

Integrative Cancer Therapies

<http://ict.sagepub.com>

Orthomolecular Oncology Review: Ascorbic Acid and Cancer 25 Years Later

Michael J. González, Jorge R. Miranda-Massari, Edna M. Mora, Angelik Guzmán, Neil H. Riordan, Hugh D. Riordan,
Joseph J. Casciari, James A. Jackson and Angel Román-Franco

Integr Cancer Ther 2005; 4; 32

DOI: 10.1177/1534735404273861

The online version of this article can be found at:
<http://ict.sagepub.com/cgi/content/abstract/4/1/32>

Published by:

 SAGE Publications

<http://www.sagepublications.com>

Additional services and information for *Integrative Cancer Therapies* can be found at:

Email Alerts: <http://ict.sagepub.com/cgi/alerts>

Subscriptions: <http://ict.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

Citations (this article cites 140 articles hosted on the
SAGE Journals Online and HighWire Press platforms):
<http://ict.sagepub.com/cgi/content/refs/4/1/32>

Orthomolecular Oncology Review: Ascorbic Acid and Cancer 25 Years Later

Michael J. González, Jorge R. Miranda-Massari, Edna M. Mora, Angelik Guzmán, Neil H. Riordan, Hugh D. Riordan, Joseph J. Casciari, James A. Jackson, and Angel Román-Franco

The effect of ascorbic acid on cancer has been a subject of great controversy. This is a follow-up review of the 1979 article by Cameron, Pauling, and Leibovitz published in *Cancer Research*. In this updated version, the authors address general aspects of ascorbic acid and cancer that have been presented before, while reviewing, analyzing, and updating new existing literature on the subject. In addition, they present and discuss their own mechanistic hypothesis on the effect of ascorbic acid on the cancer cell. The objective of this review is to provide an updated scientific basis for the use of ascorbic acid, especially intravenously as adjuvant treatment in pharmacological nutritional oncology.

Keywords: *vitamin C; intravenous ascorbic acid; cancer; tumor growth; nontoxic chemotherapy; antioxidant; prooxidant*

Twenty five years ago, an important review by Pauling, Cameron, and Leibovitz presented the scientific basis to support the use of ascorbic acid (AA) as a therapeutic agent in the treatment of cancer. A group of clinicians failed to reproduce Pauling and Cameron's earlier reports on the therapeutic effect of vitamin C on cancer patients. While this discrepancy generated controversy, the medical establishment rapidly settled the issue without further research and analysis. However, new knowledge on the pharmacokinetics and pharmacodynamics of AA and new clinical data have given a more complete understanding of the critical aspects of AA's therapeutic effect on cancer. This review will summarize these new findings and discuss our own mechanistic hypothesis on the effect of AA in the cancer cell. The objective of this review is to provide an updated scientific basis for the use of AA (intravenous route) as adjuvant treatment for cancer patients.

DOI: 10.1177/1534735404273861

AA Characteristics

Biochemistry

AA (vitamin C, ascorbate, $C_6H_{12}O_6$) is a ketolactone with a molecular weight of 176.13 g/mL. A basic identified biochemical role for AA is to accelerate hydroxylation reactions in a number of biosynthetic pathways. In many of these reactions, ascorbate directly or indirectly provides electrons to enzymes that require prosthetic metal ions in a reduced form to achieve full enzymatic activity. The best-known biochemical role of ascorbate is that of cofactor for prolyl and lysyl hydroxylase enzymes in the biosynthesis of collagen.¹ The molecular structures of AA and its oxidized form dihydroascorbic acid are similar to that of glucose. Its structure is similar to glucose because of several hydroxyl groups (OH) that are next to each other (see Figure 1).

Biological Functions

Ascorbate, present in most biological settings (pk = 4.2), is an essential vitamin for humans.² Scurvy, the deficiency disease arising from the lack of ascorbate, can reach a life-threatening level and even death.³ Most mammals synthesize ascorbate from glucose; however, humans and other primates lack the enzyme

MJG and AG are at the RECNAAC II Project, University of Puerto Rico, Medical Sciences Campus, School of Public Health, Department of Human Development, Nutrition Program, San Juan, Puerto Rico. JRM-M is at the School of Pharmacy, Department of Pharmacy Practice, University of Puerto Rico, San Juan. EMM is at the School of Medicine, Surgery Division, University of Puerto Rico, and Puerto Rico Cancer Center, San Juan, Puerto Rico. NHR is at the AiDan Incorp, Tempe, Arizona. HDR, JJC, and JAJ are at the Center for the Improvement of Human Functioning, Wichita, Kansas. AR-F is at the Department of Pathology, University of Puerto Rico, San Juan.

Correspondence: Michael J. González, University of Puerto Rico, Medical Sciences Campus, Graduate School of Public Health, Department Human Development, Nutrition Program, PO Box 365067, San Juan, PR 00936-5067. E-mail: mgonzalez@rcm.upr.edu.

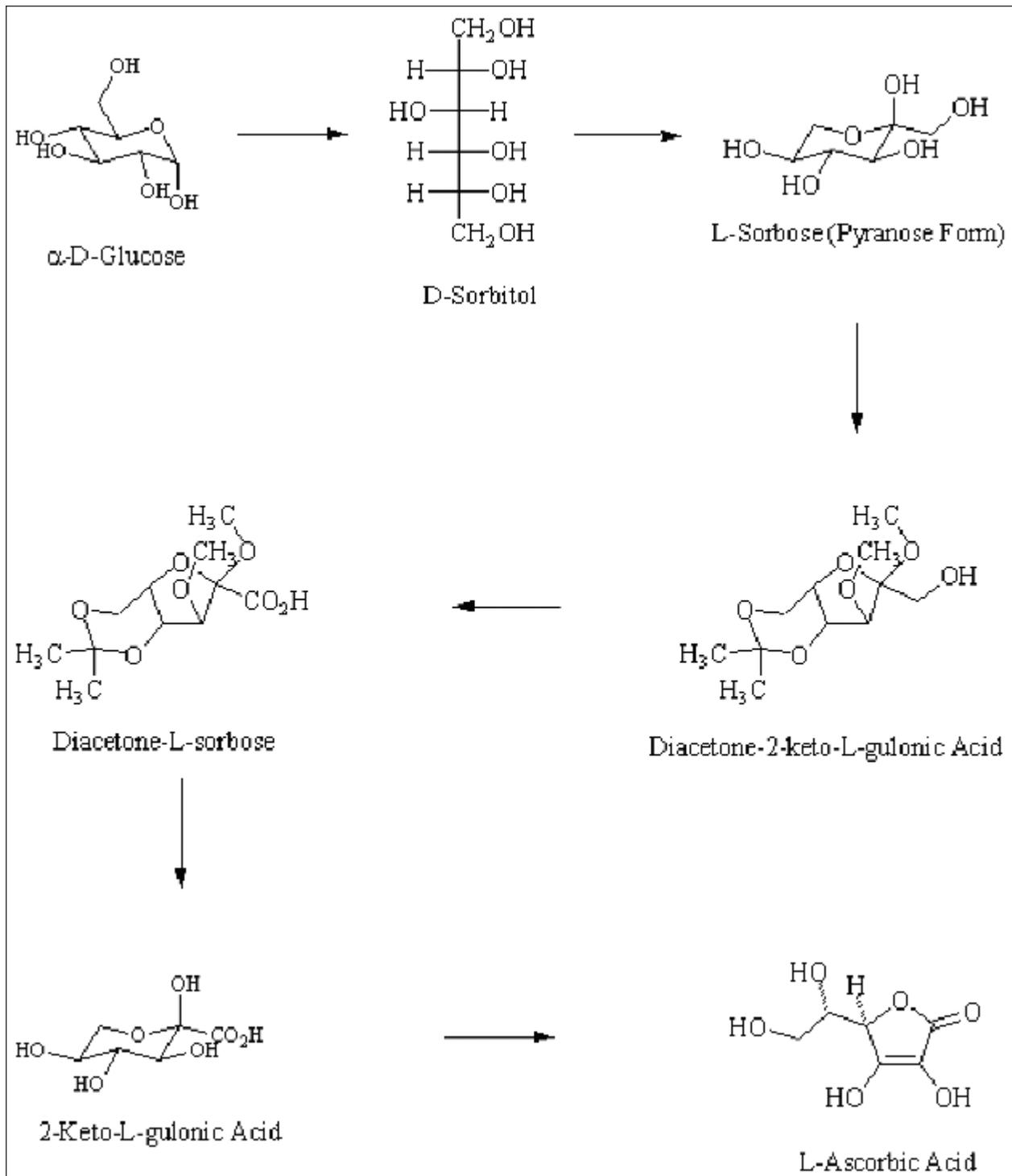


Figure 1 Glucose conversion to ascorbic acid sequence.

(L-gulonolactone oxidase) required for its synthesis.⁴ In 1965, Irwin Stone proposed that a negative mutation may have occurred in these species resulting in the loss of the ability to produce vitamin C. In cold-blooded amphibians and reptiles, the amounts of AA

produced in their small kidneys sufficed for their needs. However, with the advent of temperature regulation in highly active, warm-blooded mammals, the biochemically crowded kidneys could no longer supply AA in ample quantities.

