Multiple Dietary Antioxidants Enhance the Efficacy of Standard and Experimental Cancer Therapies and Decrease Their Toxicity

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Cancer patients can be divided into 3 groups: those receiving standard or experimental therapy, those who have become unresponsive to these therapies, and those in remission at risk for recurrence or a second new cancer. While impressive progress in standard cancer therapy has been made, the value of this therapy in the management of solid tumors may have reached a plateau. At present, there is no strategy to reduce the risk of recurrence of the primary tumors or of a second cancer among survivors. Patients unresponsive to standard or experimental therapies have little option except for poor quality of life for the remainder of life. Therefore, additional approaches should be developed to improve the efficacy of current management of cancer. In this review, the author proposes that an active nutritional protocol that includes high doses of multiple dietary antioxidants and their derivatives (vitamin C, α-tocopheryl succinate, and natural β-carotene), but not endogenously made antioxidants (glutathione- and antioxidant enzyme-elevating agents), when administered as an adjunct to radiation therapy, chemotherapy, or experimental therapy, may improve its efficacy by increasing tumor response and decreasing toxicity. This nutritional protocol can also be used when patients become unresponsive to standard therapy or experimental therapy to improve quality of life and possibly increase the survival time. The authors also propose that after completion of standard therapy and/or experimental therapy, a maintenance nutritional protocol that contains lower doses of antioxidants and their derivatives, together with modification in diet and lifestyle, may reduce the risk of recurrence of the original tumor and development of a second cancer among survivors. Experimental data and limited human studies suggest that use of these nutritional approaches may improve oncologic outcomes and decrease toxicity. This review also discusses the reasons for the current debates regarding the use of antioxidants during radiation or chemotherapy.

Keywords: ***radiation therapy; chemotherapy; hyperthermia; dietary antioxidants; endogenously made antioxidants; cancer treatment***

The incidence of new cancers in the United States 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% to 12% annually. Cancer patients can be divided into 3 groups: those scheduled to receive standard therapy or experimental therapy, those who have become unresponsive to these therapies, and those in remission carrying the risk of recurrence of primary tumors or development of a second new cancer. Except for reducing the risk of recurrence of breast cancer with tamoxifen, there is no effective strategy to reduce the risk of recurrence of the primary tumor or the development of a second new cancer induced by treatment agents. Standard cancer therapy, which includes radiation therapy, chemotherapy, and surgery (whenever feasible and needed), has been useful in producing increased cure rates in certain tumors including Hodgkin's disease, childhood leukemia, and teratocarcinoma. However, the risk of recurrence and the development of a new cancer and nonneoplastic diseases such as aplastic anemia, retardation of growth in some children, and delayed necrosis in some organs such as brain, liver, bone, and muscle exist. In addition, acute damage to normal tissue occurs during radiation therapy or chemotherapy, and in some instances, such damage becomes the limiting factor for the continuation of therapy.

The efficacy of standard cancer therapy has reached a plateau for most solid tumors despite impressive progress in radiation therapy, such as dosimetery and more efficient methods of delivery of radiation doses to tumors, and, in chemotherapy, development of novel drugs with diverse mechanisms

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of action on cell death and proliferation inhibition. Therefore, additional approaches must be developed to improve the efficacy of standard therapy and to reduce the risk of recurrence of the primary tumor and the development of a second new cancer among survivors. In addition, new approaches should be developed to improve the quality of life of those patients who become unresponsive to all standard and experimental cancer therapies.

Several laboratory experiments and limited clinical studies show that micronutrients including dietary antioxidants and their derivatives (vitamin A, vitamin C, d-α-tocopheryl succinate, and natural β-carotene) at appropriate doses, dose schedule, and treatment period may improve the outcome in all 3 groups of cancer patients. Endogenously made antioxidants such as glutathione- or antioxidant enzyme-elevating agents are not recommended during radiation or chemotherapy therapy because they may protect cancer cells against the cytotoxic effect of therapy.

This review presents laboratory and human studies that support the use of a nutritional active treatment protocol as an adjunct to standard or experimental therapy to improve the efficacy of these therapies by increasing tumor response and decreasing toxicity. The same protocol may also improve the quality of life in those patients who become unresponsive to all available therapies. A maintenance nutritional protocol together with modification in diet and lifestyle is also proposed to reduce the risk of recurrence of the primary tumor and the development of a new cancer among survivors. In addition, this review discusses the reasons for current controversies regarding the use of antioxidants during radiation or chemotherapy.

Use of Antioxidants by Cancer Patients and Recommendation of Antioxidants by Oncologists

Most oncologists do not recommend antioxidants to their patients during radiation therapy, chemotherapy, or experimental therapy. Some may recommend a multiple-vitamin preparation containing low doses of antioxidants after the completion of therapy. This recommendation may be harmful because like normal cells, cancer cells need certain amounts of micronutrients including antioxidants for growth and survival. Indeed, low doses of individual dietary antioxidants may also stimulate the proliferation of some cancer cells.[1] Therefore, it is likely that recommendation of low doses of multiple vitamins containing low doses of micronutrients including antioxidants after therapy may increase the risk of recurrence of the primary tumor among those who are in remission. On the other hand, more than 60% of cancer patients use vitamins, and the majority combine them with standard therapy, mostly without the knowledge of their oncologists.[2] This practice may also be harmful because a multiple-vitamin preparation may contain antioxidants such as glutathione-elevating agents, including α-lipoic acid and n-acetylcysteine (NAC), and antioxidant enzyme-elevating agents such as excess of selenium, which is a cofactor for glutathione peroxidase,[3] or dietary antioxidants such as vitamin E or vitamin C,[4-6] which, at low doses, may protect cancer cells against free radical damage produced by chemotherapeutic agents or x-irradiation. Neither oncologists nor patients are aware of these potential dangers of taking antioxidants without any scientific rationale.

