Review Article

High Doses of Multiple Antioxidant Vitamins: Essential Ingredients in Improving the Efficacy of Standard Cancer Therapy

Kedar N. Prasad, PhD, Arun Kumar, MD, Vinod Kochupillai, MD, and William C. Cole, PhD

Center for Vitamins and Cancer Research (K.N.P., A.K., W.C.C.), Department of Radiology, School of Medicine, University of Colorado Health Sciences Center, Denver, CO; and All India Institutes of Medical Sciences (V.K.), New Delhi, INDIA

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Numerous articles and several reviews have been published on the role of antioxidants, and diet and lifestyle modifications in cancer prevention. However, the potential role of these factors in the management of human cancer have been largely ignored. Extensive *in vitro* studies and limited *in vivo* studies have revealed that individual antioxidants such as vitamin A (retinoids), vitamin E (primarily α-tocopheryl succinate), vitamin C (primarily sodium ascorbate) and carotenoids (primarily polar carotenoids) induce cell differentiation and growth inhibition to various degrees in rodent and human cancer cells by complex mechanisms. The proposed mechanisms for these effects include inhibition of protein kinase C activity, prostaglandin E_1-stimulated adenylate cyclase activity, expression of c-myc, H-ras, and a transcription factor (E_2F), and induction of transforming growth factor-β and p^{21} genes. Furthermore, antioxidant vitamins individually or in combination enhance the growth-inhibitory effects of x-irradiation, chemotherapeutic agents, hyperthermia, and biological response modifiers on tumor cells, primarily *in vitro*. These vitamins, individually, also reduce the toxicity of several standard tumor therapeutic agents on normal cells. Low fat and high fiber diets can further enhance the efficacy of standard cancer therapeutic agents; the proposed mechanisms for these effects include the production of increased levels of butyric acid and binding of potential mutagens in the gastrointestinal tract by high fiber and reduced levels of growth promoting agents such as prostaglandins, certain fatty acids and estrogen by low fat. We propose, therefore, a working hypothesis that multiple antioxidant vitamin supplements together with diet and lifestyle modifications may improve the efficacy of standard and experimental cancer therapies.

Key Teaching Points

- Supplemental antioxidants poteniate the efficacy of chemotherapy
- Supplemental antioxidants potentiate the efficacy of x-irradiation and hyperthermia
- Supplemental antioxidants induce differentiation in cancer cells
- Supplemental antioxidants regulate gene expression in cancer cells

INTRODUCTION

At present, about 1.2 million new cases of cancer are detected annually in the US and about 600,000 people die of this disease every year. The role of antioxidant vitamins, diet and lifestyle modifications in modulating human cancer incidence has drawn significant attention from basic and clinical scientists. This issue has been critically reviewed with respect to cancer prevention in a recent publication [1]. Although the role of antioxidant vitamins in cancer treatment has been studied using tumor cells in culture, animals with induced or transplanted tumors, and patients with certain tumors, these results are scattered, and no unifying hypotheses to explain the mechanisms of action of these vitamins *in vitro* or *in vivo* that have relevance in cancer treatment have been proposed. The efficacy of standard tumor therapy (surgery, chemotherapy, and

Abbreviations: RA=retinoic acid, α-TS=α-tocopheryl succinate, α-TA=α-tocopheryl acetate, αT=α-tocopherol.

Address reprint requests to: Kedar N. Prasad, PhD, UCHSC, Dept. of Radiology, Box C278, 4200 E. 9th Avenue, Denver, CO 80262.

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radiation therapy) has reached a plateau. Therefore additional innovative approaches for the treatment of human cancer must be developed. Among such approaches, antioxidant vitamins (retinoids, vitamin E, vitamin C and carotenoids) appear to be most promising, because they at high doses not only directly inhibit the growth of various rodent and human cancer cells, but enhance the effects of standard tumor therapeutic agents *in vitro* and *in vivo*. Although high doses of individual antioxidant vitamins, primarily, retinoids are being used in the treatment of some human tumors, no well designed controlled studies on the effects of antioxidant vitamins in combination with standard therapy are in progress. The lack of enthusiasm among clinical oncologists for using high doses of antioxidant vitamins in combination with radiation therapy and chemotherapy is primarily based on fear that antioxidant vitamins may protect both normal and cancer cells against free radicals which are generated by x-irradiation and most chemotherapeutic agents. Several *in vitro* and some *in vivo* studies suggest that such concerns are not valid. Therefore, a critical review on the role of antioxidant vitamin supplements, diet, and lifestyle modifications in combination with standard cancer therapy in improving management of human cancer is needed. Based on results of our studies and others, we have proposed a hypothesis that supplementation with high doses of multiple antioxidant vitamins, together with diet modification and lifestyle changes may improve the efficacy of standard and experimental cancer therapies by reducing their toxicity on normal cells and by enhancing their growth- inhibitory effects (due to cell death, differentiation and reduced proliferation rate) on cancer cells. This review will discuss whether or not the above hypothesis can be supported by published experimental and/or clinical results.

EFFICACY OF STANDARD TUMOR THERAPY PROTOCOLS

The efficacy of standard therapy which involves a combination of surgery (when feasible), multiple chemotherapeutic agents and ionizing radiation has reached a plateau, and for most solid tumors it remains ineffective on the criterion of a 5-year survival rate (cure rate). In some cancers (Hodgkin's diseases, some childhood leukemia, testicular tumors in adolescents and breast cancer), standard therapy has produced increased cure rates, but fear of developing second cancers and late non-neoplastic diseases among survivors exists. This is due to the fact that most of the anti-tumor agents are themselves mutagens and carcinogens. In addition to late harmful effects, standard therapy produces acute toxicity during treatment, the severity of which may limit continuation of the treatments. The issue of toxicity becomes more critical for patients from developing countries, many of whom are malnourished which could further aggrevate the toxicity of standard therapy. Therefore, agents which could reduce the toxicity of standard therapy on normal cells, but not on cancer cells, would markedly improve the efficacy of standard therapy in cancer treatment. Data are presented to show that antioxidant vitamins are among such agents. Generally, human tumors are highly variable with respect to their sensitivity to standard therapy. For example, teratocarcinoma cells are extremely sensitive to standard therapy, whereas cutaneous melanoma cells are very resistant. Therefore, agents which increase the growth-inhibitory effect of standard therapy on tumor cells irrespective of tumor type but not on normal cells would improve the efficacy of standard therapy. Results are presented to show that antioxidant vitamins are among such agents. Among long-term survivors of standard therapy who have increased risk of developing new cancer and other non-neoplastic diseases, agents which can reduce these risks would improve the value of standard therapy. Data are shown that antioxidant vitamin supplements, and diet and lifestyle changes may represent such agents. Vitamin supplements, diet, and lifestyle modification protocols for cancer prevention in this high risk population has been published [1].