Ascorbate is considered the most important antioxidant in extracellular fluid.⁵ Ascorbate is a water-soluble compound distributed throughout the body, with high concentrations found in a number of tissues including the eye lens, white blood cells, adrenals, and pituitary glands.¹ Normal plasma concentrations of AA are about 0.6 to 2.0 mg/dL. These tissues (eye lens, adrenals, and pituitary) contain at least twice this amount. Ascorbate is required in the synthesis of carnitine from lysine,⁶ neurotransmitter synthesis,¹ cytochrome P-450 activity, cholesterol metabolism, detoxification of exogenous compounds,^{4,7} and as an antioxidant.⁵ In addition, when given in large doses (mainly intravenous), ascorbate may function as an ergogenic aid. To our knowledge, this biochemical role has not been previously described in the literature, although there is evidence of ascorbate increasing cell respiration and adenosine triphosphate (ATP) production in osteoblasts.⁸ This newly proposed function of ascorbate may be of great relevance to patients suffering chronic-degenerative diseases, especially those with chronic fatigue syndrome, AIDS, and cancer. We suggest that this ergogenic activity reported for large doses of ascorbate is probably due to ascorbate's oxidation reduction potential, capable of providing necessary electrons to the electron transport system in the mitochondria for increased energy production. This participation of ascorbate in electron transport reactions was postulated 71 years ago by Szent-Gyorgyi.⁹

AA and Cancer

Vast literature exists on AA and cancer. As early as 1949, ascorbate use was proposed for cancer therapy.¹⁰ Since 1952, ascorbate has been proposed as a chemotherapeutic agent.¹¹ Hundreds of articles including an array of in vitro, in vivo, cell, animal, and human studies have been published on this topic (see Padayatty et al¹² for a general review on vitamin C and Tamayo and Richardson¹³ for a review on ascorbate and cancer). However, the first comprehensive review of this topic was published in 1979 in *Cancer Research*.¹⁴ In this review, we will update (after 25 years) that seminal publication on nutritional oncology by Cameron, Pauling, and Leibovitz.¹⁴ We performed a MEDLINE search (1979-2003) using the terms *vitamin C* and *cancer*. We also searched other indexing engines, such as Index Medicus, Biological Abstracts, and Docrine. We also used the references in the searched articles. From the results, we selected the articles pertaining to the use of AA as treatment or as a potential therapeutic adjuvant to explain AA anticancer activity. We embarked on the mission of filling gaps that have arisen since the pioneering article was published. There is a

new body of data that evidences the chemotherapeutic potential of ascorbic acid.

Cancer Preventive Mechanisms of AA

Antioxidant Properties of Ascorbate

Ascorbate is considered one of the strongest reductants and radical scavengers. Ascorbate reduces unstable oxygen, nitrogen, and sulphur-centered radicals. In addition, it acts as a primary defense against aqueous radicals in blood.¹⁵ In studies with human plasma, ascorbate protected plasma lipids against detectable peroxidative damage induced by aqueous peroxy radicals.¹⁶ By efficiently trapping peroxy radicals in the aqueous phase before they can reach the lipid-rich membranes and initiate lipid peroxidation, ascorbate can protect biomembranes against primary peroxidative damage. Ascorbate may also protect membranes against peroxidation due to its synergistic antioxidant function with vitamin E. Ascorbate may enhance or reinstate the activity of tocopherol (vitamin E), the principal lipid-soluble antioxidant.¹⁵ While the occurrence of this action has been questioned in an in vivo setting,¹⁶ it seems reasonable when both vitamins are present in an environment of elevated oxidative stress. Ascorbate reacts with the tocopheroxyl (chromanoxyl) radical that arises in cell membranes as a result of vitamin E antioxidant activity and simultaneously regenerates tocopherol and transfers the oxidative challenge to the aqueous phase.¹⁷ At this point, the less reactive ascorbate radical can be enzymatically reduced back to AA by a nicotinamide adenine dinucleotide-dependent system.¹⁸⁻²¹ This probably explains how ascorbate reduces nitrates and prevents the formation of carcinogenic nitrosamines.²²

Primary Anticancer Mechanisms of AA

Oxidative, Oxidant, and Prooxidant Properties of Ascorbate

AA not only possesses antioxidant activity but also has cytotoxic effects at higher concentrations.²³⁻²⁵ It has been suggested that ascorbate promotes oxidative metabolism by inhibiting use of pyruvate for anaerobic glycolysis.²⁶ Ascorbate in high doses inhibits prostaglandins of the 2-series (arachidonic acid-derived), which have been correlated with increased cell proliferation.²⁷ Also, a growth inhibitory effect has been produced by ascorbate or its derivatives in at least 7 types of tumor cells.²⁸⁻³⁴ While this inhibitory action was not observed in normal fibroblasts by some research groups,²⁸⁻³³ other researchers have reported

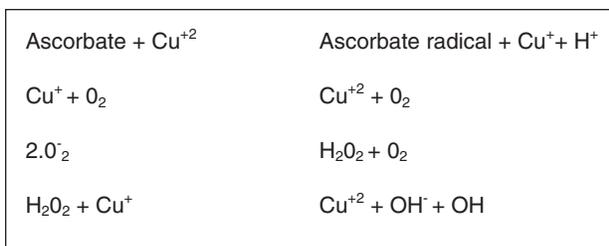


Figure 2 Ascorbate-copper interaction.

otherwise with respect to the fibroblast inhibition.³⁴⁻³⁸ Nevertheless, all reports are in accord that this cytotoxic effect produced by ascorbate in an array of cell lines (mostly malignant) has been associated with its prooxidant activity.^{23,39-45} Ascorbate and its radical potentiate the activation of transcription factor NF- κ B, which has been associated with inhibition of cell growth.⁴⁶

The Role of Hydrogen Peroxide

Ascorbate can generate hydrogen peroxide (a reactive oxygen species) on oxidation (with oxygen) in biological systems.⁴⁷⁻⁴⁹ This action can be enhanced by divalent cations such as iron and copper (see Figure 2).^{25,39,50} Hydrogen peroxide may further generate additional reactive species, such as the hydroxyl radical and secondary products of oxidation, such as aldehydes. These reactive species can compromise cell viability mainly by damaging the cell membranes of malignant cells, which are relatively deficient in catalase activity.^{42,50-55} However, these oxidative reactions may form in only minute quantities in healthy organisms. This is mainly because most transition metal ions are bound to proteins in serum, which makes them unavailable to participate in biochemical reactions.⁵⁶ Nevertheless, these oxidation reactions may take place in pathological states such as malignancy, in which cohesive forces that inhibit the liberation of the metal ion from the proteins as well as the control of the cell's replication mechanisms are drastically reduced.⁵⁶ These reactive species are capable of inducing multiple negative cellular effects such as DNA strand breaks, disruption of membrane function via lipid peroxidation, and depletion of cellular ATP.⁵⁴ The failure to maintain high ATP production (cell energy level) may be a consequence of oxidative inactivation of key enzymes, especially those related to the Krebs cycle and the electron transport system. A distorted mitochondrial function (transmembrane potential) may result. This aspect could be suggestive of an important mitochondrial involvement in the carcinogenic process. In this respect, ascorbate may serve yet another metabolic and physiological function by

providing reductive energy, that is, the electrons necessary to direct energy pathways in the mitochondria.⁵⁷⁻⁶¹ Interestingly, ascorbate has been detected within the mitochondria where it is also regenerated.⁶²

In general, the cytotoxicity induced by ascorbate seems to be primarily mediated by hydrogen peroxide.^{28-31,36,51,63-66} Of interest is the observation that in proliferating cells, very low levels of hydrogen peroxide (3-15 μ M) stimulate cell division, whereas greater concentrations induce cell growth arrest, apoptosis, and/or necrosis.⁶⁶ It has also been shown that the amount of hydrogen peroxide generated by the cells was proportionally dependent on the ascorbate concentration and inhibited by serum.^{35,67-69} Human serum, as part of its normal contents, has certain proteins such as albumin and glutathione with antioxidant capacity that may stabilize ascorbate (directly or indirectly by chelating available transition metals). In addition, serum contains antioxidant enzymes such as catalase, which decomposes hydrogen peroxide. Other antioxidant enzymes including glutathione peroxidase and superoxide dismutase complement the catalase function.

