine kinase	TGFb
Protein kinase C	Map kinase
	Caspase
	Cyclin A and D and their kinases

Summarized from reviews (8, 10).

vascularity, and uptake and subsequent intracellular metabolism of antioxidant micronutrients between normal and cancer cells.

Dietary antioxidant-induced alterations in gene expression in cancer cells

Since high-dose vitamins A, C and E and carotenoids within certain dose ranges inhibit the growth of cancer cells but not of normal cells (16–20, 22–43), the studies on the expression of genes that are involved in differentiation, growth regulation, transformation and apoptosis have been investigated only in cancer cells. These studies reveal that retinoids, vitamin E and β-carotene attenuate the levels of those cell signaling systems and gene expressions that can lead to decreased cell proliferation rate, increased differentiation and/or apoptosis. They include expression of c-myc, H-ras (49, 50), N-myc (51), mutated p53 (27), protein kinase C activity (52, 53), caspase (54), tumor necrosis factor (55), transcriptional factor E2F (25) and Fas (24). Retinoids, vitamin E and β-carotene enhance the levels of those cell signaling pathways and gene expression that can lead to reduced growth rate, increased differentiation and/or apoptosis, and they include the expression of wild type p53 (27) and p21 (32), transforming growth factor β (TGF-β) (22) and the connexin gene (28). The above changes (Table 2) in gene expression may be one of the major factors that account for the growth-inhibitory effect of these dietary antioxidant micronutrients on cancer cells. It should be pointed out that most of the effects of dietary antioxidant micronutrients such as vitamins A, C and E, and carotenoids on gene expression in cancer cells may not be due to their classical antioxidant action.

In addition to changes in gene expression, a novel mechanism of action of α-TS has been reported in an animal tumor model. α-TS inhibits the growth of tumor cells in vivo without affecting the normal cells (33). It also reduces the expression of vascular endothelial growth factor (VEGF), and thus acts as an anti-angiogenesis factor at a concentration which is not toxic to normal cells. It is unknown whether retinoids, vitamin C and β-carotene, which also inhibit the growth of cancer cells, can cause similar effects on angiogenesis in vivo.

Effect of low doses of individual dietary antioxidants

In contrast to the effect of high doses of dietary antioxidant micronutrients, low doses of these micronutirents can have no effect on the growth of cancer cells and normal cells, and may even stimulate the growth of some cancer cells without affecting the growth of normal cells. For example, vitamin C at a low dose stimulated the growth of human parotid carcinoma cells in culture (16) and human leukemic cells in culture (15), but has no effect on the growth of human melanoma cells in culture (16) or murine neuroblastoma cells (20). Polar carotenoids at low doses can stimulate the growth of human melanoma cells in culture (17). In addition, certain amounts of antioxidants are needed for the growth

of normal and cancer cells. Therefore, we do not recommend low doses of individual or multiple antioxidant during radiation therapy.

Effect of multiple dietary antioxidants

A mixture of dietary antioxidants is more effective in reducing the growth of cancer cells than the individual antioxidants. A mixture of retinoic acid, α-TS, vitamin C and polar carotenoids produced approximately 50% growth inhibition in human melanoma cells in culture at doses which produced no significant effect on growth when used individually (Table 3). Doubling the dose of vitamin C in the mixture caused a dramatic enhancement of growth inhibition. Similar observations were made on human parotid carcinoma cells in culture (16). A 50% reduction in dose of each micronutrient in the mixture did not affect the growth of human melanoma cells in culture. Each of the dietary antioxidants has different modes of action and therefore, it is essential that multiple dietary antioxidants are used in combination with radiation therapy.

Effect of individual endogenously made antioxidants

The effect of endogenously made antioxidants on cancer cells appears to be dose-dependent. For example, the over-expression of Mn-SOD reduces the growth and suppresses the malignant phenotype of glioma (56) and melanoma cells (57) in culture. Glutathione-elevating agents such as N-acetylcysteine (NAC) at high doses inhibit the growth of cancer cells in vitro and in vivo (58).