Experimental Studies Showing Protection of Cancer Cells Against Damage Produced by Standard Therapeutic Agents by Antioxidants or Their Derivatives

Several studies have shown that antioxidants protect cancer cells and normal cells, if dietary antioxidants or their derivatives or endogenously made antioxidants at doses that do not affect the proliferation of these cells are administered only one time shortly before cancer therapeutic agents. Vitamin E (α-tocopherol), vitamin C, or NAC, when given in a single low dose shortly before x-irradiation, reduced the effectiveness of irradiation on cancer cells in in vitro and in vivo models.[3-6] Concentrations used in these studies do not affect the growth of cancer cells. The importance of SH-compounds in radiation protection was further demonstrated by the fact that mitotic cells, which were most radiosensitive, had the lowest levels of SH-compounds and that S-phase cells, which were the most radioresistant, had the highest level of these compounds.[3] When the level of SH-compounds was elevated in mitotic cells prior to x-irradiation, they became radioresistant like S-phase cells.[3] Overexpression of antioxidant enzymes such as mitochondrial manganese-superoxide dismutase (Mn-SOD) enhanced the radioresistance of tumor cells.[7,8] Therefore, recommendation of low doses of dietary antioxidants or any nontoxic doses of glutathione-elevating agents such as α-lipoic acid and NAC during radiation therapy or chemotherapy may be harmful. A study has reported that NAC at a dose of 1 g/kg of body weight delivered intraperitoneally in combination with doxorubicin reduced tumorigenicity and metastasis following transplantation of B-16 murine melanoma cells in mice.[9] This dose is very high to be of any relevance in humans. NAC at concentrations greater than 500 mg/person/d increased the urinary excretion of Zn^{10} and thus can induce Zn deficiency. Selenium (sodium selenite) and vitamin E protected against radiation-induced intestinal injury.[11] However,

organic selenium at a high dose (0.2 mg/mouse/d or 10 mg/kg of body weight/d) enhances the therapeutic efficacy of some chemotherapeutic agents in athymic mice bearing human squamous cell carcinoma of the head and neck.[12] The observations could be very exciting if a similar observation is made in human cancer without unacceptable toxicity. It should be pointed out that rodents exhibit a high degree of resistance to most therapeutic agents. For example, antiangiogenesis agents, amifostine, and proteasome inhibitors that were found to be very effective in reducing the growth of tumor cells or to enhance the efficacy of cancer therapeutic agents were found to be toxic in humans; their clinical relevance therefore remains limited.

Experimental Studies Showing Protection of Normal Cells Against Damage Produced by Standard Therapeutic Agents by Antioxidants or Their Derivatives

Vitamin E given in a single dose to normal adult rodents before x-irradiation increased the survival of irradiated animals. Vitamin C and vitamin E administered in a single dose before irradiation reduced the level of DNA damage to normal cells. Tocopherol in combination with pentoxifylline decreased the level of radiation-induced fibrosis. Vitamin E reduces bleomycin-induced lung fibrosis, adriamycin-induced cardiac toxicity, and adriamycin-induced skin necrosis. Vitamin E also reduces doxorubicin-induced toxicity in liver and kidney and intestinal mucositis. Vitamin C has been shown to reduce the adverse effects of some chemotherapeutic agents on normal cells, such as reducing the adverse effects of adriamycin. Vitamin C, α-tocopheryl succinate (α-TS), and 13-*cis*-retinoic acid (RA) reduce bleomycin-induced chromosomal breakage. The above studies have been discussed in a recent review.[13] Pretreatment of rats with vitamin E or selenium protected the gut against radiation-induced injuries by similar levels.[11] The combination of selenium and vitamin E produced a similar level of protection as that produced by the individual agents. Unfortunately, this study failed to mention the form of vitamin E or selenium. This is important because the efficacy of these agents may vary depending on their respective forms.

Certain Prevention Studies Used as an Argument for Not Recommending Antioxidants as an Adjunct to Standard Therapy

There are no cancer treatment trials indicating that high doses of multiple dietary antioxidants or their derivatives when given before therapy and every day thereafter for the entire treatment period have protected cancer cells against damage produced by radiation therapy or chemotherapy. Studies often quoted are those that are either epidemiologic or intervention trials with 1 or more antioxidants in cancer prevention. Cancer prevention trials with synthetic β-carotene in which the incidence of lung cancer among male heavy smokers increased by 17%[14,15] are often quoted as evidence for not recommending any antioxidants during cancer therapy. Heavy smokers have a highly oxidative body environment; therefore, any single antioxidants including β-carotene would be oxidized to form free radicals that can increase the risk of lung cancer. Thus, the increased risk of cancer among heavy male smokers following supplementation with β-carotene could have been predicted. An epidemiologic study[16] has analyzed 90 patients with breast cancer who took vitamin/mineral regimens within 180 days of diagnosis and continued for a 2-month period whether or not they received radiation therapy, chemotherapy, or both. These treatment variables were stratified and therefore were not corrected for in data analysis. The follow-up period varied from 20 months to 133 months, with a median value of 48 months. No information was provided as to whether patients were taking vitamins/minerals during the follow-up period. Vitamin/mineral doses, number of agents, and percentage of patients taking a number of vitamins and minerals were also markedly varied. β-carotene doses varied from 0 to 250,000 IU, vitamin B3 from 0 to greater than 1 mg, vitamin C from 1 to 24 g, selenium from 0 to 1000 μg, Zn 0 to greater than 50 mg, and for coenzyme Q10, no dose was given. Among 90 patients, 2% took 3 agents (no name of agent or their doses mentioned), 23% took 4 agents (no name of agents or their doses mentioned), 56% took 5 agents (no name of agents or their doses mentioned), and 19% took all 6 agents (no doses were given). These confounding variables were not accounted for while analyzing data. Based on these data, the authors concluded that breast cancer–specific survival and disease-free survival time was not improved by the above vitamin/mineral regimes. Unfortunately, presentation of data without correcting for prognostic and demographic factors alone revealed that patients taking vitamin/mineral supplements as described in this study produced poor survival and disease-free survival time in comparison to historical controls receiving conventional therapy. Presentation of such data appears to be inconsistent with the author's conclusion and creates confusion in public and professionals. Thus, the extrapolation of data obtained from epidemiologic or cancer-prevention studies to cancer treatment may be incorrect and misleading.

Experimental Studies In Vitro and in Animals Showing the Effect of Individual or Multiple Dietary Antioxidants and Their Derivatives on Growth of Cancer Cells

Antioxidants and their derivatives such as vitamin A (including retinoids), vitamin C, α-TS, and natural β-carotene at high doses induce differentiation, proliferation inhibition, and apoptosis depending on dose and type of antioxidant, treatment schedule, and type of tumor cell, without producing similar effects on most normal cells in vitro and in vivo. These studies have been referenced in several reviews and articles.[17-24] One example of α-TS-induced differentiation in murine melanoma cells is presented in Figure 1. The selectivity of the damaging effect of high doses of antioxidants or their derivatives on cancer cells is often ignored while discussing the valuable role of antioxidants as an adjunct to standard therapy.