Hydrogen peroxide is most likely generated intracellularly during ascorbate's metabolic oxidation to dehydroascorbate. Hydrogen peroxide reduces cellular levels of thiols and can initiate membrane lipid peroxidation.^{28,34,50-52,63,70-73} As mentioned previously, the antiproliferative action of ascorbate in malignant cultured cells, animal, and human tumor xenografts has been augmented by the addition of the cupric ion, a catalyst for the oxidation of ascorbate.^{34,39-41,74-76} In addition, the combination of ascorbate and copper has been shown to inactivate lactate dehydrogenase,⁷⁷ the enzyme responsible for the reduction of pyruvate to lactate (a metabolic dead-end product prevalent in anaerobic environments such as in cancer). Copper in the form of copper sulfate may also inhibit tyrosinase activity.^{78,79} It has also been suggested that the selective toxicity of ascorbate in malignant cells may be due to reduced levels of antioxidant enzymes, catalase, superoxide dismutase, and glutathione peroxidase⁸⁰ in these cells, leading to cellular damage through the accumulation of hydrogen peroxide.^{44,74,81-85} There is a 10- to 100-fold greater content of catalase in normal cells than in tumor cells.^{44,81}

Furthermore, the addition of vitamin K3 (menadione) to AA produces a synergistic antitumor activity.^{73,86-89} Since menadione is reduced intracellularly via 1- or 2-electron transfer action (probably by AA), this may lead to formation of hydrogen peroxide and other reactive oxygen species, concomitant with the depletion of glutathione. Decreases of glutathione have also been associated with ascorbate

metabolism.⁹⁰ Interestingly, a new form of cell death (autschizis) has been described for this synergistic vitamin phenomenon (vitamins C and K) in which tumor cells undergo profound perturbations of cytoskeleton and membranes that ultimately kill the cells by a form of cell death that is distinct from apoptosis, oncosis, or necrosis.⁸⁷⁻⁹¹ For this reason, the combination of megadoses of IV ascorbate together with oxygen, vitamin K, lipoic acid, coenzyme Q10, and small doses of copper may seem logical as part of a nontoxic treatment protocol for cancer. Intravenous administration of ascorbate can yield very high plasma levels that seem to be necessary for ascorbate's toxic effect on malignant cells.⁹²⁻⁹⁵

Other AA Oxidation Products

AA oxidation products such as dehydroascorbic acid, 2,3-diketogulonic acid, and 5-methyl 1-3, 4-dehydroxytetrone, all degradation products of AA, have demonstrated antitumor activity.^{34,39-42} In addition, other compounds arising from the oxidation or degradation of ascorbate can inhibit tumor growth. The most effective ones are γ -crolactone and 3-hydroxy-2-pyrone. The available evidence suggests that these vitamin C oxidation products and/or metabolic by-products have a function in controlling mitotic activity. All active compounds consist of an unsaturated lactose ring with a double bond conjugated with a carbonyl group, suggesting that this particular structural feature of the lactose ring may be relevant in the antitumor activity.³⁴ The antitumor activity shown by these compounds could be due to their ability to produce active molecular species that inhibit tumor growth such as hydrogen peroxide and certain aldehydes. Most of these compounds are very unstable, and their growth inhibitory activities could be attributed to their chemical instability that favors the formation of reactive species. These antiproliferative mechanisms of AA and/or its oxidation products on tumor cells are probably of a very complex nature since they seem to involve a series of pleiotropic chain reactions.

Large amounts of AA intake can change the levels of certain amino acids in body fluids⁹⁶⁻⁹⁹ and may deplete the bioavailability of lysine and cysteine, 2 amino acids that are required for rapidly growing tumors.¹⁰⁰ Experiments using tissue homogenate show that the interactions between ascorbate, metal ions, and oxygen are capable of inducing structural changes in protein.⁹⁸⁻¹⁰¹ These electron transfers need a conductor to proceed, and proteins can serve as electron conductors for these reactions. Metal ions, such as copper, are good electron conductors because their valence bonds are partially filled. The resulting

molecules contain 1 or more uncoupled electrons and are very reactive free radicals.

Dehydroascorbic acid (the oxidized, nonionic, and more lipid-soluble form of ascorbate) and the semidehydroascorbic acid radical have been shown to promote lipid peroxidation.⁴² One of us (M.J.G.) has demonstrated that secondary products of lipid peroxidation have an inhibitory action on human malignant cell proliferation.^{53,56,63,70} There is evidence to suggest that dehydroascorbic acid may work as a mitotic inhibitor *in vivo*.⁹² Dehydroascorbic acid may prevent cell division by inhibiting protein synthesis at the ribosomal level.⁹² Interestingly, prolonged exposure to high concentrations of dehydroascorbic acid may cause irreparable damage resulting ultimately in complete lysis of the cells.⁹²

Intracellular Transport of Ascorbate and Its Tumor Specificity

Extracellular ascorbate is oxidized, transported as dehydroascorbic acid, and reduced intracellularly to ascorbate.¹⁰² Actually, many cell types transport ascorbate solely in its oxidized form, through facilitated glucose transporters.¹⁰³ These cells accumulate large intracellular concentrations of ascorbate by reducing dehydroascorbate to ascorbate, a form that is trapped intracellularly. Other cells can transport ascorbate in its reduced form through a sodium-dependent cotransporter.¹⁰³ To ascorbate's advantage, tumor cells have an increased requirement for glucose.¹⁰⁴ To compensate for this increased need for glucose, tumor cells increase their quantity of glucose transporters.¹⁰⁵ This action greatly enhances the entrance of either ascorbate or its oxidized form, dehydroascorbate, into the cancer cell. This facilitates the action of ascorbate as a selective, nontoxic (to normal cells) chemotherapeutic agent. These issues are very relevant in the clinical use of AA and dehydroascorbic acid. Also, dehydroascorbic acid may be further metabolized to 2, 3-diketogulonic acid or reduced back to AA. It is conceivable that ascorbate may have a preferential cytotoxicity against tumor cells, and this can be associated to its selective uptake by the cancer cell and the intracellular generation of hydrogen peroxide via redox reactions with no toxic effects on normal tissue.^{44,88,93,100} The most likely reason for this can be a quantitative difference in the content of the enzyme catalase mentioned earlier.⁸¹

It is important to recognize that ascorbate's antioxidant or prooxidant characteristics depend on the redox potential of the surrounding environment at a specific point in time and the concentration of ascorbate. It is conceivable that nutrients that have chemopreventive properties may be capable of

inhibiting the continual growth of transitory clones of cells through their antagonistic pro-oxidant activity. In contrast, uncontrolled pro-oxidant activity can generate excess free radicals (reactive oxygen species) that can be deleterious to cellular membranes and DNA.^{58,106-110} This paradoxical role of antioxidants and pro-oxidants has been analyzed previously.¹⁰⁹⁻¹¹¹ Interestingly, during differentiation, there is an increased cellular production of oxidants that appear to provide 1 type of physiological stimulation for changes in gene expression that lead to a terminal differentiated state.¹¹² In addition to this, ascorbate has been shown to stimulate differentiation in brain cells¹¹³ and redifferentiation in hepatoma cells.¹¹⁴

Oxygen, the final electron acceptor, is of great importance to the ascorbate-induced cytotoxic action on cancer cell proliferation by interfering with anaerobic respiration (fermentation), a commonly used energy mechanism of malignant cells. It would be worth investigating the status of the mitochondria of malignant cells since we believe this may be relevant to the origin of malignancy.¹¹² A problem in electron transfer activity might well be coupled to defective mitochondria, and vitamin C may help correct this electron transfer problem.¹¹⁵

Intravenous AA

The concentrations of ascorbate toxic to cancer cells *in vitro* can be achieved clinically by intravenous administration.⁹³⁻⁹⁴ It has been observed that a seemingly large dose of a 30-g infusion of AA given to a cancer patient was not adequate to raise the plasma level to a level that was toxic to tumor cells as reported *in vitro* (>200 mg/dL for dense monolayers and >400 mg/dL for hollow fiber models). Infusion of 60 g resulted in a brief (30-minute) elevation of plasma levels of vitamin C above 400 mg/dL, while 60 g infused over 60 minutes immediately followed by 20 g infused over the next 60 minutes resulted in a 240-minute period in which the vitamin C plasma concentration was near or above 400 mg/dL, a concentration proven to be cytotoxic.⁹⁴ Lipoic acid (thioctic acid), an aqueous and lipid-soluble antioxidant that recycles vitamin C, decreased the dose of vitamin C required to kill 50% of tumor cells from 700 mg/dL to 120 mg/dL.⁹⁵ Lipoic acid can mediate the reduction of dehydroascorbic acid and improves mitochondrial function.¹¹⁶ It is conceivable that other energy intermediates such as acetyl-L-carnitine, coenzyme Q10, B-complex vitamins, vitamin K3, magnesium, α -ketoglutarate-aspartate, among others, will prove of benefit against cancer either by interacting directly with ascorbate (redox) or by stimulating/improving and/or correcting aerobic metabolism in the mitochondria. This information supports the hypothesis that certain

oxidation intermediates and/or aerobic metabolism cofactors originating from nutrients or from their interaction can act as active antineoplastic agents. It seems that the cytotoxic effects of ascorbate and its derivatives are ascribed to their chemical properties related to their molecular structural characteristics and not to vitamin activity.

In general, we are proposing the pro-oxidant activity exhibited by AA as the main mechanism by which it inhibits cancerous growth and metastasis and its proposed role as an energy intermediate as a possible secondary or accessory anticancer mechanism.

Secondary Anticancer Mechanisms of AA: Host Resistance to Cancer

AA and Intracellular Matrix

AA metabolism is associated with other different mechanisms known to be involved in host resistance to malignant disease. Cancer patients are significantly depleted of ascorbate. This could indicate an increased requirement and utilization of this substance to potentiate these various resistance mechanisms. Scurvy results from the severe dietary lack of ascorbate. It is a syndrome of generalized tissue disintegration at all levels, involving the dissolution of intercellular ground substance, the disruption of collagen bundles, and the lysis of the interepithelial and interendothelial cement. This disintegration leads to ulceration with secondary bacterial colonization, to vascular disorganization with edema and interstitial hemorrhage, and to generalized undifferentiated cellular proliferation with specialized cells throughout the tissue reverting to a primitive form.¹⁴ The generalized stromal changes of scurvy are identical to the local stromal changes observed in the immediate vicinity of invading neoplastic cells.¹¹⁷ Thus, stromal resistance may be a physical line of defense against cancer by encapsulating neoplastic cells with a dense fibrous tissue. This feature can be enhanced by high doses of ascorbate. Vitamin C also enhances the resistance of the intercellular ground substance to local infiltration.