TABLE 3 Effect of a mixture of four antioxidant micronutrients on growth of human melanoma cells in culture

Treatments	Cell number (% of controls)
Vit C (50 μg/ml)	102 ± 5[a]
PC (10 μg/ml)	96 ± 2
α-TS (10 μg/ml)	102 ± 3
RA (7.5 μg/ml)	103 ± 3
Vit C (50 μg/ml) + PC (10 μg/ml) + α-TS (10 μg/ml) + RA (7.5 μg/ml)	56 ± 3
Vit C (100 μg/ml)	64 ± 3
Vit C (100 μg/ml) + PC (10 μg/ml) + α-TS (10 μg/ml) + RA (7.5 μg/ml)	13 ± 1

Data were summarized from a previous publication (17).
[a]standard error of the mean.
PC, polar carotenoids originally referred to as beta-carotene (30); Vit C, sodium ascorbate; α-TS, α-tocopheryl succinate; RA, 13-*cis*-retinoic acid.

EXPERIMENTAL BASIS OF OUR HYPOTHESIS

High doses of dietary antioxidants enhance the effect of irradiation on cancer cells

These antioxidants enhance the effect of irradiation selectively on cancer cells while protecting normal cells against some of the injuries. To observe this effect, antioxidants must be given before and after irradiation at high doses, and they must be present throughout the experimental period. The extent of enhancement of radiation damage by dietary antioxidant micronutrients depends upon the dose of radiation, dose and types of antioxidants, treatment period and type of tumor cells.

Vitamins A, C and E, and carotenoids under the above experimental conditions may protect normal cells against radiation damage, but may enhance the effect of irradiation on cancer cells. For example, retinoic acid enhances the effect of irradiation on tumor cells by inhibiting the repair of potential lethal damage in cancer cells more effectively than that produced in normal fibroblasts (59). Retinoic acid with α2a interferon enhances radiation-induced toxicity in neck and head squamous cell carcinoma cells in culture (60, 61). We have reported that the dose of vitamin E (α-TS) which inhibited the growth of human cervical cancer cells in culture, but not of normal human fibroblasts in culture, when given in a single high-dose before irradiation, enhanced the levels of radiation-induced decrease in mitotic accumulation (Fig. 2) and chromosomal damage (Fig. 3) in cancer cells. This antioxidant was present in the growth medium before and after irradiation for the entire observation period. On the other hand, the same dose of α-TS did not modify the effect of irradiation on mitotic accumulation in normal cells (42), but it protected normal cells against chromosomal damage (43). In another study, we have reported that an aqueous form of vitamin E and α-TS enhanced the level of radiation-induced growth inhibition in neuroblastoma cells (Fig. 4) (62). Vitamin C enhanced the effect of irradiation on neuroblastoma cells, but not on glioma cells in culture (20). Dehydroascorbic acid (DHA), the major metabolite of ascorbic acid, acts as a radiosensitizer for hypoxic tumor cells (63). These studies show that certain antioxidant micronutrients in a single high-dose, when given before irradiation, can protect normal cells against some of the effects of radiation damage, as well as