Treatment of cancer cells with high dose RA, α-TS, or β-carotene markedly alters expression of genes, levels of proteins, and translocation of certain proteins from one cellular compartment to another, causing differentiation, proliferation inhibition, and apoptosis, depending on the type and form of antioxidant, treatment schedule, and type of tumor cell. The alterations in gene expression and protein levels are directly related to proliferation inhibition and apoptosis. These studies have been summarized in recent reviews.[13,17,18]

The treatment schedule with high-dose antioxidants is also very important in producing a differential effect on normal and cancer cells. A short exposure time (a few hours) even at a high dose may not cause significant reduction in proliferation of cancer cells. A treatment time of at least 24 hours is needed to observe a significant reduction in proliferation of cancer cells. Therefore, it is essential that high-dose antioxidants are administered before therapy and every day thereafter for the entire treatment period. A combination of antioxidants is more effective in reducing proliferation of cancer cells than the individual agents (Table 1).[25,26]

Experimental Studies Showing the Effect of Individual Endogenously Made Antioxidants on the Growth of Cancer Cells

Endogenously made antioxidants also inhibited the proliferation of cancer cells. For example, overexpression of Mn-SOD reduces the proliferation and suppresses the malignant phenotype of glioma[27] and melanoma cells[28] in culture. Glutathione-elevating agents such as NAC at very high doses (1-2 g/kg of body weight) inhibit the proliferation of cancer cells in vitro and in vivo.[9] These doses may not be relevant to humans because of toxicity.

In Vitro Studies Showing Enhancement of Radiation-Induced Damage on Cancer Cells by Dietary Antioxidants or Their Derivatives

Dietary antioxidants and their derivatives enhance the effect of irradiation selectively on cancer cells while protecting normal cells against some injuries. 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.[29] Retinoic acid in combination with interferon-α2a enhances radiation-induced toxicity in neck and head squamous cell carcinoma cells in culture.[30] We have reported that the dose of vitamin E (α-TS) that inhibited the proliferation 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[31] and chromosomal damage[32] (Figure 2) in cancer cells. On the other hand, the same dose of α-TS did not modify the effect of irradiation on mitotic accumulation in normal cells,[31] but it protected normal cells against chromosomal damage.[32] 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 (NB) cells (Figure 3).[1,13] Vitamin C enhanced the effect of irradiation on NB cells but not on glioma cells in culture.[33] Dehydroascorbic acid, the major metabolite of ascorbic acid, acts as a radiosensitizer for hypoxic tumor cells in culture.[34]

Animal Studies Showing Enhancement of Radiation-Induced Damage on Cancer Cells by Dietary Antioxidants or Their Derivatives

Vitamin A (retinyl palmitate) or β-carotene at high doses given daily through dietary supplementation 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 2).[35] 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.[36] The administration of multiple antioxidant micronutrients (vitamins A, C, and E) protected normal cells against damage produced by radioimmunotherapy in mice without protecting cancer cells.[37]

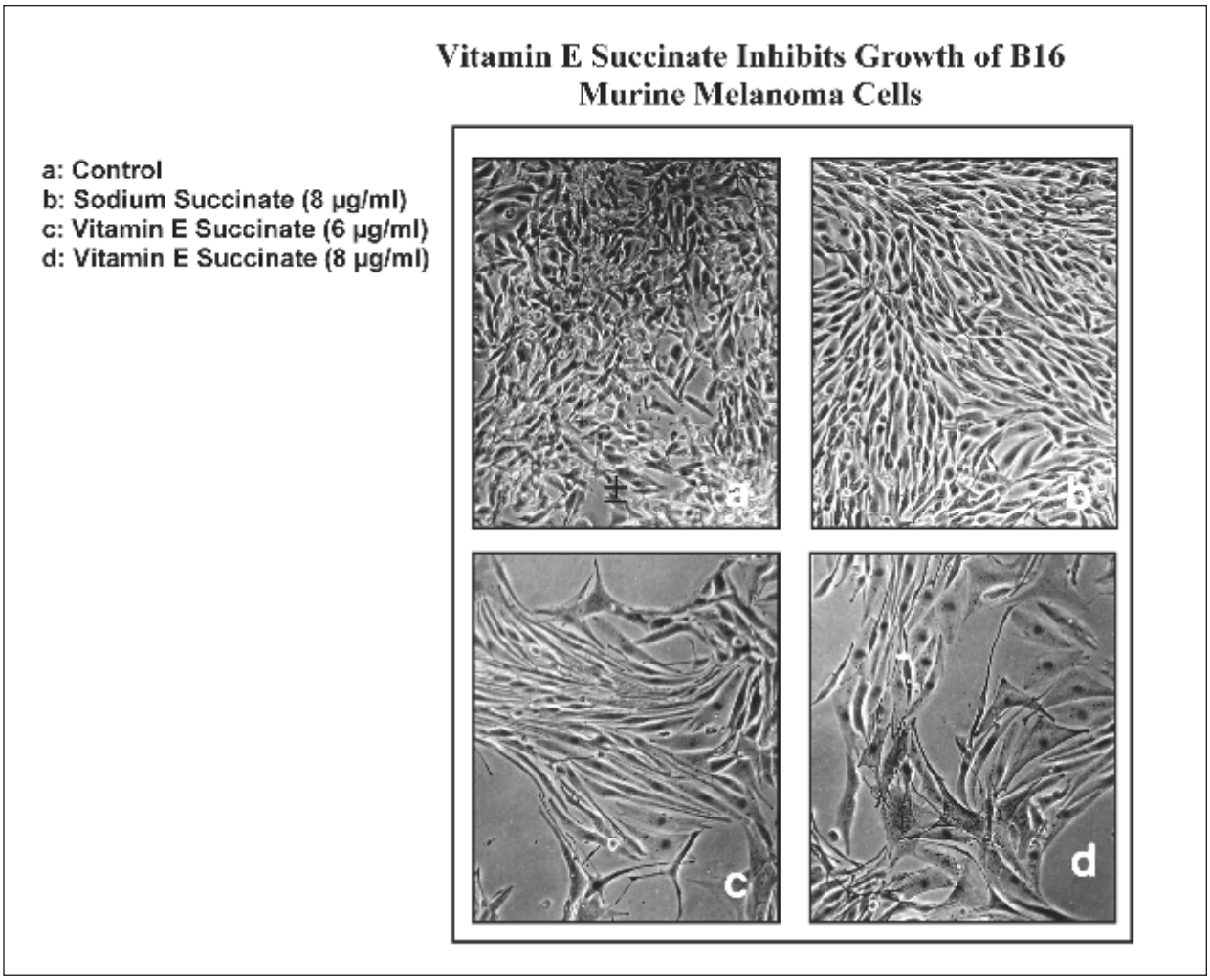

Figure 1 *Melanoma cells (10^5) were plated in tissue culture dishes (60 mm), and* d-α *tocopheryl succinate (α-TS) and sodium succinate plus ethanol were added to separate cultures 24 hours after plating. Drugs and medium were changed at 2 and 3 days after treatment. Photomicrographs were taken 4 days 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 6 µg/mL (c), and 8 µg/µL (d) showed a dramatic change in morphology. Magnification = ×300.*[13]

