Ascorbic Acid: Biologic Functions and Relation to Cancer

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Evidence continues to accumulate that ascorbic acid (vitamin C) has numerous biologic effects, including some that may relate to the prevention and treatment of cancer. Therefore, the National Cancer Institute and the National Institute of Diabetes and Digestive and Kidney Diseases sponsored a symposium on the biologic functions of vitamin C and its possible relation to cancer. The symposium was held at the National Institutes of Health (NIH) September 10-12, 1990. The antioxidant, enzymatic, immune, and cancer-related functions of ascorbic acid were discussed.

Antioxidant Functions

Since oxidation and free radicals are associated with carcinogenesis, the free-radical scavenger and antioxidant properties of ascorbic acid were initially considered. Dr Balz Frei (Harvard School of Public Health, Cambridge, Mass) reported that ascorbic acid protects plasma lipids from oxidative damage. Of all agents tested, including protein thiols, bilirubin, uric acid, beta carotene, and α-tocopherol, ascorbic acid was the most effective. Ascorbate is the only antioxidant in plasma that completely protects lipids from oxidation.

Complementary studies, reported by Dr Etsuo Niki (Department of Reaction Chemistry, University of Tokyo, Tokyo, Japan), showed that vitamin C also prevents oxidative damage to cell membranes induced by aqueous radicals. Hydrophilic ascorbic acid, however, was not able to scavenge lipophilic radicals located within the interior of membranes. Tocopherol and ubiquinol are the primary scavengers of radicals within lipid membranes. Ascorbate causes the regeneration of tocopherol from the oxidized form. As a result, tocopherol can continue to scavenge free radicals within membranes.

Dr Earl Stadtman (NIH) reported that ascorbate can also serve as a pro-oxidant in the presence of iron in vitro. Oxygen-free radicals are involved in several pathologic processes, including aging, diabetes, and cancer. Whereas ascorbate can protect cells against oxygen toxicity, when it is mixed with Fe(III), or Cu(II) and oxygen, ascorbate can promote oxidation leading to changes in the side chains of amino acids at the metal-binding site. Histidyl residues, for example, are converted to asparaginyl or aspartyl residues. It was noted that with increasing age, there is a progressive increase in levels of oxidized proteins. Several participants, however, questioned the importance of the pro-oxidant action in vivo.

The sparing effect of urate for ascorbate in serum was discussed by Dr Alex Sevanian (University of Southern California, Los Angeles). Curiously, this sparing effect may be the result of a double mutation—one affecting ascorbate synthesis and the other affecting the destruction of uric acid. In humans, the loss of the ability to synthesize ascorbate is accompanied by a loss of uricase. As a result, primates have higher serum levels of uric acid than do many other animals, a characteristic that may offset the decreased antioxidant potential that results from lower ascorbate levels. Urate not only serves as a powerful antioxidant, but also spares ascorbic acid through inhibition of iron-catalyzed pro-oxidant reactions.

Relation to the Immune System

Dr Philip Washko (NIH) described the uptake of ascorbic acid by human neutrophils, which contain very high millimolar concentrations of vitamin C. In neutrophils, ascorbic acid accumulates against a concentration gradient by means of two distinct transport mechanisms, both of which are concentration dependent and saturable. Glucose in concentrations greater than 1 m*M* inhibits both transport mechanisms. This inhibition of ascorbic acid accumulation by glucose may have significance for patients with uncontrolled diabetes, who are often susceptible to bacterial and other infections.

Dr Millicent Goldschmidt (University of Texas, Health Science Center, Houston) showed that neutrophils from scorbutic animals have reduced bactericidal activity, and the ascorbic acid content of these neutrophils is only 6% of the level in neutrophils from normal guinea pigs. This deficiency does not affect phagocytosis but distorts leukocyte nuclear morphology and reduces chemotactic response. The ability of leukocytes to kill bacteria, whether ingested, cell associated, or extracellular, is greatly reduced. These observations were confirmed in monkeys through quantitative studies on gingival infections and plaque formation. The results may have implications for the treatment of oral infections in patients who are immunocompromised. This research also has implications for the control of infections in other sites.

Dr Betty Haskell (University of Texas, Austin) showed that complement C1q is deficient in scorbutic guinea pigs. Complement C1q, the recognition unit in the classic complement path-

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way, binds to pathogens and initiates their lysis. C1q is similar to collagen and contains helical regions with hydroxyproline residues. Animals receiving tissue-saturating doses of ascorbate had significantly higher levels of C1q.