A brisk lymphocytic response is a systemic factor indicative of enhanced host resistance and is associated with a more favorable prognosis of the disease. To proliferate, cells must escape the restraint imposed by highly viscous intercellular glycosaminoglycans and they can do this by the release of the enzyme hyaluronidase.¹¹⁸ There is evidence that a physiological hyaluronidase inhibitor is an oligoglycosaminoglycan that requires AA for its synthesis.¹¹⁹ Changes in hyaluronic acid have been shown to be conducive to cell proliferation.¹²⁰ In addition, ascorbate is involved

in the synthesis of collagen. Collagen-rich extracellular matrix including the basement membrane is a major barrier to the metastatic and invasive spread of cancer cells.¹⁴ The intercellular matrix is reinforced by a tridimensional network of interlacing collagen fibers. The amount of collagen present determines the strength of the tissue and also its resistance to malignant infiltration. Lack of ascorbate sharply reduces hydroxylation of prolyl and lysyl residues into hydroxyproline and hydroxylysine, leading to instability of the triple helix of collagen,¹²¹ which is a common feature in scurvy and also in cancer. (This is also of importance in vitamin C's role in wound healing including decubital ulcers, surgery recovery, and other accidental traumatic injuries.¹²²)

Ascorbate and Immunocompetence

Ascorbate is essential to ensure the efficient working of the immune system. The immunocompetence mechanisms are a combination of humoral and cell-mediated defensive reactions with ascorbate involved in a number of ways. In terms of humoral immunocompetence, ascorbate is essential for immunoglobulin synthesis.¹²³ In cell-mediated immunity, immunocompetence is exercised overwhelmingly by lymphocytes, which contain high concentrations of ascorbate relative to other cells. In addition, ascorbate is required for active phagocytosis.¹²⁴ Ascorbate has also been shown to enhance interferon production.^{123,125,126}

AA has other identified functions related to cancer prevention. Ascorbate is required by the mixed function oxidases for the hydroxylation of amino acids.¹⁴ The mixed function oxidases are a group of closely related microsomal enzymes that metabolize many classes of compounds and are particularly important in the inactivation of chemical carcinogens. Microsomal metabolism of carcinogens yields products generally more water soluble, which greatly increases their rate of excretion. In addition, ascorbate has been shown to protect against nitrate-induced carcinogenesis.¹²⁷ Another important anticancer function of ascorbate when provided in large quantities is that it enhances the removal of sodium via the urine, thereby reducing the level of sodium ions in the serum. In cancer, there is a disturbed sodium/potassium ratio. It has been suggested that vitamin C may also have a role inhibiting prostaglandins of the 2-series in carcinoma cells.^{27,128} In the process of prostaglandin biosynthesis, the release of arachidonic acid from cell membrane phospholipids is implicated as one of the synergistic signals leading to cell proliferation. Recently, ascorbate has been shown to stabilize p53, a protein involved in cell proliferation control.¹²⁹

Safety and Toxicity Considerations of High Doses of AA

AA is remarkably nontoxic at high levels (10 to 100 times the recommended dietary allowance when taken orally). Nevertheless, some minor toxic effects have been reported. These side effects include acidosis, oxaluria, renal stones, glycosuria, renal tubular disease, gastrointestinal disturbances, sensitivity reactions, conditioned need, prothrombin and cholesterol disturbances, vitamin B₁₂ destruction, fatigue, and sterility.¹³⁰ Of these side effects, gastrointestinal disturbances are perhaps the most consistent and prevalent problem following the ingestion of large quantities of oral AA since nausea, abdominal cramps, and diarrhea are frequently mentioned as negative side effects. These effects are lessened or eliminated by taking AA as a buffered salt or immediately after meals. The amount of oral AA tolerated by a patient without producing diarrhea increases in proportion to the stress or severity of his or her ailment.¹³¹ Bowel tolerance doses of AA ameliorate the acute symptoms of many diseases. Lesser doses often have little effect on acute symptoms but assist the body in handling the stress of disease and may reduce the morbidity of the disease.¹³²

Many of the toxic effects reported for taking large amounts of vitamin C in reality are insignificant, rare, and of minor consequences. Nevertheless, a word of caution should be given for patients with glucose-6-phosphate deficiency. When given high doses of AA, these patients may be at risk of developing hemolysis.¹³³ Before applying AA therapy, patients should be screened for this deficiency. Also while on AA therapy, intake of inorganic selenium (Na selenite) should be avoided. A possibility exists that AA may reduce selenite and render it unavailable for tissue uptake.¹³⁴ In relation to kidney stones, these are formed mostly in alkaline urine (calcium oxalate stones). High doses of ascorbate make the urine acidic, thus preventing stone formation. There are various studies that have addressed this issue¹³⁵⁻¹⁴⁰ and found no evidence of ascorbate increasing the risk of kidney stone formation.

In relation to AA given intravenously, no ill effects have been reported with doses as high as 150 to 200 g over a 24-hour period.^{44,92-95,141,142} Ascorbate is more efficient when administered intravenously than when given orally because it bypasses the gut and higher circulating levels are achieved for longer periods of time. Another valid concern when applying ascorbate intravenously is a rapid tumor hemorrhage and necrosis.¹⁴³ However, in the 28 years of administering intravenous vitamin C at the Center for the Improvement of

Human Functioning, we have never had an episode of tumor necrosis. Patients may become very ill because their bodies cannot cope with the sudden task of getting rid of such a large mass of dead tissue. This is a concern mainly for patients suffering from end-stage disease with a considerable tumor load and highly aggressive, rapidly dividing tumors. This might be the main reason not to overload the body's detoxification systems (skin, kidneys, colon, and liver) while on ascorbate therapy. AA has a unique advantage relative to other currently used remedies for cancer: it is generally harmless and safe even at sustained high doses for prolonged periods of time. Evidence supports the concept of using high-dose intravenous AA for extended periods, in doses high enough to achieve and maintain plasma levels above those that have been found to be preferentially cytotoxic to tumor cells.^{92,93} AA is one of the safest and most valuable substances available to the physician for treating cancer.

Contradictory Data on Vitamin C and Cancer

In our comprehensive search, we encountered a few contradictory studies regarding the effect of vitamin C and its effect on cancer cells. AA has been reported to enhance chemical carcinogenesis in a rodent model.¹⁴⁴⁻¹⁴⁶ This action may be due to the pro-oxidant activity of AA and the subsequent enhancement of free radical formation by the chemical carcinogen 7,12-dimethylbenz[a]anthracene. In another study, AA in low concentrations was found to be an essential requirement for the growth of murine myeloma cells in cell culture.¹⁴⁷ In contrast, further studies by the same group reported that AA inhibited growth at higher concentrations.¹⁴⁸ Also, vitamin C at low doses (± 25 $\mu\text{g}/\text{mL}$) without any other added antioxidants has been reported to stimulate growth of malignant cells while inhibiting their growth at high doses (± 200 $\mu\text{g}/\text{mL}$).¹⁴⁹ These studies constitute a very important contribution in terms of understanding AA effect on malignant cells. In addition, they help determine a therapeutic dosing range of AA for cancer, specifically, the proper dose and the concomitant use of synergistic nutrients. Therefore, instead of being contradictory, these studies actually reinforce the importance of using high-dose AA to achieve a chemotherapeutic effect.

Rethinking the Classical Vitamin C and Cancer Controversy

In the late 1970s and early 1980s, a debate ensued between Dr Linus Pauling (Linus Pauling Institute) and Dr Charles Moertel (Mayo Clinic) due to conflicting results on studies on vitamin C and cancer.¹⁵⁰⁻¹⁵³ To

make the story short, the Pauling and Cameron studies used historical controls and were positive, while the Mayo Clinic studies were done in a prospective randomized double-blinded fashion and had negative results. The Mayo Clinic studies were done with the accepted experimental design used to clarify initial observations but did not truly replicate the Cameron and Pauling studies (used a lesser dosage, less time). This issue has been reviewed elsewhere.¹⁵⁴

A critical point of both studies (Mayo Clinic and Pauling's) is that they used oral doses of ascorbate of about 10 g. Given the saturable gastrointestinal absorption and the nonlinear renal clearance,¹⁵⁵ oral absorption of AA cannot achieve plasma concentrations comparable to those obtained by intravenous administration.⁴⁴ Plasma concentrations of AA rise as the dose ingested increases until a plateau is reached with doses of about 150 to 200 mg daily.

Moreover, there is a recent report on AA as a toxic agent against cancer cells when given intravenously.⁹⁴ The doses we are advocating for therapy are substantially higher doses (25-200 g) and, most important, are given intravenously. We believe intravenous administration is more effective because plasma levels of ascorbate can reach higher levels than those attained by oral intakes, and these higher levels can be sustained for longer periods of time. These 2 aspects seem necessary to produce a selective toxic effect by AA on cancer cells. We are attempting to reach plasma levels that are 100 times higher than those that can be achieved by oral administration.

