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# 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.

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## 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.<sup>1</sup> 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.<sup>2</sup> Scurvy, the deficiency disease arising from the lack of ascorbate, can reach a life-threatening level and even death.<sup>3</sup> Most mammals synthesize ascorbate from glucose; however, humans and other primates lack the enzyme

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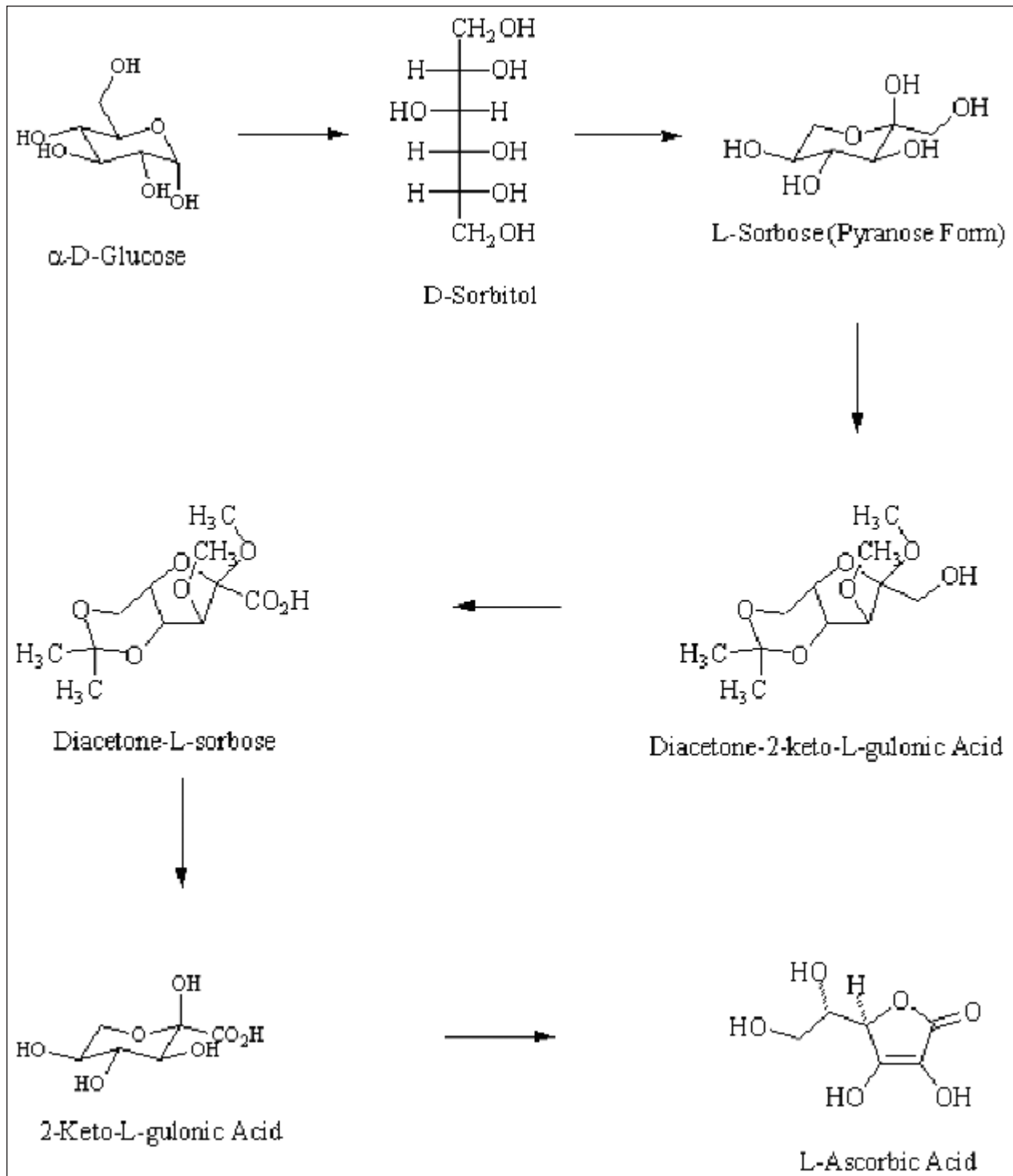


Figure 1 Glucose conversion to ascorbic acid sequence.