EFFICACY OF EXPERIMENTAL CANCER THERAPY PROTOCOLS

Among experimental cancer therapeutic approaches, hyperthermia, immunotherapy, biological response modifiers, and gene therapy are being used to treat human cancers on a limited scale. The clinical results of each of the modalities have been variable and unimpressive. Very few studies have been performed to evaluate the role of antioxidant vitamins in modifying the growth-inhibitory effects of experimental therapeutic agents in any tumor cells in culture or *in vivo*. Experimental data show that certain biological response modifiers induce cell differentiation and/or growth inhibition in some tumor cells in culture. They include interleukins, interferon, tumor necrosis factor, cAMP and butyric acid. Except for butyric acid, all of these substances produce acute toxicity in humans. Antioxidant vitamins may enhance the effect of above biological response modifiers on cancer cells *in vitro* and *in vivo*.

Before discussing the rationale for using multiple antioxidants vitamins together with modifications in diet and lifestyle in combination with standard therapy or experimental therapy in cancer treatment, it is essential to briefly describe the anticancer properties of individual antioxidant vitamins.

ANTI-CANCER PROPERTIES OF INDIVIDUAL ANTIOXIDANT VITAMINS

The effects of individual antioxidant vitamins such as retinoids, vitamin C, vitamin E and carotenoids have been extensively investigated with respect to cancer prevention. A recent comprehensive review on this issue has been published [1]. It is now essential to examine the role of these vitamins in cancer

treatment. The effect of antioxidants on tumor cell growth, differentiation and apoptosis have been studied in cell culture models, transplanted tumors in syngeneic animals and in athymic mice, and in patients with certain tumors. These studies have revealed that vitamins, when used individually, can induce apoptosis selectively in cancer cells within certain dose ranges, or can inhibit or stimulate the growth of cancer cells, depending on the dose. The above vitamin effects depend on the type of vitamins, form of vitamins (physiological vs. analogs, physiological vs. type of ester), concentration of vitamins, and type of tumor and type of clone of the same tumor.

ANTI-CANCER PROPERTIES OF VITAMIN A AND ITS ANALOGS

Commercially, vitamin A (retinol) is sold as retinyl palmitate or retinyl acetate, and they have primarily been used in animal and human studies; however, these forms of vitamin A are inactive in tissue culture because of their poor aqueous solubility. Therefore, a vitamin A metabolite, retinoic acid or its analogs, which is readily soluble in ethanol is used in all tissue culture experiments. Retinoic acid possesses all the biological functions of vitamin A except in vision. Several analogs of vitamin A have been produced in order to make them more effective against cancer cells *in vitro* and *in vivo*. The most commonly used vitamin A analog in experimental systems is 13-cis retinoic acid (13-cisRA). Retinoic acid or its derivatives can induce cell differentiation, can inhibit growth, or can have no effect on some rodent and human cancer cells *in vitro* [2–5]. The type and extent of effect depends on the type, form and concentration of retinoic acid, and the tumor cell type. For example, retinoic acid and its derivatives within a certain dose range induce cell differentiation and growth inhibition in some cancer cells *in vitro* [2,3,5], but not in others [4]. The mechanisms of retinoid-induced growth inhibition in cancer cells are unknown; however, it increases the levels of growth-inhibitory signals in cancer cells. For example, retinoic acid inhibits protein kinase C activity in cancer cells [6] and reduces the expression of *c-myc* and *H-ras* oncogenes and other cellular genes in certain cancer cells *in vitro* [7]. Some studies have shown that vitamin A at high doses reduces the growth of transplanted tumors in animals [8]. There was no evidence of toxicity in proliferating organ systems, indicating that the growth inhibitory effect of vitamin A is relatively selective for cancer cells. A recent review on selectivity of vitamin-induced apoptosis for cancer cells has been published [9]. There have been extensive studies on the effects of vitamin A derivatives on the growth of human tumors *in vivo* (Table 1). At high doses, variable extents of tumor size reduction have been reported [10–11]. Retinoids have been shown to have very little or no effect on several human tumors which included melanoma, non-small cell lung carcinoma, prostate cancer, breast cancer and neuroblastoma [10]. Some of the analogs of retinoids produce extreme toxicity. Therefore the use of single antioxidant vitamins which require very high doses for its effectiveness has no significant value in the treatment of cancer, even though such doses may cause tumor regression of variable degrees.

Table 1. Efficacy of Retinoids in the Treatment of Human Tumors

Tumor type	Design	Agents	Patient no.	Response
Actinic keratoses	Phase III (randomized)	Etretinate	54	84%
Advanced squamous cell carcinoma	Phase II	13 cRA	4	50%
		13 cRA	28	80%
Mycosis fungoides	No record	13 cRA	123	60%
Laryngeal papillomatosis	Phase II	13 cRA	6	60%
Oral leukoplakia	Phase II	b-carotene (synthetic)	24	71%
	Phase III randomized	13 cRA (high dose)	44	Marked regression
Cervical cancer (CIN II or III)	Phase II	tRA (topical)	20	50%
“	Phase III randomized	tRA (topical)	301	Increased regression of CIN II but not CIN III
“	Phase II	13 cRA+INFa	23	53%
Advanced cervical cancer	“	13 cRA+INFa	26	50%
Advanced renal cell carcinoma	“	“	24	30%

Data were summarized from Tables 1–7 of a previous publication [10].
13 cRA=13-cis Retinoic acid.
INFa=Interferon a.

Anti-Cancer Properties of Vitamin C

Commercially, vitamin C is sold as ascorbic acid, sodium ascorbate, calcium ascorbate, potassium ascorbate and ascorbyl-acetate (fat-soluble vitamin C). Ascorbic acid and sodium ascorbate have been commonly used in tissue culture, animal and human studies. Vitamin C inhibits the growth of several rodent and human tumor cells in culture, in a concentration-dependent manner [12,13]. However, at lower doses, it can stimulate the growth of human parotid carcinoma cells, but not of human parotid adenoma cells *in vitro* [5]. It also stimulates the growth of human leukemic cells *in vitro* [14] and chemical-induced tumors in animal models [15]. These results suggest that the effect of vitamin C on tumor cells depends on the dose of vitamin C and the type of cancer cells. Vitamin C, at high

doses, inhibits the growth of tumor cells in animal models [15] and human tumors [16]. Effects of vitamin C alone on human tumors *in vivo* have been controversial. Pauling et al reported that high doses (10 g or more) reduced the growth of several human tumors and increased the survival time and provided better quality of life in several patients [16]. This was disputed by another group of clinical investigators [17]. The discordance between the two studies may, in part, be due to differences in the form of vitamin C (ascorbic acid vs. sodium ascorbate) and differences in the form and stage of the tumors. There was no evidence of toxicity at high doses of vitamin C for the period of tumor therapy by the criteria of a reduction in cell proliferation rate in dividing organ systems. There was also no evidence of any other clinical toxicity in any of the above studies except for occasional gastric upset with high doses of ascorbic acid. From the results of these studies, it is clear that the use of vitamin C alone may inhibit or stimulate the growth of tumors, or may have no effect. Therefore, the use of vitamin C alone in the treatment of cancer cannot be justified on a scientific basis.

