Rethinking Vitamin C and Cancer: An Update on Nutritional Oncology

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The effect of vitamin C on cancer, especially on solid tumor malignancies, has been a subject of great controversy. In this article we address general aspects of vitamin C and cancer, while reviewing and analyzing existing literature on the subject. In addition, we present and discuss our own hypothesis on the effect of vitamin C on cancer (solid tumors). This article is an attempt to ease the existing controversy while providing an updated scientific basis for the use of vitamin C in nutritional oncology.

Vitamin C   Solid tumors

Vitamin C Characteristics

Biochemistry

Vitamin C is an essential vitamin for humans (1). Without vitamin C the deficiency disease arising (scurvy) can be life threatening (2). Most mammals synthesize vitamin C from glucose; however, humans and other primates lack some of the enzymes required for its synthesis (3). Therefore, vitamin C needs to be obtained from dietary sources. Vitamin C (ascorbic acid, $C_6H_{12}O_6$) is a ketolactone with a molecular weight of 176.13 g/mol. A basic identified biochemical role for vitamin C is to accelerate hydroxylation reactions in a number of biosynthetic pathways. In many of these reactions, vitamin C directly or indirectly provides electrons to enzymes that require pros-

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thetic metal ions in a reduced form to achieve full enzymatic activity. The best known role of vitamin C is that of cofactor for prolyl and lysyl hydroxylase enzymes in the biosynthesis of collagen (4). Vitamin C is also required in the synthesis of carnitine from lysine (5), in neurotransmitter synthesis (4), in cytochrome P-450 activity, cholesterol metabolism, and detoxification of exogenous compounds (6,7), and as an antioxidant.

Biological Functions

Vitamin C is present as ascorbate in most biological settings (pk = 4.2) and is considered the most important antioxidant in extracellular fluid (8). Vitamin C is a water-soluble compound distributed throughout the body, with high concentrations found in a number of tissues including the eye lens and adrenal and pituitary glands (4).

Primary Cancer Preventive Mechanism of Ascorbic Acid

Antioxidant Properties

Vitamin C is considered one of the strongest reductants and radical scavengers. Vitamin C reduces unstable oxygen, nitrogen, and sulphur centered radicals; in addition, it acts as primary defense against aqueous radicals in blood (9). In studies with human plasma, ascorbate protected plasma lipids against detectable peroxidative damage induced by aqueous peroxyl radicals (10). Thus, by efficiently trapping peroxyl radicals in the aqueous phase before they can initiate lipid peroxidation, ascorbate can protect biomembranes against primary peroxidative damage. Ascorbate may also protect membranes against peroxidation by enhancing or reinstating the activity of tocopherol (vitamin E), the principal lipid-soluble antioxidant (9). Although this action has been questioned in an in vivo healthy state (10), it may be reasonable in pathological states where an enhanced oxidative stress is present. Ascorbate reacts with the tocopheroxyl radical that arises in cell membranes as a result of vitamin E's antioxidant activity and regenerates tocopherol, at the same time transferring the oxidative challenge to the aqueous phase (11,12). At this point, the less reactive ascorbate radical can be enzymatically reduced back to ascorbic acid by a NADH-dependent system (13). This is probably why vitamin C also reduces nitrates, because this reaction blocks the formation of carcinogenic nitrosamines (14).

Primary Anticancer Mechanisms of Ascorbic Acid and Hypothesis

Oxidant Properties

In massive amounts (50–150 g and especially given IV), vitamin C may have an oxidizing effect because of its reducing power. This action is enhanced by divalent cations such as copper and iron. We should mention that constituents of ascorbic acid

derivatives formed in vivo could be different from those formed in vitro, because chemicals react differently in different environments. In the presence of free transition metal catalysts, ascorbic acid oxidation can yield highly reactive breakdown products capable of initiating oxidation or free radical reactions (16,17), especially in the presence of highly unsaturated lipids. However, these breakdown products can only be formed in minute quantities in vivo (healthy organisms) because most transition metal ions are attached to binding proteins, which makes them unavailable to participate in chemical reactions (18). Nevertheless, these reactions may take place in a pathogenic state such as malignancy, in which cohesive forces that inhibit replication are reduced (19).