(L-gulonolactone oxidase) required for its synthesis.<sup>4</sup> 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.<sup>5</sup> 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.<sup>1</sup> 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,<sup>6</sup> neurotransmitter synthesis,<sup>1</sup> cytochrome P-450 activity, cholesterol metabolism, detoxification of exogenous compounds,<sup>4,7</sup> and as an antioxidant.<sup>5</sup> 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.<sup>8</sup> 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.<sup>9</sup>

### AA and Cancer

Vast literature exists on AA and cancer. As early as 1949, ascorbate use was proposed for cancer therapy.<sup>10</sup> Since 1952, ascorbate has been proposed as a chemotherapeutic agent.<sup>11</sup> 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 al<sup>12</sup> for a general review on vitamin C and Tamayo and Richardson<sup>13</sup> for a review on ascorbate and cancer). However, the first comprehensive review of this topic was published in 1979 in *Cancer Research*.<sup>14</sup> In this review, we will update (after 25 years) that seminal publication on nutritional oncology by Cameron, Pauling, and Leibovitz.<sup>14</sup> 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.<sup>15</sup> In studies with human plasma, ascorbate protected plasma lipids against detectable peroxidative damage induced by aqueous peroxy radicals.<sup>16</sup> 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.<sup>15</sup> While the occurrence of this action has been questioned in an in vivo setting,<sup>16</sup> 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.<sup>17</sup> At this point, the less reactive ascorbate radical can be enzymatically reduced back to AA by a nicotinamide adenine dinucleotide-dependent system.<sup>18-21</sup> This probably explains how ascorbate reduces nitrates and prevents the formation of carcinogenic nitrosamines.<sup>22</sup>

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

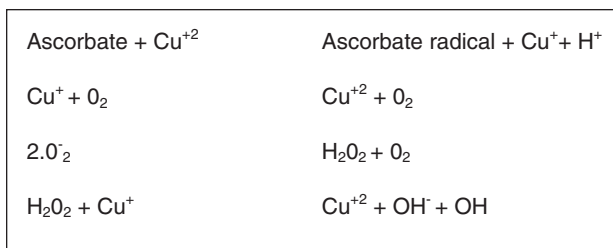


Figure 2 Ascorbate-copper interaction.

otherwise with respect to the fibroblast inhibition.<sup>34-38</sup> 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.<sup>23,39-45</sup> Ascorbate and its radical potentiate the activation of transcription factor NF-κB, which has been associated with inhibition of cell growth.<sup>46</sup>

### The Role of Hydrogen Peroxide

Ascorbate can generate hydrogen peroxide (a reactive oxygen species) on oxidation (with oxygen) in biological systems.<sup>47-49</sup> This action can be enhanced by divalent cations such as iron and copper (see Figure 2).<sup>25,39,50</sup> 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.<sup>42,50-55</sup> 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.<sup>56</sup> 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.<sup>56</sup> 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.<sup>54</sup> 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.<sup>57-61</sup> Interestingly, ascorbate has been detected within the mitochondria where it is also regenerated.<sup>62</sup>

In general, the cytotoxicity induced by ascorbate seems to be primarily mediated by hydrogen peroxide.<sup>28-31,36,51,63-66</sup> Of interest is the observation that in proliferating cells, very low levels of hydrogen peroxide (3-15 μM) stimulate cell division, whereas greater concentrations induce cell growth arrest, apoptosis, and/or necrosis.<sup>66</sup> 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.<sup>35,67-69</sup> 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.<sup>28,34,50-52,63,70-73</sup> 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.<sup>34,39-41,74-76</sup> In addition, the combination of ascorbate and copper has been shown to inactivate lactate dehydrogenase,<sup>77</sup> 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.<sup>78,79</sup> 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 peroxidase<sup>80</sup> in these cells, leading to cellular damage through the accumulation of hydrogen peroxide.<sup>44,74,81-85</sup> There is a 10- to 100-fold greater content of catalase in normal cells than in tumor cells.<sup>44,81</sup>

Furthermore, the addition of vitamin K3 (menadione) to AA produces a synergistic antitumor activity.<sup>73,86-89</sup> 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.<sup>90</sup> 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.<sup>87-91</sup> 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.<sup>92-95</sup>

### 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.<sup>34,39-42</sup> In addition, other compounds arising from the oxidation or degradation of ascorbate can inhibit tumor growth. The most effective ones are  $\gamma$ -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.<sup>34</sup> 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 fluids<sup>96-99</sup> and may deplete the bioavailability of lysine and cysteine, 2 amino acids that are required for rapidly growing tumors.<sup>100</sup> Experiments using tissue homogenate show that the interactions between ascorbate, metal ions, and oxygen are capable of inducing structural changes in protein.<sup>98-101</sup> 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.<sup>42</sup> One of us (M.J.G.) has demonstrated that secondary products of lipid peroxidation have an inhibitory action on human malignant cell proliferation.<sup>53,56,63,70</sup> There is evidence to suggest that dehydroascorbic acid may work as a mitotic inhibitor *in vivo*.<sup>92</sup> Dehydroascorbic acid may prevent cell division by inhibiting protein synthesis at the ribosomal level.<sup>92</sup> Interestingly, prolonged exposure to high concentrations of dehydroascorbic acid may cause irreparable damage resulting ultimately in complete lysis of the cells.<sup>92</sup>