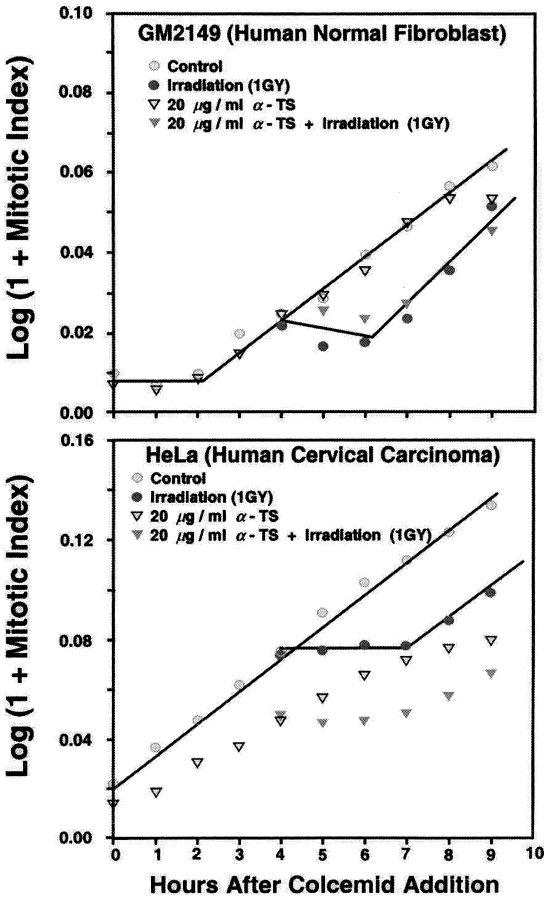

Figure 2 Effect of α-TS on mitotic accumulation in 3 human cancer cell lines and 3 normal human fibroblasts. Decreased mitotic accumulation is seen in cancer cells (A–C), but not in normal fibroblasts (D–F). Each point represents an average of 6 samples. Significant difference at $p = 0.05$ was observed in all tumor cell lines (A–C) at 20 μg/ml of α-TS at all points in comparison to controls (42).

enhance the effect of irradiation on cancer cells in culture provided they are present throughout the experimental period. A similar observation has been made in an animal model. For example, vitamin A (retinyl palmitate) or β-carotene at high doses given daily through dietary supplement before x-irradiation and throughout the experimental period enhanced the levels of radiation damage on transplanted breast adenocarcinoma in mice, and protected normal tissue against some of the toxicity of local irradiation (Table 4) (34). The administration of vitamin C through drinking water before and after x-irradiation decreased the survival of ascites tumor cells in mice without causing a similar effect on normal cells (64). The administration of multiple antioxidant micronutrients (vitamins A, C and E) protected normal cells against damage produced by radio-immunotherapy in mice without protecting cancer cells (65). The administration of these doses of multiple antioxidants may have been low; and therefore, the radiosensitizing effect of these micronutrients could not be observed. A few human studies have confirmed the differential modification of radiation injuries on normal and cancer cells by these micronutrients. For example, retinoic acid and interferon α2a enhanced the efficacy of radiation therapy of locally advanced cervical cancer (36).

Antioxidants selectively protect normal cells against radiation damage

Several studies have established the radioprotective value of vitamins A, C, and E, and carotenoids in protecting normal cells (2, 36, 65–73) but not cancer cells (65, 72). Treatment with antioxidant micronutrients also reduced the effect of irradiation on normal tissues in patients with small cell lung carcinoma (39, 40). β-Carotene reduced radiation-induced mucositis without interfering with the efficacy of radiation therapy in patients with cancer of the head and neck (74). Administration of vitamin E in a single dose to normal adult rodents before x-irradiation increased the survival of irradiated animals (66–68). The addition of vitamin C and vitamin E in a single dose before irradiation reduced the level of DNA damage to normal cells (73). Tocopherol in combination with pentoxifylline decreased the level of radiation-induced fibrosis (69).

Thus, most of the studies clearly demonstrate that high-dose dietary antioxidant micronutrients (vitamins A, C and E, and carotenoids) selectively enhance the growth-inhibitory effect of irradiation on cancer cells, and in some cases they protect normal cells against such damage. There are no published studies which show that high doses of these micronutrients can protect cancer cells against radiation damage.

Mechanisms of action of dietary antioxidant micronutrient-induced enhancement of radiation damage in cancer cells

The exact reasons for the dietary antioxidant micronutrient-induced enhancement of radiation damage on cancer cells are unknown. We propose the following: (a) the treatment of tumor cells with high doses of these micronutrients before irradiation can initiate changes in expressions of those genes which can cause differentiation, growth inhibition and/or