Table 1. Effect of a Mixture of 4 Antioxidant Micronutrients on Growth of Human Melanoma Cells in Culture

Treatment	*Cell Number (% of controls)*
Vitamin 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
Vitamin C (50 µg/mL) + PC (10 µg/mL) + α-TS (10 µg/mL) + RA (7.5 µg/mL)	56 ± 3
Vitamin C (100 µg/mL)	64 ± 3
Vitamin C (100 µg/mL) + PC (10 µg/mL) + α-TS (10 µg/mL) + RA (7.5 µg/mL)	13 ± 1

PC = polar carotenoids, originally referred to as β-carotene.[1] This is a more soluble fraction of carotenoids without the presence of β-carotene. Vitamin C = sodium ascorbate; α-TS = α-tocopheryl succinate; RA = 13-*cis*-retinoic acid. Data were summarized from a previous publication.[23]

a. Standard error of the mean.

Clinical Studies Showing Enhancement of Radiation-Induced Damage on Cancer Cells by Dietary Antioxidants or Their Derivatives

Retinoic acid and interferon-α2a enhanced the efficacy of radiation therapy of locally advanced cervical cancer.[30] Treatment with dietary antioxidants reduced the effect of irradiation on normal tissues in patients with small-cell lung carcinoma.[38,39] β-carotene reduced radiation-induced mucositis without interfering with the efficacy of radiation therapy in patients with cancer of the head and neck.[40] A recent review has discussed the pros and cons of using antioxidants in combination with radiation therapy.[41]

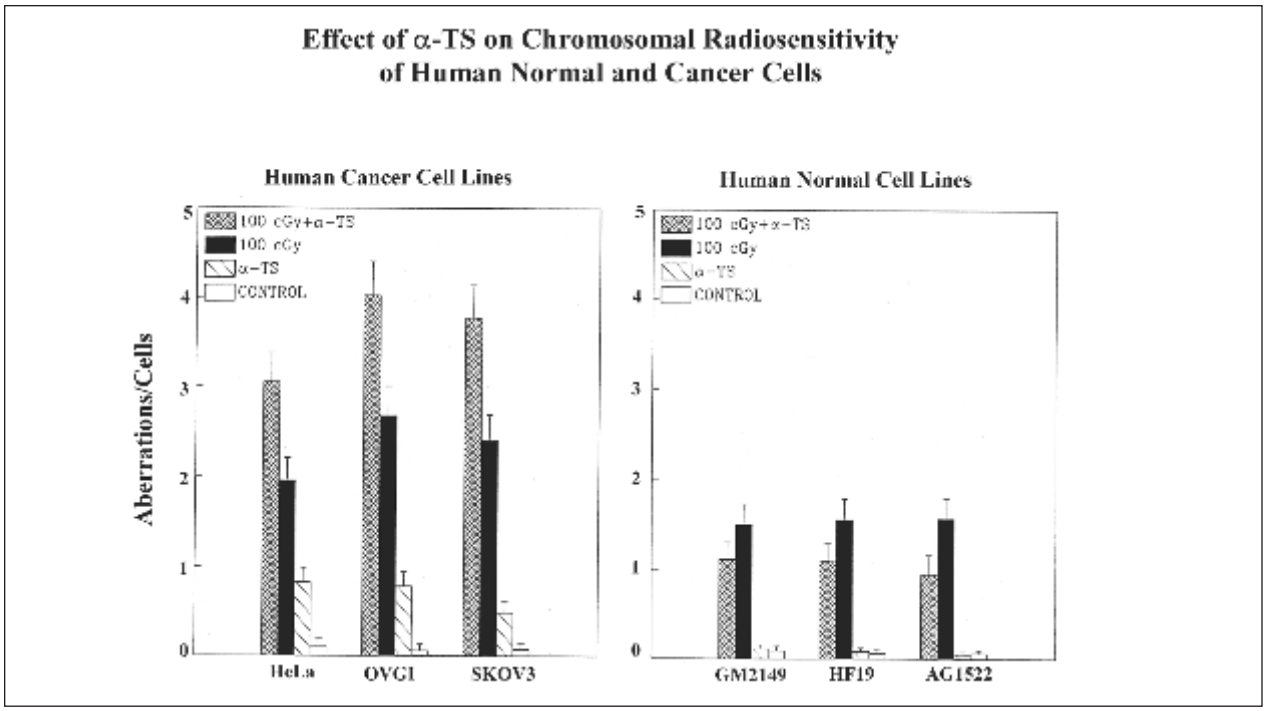

Figure 2 Effect of d-α*-tocopheryl succinate (α-TS) on the level of radiation-induced chromosomal damage in human cervical cancer (HeLa cells), ovarian carcinoma cell lines (OVG1 and SKOV3), and human normal skin fibroblasts (GM2149, HF19, and AG1522). α-TS treatment alone increased chromosomal damage in all 3 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 < *.05.*[32]

In Vitro Studies Showing Enhancement of Chemotherapeutic Agent-Induced Damage on Cancer Cells by Dietary Antioxidants or Their Derivatives

The effect of direct interaction between antioxidants and cancer therapeutic agents can initially best be tested on cancer cells in culture because it is simple and cost- and time-effective without any interference from complex molecules that are present in vivo. This is especially pertinent to conditions in which the modification of the effects of free radicals by pharmacological agents are studied. Several studies have revealed that vitamin C, α-TS, α-tocopheryl acetate, vitamin A (including retinoids), and polar carotenoids including β-carotene enhance the growth-inhibitory effect of most of the chemotherapeutic agents on some cancer cells in culture.[1] Chemotherapeutic agents used in these studies include 5-fluouracil (5-FU), vincristine, adriamycin, bleomycin, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboximide (DTIC), cisplatin, tamoxifen, cyclophosphamide, mutamycin, chlorozotocin, and carmustine. The extent of this enhancement depends on dose and form of antioxidant, treatment schedule, dose and type of chemotherapeutic agent, and type of tumor cell.