Solving the Modern Controversy: Vitamin C (Antioxidants) and Cancer Chemotherapy and Radiation

There has been a recent concern that antioxidants might reduce the effectiveness of chemotherapy and radiation by reducing the potency of free radicals necessary for cell killing. This misconception is important because it may prevent clinicians from using ascorbate as adjuvant therapy for cancer. In relation to vitamin C, this misconception was due in part to an article published by Agus et al in 1999,^{156,157} in which they described how cancer cells acquire and concentrate vitamin C.

The authors suggest that this increased intracellular concentration of ascorbate may provide malignant cells with a metabolic advantage. This suggestion has been embraced by most practitioners without question, resistance, or further evaluation. There are important details that need to be discussed to better understand this modern controversy.

Cancer cells use glucose as a main energy fuel. To provide enough glucose, the cancer cell increases the

number of facilitative glucose transporters (GLUTs). Since AA and glucose have similar molecular structures, cellular intake of vitamin C is favored in malignant cells. Certain specialized cells can transport AA directly through a sodium ascorbate cotransporter, but in most cells, vitamin C enters through GLUTs in the form of dehydroascorbic acid, which is then reduced intracellularly and retained as AA.¹⁵⁶ AA not only acts as an antioxidant but also has cytotoxic effects at higher concentrations in cancer cells since AA at high concentrations has pro-oxidant effects (please refer to the section on primary anticancer mechanisms of AA in this article). In vitro studies have shown that vitamin C in high concentrations (but not low concentrations) enhances the cytotoxicity of 5-FU in a dose-dependent manner in mouse lymphoma.¹⁵⁸ Data show that dietary antioxidants administered in high doses that inhibit the growth of cancer cells, but not normal cells, may improve the efficacy of radiation therapy.¹⁵⁹ However, results also show that dietary antioxidants given in a single low dose (that does not affect the growth of cancer cells) shortly before irradiation may protect cancer cells during radiation therapy.¹⁵⁹ A case report was recently published in which 2 patients with ovarian cancer stage IIIC responded well to treatment of chemotherapy along with high-dose antioxidants. Both patients received intravenous AA at a dose sufficient to maintain a plasma concentration above 200 mg/dL. Both had normalization of their CA-125 during the first cycle of chemotherapy, and 3 years after diagnosis, there was no evidence of recurrence of the disease. One of the patients declined further chemotherapy after the first round and continued on intravenous vitamin C and oral supplementation of several antioxidants and a multivitamin/mineral.¹⁶⁰

A vast body of literature exists on this topic and is summarized in a series of articles by Lamson and Brignall.¹⁶¹⁻¹⁶³ These articles indicate that antioxidants, including ascorbate, provide beneficial effects in various types of cancers without reduction of efficacy of chemotherapy or radiation. In addition, the data show increased effectiveness of conventional cancer therapeutic agents when given with antioxidants as well as a decrease in adverse effects.¹⁶⁰⁻¹⁶³ Moreover, an article by Prasad et al¹⁶⁴ shows similar positive results for the combination of antioxidants and conventional treatment. For a complete review of the topic, see the article by Moss.¹⁶⁵

It has been demonstrated that chemotherapy as well as radiotherapy induce a fall in plasma antioxidants in cancer patients.¹⁶⁶⁻¹⁶⁸ Extensive in vitro studies and limited in vivo studies have revealed that individual antioxidants induce cell differentiation and growth inhibition to various degrees in rodent and human cancer cells.¹⁶⁴ In addition, Prasad and

Kumar¹⁴⁹ have shown that high-dose multiple antioxidants work synergistically in reducing tumor growth of human parotid acinar cells in vitro. Finally, a clinical trial in Japan with 99 patients showed that terminal cancer patients receiving large doses of AA had much longer survival time (43 vs 246 days) than patients using low AA doses.¹⁶⁹

Conclusion

There are a wide variety of mechanisms by which ascorbate prevents and inhibits malignant growth. We have described the ones we believe are most important, most scientifically logical, and for which there is the most evidence. It is very likely that many of these mechanisms interplay in ascorbate's anticancer action. The collective evidence supports the notion of increasing ascorbate intake in patients suffering malignancies, especially provided by intravenous route. Ascorbate may produce benefits in both prevention and treatment of cancer, by inhibiting malignant cell proliferation, and inducing differentiation¹¹³ and redifferentiation.¹¹⁴ In addition, ascorbate has been of value in the palliation of pain^{170,171} and as an ergogenic agent,^{8,172} which has substantially improved the quality of life of terminal cancer patients.

The ideal anticancer agent is obviously one that specifically interferes with tumor growth, prolongs survival time, and improves quality of life. There is evidence that ascorbate might fit this description. A protocol for the proper administration of intravenous AA has been published recently.¹⁷³ Based on the evidence reviewed herein, we suggest the use of intravenous AA as adjuvant therapy in cancer treatment and the exploration of new cancer therapies based on modulation of the cellular redox state.

References

1. Levine M. New concepts in the biology and biochemistry of ascorbic acid. *N Engl J Med.* 1986;314:892-902.
2. Food and Nutrition Board, National Research Council. *Recommended Dietary Allowances.* 10th ed. Washington, DC: National Academy Press; 1989.
3. Guthrie H. *Introductory Nutrition.* 7th ed. St. Louis, Mo: Mosby College Publishing; 1989:381-382.
4. Shils M. Modern nutrition in health and disease. In: Shils M, Olson J, Shike M, eds. *Modern Nutrition in Health and Disease.* 8th ed. Philadelphia, Pa: Lea and Febiger; 1994:444-445.
5. Sies H, Stahl W, Sundquist A. Antioxidant functions of vitamins. *Ann N Y Acad Sci.* 1992;669:7-20.
6. Leibovitz B, Mueller J. Carnitine. *J Optimal Nutr.* 1993;2:90-109.
7. Block G. Vitamin C and cancer prevention: the epidemiologic evidence. *Am J Clin Nutr.* 1991;53:270s-282s.
8. Komarova SV, Ataulkhanov FI, Globus RK. Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts. *Am J Physiol Cell Physiol.* 2000;279:c1220-1229.
9. Szent-Gyorgyi A. Introduction to submolecular biology. *J Biol Chem.* 1960;90:385.