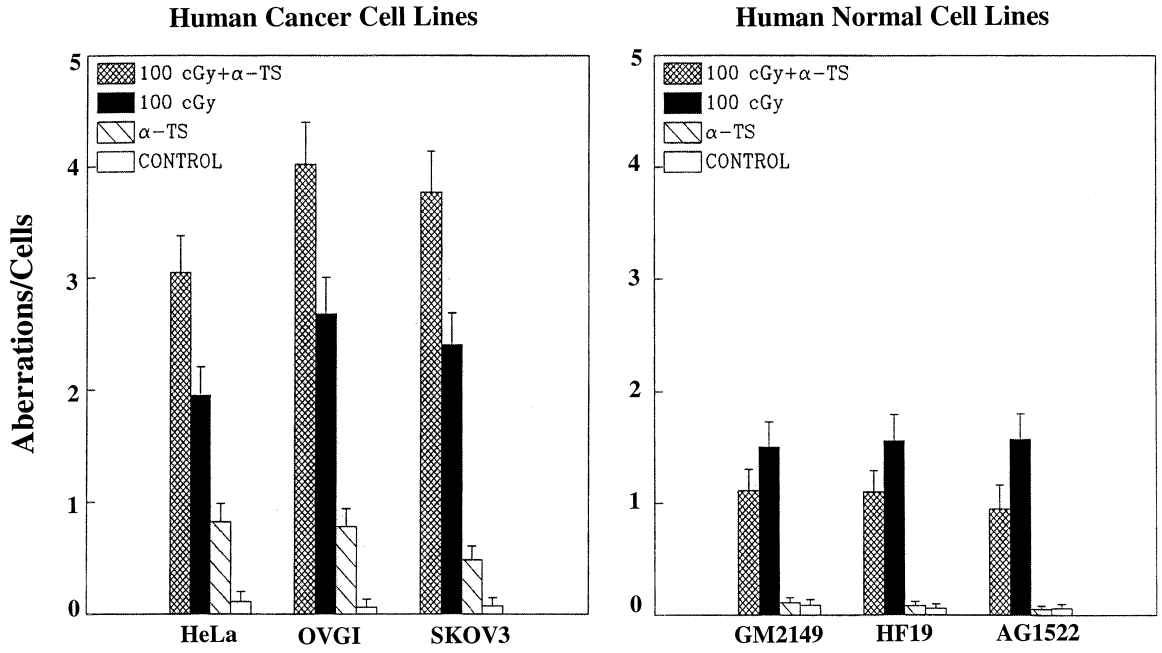

Figure 3 Effect of *d*-α-tocopheryl succinate (α-TS) on the level of radiation-induced chromosomal damage in human cervical cancer (HeLa cells), ovarian carcinoma cell lines (OVG1 and SKOV3) and in human normal skin fibroblasts (GM2149, HF19 and AG1522). α-TS treatment alone increased chromosomal damage 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; and 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 $p = 0.05$ (43).

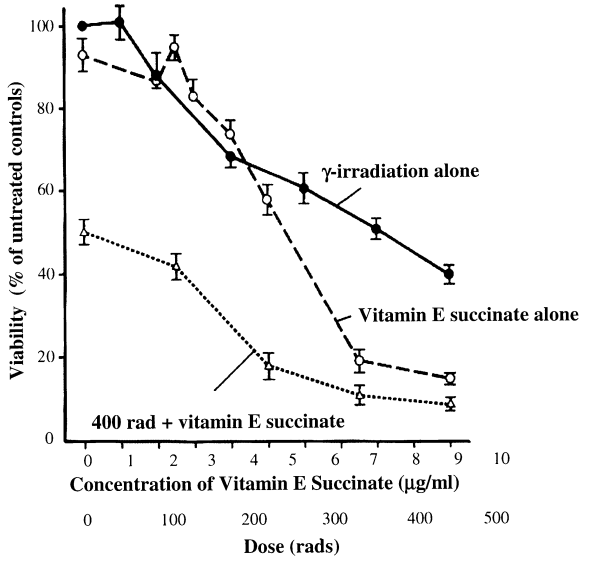

Figure 4 Neuroblastoma cells (NBP2) were plated in tissue culture dishes (60 mm), and the cells were gamma-irradiated 24 h after plating. Vitamin E succinate or the solvent (ethanol 0.25% and sodium succinate 5 μg/ml) was added immediately before irradiation. The drugs and medium were changed after 2 days of treatment. The number of cells per dish was determined after 3 days of treatment. Each experiment was repeated at least twice involving 3 samples per treatment. The average value ($172 \pm 7 \times 10^4$) of untreated control neuroblastoma cells was considered 100%, and the growth in treated cultures was expressed as a percentage of untreated controls. The bar at each point is standard error of the mean (62).