Some examples of antioxidant-induced enhancement of the effect of chemotherapeutic agents are described below. An aqueous form of vitamin E, α-tocopheryl acetate, enhanced the effect of vincristine on neuroblastoma cells in culture.[1] Vitamin C enhanced the effect of 5-FU on neuroblastoma cells in culture[33] (Figure 4).

Vitamin C enhanced the antitumor activity of doxorubicin, cisplatin, and paclitaxel in human breast cancer cells in culture.[42] Vitamin C also increased drug accumulation and reversed vincristine resistance of human non-small-cell lung carcinoma cells.[43] α-TS increased the effect of adriamycin on human prostate carcinoma cells in culture.[44] Recently, we have found that α-TS increased the effect of adriamycin on human cervical cancer cells (HeLa) without modifying the effect of adriamycin on normal human fibroblasts in culture (unpublished observations; Table 3). α-TS also enhanced the effect of carmustine on rat glioma cells in culture (unpublished observations). α-tocopherol protected cisplatin-induced toxicity without interfering with its antitumor activity in human melanoma transplanted in athymic mice.[45]

A mixture of antioxidants containing retinoic acid, vitamin C, α-TS, and polar carotenoids in combination with DTIC, tamoxifen, cisplatin, or interferon-α2a inhibited the proliferation of human melanoma cells in culture more than the growth inhibition produced by the individual agents[25] (Table 4). Another

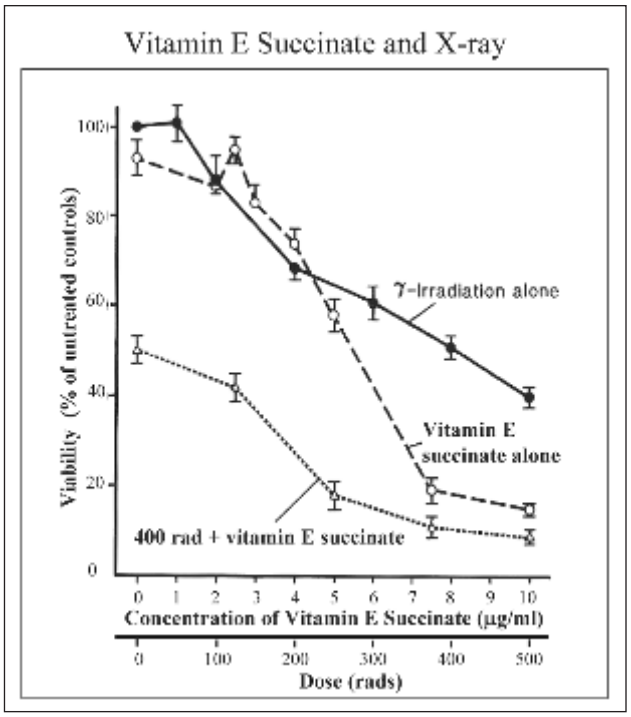

Figure 3 Neuroblastoma cells (NBP2) were plated in tissue culture dishes (60 mm), and the cells were γ-irradiated 24 hours after plating. Vitamin E succinate or solvent (ethanol 0.25% and sodium succinate 5 μg/mL) was added immediately before irradiation. The drugs and medium were changed after 2 days 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 NB cells was considered 100%, and the growth in treated cultures was expressed as a percentage of untreated controls. The bar at each point is the standard error of the mean.[13]

Table 2. Effect of Vitamin A, β-Carotene, and Local x-Irradiation on Survival of Mice With Transplanted Breast Adenocarcinoma

Treatment	*Number of Mice*	*1-Year Survival (number of mice)*
Control	24	0
3000 rads, single dose	24	0
Vitamin A	24	0
β-carotene	24	0
Vitamin A plus x-ray	24	22
β-carotene plus x-ray	24	22

Data were summarized from a previous study.[35] Diets were supplemented with vitamin A (3000 IU/mouse) and β-carotene (270 μg/mouse), and these doses were about 10 times greater than the recommended dietary allowance for mouse.

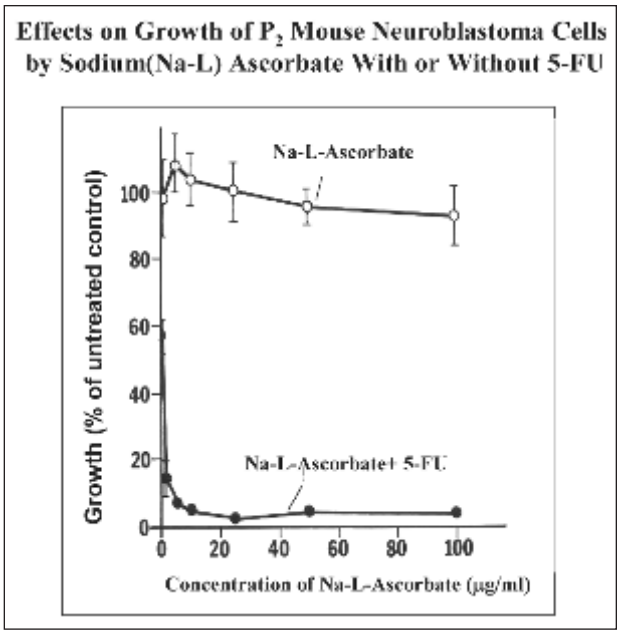

Figure 4 Neuroblastoma cells (50,000 per dish) were plated in tissue culture dishes (60 mm), and 5-fluorouracil (5-FU; 0.08 μg/mL) plus sodium ascorbate or sodium ascorbate alone was added 24 hours after plating. The drug and medium were changed every day, and the number of cells per dish was determined 3 days after treatment. Each value represents the mean of 6 to 9 samples ± standard deviation.[33]

Table 3. Modification of Adriamycin Effect on Human Cervical Cancer Cells (HeLa) and Human Normal Skin Fibroblasts in Culture by d-α-Tocopheryl Succinate

Treatment	*HeLa Cells*	*Normal Fibroblasts*
Solvent control	99 ± 2.6	104 ± 3.4
Adriamycin (0.1 μg/mL)	57 ± 6.2	77 ± 2.4
α-TS (10 μg/mL)	99 ± 1.6	101 ± 3.7
Adriamycin (0.1 μg/mL) + α-TS	20 ± 7.9	77 ± 1.7
Adriamycin (0.25 μg/mL)	14 ± 2.9	68 ± 1.0
Adriamycin (0.25 μg/mL) + α-TS	5 ± 0.8	62 ± 1.8