10. Klenner FR. The treatment of polyomyelitis and other virus diseases with vitamin C. *South Med Surg*. 1949;3:7-12.
11. McCormick WJ. Ascorbic acid as a chemotherapeutic agent. *Arch Pediat*. 1952;69:151-155.
12. Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr*. 2003;22:18-35.
13. Tamayo C, Richardson MA. Vitamin C as a cancer treatment: state of the science and recommendation for research. *Altern Ther*. 2003;9:94-102.
14. Cameron E, Pauling L, Leibovitz B. Ascorbic acid and cancer: a review. *Cancer Res*. 1979;39:663-681.
15. Niki E. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am J Clin Nutr*. 1991;54:1119s-1124s.
16. Frei B, England L, Ames B. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci U S A*. 1989;86:6377-6381.
17. van den Berg JJ, Kuypers FA, Roelofsen B, Op den Kamp JA. The cooperative action of vitamins E and C in the protection against peroxidation of parinaric acid in human erythrocyte membranes. *Chem Phys Lipids*. 1990;53:309-320.
18. Chan A. Partners in defense: vitamin E and vitamin C. *Can J Physiol Pharmacol*. 1993;71:725-731.
19. Levine M, Dhariwal K, Washko PW, et al. Ascorbic acid and in situ kinetics: a new approach to vitamin requirements. *Am J Clin Nutr*. 1991;54:1157s-1162s.
20. Packer J, Slater T, Wilson R. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature*. 1979;278:737-738.
21. Burton GW, Wronska U, Stone L. Biokinetics of dietary RRR-OC-tocopherol in the male guinea pig at three dietary levels of vitamin C does not spare vitamin E in vivo. *Lipids*. 1990;25:199-210.
22. Mirvish S. Experimental evidence for inhibition of N-nitroso compound formation as a factor in a negative correlation between vitamin C consumption and the incidence of certain cancers. *Cancer Res*. 1974;34:1948s-1951s.
23. González MJ, Mora E, Riordan NH, Riordan HD, Mojica P. Rethinking vitamin C and cancer: an update on nutritional oncology. *Cancer Prev Int*. 1998;3:215-224.
24. Yamamoto K, Takahashi M, Niki E. Role of iron and ascorbic acid in the oxidation of methyl linoleate micelles. *Chem Lett*. 1987;1:49-52.
25. Rowly DA, Halliwell B. Superoxide-dependents and ascorbate-dependent formation of hydroxy radicals in the presence of copper salts: a physiologically significant reaction? *Arch Biochem Biophys*. 1983;225:279-284.
26. Ramp WK, Thornton PA. The effects of ascorbic acid on the glycolytic and respiratory metabolism of embryonic chick tibias. *Calcif Tissue Res*. 1968;2:77-82.
27. Beetens JR, Hermen AG. Ascorbic acid and prostaglandin formation. *Int J Vitam Nutr Res*. 1983;24(suppl):131s-144s.
28. Mikino Y, Sakagami H, Takeda M. Induction of cell death by ascorbic acid derivatives in human renal carcinoma and glioblastoma cell lines. *Anticancer Res*. 1999;19:3125-3132.
29. Nakamura Y, Yamafuji K. Antitumor activities of oxidized products of ascorbic acid. *Sci Bull Fac Kyushu Univ*. 1968;23:119-125.
30. Yamafuji K, Nakamura Y, Omura H, Soeda T, Gytoku K. Antitumor potency of ascorbic, dehydroascorbic or 2, 3-diketogulonic acid and their action on deoxyribonucleic acid. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol*. 1971;76:1-7.
31. Omura H, Tomita Y, Yasuhiko N. Antitumor potentiality of some ascorbate derivatives. *J Fac Agr Kyushu Univ*. 1974;18:181-189.
32. Tomita Y, Eto M, Lio M. Antitumor potency of 3-methyl-3,4-dihydroxytetrone. *Sci Bull Fac Agr Kyushu Univ*. 1974;28:131-137.
33. Poydock ME, Reikert D, Rice J, Aleandri L. Inhibiting effect of dehydroascorbic acid on cell division in ascites tumors in mice. *Exp Cell Biol*. 1982;50:34-38.
34. Leung PY, Miyashita K, Young M, Tsao CS. Cytotoxic effect of ascorbate and its derivative on cultured malignant and non-malignant cell lines. *Anticancer Res*. 1993;13:47-80.
35. Avakawa N, Nemoto S, Suzuki E, Otsuka M. Role of hydrogen peroxide in the inhibitory effect of ascorbate on cell growth. *J Nutr Sci Vitaminol*. 1994;40:219-227.
36. Peterkofsky B, Prather W. Cytotoxicity of ascorbate and other reducing agents towards cultured fibroblasts as a result of hydrogen peroxide formation. *J Cell Physiol*. 1971;90:61-70.
37. Yve B, Niedra JT, Baum JL. Effects of ascorbic acid on cultured rabbit endothelial cells. *Invest Ophthalmol Mol Vis Sci*. 1980;19:1471-1476.
38. Jampel HD. Ascorbic acid is cytotoxic to dividing human Tenon's capsule fibroblasts. *Arch Ophthalmol*. 1990;108:1323-1325.
39. Tsao CS, Dunhan WB, Leung PY. In vivo antineoplastic activity of ascorbic acid for human mammary tumor. *In Vivo*. 1988;2:147-150.
40. Tsao CS, Dunhan WB, Leung PY. Effect of ascorbic acid and its derivatives on the growth of human mammary tumor xenografts in mice. *Cancer J*. 1989;5:53-59.
41. Poydock ME. Effect of combined ascorbic acid and B12 on survival of mice implanted with Ehrlich carcinoma and L1210 leukemia. *Am J Clin Nutr*. 1982;54:1261s-1265s.
42. Edgar JA. Dehydroascorbic acid and cell division. *Nature*. 1970;227:24-26.
43. Bram S, Froussard P, Guichard M, et al. Vitamin C preferential toxicity for malignant melanoma cells. *Nature*. 1980;284:629-631.
44. Riordan NH, Riordan HD, Meng XL, Li Y, Jackson JA. Intravenous ascorbate as a tumor cytotoxic chemotherapeutic agent. *Med Hypotheses*. 1995;44:207-213.
45. Sakagami H, Satoh K. Pro-oxidant action of two antioxidants: ascorbic acid and gallic acid. *Anticancer Res*. 1997;17:221-224.
46. Muñoz E, Blazquez MV, Ortiz C, Gómez-Díaz C, Navas P. Role of ascorbate in the activation of NF- κ B by tumour necrosis factor- α in T-cells. *Biochem J*. 1997;325:23-28.
47. Halliwell B. Vitamin C: antioxidant or pro-oxidant in vivo? *Free Radic Res*. 1996;25:439-454.
48. Alcaín FJ, Buron MI. Ascorbate on cell growth and differentiation. *J Bioenerg Biomembr*. 1996;26:393-398.
49. Asano K, Satoh K, Hosaka M, et al. Production of hydrogen peroxide in cancerous tissue by intravenous administration of sodium 5,6 benzylidene-L-ascorbate. *Anticancer Res*. 1999;19:229-236.
50. Jonas SK, Riley PA, Willson RL. Hydrogen peroxide cytotoxicity. *Biochem J*. 1989;264:651-655.
51. Clement MV, Ramalingam J, Long LH, Halliwell B. The in vitro cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves hydrogen peroxide. *Antiox Redox Signal*. 2001;3:157-163.
52. Sakagami H, Satoh K, Kochi M. Comparative study of the antitumor action between sodium 5,6 benzylidene-L-ascorbate and sodium ascorbate. *Anticancer Res*. 1997;17:4401-4452.
53. González MJ, Schemel RA, Gray JI, Dugan LJR, Sheffield LG, Welsh CW. Effect of dietary fat growth of MCF-7 and MDA-MB231 human breast carcinomas in athymic nude mice: relationship between carcinoma growth and lipid peroxidation products level. *Carcinogenesis*. 1991;12:1231-1235.
54. González MJ. Lipid peroxidation and tumor growth: an inverse relationship. *Med Hypotheses*. 1992;38:106-110.
55. González MJ, Riordan NH. The paradoxical role of lipid peroxidation on carcinogenesis and tumor growth. *Med Hypotheses*. 1996;46:503-504.