Anti-Cancer Properties of Vitamin E (α-Tocopherol)

Commercially, the forms of vitamin E available include *d*-α-tocopherol (α-T) (natural) or *dl*-α-tocopherol (synthetic), mixed tocopherols, α-tocopheryl acetate (α-TA), α-tocopheryl succinate (α-TS) and α-tocopheryl nicotinate (α-TN). A water-soluble vitamin E analog (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, vitamin E) has become available for investigational use [18]. In addition, a synthetic preparation of α-tocopheryl hemisuccinate [19], which is not hydrolyzed, has also become available for experimental use.

Before 1980, all animal and human studies utilized α-T or α-TA to investigate the effects of vitamin E on a given biological or physiological function primarily *in vivo*. Because of its relative lack of aqueous solubility, the mechanistic effect of this vitamin could not be investigated in cell culture systems. In 1982, we demonstrated, for the first time, that α-TS is the most active form of vitamin E for inducing cell differentiation (Fig. 1), growth inhibition and cell death in murine melanoma cells in culture [20]. The potency of α-TS was further confirmed on several human and rodent tumor cells in culture which include human promyoletic leukemia [21], murine and human neuroblastoma [22,23], rat glioma [22], murine and human melanoma [4,20], human parotid carcinoma [5], and human breast carcinoma [21,24]. This observation was also confirmed in animal cancer *in vivo* [25,26]. Even as an antioxidant, α-TS was more potent than α-tocopherol [27]. Water soluble preparations of vitamin E also reduce the growth of human colorectal cancer cells *in vitro* and *in vivo* [18] and in murine neuroblastoma and rat glioma cells in culture [28]. The exact reasons for the effectiveness of α-TS or water soluble preparations of vitamin E on tumor cells *in vitro* are unknown. It may be due

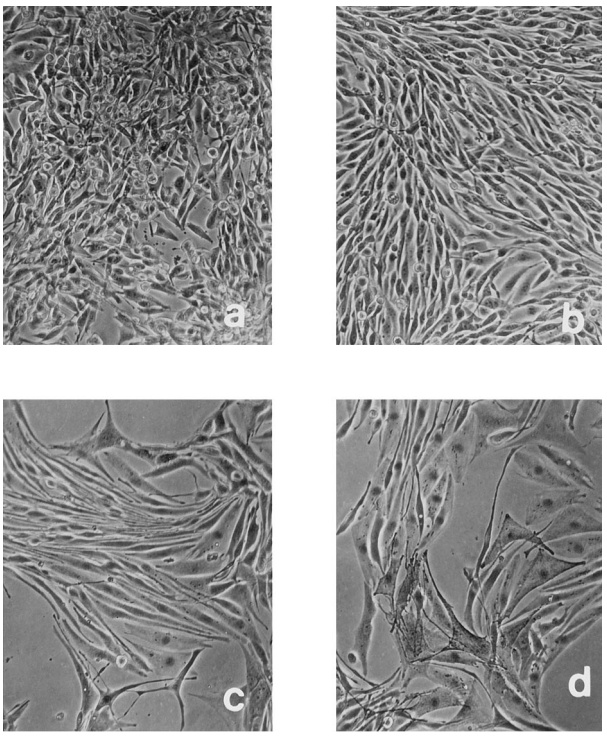

Fig. 1. Melanoma cells (10^5) were plated in tissue culture dishes (60 mm), and d-α-tocopheryl succinate (α-TS) and sodium succinate plus ethanol were added to separate cultures 24 hours after plating. Drugs and medium were changed at 2 and 3 days after treatment. Photomicrographs were taken 4 days after 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 c) 5 μg/mL, and d) 6 mg/mL, showed a dramatic change in morphology [20] $\times$300.

to increased intracellular accumulation of these forms of vitamin E in cancer cells. Indeed, when tumor cells were incubated in the presence of α-TS, α-TA, or α-T for a period of 1 or 24 hours, only α-TS was observed in an extraction for vitamin E by high performance liquid chromatography [22]. This suggested that α-TS crossed cell membranes more readily than α-TA or α-T. For most tumors, natural and synthetic forms of α-TS were equally effective, but for rat glioma (C-6) cells, the natural form of α-TS was more effective than the synthetic form [22]. The higher biological efficacy of the natural form of vitamin E was further documented by the observation that various organs in the rat selectively accumulate the natural form of vitamin E more than the synthetic form when administered simultaneously [29].

The use of α-TS in cancer therapy was criticized on the grounds that all α-TS will be hydrolyzed in the gut before absorption, and will therefore have the same biological effectiveness as α-T. This conventional thinking was found to be incorrect by experiments with humans. Human melanoma patients who were consuming 800 I.U. of d-α-TS daily showed

increased levels of α-T (60 μg/ml) and significant amounts of α-TS (6 μg/ml) in plasma (Prasad and Robinson, unpublished observation). The basal level of α-T in human blood is about 6 to 10 μg/ml. This suggested that a portion of α-TS is absorbed without hydrolysis and can become available to tumor cells for increased accumulation. A direct administration of α-TS near the site of buccal carcinoma caused regression of the tumor, whereas α-TA was ineffective [25,26]. α-TS also inhibited the growth of human neuroblastoma tumor transplanted into athymic mice [23]. There was no evidence of damage to normal tissue in any of the *in vivo* studies with α-TS. Thus, a few *in vivo* studies suggest that α-TS selectively inhibits the growth of tumor cells. The exact mechanisms of action of α-TS on cancer cells are unknown. However, increased accumulation of α-TS intracellularly can increase the level of growth inhibitory signals. Indeed, α-TS [30] and α-T [31] inhibit protein kinase C activity and expression of *c-myc* and *H-ras* oncogenes [32], both of which are considered one of the growth regulatory signals. Alpha TS also increases the synthesis and release of transforming growth factor-β which is considered one of the growth inhibitory signals [21] and inhibits the phophorylation and transactivation of E_2F, which is considered one of the important components in the regulation of cell proliferation [24]. There is no evidence that α-TS inhibits the growth of any normal dividing cells *in vivo*. This may be due to the fact that normal cells are considered to have strict homeostatic control for the uptake of antioxidant vitamins; and therefore, α-TS does not accumulate in amounts which can inhibit the growth of these cells. The differential uptake of α-TS between normal cells and cancer cells must be confirmed by additional experiments *in vivo*. Based on these results, we favor the use of *d*-α-TS for any clinical studies with vitamin E.