Cells (20,000) were plated in a 24-well chamber, and adriamycin and α-tocopheryl succinate (α-TS) were added one after another at the same time. Drug, α-TS, and fresh growth medium were changed at 2 d after treatment, and the viability of cells was determined by MTT assay. Growth in experimental groups was expressed as percentage of untreated control. Each experiment was repeated at least twice, and each value represents an average of 6 to 9 samples ± SEM (our unpublished observation).

study has reported that a mixture of dietary antioxidants (vitamin C, 100 μg/mL; α-tocopherol, 10 μg/mL; and β-carotene, 10 μg/mL) by itself increased cytotoxicity from 4% to 15%, whereas carboplatin (0.5 μg/mL) and paclitaxel (0.05 μmol) increased it to 22% and 87%, respectively. However, the mixture of dietary antioxidants enhanced the apoptotic effect of paclitaxel and carboplatin.[46] The most pronounced effect was observed when the antioxidant mixture was given before treatment with chemotherapeutic agents, followed by paclitaxel treatment for 24 hours and then followed by carboplatin treatment for 24 hours (Table 5). This suggests that multiple antioxidants are also effective in enhancing the effect of

Table 4. Enhancement of the Effect of Certain Chemotherapeutic Agents by a Mixture of 4 Antioxidants on Human Melanoma Cells in Culture

Treatment	*Cell Number (% of controls)*
Solvent	101 ± 4[a]
Cisplatin (1 μg/mL)	67 ± 4
Antioxidant mixture	56 ± 3
Cisplatin + antioxidant mixture	38 ± 2
Tamoxifen (2 μg/mL)	81 ± 3
Tamoxifen + antioxidant mixture	30 ± 2
DTIC (100 μg/mL)	71 ± 2
DTIC + antioxidant mixture	38 ± 2
Interferon-α2b	82 ± 5
Interferon-α2b + antioxidant mixture	29 ± 1

DTIC = 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboximide Data were summarized from a previous publication.[23] Polar carotenoids were originally referred as β-carotene.[1] This is a more soluble fraction of carotenoids without the presence of β-carotene. Vitamin C 50 μg/mL, polar carotenoids 10 μg/mL, α-tocopheryl succinate 10 μg/mL, and 13-*cis*-retinoic acid 7.5 μg/mL were added simultaneously.[25]
a. Standard error of the mean.

certain chemotherapeutic agents on cancer cells. Clinical studies on these issues are in progress.

Animal Studies Showing Enhancement of Chemotherapeutic Agent-Induced Damage on Cancer Cells by Dietary Antioxidants or Their Derivatives

A few in vivo studies support the concept that antioxidants selectively enhance the effect of chemotherapeutic agents on tumor cells by increasing tumor response. For example, vitamin A (retinyl palmitate) or synthetic β-carotene at doses that were 10-fold higher than the recommended dietary allowance for these nutrients, in combination with cyclophosphamide, increased the cure rate from 0% to more than 90% in mice with transplanted adenocarcinoma of the breast.[35] A study using a thiol-containing antioxidant, pyrrolidinedithiocarbamate, and a water-soluble vitamin E analogue (6-hydroxy-2,5,7,8-tetrameythylchroman-2-carboxylic acid; vitamin E) showed that antioxidant treatment enhanced the antitumor effects of 5-FU in athymic mice implanted with human colorectal cancer.[21] The synthetic retinoid (fenretinide) was effective against a human ovarian carcinoma xenograft and potentiated cisplatin activity.[47]

In Vitro Studies Showing Enhancement of Hyperthermia-Induced Damage on Cancer Cells by α-Tocopheryl Succinate

Hyperthermia (43°C to 45°C) alone or in combination with radiation is primarily used in the management of local tumors when all other therapeutic modalities have failed. This approach has not been effective for the long-term management of tumors. Therefore, the current treatment approaches must be altered from local hyperthermia at higher temperatures to whole-body hyperthermia at lower temperatures that could be tolerated without side effects. We have reported[48] that α-TS markedly increased the growth-inhibitory effect of low temperature (41°C) and high temperature (43°C) hyperthermia on neuroblastoma cells in culture (Table 6). We propose that multiple antioxidants in combination with hyperthermia (local or whole body) may further improve the efficacy of hyperthermia in the treatment of human cancer.

In Vitro Studies Showing Enhancement of Sodium Butyrate- and Interferon-Induced Damage on Cancer Cells by α-Tocopheryl Succinate and Retinoic Acid

Butyric acid, a 4-carbon fatty acid,[49] or its analog, phenylbutyrate,[50,51] exhibits strong anticancer properties on tumor cells in culture. However, clinical studies with these agents produced minimal benefits in cancer patients.[52,53] Therefore, any agents that can enhance the effect of sodium butyrate or phenylbutyrate would enhance the value of these agents in clinical studies. We have reported that α-TS enhanced the growth-inhibitory effect of sodium butyrate on certain tumor cells in culture (Figure 5).[54] Retinoic acid increased the effect of phenylbutyrate on human prostate cancer cell growth and angiogenesis in athymic mice.[55] α-TS[25] and retinoids[30] also enhance the effect of interferon in cell culture and in vivo, respectively.

Clinical Studies on the Efficacy of Multiple Dietary Antioxidants or Their Derivatives as an Adjunct to Radiation Therapy and/or Chemotherapy

Eighteen nonrandomized patients with small-cell lung cancer received multiple antioxidant treatments with chemotherapy and/or radiation. The median survival time was markedly enhanced, and patients tolerated chemotherapy and irradiation well.[38] Similar observations were made in several private practice settings.[39] A randomized pilot trial (phase I/II) with high-dose multiple micronutrients including dietary antioxidants and their derivatives (SEVAK, a multiple vitamin preparation, 8 g vitamin C as calcium ascorbate, 800 IU vitamin E as α-TS, and 60 mg natural β-carotene, orally, divided into 2 doses, half in the morning and half in the evening) in patients with stage 0 to III breast cancer receiving radiation therapy has been completed.[56] There were 25 patients in the radiation arm and 22 patients in the combination arm. A follow-up period of 22 months during which no maintenance supplements were given showed that 1 patient in the radiation arm developed a new cancer

Table 5. Flow-Cytometric Analysis of the Effect of the Combination of the Agents (Paclitaxel, Carboplatin, and Antioxidant Mixture) on Apoptosis in H520 Cells