### Intracellular Transport of Ascorbate and Its Tumor Specificity

Extracellular ascorbate is oxidized, transported as dehydroascorbic acid, and reduced intracellularly to ascorbate.<sup>102</sup> Actually, many cell types transport ascorbate solely in its oxidized form, through facilitated glucose transporters.<sup>103</sup> 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.<sup>103</sup> To ascorbate's advantage, tumor cells have an increased requirement for glucose.<sup>104</sup> To compensate for this increased need for glucose, tumor cells increase their quantity of glucose transporters.<sup>105</sup> 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.<sup>44,88,93,100</sup> The most likely reason for this can be a quantitative difference in the content of the enzyme catalase mentioned earlier.<sup>81</sup>

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.<sup>58,106-110</sup> This paradoxical role of antioxidants and pro-oxidants has been analyzed previously.<sup>109-111</sup> 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.<sup>112</sup> In addition to this, ascorbate has been shown to stimulate differentiation in brain cells<sup>113</sup> and redifferentiation in hepatoma cells.<sup>114</sup>

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.<sup>112</sup> A problem in electron transfer activity might well be coupled to defective mitochondria, and vitamin C may help correct this electron transfer problem.<sup>115</sup>

### ***Intravenous AA***

The concentrations of ascorbate toxic to cancer cells *in vitro* can be achieved clinically by intravenous administration.<sup>93-94</sup> 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.<sup>94</sup> 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.<sup>95</sup> Lipoic acid can mediate the reduction of dehydroascorbic acid and improves mitochondrial function.<sup>116</sup> It is conceivable that other energy intermediates such as acetyl-L-carnitine, coenzyme Q10, B-complex vitamins, vitamin K3, magnesium,  $\alpha$ -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.<sup>14</sup> The generalized stromal changes of scurvy are identical to the local stromal changes observed in the immediate vicinity of invading neoplastic cells.<sup>117</sup> 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.<sup>118</sup> There is evidence that a physiological hyaluronidase inhibitor is an oligoglycosaminoglycan that requires AA for its synthesis.<sup>119</sup> Changes in hyaluronic acid have been shown to be conducive to cell proliferation.<sup>120</sup> 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.<sup>14</sup> 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,<sup>121</sup> 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.<sup>122</sup>)

### **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.<sup>123</sup> 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.<sup>124</sup> Ascorbate has also been shown to enhance interferon production.<sup>123,125,126</sup>

AA has other identified functions related to cancer prevention. Ascorbate is required by the mixed function oxidases for the hydroxylation of amino acids.<sup>14</sup> 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.<sup>127</sup> 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.<sup>27,128</sup> 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.<sup>129</sup>

### **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 B<sub>12</sub> destruction, fatigue, and sterility.<sup>130</sup> 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.<sup>131</sup> 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.<sup>132</sup>

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.<sup>133</sup> 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.<sup>134</sup> 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 issue<sup>135-140</sup> 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.<sup>44,92-95,141,142</sup> 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.<sup>143</sup> 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.<sup>92,93</sup> 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.<sup>144-146</sup> 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.<sup>147</sup> In contrast, further studies by the same group reported that AA inhibited growth at higher concentrations.<sup>148</sup> Also, vitamin C at low doses ( $\pm 25$   $\mu\text{g}/\text{mL}$ ) without any other added antioxidants has been reported to stimulate growth of malignant cells while inhibiting their growth at high doses ( $\pm 200$   $\mu\text{g}/\text{mL}$ ).<sup>149</sup> 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.<sup>150-153</sup> 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.<sup>154</sup>

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,<sup>155</sup> oral absorption of AA cannot achieve plasma concentrations comparable to those obtained by intravenous administration.<sup>44</sup> 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.<sup>94</sup> 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,<sup>156,157</sup> 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.<sup>156</sup> 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.<sup>158</sup> 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.<sup>159</sup> 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.<sup>159</sup> 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.<sup>160</sup>

A vast body of literature exists on this topic and is summarized in a series of articles by Lamson and Brignall.<sup>161-163</sup> 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.<sup>160-163</sup> Moreover, an article by Prasad et al<sup>164</sup> shows similar positive results for the combination of antioxidants and conventional treatment. For a complete review of the topic, see the article by Moss.<sup>165</sup>

It has been demonstrated that chemotherapy as well as radiotherapy induce a fall in plasma antioxidants in cancer patients.<sup>166-168</sup> 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.<sup>164</sup> In addition, Prasad and

Kumar<sup>149</sup> 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.<sup>169</sup>

## 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 differentiation<sup>113</sup> and redifferentiation.<sup>114</sup> In addition, ascorbate has been of value in the palliation of pain<sup>170,171</sup> and as an ergogenic agent,<sup>8,172</sup> 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.<sup>173</sup> 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.

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