TABLE 4 Effect of vitamin A, beta-carotene and local x-irradiation on survival of mice with transplanted breast adenocarcinoma.

Treatments	No. of mice	One year survival (no. of mice)
Control	24	0
3000 rads, single dose	24	0
Vitamin A	24	0
Beat-carotene	24	0
Vitamin A plus x-ray	24	22
Beta-carotene plus x-ray	24	22

Data were summarized from Seifter, et al. (34). Diets were supplemented with vitamin A (3000 IU/mouse) and beta-carotene (270 μg/mouse), and these doses were about 10 times greater the RDA for mouse.

apoptosis (22, 24–28, 33, 49–55), and this damage will continue to progress during the entire period of radiation therapy; and (b) micronutrients such as retinoic acid can inhibit the repair of radiation damage in cancer cells more than that in normal cells (59). In contrast to cancer cells, these dietary micronutrients do not enhance radiation damage in normal cells; therefore, they can protect them against such damage at least on certain criteria.

Modification of radiation damage by endogenously made antioxidants

Extensive studies have been published to show that endogenously made antioxidants such as SH-compounds and their derivatives (cysteamine, glutathione, and others), when given before x-irradiation at doses which do not cause toxicity, protected normal and cancer cells in vitro and in vivo (1, 2). As a matter of fact, differential radiosensitivity, which is commonly observed during various phases of the cell-cycle, is related to a difference in the levels of SH-compounds. It has been shown that mitotic cells, which are most radiosensitive, have the lowest levels of SH-compounds, and that S-phase cells, which are the most radioresistant, have the highest level of these compounds (75).

Antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase play an important role in protecting normal cells against damage produced by reactive oxygen species. Therefore, one would predict that they play a similar role in cancer cells.

Indeed, overexpression of antioxidant enzymes increased the radioresistance of tumor cells (76–78). For example, some studies have revealed that overexpression of mitochondrial manganese-superoxide dismutase (Mn-SOD) suppressed the growth of some tumor cells (77) and enhanced the radioresistance of hepatocellular carcinoma cell line (78) and other tumor cells (76–79). However, it does not modify radiation damage on hypoxic cancer cells (79). For these reasons, we do not recommend endogenously made antioxidants such as glutathione-elevating agents (NAC and α-lipoic acid) or selenium, a co-factor for glutathione peroxidase, over the RDA amount during radiation therapy.

EXPERIMENTAL BASIS OF OTHER HYPOTHESIS

Dietary antioxidants such as vitamin E (α-tocopherol) when given in a single low dose shortly before irradiation reduces the effectiveness of x-irradiation in an animal tumor model (14). Similarly, when vitamin C, E or NAC, a glutathione-elevating agent, is added to tumor cell culture in a single low dose that does not affect the growth of cancer cells, shortly before irradiation, it protected cancer cells against radiation damage (11, 13, 14). These data have been used to suggest that antioxidants should not be used during radiation therapy.

Comments on current controversies

The data presented in support of each of the proposed hypotheses suggest that the primary basis of the current debate on the use of antioxidants during radiation therapy is due to extrapolation of two opposing conceptual frame works. The first is based on specific experimental designs while the other is based on experimental conditions that are entirely different with respect to dose, treatment time and type of antioxidants. Data show that high doses of dietary antioxidants that inhibit the growth of cancer cells but not normal cells when given before and after irradiation for the entire observation period may improve the efficacy of radiation therapy. However, results also show that low doses of dietary or endogenously made antioxidants given in a single low dose that does not affect the growth of cancer cells shortly before irradiation may protect cancer cells against radiation damage.

Some oncologists recommend a multiple vitamin preparation containing low doses of antioxidants after completion of radiation therapy. This practice may be counterproductive, because like normal cells, tumor cells need certain amounts of antioxidants for growth and survival, and because low doses of antioxidants may stimulate the growth of residual tumor cells. Thus, it is essential that these factors should be taken into account while designing a clinical trial to test the efficacy of antioxidants in combination with radiation therapy. In addition, data on antioxidants that are obtained from cancer prevention studies should not be used in designing cancer treatment investigations, because they could be harmful. For example, low doses of NAC or high levels of antioxidant enzymes may be very useful in cancer prevention, but could be harmful when used in combination with radiation therapy, because they would protect cancer cells against radiation damage.