	Treatment of Cells				
Serial Number	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Apoptosis (% cells)*[a]
1	Cells plated	—	—	—	20.6 ± 1.2
2	Cells plated	Paclitaxel + carboplatin	—	—	40.3 ± 3.1
3	Cells plated	Paclitaxel	Carboplatin	—	54.3 ± 2.2
4	Cells plated	Vitamins + paclitaxel	Carboplatin	—	70.11 ± 3.7
5	Cells plated	Vitamins	Paclitaxel	Carboplatin	89.15 ± 4.3

Cells were plated on day 1, and flow cytometry was performed on day 5. Control = serial number 1. Doses: paclitaxel, 0.05 μmol/mL; carboplatin, 0.5 μg/mL; vitamin C, 100 μg/mL; vitamin E, 10 μg/mL; β-carotene, 10 μg/mL.
a. Mean ± SE, day 5. Results are of 3 separate experiments, each performed in duplicate.[46]

Table 6. Effect of α-Tocopheryl Succinate (α-TS) on Hyperthermia-Induced Growth Inhibition in Neuroblastoma Cells in Culture

Treatment	*Cell Number % of controls)*
Solvent (ethanol 0.25%) + sodium succinate (5 μg/mL)	102 ± 3
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 h)	55 ± 2
α-TS + 40°C	30 ± 2

Data were summarized from a previous publication. Values are mean ± SEM.[48]

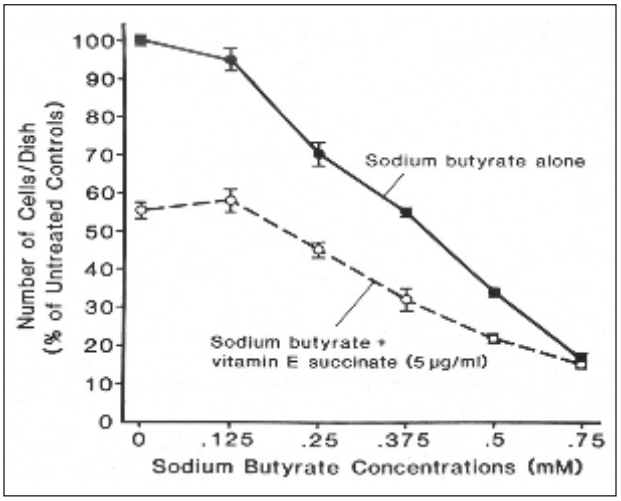

Figure 5 Effect of d-α*-tocopheryl succinate (vitamin E succinate) in combination with sodium butyrate on the growth of neuroblastoma cells in culture. Cells (50,000 cells/60 mm dish) were plated in tissue culture dishes, and vitamin E succinate and sodium butyrate were added one after another 24 hours later. Fresh growth medium and agents were changed at 2 days after treatment, and growth was determined at 3 days after treatment. Each value represents an average of 6 samples. The bar at each point is the standard error of the mean.*[13]

in the contralateral breast, and another patient in the same arm developed lobular carcinoma in situ in the opposite breast. In the combination arm, no new tumor has developed. A randomized trial with high-dose antioxidants (6 g vitamin C as ascorbic acid, 800 IU α-TS, 60 mg β-carotene, 900 μg selenium) in combination with chemotherapeutic agents (cisplatin and paclitaxel) in patients with advanced non-small-cell carcinoma of the lung (34 patients in the chemotherapy arm and 31 patients in the combination arm) reported beneficial effects on tumor response and tolerance to chemotherapeutic agents for a follow-up period of 1 year (Table 7).[57] The addition of selenium in this trial may have reduced the efficacy of the protocol. Based on the beneficial effects of multiple antioxidants in combination with standard therapy on 2 patients with ovarian cancer,[58] Drisko has started a new trial with multiple antioxidants on ovarian cancers. Thus, in vitro, in vivo (animal), and limited human studies suggest that a well-designed trial with multiple antioxidants and their derivatives administered before, during, and after standard therapy is urgently needed.

Mechanisms of Enhancement of the Effect of Standard Therapeutic Agents on Cancer Cells by High-Dose Dietary Individual Antioxidants and Their Derivatives

The exact reasons for the dietary antioxidant-induced enhancement of damage produced by standard therapeutic agents on cancer cells are unknown. We propose that treatment of tumor cells with high doses of dietary antioxidants before standard therapy can initiate damage in cancer cells but not in normal cells. Free radicals generated by therapeutic agents, even if completely quenched by antioxidants, become irrelevant because damaged cancer cells suffer further injuries by mechanisms other than free radicals associated with therapeutic agents. The damage to cancer cells is further enhanced by the fact that micronutrients such

Table 7. Preliminary Results of a Randomized Clinical Trial Using High-Dose Multiple Antioxidants as an Adjunct to Chemotherapy

Tumor Response and Survival	*Chemotherapy Arm (number of patients = 34)*	*Chemotherapy + Antioxidant Arm (number of patients = 31)*
Complete response	0	1
Partial response, %	32	45
Overall survival at 1 year, %	33	54
Median survival time, mo	8	13

Ascorbic acid, 6100 mg; α-tocopherol, 1050 mg; β-carotene (synthetic), 60 mg; copper sulfate, 6 mg; manganese sulfate, 9 mg; zinc sulfate, 45 mg; and selenium, 900 mg per d. Antioxidant was started 48 h before chemotherapy and continued 1 mo after completion of therapy, then reduced to half the dose as a maintenance regimen.[57]

as retinoic acid can inhibit the repair of radiation damage in cancer cells.[29] It has been reported that α-TS-induced apoptosis in cancer cells is independent of p53 and p21,[59] whereas 5-FU-induced apoptosis is mediated via p53 and p21.[21] Therefore, the combination of 2 agents may be more effective than the individual agents. High expression of c-myc and H-ras oncogenes increased radioresistance of cancer cells,[3] whereas the expression of c-myc and H-ras was reduced by α-TS treatment.[13] The combination of the 2 agents causes more cell death than the individual agents alone. α-TS in vivo acts as an antiangiogenesis agent,[24] whereas radiation or chemotherapeutic agents do not; therefore, the combination of α-TS with these therapeutic agents may be more effective than the individual agents. The effect of high-dose dietary antioxidants in enhancing the level of damage in cancer cells produced by x-irradiation or chemotherapeutic agents is not due to their antioxidant activity or their differential accumulation in the cells.[32] These dietary antioxidants and their derivatives do not initiate damage in normal cells before standard therapy; therefore, when these cells are treated with radiation or chemotherapeutic agents, they can be protected by antioxidants through their classical antioxidant activity. The exact mechanisms of enhancement of radiation- or chemotherapeutic agent–induced damage on cancer cells remain unknown.