Anti-Cancer Properties of Carotenoids

There are over 1000 carotenoids found, so far, in nature; however, only a few of them occur in abundance in fruit and vegetables. These include β-carotene (carrots), lycopene (tomatoes) and lutein (spinach). Among these, the most widely studied carotenoid is β-carotene. Beta-carotene is available in synthetic and natural forms. Most of the clinical and experimental studies have been conducted with the synthetic form because it is cheaper and thought to be a more purified form of β-carotene, exhibiting a single peak on HPLC analysis. On the other hand, natural β-carotene might contain other carotenoids which would make the interpretation of any β-carotene effect difficult. Recently, we observed that commercial preparations of synthetic β-carotene may contain contaminants of relatively polar substances which absorb at 450 nm, and therefore are considered putative polar carotenoids [33]. In some preparations of synthetic β-carotene, no peak corresponding to β-carotene could be observed, but instead there was a multiplicity of peaks corresponding to putative polar carotenoids. Such polar carotenoid peaks were also present in preparations of natural β-carotene. Our earlier studies [1,4,33] with synthetic β-carotene preparations showed that β-carotene can induce differentiation in murine melanoma cells, but can inhibit or stimulate the growth of human melanoma cells. It can also inhibit the growth of several other tumor cells in culture, and can stimulate the level of cAMP-induced differentiation of murine neuroblastoma cells in culture. However, HPLC analysis of the synthetic β-carotene preparation which was used for these studies showed only the aforementioned polar carotenoids with the total absence of β-carotene. Freshly dissolved and a 6-month-old sample of the preparation gave similar HPLC profiles and similar biological activity [33], suggesting that these polar carotenoids are very stable in ethanol solution. Synthetic forms of β-carotene have been used by other investigators and they have shown to increase the expression of connexin gene, a gene which codes for the gap junction protein [34]. Several animal studies have utilized synthetic β-carotene in cancer treatment experiments. For example, supplementation with high doses of β-carotene caused slight regression of transplanted adenocarcinoma of breast in rat [8]. Administration of β-carotene directly adjacent to the tumor site markedly inhibited the growth of oral carcinoma in hamsters [25,26]. Synthetic β-carotene has been used in the treatment of oral leukoplakia in humans, and a marked regression was reported [35]. None of these studies measured their synthetic β-carotene preparation by analytical HPLC for purity. Therefore, whether or not the above effects were due to β-carotene and/or contaminants present in the preparation cannot be ascertained. On the contrary, they suggest that the role of polar carotenoids as contaminants in β-carotene preparations in inducing growth inhibition or growth stimulation must be considered. Homogeneous preparations of synthetic β-carotene dissolved in tetrahydrofuran has shown no effect on the growth of murine neuroblastoma cells in culture [33]. Preparations of natural β-carotene reduced the radiation-induced transformation of mammalian cells *in vitro*, whereas a synthetic one did not [36]. This study also provided no evidence of the purity of the β-carotene preparations. In our experience, natural β-carotene preparations exhibited several peaks in addition to the β-carotene as analyzed by HPLC [33]. Thus all experimental and clinical studies published on synthetic or natural β-carotene in which the purity of the preparation before use was not reported must be considered invalid with respect to the role β-carotene in modulating published biological functions. Nevertheless, β-carotene, being an important antioxidant, should be used in the treatment of cancer and we favor the use of natural β-carotene for this purpose.

Anti-Cancer Properties of B Vitamins

Most of the B vitamins have shown no direct anti-cancer activity. Supplementation with high doses of B_6 can stimulate the growth of transplanted human lung cancer cells in nude mice [37]. Others have reported that B_6 inhibits the growth of some tumor cells *in vitro* [38]. Nicotinamide (vitamin B_3)

increases radiation response of tumors in an animal model by increasing blood flow to tumor tissue [39,40]. These studies further suggest that the use of single vitamins in cancer treatment could be counter productive. High doses of B_6 (50 mg or more) could cause peripheral neuropathy. Nevertheless, supplementation with moderate doses of B vitamins is essential for maintaining optimal health.

Efficacy of a Mixture of Antioxidant Vitamins

Individual antioxidant vitamins produce varying degrees of tumor regression *in vivo* only at very high doses which frequently cause toxicity, especially with retinoid derivatives. At lower doses, they may be ineffective or stimulate the growth of cancer cells. Therefore, the use of single vitamins in cancer treatment has no biological or clinical merit. This led to the investigation of the effects of multiple antioxidants on the growth of cancer cells *in vitro* in order to demonstrate whether the individual vitamins can interact with each other to produce a higher degree of growth inhibition selectively on cancer cells than can be achieved by single vitamins at the same doses. We have reported (Table 2) that a mixture of four antioxidants (13-cis-retinoic acid, sodium ascorbate, *d*-α-tocopheryl succinate and polar carotenoids without any β-carotene) markedly inhibited the growth of human melanoma cells in culture at doses where each component individually had no effect on growth [4]. This observation was considered important because it suggested, for the first time, that a mixture of vitamins could be more effective than single vitamins in reducing tumor growth. This study also revealed the possibility that lower doses of individual vitamins as part of a mixture can be used in cancer treatment and thereby avoid the possibility of the toxicity seen with the single vitamins at higher doses, or growth stimulation at lower doses. The mixture of four antioxidants produced similar results on human parotid carcinoma cells in reducing their growth [5] (Table 3). Vitamin C (100 μg), α-TS (10 μg/ml), and RA (7.5 μg/ml) individually have no significant effect on the growth of tumor cells; however, polar carotenoids (10 μg/ml) reduced the growth to 60% of control. Doubling of the doses of one of the four vitamins in a vitamin mixture further reduced the growth of tumor cells *in vitro*. No well controlled studies on the effect of a mixture of four vitamins on the growth of animal tumors or human tumors have been performed. Therefore, preclinical and clinical studies using multiple vitamins at appropriate doses must be initiated.