56. Gutteridge JMC, Richmond R, Halliwell B. Oxygen free-radicals and lipid peroxidation: inhibition by the protein caeruloplasmin. *FEBS Lett.* 1980;112:269-272.
57. Szent-Gyorgyi A. The living state and cancer. *Physiol Chem Phys.* 1980;12:99-110.
58. Schwarz JL. The dual roles of nutrients as antioxidants and pro-oxidants: their effects on tumor cell growth. *J Nutr.* 1996; 126:1221S-1227S.
59. Sigal A, King CG. The relationship of vitamin C to glucose tolerance in the guinea pig. *J Biol Chem.* 1936;166:489-492.
60. Landauer W, Sopher D. Succinate, glycerophosphate and ascorbate as sources of cellular energy as antiteratogens. *J Embryol Exp Morph.* 1970;24:187-202.
61. Cathcart RF. A unique function for ascorbate. *Med Hypotheses.* 1991;35:32-37.
62. Li Y, Cobb CE, Hill KE, Burk RF, May JM. Mitochondrial uptake and recycling of ascorbic acid. *Arch Biochem Biophys.* 2001;387:143-153.
63. González MJ, Schemmel RA, Dugan L Jr, Gray JI, Welsch CW. Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. *Lipids.* 1993;28:827-832.
64. Sakagami H, Satoh K, Hakeda Y, Kumegawa M. Apoptosis-inducing activity of vitamin C and vitamin K. *Cell Mol Biol.* 2000; 46:129-143.
65. Iwasaka K, Koyama N, Nogaki A, et al. Role of hydrogen peroxide in cytotoxicity induction by ascorbates and other redox compounds. *Anticancer Res.* 1998;18:4333-4337.
66. Davies KJA. The broad spectrum of responses to oxidants in proliferating cells: a new paradigm for oxidative stress. *Life Sci.* 1999;48:41-47.
67. Dasgupta A, Zdunek T. In vitro lipid peroxidation of human serum catalyzed by cupric ion: antioxidant rather than pro-oxidant role of ascorbate. *Life Sci.* 1992;50:875-882.
68. Sakagami H, Satoh K, Sugaya K, et al. Effect of the type of serum in the medium on sodium ascorbate-induced toxicity. *Anticancer Res.* 1996;16:1937-1942.
69. Sakagami H, Satoh K, Taguchi S, Takeda M. Inhibition of cytotoxic activity of ascorbate by human cancer patient sera. *Anticancer Res.* 1997;17:425-428.
70. González MJ. Fish oil, lipid peroxidation and mammary tumor growth. *J Am Coll Nutr.* 1995;14:325-335.
71. Sestili P, Brandi G, Brambilla L, Cattabeni F, Cantoni O. Hydrogen peroxide mediates the killing of U937 tumor cells elicited by pharmacologically attainable concentrations of ascorbic acid cell death prevention by extracellular catalase from cultured erythrocytes of fibroblasts. *J Pharmacol Exp Ther.* 1996; 277:1719-1725.
72. Iyanagi T, Yamazaki I, Anan KF. One electron oxidation-reduction properties of ascorbic acid. *Biochem Acta.* 1985;806:255-261.
73. Venugopal M, Jamison JM, Gilloteaux J, et al. Synergistic antitumor activity of vitamins C and K 3 on human urologic tumor cell lines. *Life Sci.* 1996;59:1389-1400.
74. Satoh K, Kadofuku T, Sakagami H. Copper but not iron, enhances apoptosis-inducing activity of antioxidants. *Anticancer Res.* 1997;17:2487-2490.
75. González MJ, Miranda-Massari JR, Mora EM, et al. Orthomolecular oncology: a mechanistic view of intravenous ascorbate's chemotherapeutic activity. *PR Health Sci J.* 2002;21: 39-41.
76. González MJ, Mora EM, Miranda-Massari JR, Matta J, Riordan HD, Riordan NH. Inhibition of human breast carcinoma cell proliferation by ascorbate and copper. *PR Health Sci J.* 2002;21:21-30.
77. Nelson SR, Pazdernik TL, Samson FE. Copper plus ascorbate inactivates lactate dehydrogenase: are oxygen radicals involved? *Proc West Pharmacol Soc.* 1992;35:37-41.
78. Powers HJ, Gibson AT, Bates CJ, Primhak RA, Beresford J. Does vitamin C intake influence the rate of tyrosine catabolism in premature babies? *Ann Nutr Metab.* 1994;38:166-173.
79. Palumbo A, Misuraca G, D'Ischia M, Prota G. Effect of metal ions on the kinetics of tyrosine oxidation catalysed by tyrosinase. *Biochem J.* 1985;288:647-651.
80. Sun Y, Oberley LW, Oberley TD, Elwell JH, Sierra-Rivera E. Lowered antioxidant enzymes in spontaneously transformed embryonic mouse liver cells in culture. *Carcinogenesis.* 1993;14:1437-1446.
81. Benade L, Howard T, Burk D. Synergistic killing of Ehrlich ascites carcinoma cells by ascorbate and 3-amino-1,2,4-triazole. *Oncology.* 1969;23:33-43.
82. Punnonen K, Ahotupa M, Asaishi K, Hyoty M, Kudo R, Punnonen R. Antioxidant enzyme activities and oxidative stress in human breast cancer. *J Cancer Res Clin Oncol.* 1994; 120: 374-377.
83. Jaruga P, Olinste R. Activity of antioxidant enzymes in cancer diseases. *Postępy Hig Med Dosw.* 1994;48:443-455.
84. Sun Y, Colburn NH, Oberley LW. Depression of catalase gene expression after immortalization and transformation of mouse liver cells. *Carcinogenesis.* 1993;14:1505-1510.
85. Bozzi A, Mavelli I, Mondovi B, Strom R, Rotilio G. Differential sensitivity of tumor cells to externally generated hydrogen peroxide: role of glutathione and related enzymes. *Cancer Biochem Biophys.* 1979;3:135-141.
86. Noto V, Taper HS, Jiang YH, Janssens J, Bonte J, De Loeker W. Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4 naphthoquinone (vitamin K 3) treatment of human tumor cell growth in vitro. *Cancer.* 1989;63:901-906.
87. Gilloteaux J, Jamison JM, Arnold D, et al. Cancer cell necrosis by autschizis: synergism of antitumor activity of vitamin C—vitamin K 3 on human bladder carcinoma T-24 cells. *Scanning.* 1998;20:564-575.
88. Gilloteaux J, Jamison JM, Ervin E, Arnold D, Summers JL. Scanning electron microscopy and transmission electron microscopy aspects of the synergistic antitumor activity of vitamin C/vitamin K 3 combinations against human T-24 bladder carcinoma: another kind of cell death. *Scanning.* 1998;20:208-209.
89. Gilloteaux J, Jamison JM, Arnold D, Taper HS, Summers JL. Ultrastructural aspects of autschizis: a new cancer cell death induced by the synergistic action of ascorbate/menadione on human bladder carcinoma cells. *Ultrastructural Pathol.* 2001;25:183-192.
90. Grad JM, Bahlis NJ, Reis I, Oshiro MM, Dalton WS, Boise LH. Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. *Blood.* 2001;98:805-813.
91. Jamison JM, Gilloteaux J, Taper HS, Calderón PB, Summers JL. Autoschizis: a novel cell death. *Biochem Pharmacol.* 2002; 63:1773-1783.
92. Riordan HD, Jackson JA, Schultz M. Case study: high-dose intravenous vitamin C in the treatment of a patient with adenocarcinoma of the kidney. *J Orthomolec Med.* 1990;5:5-7.
93. Jackson JA, Riordan HD, Hunninghake RE, Riordan NH. High dose intravenous vitamin C and long time survival of a patient with cancer of the head of the pancreas. *J Orthomolec Med.* 1995;10:87-88.
94. Riordan NH, Riordan HD, Casciari JJ. Clinical and experimental experiences with intravenous vitamin C. *J Orthomolec Med.* 2000;15:201-213.
95. Casciari JJ, Riordan NH, Schmidt TL, Meng XL, Jackson JA, Riordan HD. Cytotoxicity of ascorbate, lipoic acid and other

- antioxidants in hollow fiber in vitro tumours. *Br J Cancer*. 2001;84:1544-1550.
96. Lykkesfeldt J, Hagen TM, Vinarsky V, Ames BN. Age associated decline in ascorbic acid concentration, recycling and biosynthesis in rat hepatocytes-reversal with (R)- α -lipoic acid supplementation. *FASEB J*. 1998;12:1183-1189.
 97. Tsao CS, Miyashita K. Effects of high intake of ascorbic acid on plasma levels of amino acids. *IRCS Med.Sci.* 1984;12:1052-1053.
 98. Tsao CS, Miyashita K. Effects of large intake of ascorbic acid on the urinary excretion of amino acids and related compounds. *IRCS Med.Sci.* 1985;13:855-856.
 99. Basu TK. Possible role of vitamin C in cancer therapy. In Hanck A, Ritzel G., eds. *Vitamin C: Recent Advances and Aspects in Virus Disease, Cancer and in Lipid Metabolism*. Bern, Switzerland: Hans Huber; 1979:95-102.
 100. Bensch KG, Fleming JE, Lohman W. The role of ascorbic acid in senile cataracts. *Proc Natl Acad Sci USA*. 1985;82:7193-7196.
 101. Garland D, Zigler JS, Kinoshita J. Structural changes in bovine lens crystallins induced by ascorbate, metal and oxygen. *Arch Biochem Biophys*. 1986;251:771-776.
 102. Moss RW. *Questioning Chemotherapy*. New York, NY: Equinox Press; 1995.
 103. Wang Y, Russo TA, Kwon O, Chanok S, Rumsey SC, Levine M. Ascorbate recycling in human neutrophils: induction by bacteria. *Proc Natl Acad Sci U S A*. 1997;94:13816-13819.
 104. Spielholz C, Golde DW, Houghton AN, Nualart F, Vera JC. Increased transport of dehydroascorbic acid without changes in sodium dependent ascorbate transport in human melanoma cells. *Cancer Res*. 1997;57:2529-2537.
 105. Warburg O. On the origin of cancer cells. *Science*. 1956;123:309-314.
 106. Younes M, Lechago LV, Somoano JR, Mosharaf M, Lechago J. Nude expression of the human erythrocyte glucose transporter Glut-1 in human cancers. *Cancer Res*. 1996;56:1164-1167.
 107. Alcain FJ, Buron I, Rodríguez-Aguilera JC, Villalba JM, Navas P. Ascorbate free radical stimulates the growth of a human promyelocytic leukemia cell line. *Cancer Res*. 1990;50:5887-5891.
 108. Arnold RS, Shi J, Murad E, et al. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. *Proc Natl Acad Sci U S A*. 2001;98:5550-5555.
 109. Djuric Z, Everett CK, Luongo DA. Toxicity, single-strand breaks and 5-hydroxymethyl-2-deoxyuridine formation in human breast epithelial cells treated with hydrogen peroxide. *Free Radic Biol Med*. 1993;14:541-547.
 110. González MJ, López D, Argüelles M, Riordan NH. Antioxidants and pro-oxidants: a commentary about their discrepant role in carcinogenesis. *Age*. 1996;19:17-18.
 111. González MJ, Riordan NH, Matos MI, Argüelles M. Antioxidants and cancer: a brief discussion on controversies, contradictions and paradoxes. *J Orthomolec Med*. 1997;12:145-148.
 112. Allen RG, Venkatraj VS. Oxidants and antioxidants in development and differentiation. *J Nutr*. 1992;22:631-635.
 113. Lee JY, Chang MY, Park CH, et al. Ascorbate induced differentiation of embryonic cortical precursors into neurons and astrocytes. *J Neurosci Res*. 2003;73:156-165.
 114. Kang JH, Shi YM, Zheng RL. Effects of ascorbic acid in human hepatoma cell proliferation and redifferentiation. *Acta Pharmacol Sin*. 1999;20:1019-1024.
 115. John AP. Dysfunctional mitochondria, not oxygen insufficiency, cause cancer cells to produce inordinate amounts of lactic acid: the impact of this on the treatment of cancer. *Med Hypotheses*. 2000;57:429-431.
 116. Ingebretsen OC, Normann PT. Transport of ascorbate into guinea pig liver mitochondria. *Biochem Biophys Acta*. 1982;684:21-26.
 117. McCormick WJ. Cancer: a collagen disease, secondary to a nutritional deficiency? *Arch Pediatr*. 1959;76:166-171.
 118. Dresden MH, Heilman SA, Schmidt JD. Collagenolytic enzymes in human neoplasms. *Cancer Res*. 1972;32:993-996.
 119. Cameron E, Pauling L. Ascorbic acid and the glycosaminoglycan: an orthomolecular approach to cancer and other diseases. *Oncology*. 1973;27:181-192.
 120. Yoneda M, Shimizu S, Nishi Y, Yamagata M, Suzuki S, Kimata K. Hyaluronic acid dependent change in the extracellular matrix of mouse dermal fibroblasts that is conducive to cell proliferation. *J Cell Sci*. 1998;90:275-286.
 121. Kennedy JF. Chemical and biochemical aspects of the glycosaminoglycans and proteoglycans in health and disease. *Adv Clin Chem*. 1976;18:1-101.
 122. Ringsdorf WM JR, Cheraskin E. Vitamin C and human wound healing. *Oral Surg*. 1982;53:231-236.
 123. Lewin S. *Vitamin C: Its Molecular Biology and Medical Potential*. New York, NY: Academic Press; 1976.
 124. Goetzl EJ, Wasserman SI, Gigli I, Austen KF. Enhancement of random migration and chemotactic response of human leukocytes by ascorbic acid. *J Clin Invest*. 1974;53:813-818.
 125. Siegel BV. Enhancement of interferon production by poly (rI), poly (rC) in mouse cell cultures by ascorbic acid. *Nature*. 1975;254:531-532.
 126. Dahl H, Degre M. The effect of ascorbic acid on production of human interferon and the antiviral activity in vitro. *Acta Pathol Scand Sect B*. 1976;84:280-284.
 127. Mirvish SS, Wallcave L, Eagen M, Shubik P. Ascorbate-nitrate reaction: possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science*. 1972;177:65-68.
 128. El Attar TMA, Lin HS. Effect of vitamin C on prostaglandin synthesis by fibroblasts and squamous carcinoma cells. *Prostaglandins Leukot Essent Fatty Acids*. 1992;47:253-257.
 129. Reddy VG, Khanna D, Singh N. Vitamin C augments chemotherapeutic response of cervical carcinoma beta cells by stabilizing p53. *Biochem Biophys Res Comm*. 2001;282:409-415.
 130. Barnes LA. Safety consideration with high ascorbic acid dosage. *Ann N Y Acad Sci*. 1974;258:253-258.
 131. Carthcart RE. Vitamin C, titrating to bowel tolerance, anascorbemia and acute induced scurvy. *Med Hypotheses*. 1981;7:1359-1376.
 132. Carthcart RE. The method of determining proper doses of vitamin C for the treatment of diseases by titrating to bowel intolerance. *Australas Nurses J*. 1981;9:9-13.
 133. Rees DC, Kelsey H, Richards JDM. Acute hemolysis induced by high dose ascorbic acid in glucose-6-phosphate dehydrogenase deficiency. *Br Med J*. 1993;306:841-842.
 134. González MJ. Ascorbic acid and selenium interaction: its relevance in carcinogenesis. *J Orthomolec Med*. 1990;5:67-69.
 135. Heinz-Schmidt K, Hagmaier V, Hornig DH, Vuilleumier JP, Rutishauser G. Urinary oxalate excretion after large intakes of ascorbic acid in man. *Am J Clin Nutr*. 1981;34:305-311.
 136. Sutton JL, Basu TK, Dickerson JWT. Effect of large doses of ascorbic acid in man on some nitrogenous components of urine. *Human Nutr*. 1983;37A:136-140.
 137. Erden F, Hacisalihoglu A, Kocer Z, Simsek B, Nebioglu S. Effects of vitamin C intake on whole blood plasma, leukocyte and urine ascorbic acid and urine oxalic acid levels. *Acta Vitaminol Enzymol*. 1985;7:123-130.
 138. Tsao CS, Leung PY. Urinary ascorbic acid levels following the withdrawal of large doses of ascorbic acid in guinea pigs. *J Nutr*. 1988;118:895-900.
 139. Gerster H. No contribution of ascorbic acid to renal calcium oxalate stones. *Ann Nutr Metab*. 1997;41:269-282.
 140. Wandzilak TR, D'Andre SD, Davis PA, Williams HE. Effect of high dose vitamin C on urinary oxalate levels. *J Urol*. 1994;151:834-837.