Altered Gene Expression in Cancer Cells After Treatment With Antioxidants or Their Derivatives

These studies reveal that retinoids, vitamin E (α-TS and α-tocopherol), and β-carotene inhibit as well as stimulate the levels of some cell-signaling systems and gene expressions that can lead to decreased cell proliferation rate and increased differentiation and/or apoptosis in cancer cells. The inhibitory events include decreased expression of c-myc, H-ras,[60] N-myc,[61] mutated p53,[23] the activity of protein kinase C,[62,63] caspase,[64] tumor necrosis factor,[65] transcriptional factor E2F,[66] and Fas.[67] The stimulatory events include increased expression of wild-type p53[23] and p21,[21] transforming growth factor b,[68] and the connexin gene.[69] Marked changes in gene expression have been observed as early as 30 minutes after treatment of neuroblastoma cells with a growth-inhibitory concentration of α-TS (Figure 6). The above changes in gene expression may be one of the major factors that account for the growth-inhibitory effect of these dietary antioxidants and their derivatives on cancer cells.

Antioxidant Deficiency-Induced Enhancement of the Effect of Therapeutic Agents on Cancer Cells

Using transgenic mice with brain tumors, it was reported[70] that a diet deficient in vitamins A and E increased apoptosis by about 5-fold and reduced tumor volume by about 50% after 4 months on the diet in comparison to a standard diet or a diet rich in vitamins A and E (2-fold more than that in standard diet). No evidence of apoptosis was found in spleen, small intestine, or liver. From results such as this, it is inferred that if the deficiency of antioxidants reduces the growth of cancer cells, then an excess of them may stimulate their growth. This inference is not applicable to high doses of dietary antioxidants and their derivatives that cause proliferation inhibition and/or apoptosis in cancer cells without affecting most normal cells. It should be pointed out that a deficiency in vitamins A and E may induce irreversible neurological and neuromuscular damage and other toxicities. Furthermore, such an antioxidant-deficient diet before treatment may enhance the effect of x-irradiation or chemotherapeutic agents on both cancer cells and normal cells. Therefore, creating an antioxidant deficiency before standard therapy does not appear to be a rational choice.

Proposed Nutritional Protocols

Based on the studies presented in this review, we have developed nutritional protocols that are divided into 2 categories: active cancer treatment protocol and maintenance protocol.

Active treatment protocol. This protocol is in clinical trial[56] and consists of SEVAK (Premier Micronutrient Corporation, Nashville, Tenn), which contains multiple micronutrients including vitamins A, C, and E and natural β-carotene; vitamin D; B vitamins; and appropriate minerals, but no iron, copper, or manganese. A preparation of multiple micronutrients such as SEVAK is suggested because some of the

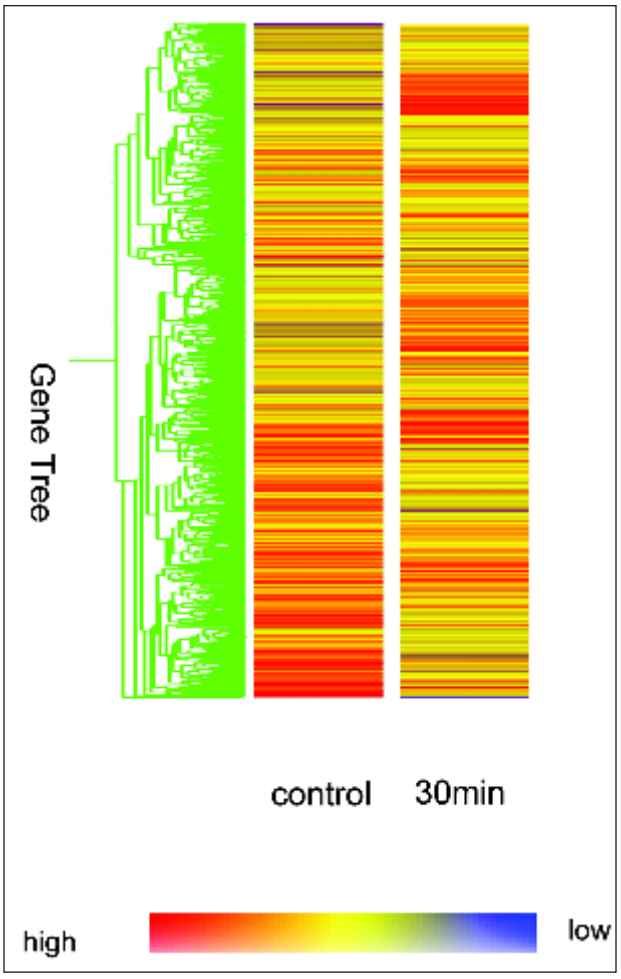

Figure 6 Hierarchical clustering analysis of gene array data 30 minutes after treatment with α-TS shows a marked alteration in the levels of gene expression. Green lines represent the relatedness of the overall global gene expression pattern based on measures of gene similarity.

micronutrients may be depleted during radiation therapy or chemotherapy due to extensive cellular death, loss of appetite, and other side effects. In addition to SEVAK, an additional 8 g of vitamin C in the form of calcium ascorbate, 800 IU vitamin E as α-TS, and 60 mg of natural β-carotene is recommended. All micronutrient supplements described above should be taken orally and in 2 divided doses, one-half dose in the morning and one-half dose in the evening. The rationale for taking antioxidants twice a day is that the biological half-life of most of them is about 6 to 12 hours. Active treatment protocol should be started at least 48 hours prior to standard or experimental therapy and should be continued for 1 month after completion of therapy. This protocol can also be used for those patients who have become refractory to all conventional treatments to improve the quality of life. A phase I study is needed to determine the value of the proposed approach.

Maintenance protocol. A month after completion of standard therapy or experimental therapy, the maintenance protocol begins. This protocol contains SEVAK and an additional 4 g of vitamin C, 400 IU of α-TS, and 30 mg of β-carotene. Such maintenance doses of micronutrients may reduce the risk of recurrence of tumors as well as the risk of a second malignancy among survivors. Diet (low fat and high fiber) and lifestyle (no tobacco smoking or tobacco products, reduce physical and mental stress, moderate exercise) are equally important to improve the efficacy of the proposed nutritional protocols.

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