Table 2. Effect of a Mixture of Four Vitamins on Growth of Human Melanoma Cells in Culture

Treatments	Cell number (% of controls)
Vit C (100 μg/ml)+PC (10 μg/ml)+α-TS (10 μg/ml)+RA (7.5 μg/ml)	13±1[a]
Vit C (50 μg/ml)+PC (10 μg/ml)+α-TS (10 μg/ml)+RA (7.5 μg/ml)	56±3
Vit C (50 μg/ml)+PC (5 μg/ml)+α-TS (5 μg/ml)+RA (3.8 μg/ml)	98±4
Vit C (100 μg/ml)	64±3
Vit C (50 μg/ml)	102±5
PC (10 μg/ml)	96±2
α-TS (10 μg/ml)	102±3
RA (7.5 μg/ml)	103±3

Data were summarized from a previous publication [4].

[a] Standard error of the mean.

PC=Polar carotenoid (PC) originally referred to as beta-carotene [52].

Table 3. Effect of a Mixture of Four Vitamins on Growth of Human Tumorigenic Parotid Acinar Cells in Culture

Treatments	Cell number (% of controls)
Vit C (100 μg/ml)+PC (10 μg/ml)+α-TS (10 μg/ml)+RA (7.5 μg/ml)	40±2[a]
Vit C (100 μg/ml)+PC (20 μg/ml)+α-TS (10 μg/ml)+RA (7.5 μg/ml)	19±2
Vit C (100 μg/ml)+PC (10 μg/ml)+α-TS (20 μg/ml)+RA (7.5 μg/ml)	10±1
Vit C (100 μg/ml)+PC (10 μg/ml)+α-TS (10 μg/ml)+RA (15 μg/ml)	25±3
Vit C (100 μg/ml)	98±3
PC (10 μg/ml)	60±5
PC (20 μg/ml)	30±1
α-TS (10 μg/ml)	90±5
α-TS (20 μg/ml)	62±4
RA (7.5 μg/ml)	89±2
RA (15 μg/ml)	55±2

[a] Standard error of the mean.

PC=polar carotenoid, originally referred to as beta-carotene [52].

α-TS=α-tocopheryl succinate (vitamin E).

Vit C=Vitamin C (sodium ascorbate).

RA=13-cis Retinoic acid.

Data were summarized from a previous publication [5].

EFFICACY OF ANTIOXIDANTS IN COMBINATION WITH STANDARD TUMOR THERAPEUTIC AGENTS

Several *in vitro* studies have revealed that vitamin C [12,13], α-TS [22,41,42], water soluble preparations of vitamin E [18,28], vitamin A and its derivatives [8,44] and β-carotene [8,45] enhance the growth inhibitory effect of most of the currently used chemotherapeutic agents on some cancer cells. Some examples of the effect of vitamins C and E in combination with standard therapeutic agents have been summarized in Tables 4 and 5, and Fig. 2 to 4. The extent of this enhancement depends on the dose and form of vitamin, the dose and type of chemotherapeutic agent and the type of tumor cells. However, vitamin C alone failed to enhance the effect of vincristine, 6-thioguanine, 1(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), adriamycin [41], and it reduced the effect of methotrexate and 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) on neuroblastoma cells in culture [12]. A mixture of vitamins

Table 4. Effect of Vitamin C in Combination with x-Irradiation and Certain Chemotherapeutic Agents on Murine Neuroblastoma Cells in Culture

Treatments	Cell number (% of controls)
Sodium (Na) ascorbate (5 or 200 μg/ml)	105±9[a]
5-Fluorouracil (0.08 μg/ml)	62±5
Na ascorbate (5 μg/ml)+5-Fluorouracil	4.5±2.1
X-irradiation (4 Gy)	28±3
Na ascorbate (5 μg/ml)+X-irradiation	1.8±0.4
Bleomycin (0.004 unit/ml)	27±2
Na ascorbate (200 μg/ml)+bleomycin	8±1
Sodium butyrate (0.5 mM)	13±2
Na Ascorbate (200 μg/ml)+sodium butyrate	4.6±1

Data were summarized from previous publications [12].
[a] Standard deviation.

Table 5. Effect of Aqueous Preparation of Vitamin E (Alpha Tocopheryl Acetate) in Combination with Certain Chemotherapeutic Agents on Murine Neuroblastoma Cells in Culture

Treatments	Cell number (% of controls)
Vitamin E (0.02 IU/ml)	53±5[a]
Bleomycin (0.002 Unit/ml)	54±4
Vitamin E+bleomycin	29±3[b]
5-Fluorouracil (0.03 μg/ml)	63±4
5-Fluorouracil+vitamin E	15±3[c]
Adriamycin (0.001 μg/ml)	42±3
Adriamycin+vitamin E	12±2[c]
CCNU (10 μg/ml)	55±6
CCNU+vitamin E	38±3
DTIC (4 μg/ml)	60±5
DTIC+vitamin E	31±4[b]
Cis-platin (0.2 μg/ml)	43±5
Cis-platin+vitamin E	18±5[b]
Mutamycin (0.01 μg/ml)	48±4
Mutamycin+vitamin E	24±4[b]
Chlorozotocin (2 μg/ml)	82±4
Chlorozotocin+vitamin E	36±3[c]

Adopted from a previous publication [28].
[a] Standard deviation.
[b] Additive effect.
[c] Synergistic effect.

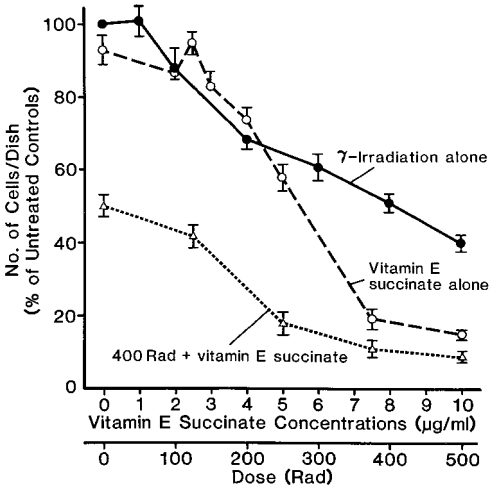

Fig. 2. Neuroblastoma cells (NBP2) were plated in tissue culture dishes (60 mm), and the cells were gamma-irradiated 24 hours after plating. Vitamin E succinate or the solvent (ethanol 0.25% and sodium succinate 5 μg/mL) was added immediately before irradiation. The drugs and medium were changed after 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 three samples per treatment. The average value ($172\pm7\times10^4$) of untreated control NB cells was considered 100%, and the growth in treated cultures was expressed as a % of untreated controls. The bar at each point is standard error of the mean [41].