141. Klenner FR. Observations on the dose and administration of ascorbic acid when employed beyond the range of a vitamin in human pathology. *J Appl Nutr*. 1971;23:61-88.
142. Cathcart RF. Vitamin C: the non-toxic, non-rate-limited antioxidant free radical scavenger. *Med Hypotheses*. 1985;18:61-77.
143. Campbell A, Jack T. Acute reactions to mega ascorbic acid therapy in malignant disease. *Scott Med J*. 1979;24:151-153.
144. Schwartz J, Shklar G, Trickler D. Vitamin C enhances the development of carcinoma in the hamster buccal pouch experimental model. *Oral Surg Oral Med Oral Pathol*. 1993;76:718-722.
145. Shklar G, Schwartz J, Trickler D, Cheverie SR. The effectiveness of a mixture of beta carotene, alpha tocopherol, glutathione and ascorbic acid for cancer prevention. *Nutr Cancer*. 1993;20:145-151.
146. Cohen M, Bhagavan HN. Ascorbic acid and gastrointestinal cancer. *J Am Coll Nutr*. 1995;14:565-578.
147. Park CH. Vitamin C and leukemia and preleukemia cell growth. *Prog Clin Biol Res*. 1988;259:321-330.
148. Koh WS, Lee SJ, Lee H, et al. Differential effects and transport kinetics of ascorbate derivatives in leukemic cell lines. *Anticancer Res*. 1998;18:2487-2493.
149. Prasad KN, Kumar R. Effect of individual and multiple antioxidant vitamins on growth and morphology of human non-tumorigenic and tumorigenic parotid acinar cell in cultures. *Nutr Cancer*. 1996;26:11-19.
150. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci U S A*. 1976;73:3685-3689.
151. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci U S A*. 1978;75:4538-4542.
152. Creagan ET, Moertel CG, O'Fallon JR, et al. Failure of high-dose vitamin C (ascorbic acid) to benefit patients with advanced cancer: a controlled trial. *N Engl J Med*. 1979;301:687-690.
153. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Aves MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who had no prior chemotherapy. *N Engl J Med*. 1985;312:137-141.
154. Richards E. The politics of therapeutic evaluation: the vitamin C and cancer controversy. *J Nutr Med*. 1994;4:215-246.
155. Blanchard J, Tozer TN, Rowland M. Pharmacokinetic perspective on megadoses of ascorbic acid. *Am J Clin Nutr*. 1997;66:1165-1171.
156. Agus DB, Vera JC, Golde DW. Stand allocation: a mechanism by which tumors obtain vitamin C. *Cancer Res*. 1999;59:4555-4558.
157. Langemann H, Torhorst J, Kabiersch A, Krenger W, Honegger CG. Quantitative determination of water- and lipid-soluble antioxidants in neoplastic and non-neoplastic human breast tissue. *Int J Cancer*. 1989;43:1169-1173.
158. Nagy B, Mucsi I, Molnar J, Varga A, Thurzo L. Chemosensitizing effect of vitamin C in combination with 5-FU. *In Vitro*. 2003;17:289-292.
159. Prasad KN, Cole WC, Kumar B, Prasad KC. Pros and cons of antioxidant use during radiation therapy. *Cancer Treat Rev*. 2002;28:79-91.
160. Drisko JA, Chapman J, Hunter VJ. The use of antioxidants with first line chemotherapy in two cases of ovarian cancer. *J Am Coll Nutr*. 2003;22:118-123.
161. Lamson DW, Brignall MS. Antioxidants in cancer therapy: their actions and interactions with oncologic therapies. *Alt Med Rev*. 1999;4:304-329.
162. Lamson DW, Brignall MS. Antioxidants in cancer therapy II: quick reference guide. *Altern Med Rev*. 2000;5:152-163.
163. Lamson DW, Brignall MS. Antioxidants in cancer III. *Altern Med Rev*. 2000;5:196-208.
164. Prasad KN, Kumar A, Kochupillai V, Cole WC. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. *J Am Coll Nutr*. 1999;18:13-25.
165. Moss RW. *Antioxidants Against Cancer*. New York, NY: Equinox Press; 2000.
166. Weijl NI, Hopman GD, Wipkink-Bakker A, et al. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. *Ann Oncol*. 1998;9:1331-1337.
167. Clemens MR, Muller-Ladner CI, Gey KF. Vitamins during high dose chemo and radio therapy. *Z Ernährungswiss*. 1992;31:110-120.
168. Schreurs WH, Odink J, Egger RJ, Wedel M, Brunning PF. The influence of radiotherapy and chemotherapy on the vitamin status of cancer patients. *Int J Vitam Nutr Res*. 1985;55:425-432.
169. Murata A, Murata A, Morishige F, Yamaguchi H. Prolongation of survival times of terminal cancer patients by administration of large doses of ascorbate. *Int J Vitam Nutr Suppl*. 1982;23:103-113.
170. Ringsdorf WM. Vitamin C supplementation and relief from pain. *J Ala Dent Assoc*. 1969;68:47-50.
171. Jensen NH. Reduced pain from osteoarthritis in hip joint or knee joint during treatment with calcium ascorbate. *Ugeskr Laeger*. 2003;165:2563-2566.
172. Luo G, Xie ZZ, Liu FY, Zhang GB. Effect of vitamin C on mitochondrial function and ATP content in hypoxic rats. *Zhongguo Yao Li Xue Bao*. 1998;19:351-355.
173. Riordan HD, Hunnighake RB, Riordan NR, et al. Intravenous ascorbic acid: protocol for its application and use. *PR Health Sci J*. 2003;22:287-290.