We are proposing this pro-oxidant activity as one of the main mechanisms by which vitamin C fights cancer. The antitumor action of ascorbic acid in cultured cells, in animals and in human tumor xenografts, has been increased with the addition of cupric ion, a catalyst for the oxidation of ascorbic acid (20,21). These results support the hypothesis that certain oxidation intermediates resulting from the interaction of dietary constituents (ascorbic acid, omega-3 fats) can act as active antineoplastic agents. Furthermore, ascorbic acid oxidation products, such as dehydroascrobic acid, 2,3-diketogulonic acid, and 5-methyl-3,4-dihydroxytetrone, a degradation product of ascorbic acid, have shown antitumor activity (22–27). In addition, other compounds formed by the oxidation or degradation of ascorbate can inhibit tumor growth. The most effective ones are gamma-cronolactone and 3-hydroxy-2-pyrone, in addition to 5-methyl-3,4-dihydroxytetrone. The available evidence suggests that these vitamin C oxidation products and/or metabolic by-products have a function controlling mitotic activity. All active compounds consist of an unsaturated lactone ring with a double bond conjugated with a carbonyl group. This might suggest that the particular structural feature of the lactone ring is responsible for the antitumor activity (21). The antitumor activity of 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. These antiproliferative mechanisms for the action of ascorbic acid and oxidation products on tumor cells are probably of a very complex nature, because they seem to involve a series of pleiotropic reactions. Large amounts of ascorbic acid intake can change the levels of certain amino acids in body fluids (28,29) and may deplete the bioavailability of lysine and cysteine, which are required for rapidly growing tumors (30). Experiments using tissue homogenates show that the interactions between ascorbate, metal ions, and oxygen are capable of inducing structural changes in animal proteins (31,32). These electron-induced or charge transfer changes need a conductor, and proteins can serve as electronic conductors for these reactions. Metal ions such as copper are good electric conductors because their valence bonds are but partially filled and there is plenty of space for interactions. The resulting molecules containing one or more uncoupled electrons are very reactive free radicals.

We should remember that cells, in order to be able to divide, need to reduce cohesiveness and dismount part of their structure; in other words, differentiate. This unstable state of cellular organization facilitates free radical damage in the malignant cell. This principle is used in chemotherapy treatment as with the drug doxorubicin

(Adriamycin). The difference is that the free radicals formed by the drug are much more potent, thus more toxic, to such extent that is more damaging to normal tissue and detrimental to the cancer patient. Doxorubicin causes cardiac toxicity, nausea, vomiting, and hair loss [for a detailed outlook on chemotherapy and cancer, see (33)].

Oxygen, the final electron acceptor, is of great importance in the ascorbate-induced inhibitory action, because oxygen produces a radical out of the ascorbic acid. Oxygen by itself has an inhibitory action on cancer cell proliferation by interfering with anaerobic respiration (fermentation), a common energy mechanism utilized by malignant cells. It would be worth investigating the status of the mitochondria of malignant cells because we believe this may be relevant to the origin of malignancy. A problem in electron transfer might well be coupled to a defective mitochondria, and vitamin C may help correct this electron transfer problem. This inhibitory action of ascorbic acid has been described since 1952 (34).

Dehydroascorbic acid and the semi-dehydroascrobic acid radical have been shown to promote lipid peroxidation (23). One of us (M.J.G.) has demonstrated that secondary products of lipid peroxidation have an inhibitory action on human malignant cell proliferation (35,36). There is evidence to suggest dehydroascorbic acid functions as a mitotic inhibitor in vivo (37). Dehydroascorbate may prevent cell division by inhibiting protein synthesis at the ribosomal level. Interestingly, prolonged high concentrations of dehydroascorbic acid may cause irreparable damage resulting ultimately in complete lysis of the cells. We should note that dehydroascorbic acid is unstable and must be produced constantly to be maintained in high concentrations. This issue is very important for clinical use of ascorbate. Dehydroascorbic acid is further metabolized to 2,3-diketogluonic acid or reduced back to ascorbic acid. It is conceivable hat ascorbate may have a preferential cytotoxicity against tumor cells and this can be associated to intracellular generation of hydrogen peroxide with no toxic effects on normal tissue (38–40). The main probable reason for this can be a quantitative difference in the content of the enzyme catalase (41). There is a 10- to 100-fold greater content of catalase in normal cells than in tumor cells (38). For this reason, the combination of mega-doses of ascorbate, oxygen, and copper ion seems logical as part of a treatment protocol against cancer, especially solid malignant tumor. Copper ions react with ascorbate and generate free radicals in solution (42) (Fig. 1). In addition, ascorbate can generate hydrogen peroxide upon oxidation by oxygen gas in biological systems (40). Also, there is evidence that copper may facilitate oxidative tissue injury through a free radical pathway analogous to the Fenton reaction in rats (in vivo) treated simultaneously with copper and ascorbic acid (43).