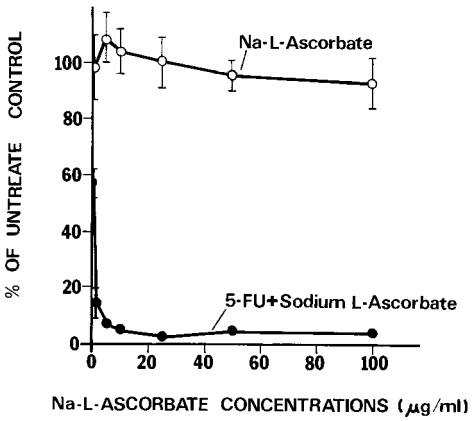

Fig. 3. Neuroblastoma cells (50,000 per dish) were plated in tissue culture dishes (60 mm), and 5-Fluorouracil (5-FU) (0.08 μg/mL) plus sodium ascorbate or sodium ascorbate alone was added 24 hours after plating. The drug and medium were changed every day, and the number of cells per dish was determined 3 days after treatment. Each value represents the mean of six to nine samples±standard deviation [12].

containing vitamin C failed to produce such an effect in combination with DTIC on human melanoma cells in culture [4]. A few *in vivo* studies support the concept that antioxidant vitamins selectively enhance the effect of standard therapy on tumor cells and thereby increase cure rates. For example, vitamin A (retinyl palmitate) or synthetic β-carotene in combination with x-irradiation or cyclophosphamide, increased the cure rate from 0 to over 90% in rats with transplanted adenocarcinoma of the breast [8]. The purity of β-carotene prior to administration was not reported; therefore, the above effect of β-carotene cannot be attributed to β-carotene alone. In contrast to the above effect, synthetic β-carotene reduced the growth-inhibitory effect of 5-FU, but it enhanced the growth-inhibitory effect of adriamycin and an alkylating agent [45]. A thio-containing antioxidant, pyrrolidinedithiocarbamate (PDTC), and a water-soluble vitamin E analogue (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; vitamin E), enhanced antitumor effects of 5-FU and doxorubicin *in vitro* against

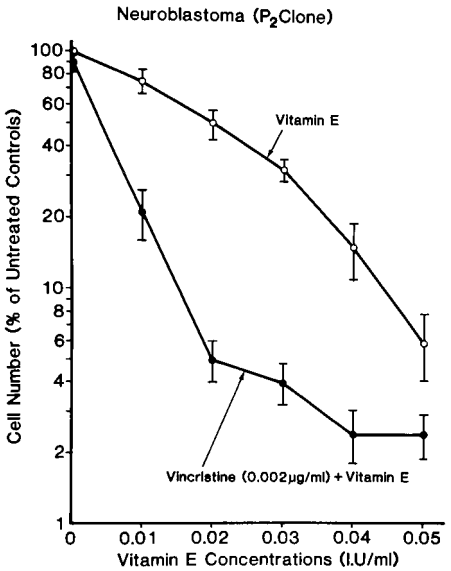

Fig. 4. Neuroblastoma cells (NBP2) (50,000 per dish) were plated in tissue culture dishes (60 mm), and vincristine and aqueous preparation of vitamin E (dl-α tocopheryl acetate) were added 24 hours later. Drugs and medium were changed 2 days after treatment. The cell number and the number of trypan blue-stained cells were determined 3 days after treatment. The number of stained cells was subtracted from the total number of cells to obtain viable cells per dish. The average of control cultures was considered 100%. Each value represents an average of at least six samples. The bar of each point is standard deviation. The bars not shown in figure were equal to sizes of symbols [28].

several cancer cell lines, and the effect of 5-FU *in vivo* against two colorectal cancer cell lines [18]. Similar results were reported earlier with vitamin C and 5-FU [12], and a water soluble preparation of vitamin E and 5-FU [28] on neuroblastoma cells. The effect of individual antioxidant vitamins in combination with x-irradiation or chemotherapeutic agents has not been tested in human tumors *in vivo* in a systematic manner. Most standard therapeutic agents mediate their effects, in part, by generating free radicals which damage both normal and cancer cells. Therefore, clinical oncologists fear that the use of high doses of antioxidant vitamins during standard cancer therapy might be harmful since they might protect both normal and cancer cells against free radical damage produced by tumor therapeutic agents. The available experimental data suggest that such fear has no scientific basis. For example, vitamin C [12,13], α-TS [22,41,42], water soluble preparations of vitamin E [18,28], vitamin A and retinoids [8,44] and β-carotene [8,45] individually enhance the growth inhibitory effect of x-irradiation and certain chemotherapeutic agents on tumor cells *in vitro* and *in vivo*. Some examples are illustrated in Fig. 2 to 4. This is a direct demonstration that antioxidants do not protect cancer cells against free radical and growth-inhibitory effects of standard therapy. On the contrary, they enhance its growth-inhibitory effects on tumor cells, but protect normal cells against its adverse effects. The failure of antioxidants to protect cancer cells and the success of antioxidants in protecting normal cells against the growth-inhibitory effects of standard tumor therapeutic agents can be attributed to the following: a) cancer cells can accumulate high intracellular levels of vitamins [22] due to altered homeostatic controls for vitamin uptake, whereas normal cells cannot accumulate high levels of vitamins due to intact homeostatic controls; and b) an excessive intracellular accumulation of antioxidant vitamins affects a variety of biochemical events in cancer cells, including, inhibition of protein kinase C in tumor cells [30,31,46], reduction of the expression of *c-myc* and *H-ras* genes in tumor cells [7,32], reduction of phosphorylation and transactivation of E_2F [24], enhancement of the synthesis and secretion of transforming growth factor-β [21], and induction P^{21}, a powerful inhibitor of the cell cycle [18]. All of these intracellular signals are considered growth-inhibitory signals for most tumor cells. Therefore, the initial protection of cancer cells from free radical damage by antioxidant vitamins becomes irrelevant because of the above subsequent intracellular events which are hypothesized to lead to growth inhibition, cell differentiation and cell death. The normal cells, which were initially protected from free radical damage by antioxidants, continued to be viable because antioxidant vitamins, presumably by virtue of their lack of intracellular accumulation, failed to influence growth inhibitory signals.