Clinical studies with multiple dietary antioxidant micronutrients in combination with standard therapy

A randomized placebo control trial on the use of antioxidants during radiation therapy has not yet been performed. Dr Jae Ho Kim of Henry Ford Hospital in Detroit, Michigan, has completed a randomized pilot trial with high-dose multiple micronutrients including dietary antioxidants (vitamins C and E, and natural β-carotene) in cancer patients receiving chemotherapy and radiation therapy.

Results showed that all patients tolerated high-dose micronutrients well, and that quality of life was improved during radiation therapy with no adverse effect on the efficacy of standard therapy (personal communication). A few oncologists are using high dose multiple antioxidants in combination with standard cancer therapy and improved outcomes have been reported (39, 40).

PROPOSED DOSES OF MICRONUTRIENTS TO BE USED AS AN ADJUNCT TO RADIATION THERAPY

The scientific rationale for the proposed micronutrient protocol has been discussed in detail in recent publications (8, 10). The micronutrient supplement protocol is divided into two categories: active treatment phase and maintenance phase. During the active treatment phase, daily micronutrients are given orally at least 48 h before irradiation, and continued for the entire treatment period. After the completion of treatment, doses of additional micronutrients are reduced to half over a 4-week period, and maintained during the maintenance phase (lifetime). The rational for giving micronutrients at least 48 h before irradiation is that micronutrient-induced damage is initiated in cancer cells prior to irradiation. The rational for continuing micronutrient supplement through out the treatment period is that these micronutrients continue to cause additional damage in cancer cells as well as inhibiting the repair of radiation damage.

The micronutrient protocol contains supplements described below. A baseline supplement contains multiple micronutrients vitamin A, C and E, and natural β-carotene, vitamin D, B-vitamins and appropriate minerals but not iron, copper and manganese, because these three trace minerals interact with vitamin C to produce free radicals. A brand name, Sevak (Premier Micronutrient Corporation, Nashville, TN), which contains the above ingredients, is being sold commercially and is in a clinical trial. A preparation of multiple micronutrients is suggested because some of them may be depleted during radiation therapy due to extensive cellular death, loss of appetite and other side effects. An additional 8 grams of vitamin C in the form of calcium ascorbate is recommended. Doses of vitamin C at 10 grams or more have been used in human cancer treatment without toxicity (38, 80). This form of vitamin C was selected because ascorbic acid at high doses can cause an upset stomach in some patients (80). Calcium ascorbate rather than sodium ascorbate was selected because sodium ascorbate at high doses can increase the molarity of urine in the bladder and increase the risk of chemical induced bladder cancer in animals due to chronic irritation (81). An additional 800 IU of natural vitamin E in the form of α-TS is recommended. This form of vitamin E is the most potent form of vitamin E both in vitro and in vivo (8, 10). The natural form of vitamin E is used because animal studies demonstrate that various organs selectively pick up the natural form of vitamin E over the synthetic form (82). An additional 60 mg/day of natural β-carotene is recommended. The natural form of β-carotene was selected because it is more effective than the synthetic form. For example, natural β-carotene protects against radiation-induced transformation in vitro, whereas synthetic β-carotene is ineffective (83).

All micronutrient supplements described above should be taken orally and in two divided doses, one-half dose in the morning and one-half dose in the evening. The rationale for taking micronutrients twice a day is that the biological half-life of most micronutrients is about 6–12 h. Micronutrient supplements should be started at least 48 h prior to standard therapy and continued for one month after completion of standard therapy. Thereafter, the maintenance phase begins in which additional doses of vitamin C, vitamin E and β-carotene can gradually (over a 4-week period) be reduced to half the levels of the active treatment phase. Such maintenance doses of micronutrients may reduce the risk of recurrence of tumors as well as the risk of a second malignancy among cancer survivors.

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