It is important to note that antioxidant or pro-oxidant characteristics depend on the redox potential of the individual molecule and the environment surrounding the cell (its inorganic chemistry). It is conceivable that nutrients acting as chemopreventives are capable of inhibiting the continual growth of transformed clones of cells through their pro-oxidant activity. In contrast, antioxidant activity in malignant tissue may result in enhanced growth of transformed cells. Furthermore, uncontrolled pro-oxidant activity in normal cells can generate free radicals (reactive oxygen species) that could damage cellular membranes and DNA (35,36,44). This is likely to be one of the
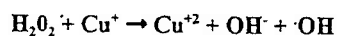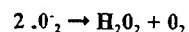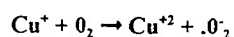
$$\text{Ascorbate} + Cu^{+2} \rightarrow \text{Ascorbate radical} + Cu^+ + H^+$$

$$Cu^+ + O_2 \rightarrow Cu^{+2} + .O_2^-$$

$$2 .O_2^- \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + Cu^+ \rightarrow Cu^{+2} + OH^- + \cdot OH$$

Figure 1. Ascorbate–copper interaction.

mechanisms by which antioxidants protect normal cells from damaging free radicals (45–47). Interestingly, during differentiation there is an increased cellular production of oxidants that appears to provide one type of physiological stimulation for changes in gene expression that lead to a terminal differentiated state (48).

Secondary Anticancer Mechanisms of Ascorbic Acid: Host Resistance to Cancer

Ascorbic Acid and Intracellular Matrix

Ascorbic acid 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.

The basic function of vitamin C is the prevention of scurvy. Scurvy results from the severe dietary lack of ascorbate. It is a syndrome of generalized tissue disintegration at all levels, involving the dissolution of intracellular ground substance, the disruption of collagen bundles, and the lysis of the interepithelial and interendothelial cements, leading to ulceration with secondary bacterial colonization and to vascular disorganization with edema and interstitial hemorrhage, in addition to generalized undifferentiated cellular proliferation, with specialized cells throughout the tissue reverting to a primitive form (49). The generalized stromal changes of scurvy are identical to the local stromal changes observed in the immediate vicinity of invading neoplastic cells (50). 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. Other important stromal factors in resistance that are enhanced by vitamin C are: 1) resistance of the intercellular ground substance to local infiltration, and 2) degree of lymphocytic response. A brisk lymphocytic response is a systemic factor indicative of enhanced host resistance and is associated with a more favorable prognosis of the disease. In order to proliferate cells must escape the restraint imposed by highly viscous intercellular glycosaminoglycans and must do this by the release of the enzyme hyaluronidase (51). There is evidence that

a physiological hyaluronidase inhibitor is an oligoglycosaminoglycan that requires ascorbic acid for its synthesis (52). Changes in hyaluronic acid have been shown to be conducive to cell proliferation (53). In addition to this, 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 (49). 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 hydorxyproline and hydroxylysine, leading to instability of the triple helix of collagen (54), which is a common feature in scurvy and also in cancer. This is also of importance in vitamin C's role on wound healing, including decubital ulcers, surgery recovery, and other accidental traumatic injuries (55).

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 (56). 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 (57). Ascorbate has also been shown to enhance interferon production (58,59).

Ascorbic acid has other identified functions related to cancer prevention. Ascorbate is required by the mixed function oxidases for the hydroxylation of amino acids (49). 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 (60). Another important anticancer function of ascorbate when provided in large quantities is that is 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. Also, it has been suggested that vitamin C may have a role inhibiting prostaglandins of the two-series in carcinoma cells (61). 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.

Conclusion

There are definitely various mechanisms by which vitamin C attacks malignant growth. We have described the ones we believe more important, scientifically logical,

and for which we have more 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 for patients suffering malignancies. Ascorbate may produce benefits in both prevention and treatment of cancer.

Acknowledgments

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