The effects of a mixture of antioxidant vitamins in combination with tumor therapeutic agents commonly used in the treatment of human melanoma have also been investigated using human melanoma cells in culture as an experimental model. Results showed that a mixture of antioxidant vitamins in combination with DTIC, cisplatin, tamoxifen and interferon α-2b produced a higher degree of growth inhibition than that caused by vitamins alone or chemotherapeutic agents alone (Table 6). It is interesting to note that vitamin C, when used

Table 6. Enhancement of the Effect of Certain Chemotherapeutic Agents by a Mixture of Four Vitamins on Human Melanoma Cells in Culture

Treatments	Cell number (% of controls)
Solvent	101±4[a]
Cis-platin (1 μg/ml)	67±4
Vitamin mixture	56±3
Cis-Platin+vitamin mixture	38±2
Tamoxifen (2 μg/ml)	81±3
Tamoxifen+vitamin mixture	30±2
DTIC (100 μg/ml)	71±2
DTIC+vitamin mixture	38±2
Interferon a2b	82±5
Interferon a2b+vitamin mixture	29±1

Data were summarized from a previous publication [4].

[a] Standard error of the mean.

Polar carotenoid was originally referred as beta-carotene [52].

Vitamin C, 50 μg/ml; polar carotenoid, 10 μg/ml; a tocopheryl succinate, 10 μg/ml and 13-cis-retinoic acid, 7.5 μg/ml were added simultaneously.

individually, blocked that action of DTIC on murine neuroblastoma tumor cells *in vitro* [12]; however, when it is used in a mixture of antioxidant vitamins, produced no such effect on human melanoma cells in culture. On the contrary, such a mixture of vitamins enhanced the effect of DTIC on melanoma cells *in vitro* [4]. This observation further suggests that the use of single vitamins in the treatment of human cancer has no biological rationale.

EFFICACY OF ANTIOXIDANT VITAMINS IN COMBINATION WITH HYPERTHERMIA

Hyperthermia alone or in combination with radiation, is primarily used in the management of local tumors when all other standard modalities have failed. Variable improvements in transient tumor control have been observed. The temperatures used range from 43°C to 45°C. Hyperthermia, for the most part, has not proven to be of any significant value in improving the cure rate or survival time, but has proven to be of some value in improving quality of life for a variable period of time. Antioxidant vitamins such as α-TS [43,47,48] enhances the effect of hyperthermia at 43°C as well as 41°C on murine melanoma [47] and murine neuroblastoma [48] cells in culture (Table 7). The mechanisms of their interaction are unknown; the effect of other antioxidants in combination with hyperthermia on the growth of tumor cells has not been studied. It is important to observe that antioxidants such as α-TS can enhance the growth-inhibitory effect of hyperthermia on tumor cells *in vitro*. Therefore, antioxidant vitamin supplements may improve the efficacy of hyperthermia in the management of human cancer.

Table 7. Effect of Alpha Tocopheryl Succinate (α-TS) on Hyperthermia-Induced Growth Inhibition in Neuroblastoma Cells in Culture

Treatments	Cell number (% of controls)
Solvent (ethanol 0.25%)+Sodium Succinate (5 μg/ml)	102±3[a]
α-TS (5 μg/ml)	50±3
43°C (20 min)	43±1
α-TS+43°C	9±1
41°C (45 min)	56±3
α-TS+41°C	21±2
40°C (8 hr)	55±2
α-TS+40°C	30±2

Data were summarized from previous publications [43,48].
[a] Standard error of the mean.

ANTIOXIDANT VITAMINS IN COMBINATION WITH CERTAIN BIOLOGICAL RESPONSE MODIFIERS (BUTYRIC ACID, cAMP AND INTERFERON)

Butyric acid, a 4-carbon fatty acid, exhibits strong anticancer properties *in vitro* and *in vivo* [49]. We have shown [50] that antioxidant vitamins such as α-TS enhance the growth-inhibitory effect of butyric acid on certain tumor cells in culture. cAMP is known to induce terminal differentiation in neuroblastoma cells in culture [51]. α-TS and polar carotenoids [33], originally referred to as β-carotene [52], enhance the level of cAMP-induced differentiation in these cells [53] (Fig. 5). In addition, α-TS [54] also enhances the level of cAMP-induced differentiation in murine melanoma cells in culture (Fig. 6).

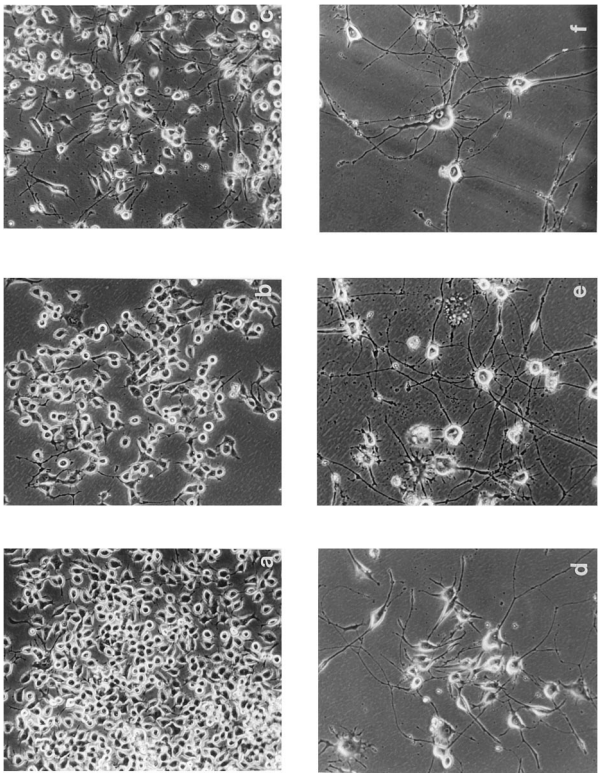

Fig. 5. Photomicrographs of neuroblastoma cells (NBP2) in culture after treatment with RO20-1724, an inhibitor of cyclic nucleotide phosphodiesterase, and a polar carotenoid originally referred to as beta-carotene [52]. Control a) 4 days after plating (50,000 cells/60-mm dish) showing mostly round cells; polar carotenoid (20 μg/mL-treated cells; b) 4 days after treatment showing no significant change in morphology; RO20-1724 (200 μg/mL)-treated cells; c) 4 days after treatment revealing increased number of cells with neurites; cells treated with RO20-1724 plus polar carotenoid d) for a period of 4 days showing more differentiated cells than those produced by RO20-1724 treatment alone; cells treated with RO20-1724 plus polar carotenoid for a period of 8 days e) and f) 11 days showing extensive network of neurites [53] ×200.

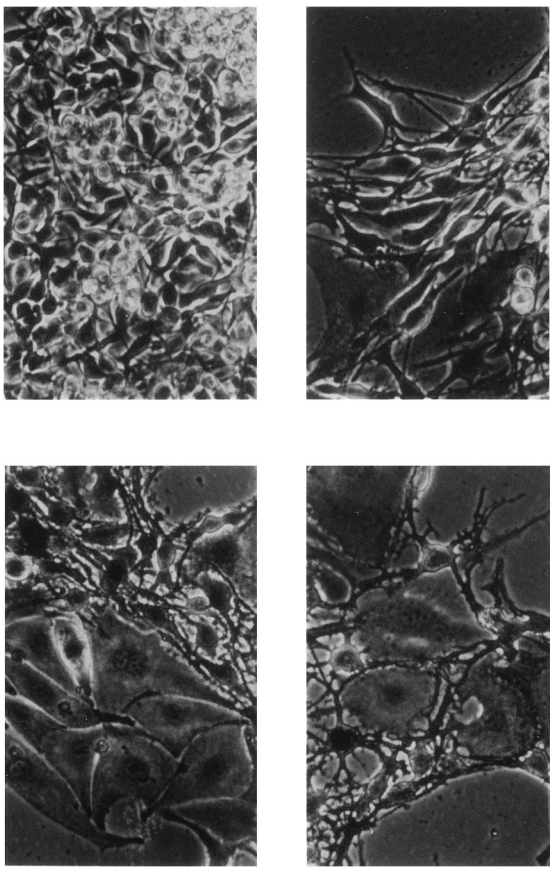

Fig. 6. Photomicrographs of murine melanoma (B16) cells were taken 4 days after treatment. Control culture contained cells with varied morphology (a). The melanoma cells treated with vitamin E succinate (4 μg/mL) were elongated, had long cytoplasmic processes, and were arranged alongside each other (b). Melanoma cells treated with RO20-1724, an inhibitor of cyclic nucleotide phosphodiesterase (100 μg/mL) were large and elongated, had some long processes, and were arranged alongside each other (c). A combination of RO20-1724 and vitamin E succinate (d) increased the level morphologic differentiation more than that produced by individual agents [54] $\times$450.

α-TS [4] and retinoids [55] also enhance the effect of interferon *in vitro* and *in vivo*, respectively. These results suggest that antioxidant vitamins can enhance the efficacy of biological response modifiers on tumor cells. The experimental results discussed above strongly support the first part of our hypothesis: supplementation with multiple antioxidant vitamins may selectively enhance the growth-inhibitory effects of standard and experimental cancer therapies.

REDUCTION OF TOXICITY OF STANDARD TUMOR THERAPEUTIC AGENTS BY ANTIOXIDANT VITAMINS

The second part of our proposed hypothesis is that antioxidant vitamins in combination with standard therapeutic agents may reduce the toxicity of these agents on normal cells. Several studies using animal models (primarily rats and rabbits) support this hypothesis. Vitamin E reduces bleomycin-lung fibrosis [56], adriamycin-induced cardiac toxicity [57–60] and skin necrosis [61]. Vitamin E also reduces adriamycin-induced toxicity in liver, kidney and intestinal mucosa [62]. Some studies have shown that vitamin E protects normal tissue, *in vivo*, against radiation damage [63–65], whereas others have reported no such effects on normal or tumor tissue [66,67]. Another study has reported that β-carotene and vitamin A (retinyl palmitate) reduces adverse effects of x-irradiation and cyclophosphamide in rats [9]. Vitamin C has been shown to reduce the adverse effects of adriamycin on normal animal cells [68]. Vitamin C, α-TS and RA reduce bleomycin-induced chromosomal breakage [69].

Based on the data discussed above and safety issues, the following vitamin supplements are recommended during and after standard therapy: Antioxidants recommended during treatment are based on the following rationale as described below. a) Multiple antioxidant vitamins including B-vitamins and appropriate minerals but without iron, copper and manganese, since these three minerals interact with vitamin C to produce free radicals. b) Additional 8 grams of vitamin C in the form of calcium ascorbate. Doses of vitamin C at 10 g or more have been used in human cancer treatment without toxicity [16]. This form of vitamin C was selected because ascorbic acid at high doses can cause upset stomach in some patients. Calcium ascorbate rather than sodium ascorbate was selected, because sodium ascorbate at high doses can increase molarity of urine in the bladder and increase the risk of chemical-induced bladder cancer in animals due to chronic irritation [70]. c) Additional 800 IU of natural vitamin E in the form of α-TS. This dose was selected because it has been used in patients with melanoma for over 1 year without toxicity (Prasad and Robinson, unpublished observation). This form of vitamin E is the most potent form of vitamin E *in vitro* and *in vivo*. The natural form of vitamin E is selected, because various organs at least in rats selectively pick up the natural form of vitamin E more than the synthetic form. d) Additional natural β-carotene 60 mg/day. The natural form of β-carotene was selected because it has been shown to be more active. For example, natural β-carotene protects against radiation-induced transformation *in vitro*, whereas synthetic β-carotene was ineffective [36]. All vitamin doses described above should be taken orally and divided into two doses, one in the morning and one in the evening, before meals. The rationale for taking twice a day is that the biological half-life of antioxidant vitamins is about 6 to 12 hours. The above vitamin supplements should be started 48 hours prior to standard therapy and should be continued until 1 month after the completion of standard therapy. After that, doses of vitamin C, vitamin E and β-carotene should be reduced by one-half gradually over a 4-week period. The multiple vitamins together with reduced doses of vitamin C, vitamin E and β-carotene

should be continued throughout one's lifetime (for patients in remission).

DIET AND LIFESTYLE MODIFICATIONS

A low fat (10% of calories from fat) and high fiber (25 to 30 g from fruits, vegetables and cereals) diet should be continued during and after standard treatment. The fat content in the diet may be increased to 15% of total calories during complete remission, but high fiber intake should be continued throughout one's life. A low fat diet may interfere with tumor growth by reducing tumor growth stimulating signals such as prostaglandins [71] and estrogens. A high fiber diet can reduce the rate of tumor growth by generating millimolar levels of butyric acid [1], a powerful anti-cancer agent [49]. Lifestyle changes include no tobacco smoking or consumption of tobacco products, reduced consumption of caffeine, alcoholic beverages (not more than two glasses of wine per week), daily exercise, reduced physical and mental stress.

The proposed recommendations discussed above will test our hypothesis that vitamin supplements, diet, and lifestyle modifications may markedly improve the efficacy of standard and experimental therapies by enhancing their growth-inhibiting effects selectively on tumor cells, and by reducing their toxicity to normal cells. The proposed recommendation for vitamin supplements and diet and lifestyle modifications may also reduce the risk of second malignancies, which are being detected at increased rates among survivors of standard cancer treatment.

ACKNOWLEDGMENT

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