In Vitro Effects on Tumor Growth

Investigators also discussed the role of ascorbic acid in tumor cell growth and malignant transformation. Dr Luminita Ibric (Children's Hospital, Los Angeles, Calif) reported that ascorbic acid has been shown to irreversibly suppress methylcholanthrene-induced transformation of C3H/10t1/2 cells. The effect appears to be related to the redox potential of the cells and perhaps to changes in lipid metabolism with ascorbate treatment.

The effect of ascorbate is not limited to chemically induced transformation. Dr Richard Schwarz (Lawrence Berkeley Laboratory, Berkeley, Calif) has shown that ascorbate prevents oncogenic transformation of cells infected with the Rous sarcoma virus. In his study, the ascorbate reduced virus production and promoted synthesis of proteins associated with differentiation. Dr Schwarz was not able to explain the mechanism of this inhibitory effect on Rous virus production.

The effect of ascorbate on human immunodeficiency virus (HIV) replication was described by Dr Raxit Jariwalla (Linus Pauling Institute, Palo Alto, Calif). In chronically infected T lymphocytes exposed to high but nontoxic levels of sodium ascorbate, reverse transcriptase activity was reduced by more than 99%. Levels of p24 HIV core antigen in the culture supernatant were reduced by 90%. In acutely infected cells, ascorbate reduced syncytium formation by 93%. The continuous presence of ascorbate was necessary for HIV suppression. Ascorbate did not inactivate extracellular virus. Under the same conditions, there were no detectable inhibitory effects of ascorbate on growth, metabolic activity, or rate of protein synthesis in uninfected T lymphocytes.

Additional studies with results that may have clinical applications were presented by Dr Chan Park (Texas Tech University, Lubbock). Dr Park found that leukemic colony-forming cells from patients with acute myeloid leukemia or the myelodysplastic syndrome can be divided into three groups: one stimulated, another suppressed, and the third not affected by ascorbate. Of approximately 500 cases studied, ascorbic acid enhanced colony growth in 35% and suppressed growth in 15%. These effects were not seen in normal controls or in patients with chronic myelogenous leukemia. Other redox agents, including vitamin E, were ineffective. D-Ascorbic acid was much less effective than L-ascorbic acid. These observations had prognostic value. Patients with myelodysplastic syndrome whose colonies were stimulated by ascorbate had shorter survival times than patients whose colonies were not affected.

In Vivo Effects on Tumor Growth

There was considerable interest in the session on tumor growth and vitamin C. The first speaker was Dr Linus Pauling (Linus Pauling Institute), who reviewed two large studies using animals. Skin tumors in hairless mice exposed to UV radiation were reduced in ascorbate-treated animals. By 20 weeks, five times more mice in the control group had developed large lesions than in the group given high doses of ascorbate. In RIII mice, the spontaneous appearance of mammary tumors was significantly delayed in animals treated with ascorbate. The median age at appearance of first tumor was 83 weeks in controls and 125 weeks in the group given high doses of ascorbate. The highest dose these animals received was approximately 10 g of vitamin C per kilogram of body weight.

Dr Joachim Liehr (University of Texas Medical Branch, Galveston) showed that ascorbic acid inhibits estrogen-induced renal tumors by 50% in hamsters by reducing the concentration of estrogen quinone metabolites and their DNA adducts. He also described the biochemical mechanism involved in the development of estrogen-induced cancers through nonhormonal actions. Dr Eymard Poydock (Mercyhurst College, Erie, Pa) described the inhibitory effect of ascorbate and vitamin B_{12} on implanted Ehrlich carcinoma and L1210 leukemia in mice. All control mice had died by day 19, whereas more than 50% of treated mice were still free of tumors after 60 days. In the presence of vitamin B_{12}, the cobalt nucleus is released and attaches to ascorbic acid, possibly forming cobalt ascorbate. The cobalt-vitamin C complex prevents mitoses in several transplantable murine tumors without damage to normal cells.

Dr Robert Smart (North Carolina State University, Raleigh) reported on the effect of ascorbic acid and its synthetic lipophilic derivative ascorbyl palmitate on phorbol ester-induced skin tumor promotion in CD-1 mice. Large topical doses of ascorbic acid and small doses of ascorbyl palmitate inhibited tumor promotion and ornithine decarboxylase activity. Ascorbyl palmitate also inhibited DNA synthesis. The number of tumors per mouse was reduced by 90%, and the number of mice with tumors was reduced by 86%. Dietary ascorbic acid inhibited the induction of ornithine decarboxylase but did not prevent tumor promotion or epidermal DNA synthesis.

The inhibiting effect of ascorbic acid on the growth of human mammary tumor xenografts in mice was described by Dr Constance Tsao (Linus Pauling Institute). Ascorbic acid, along with cupric sulfate, inhibited the growth of tumor fragments implanted beneath the renal capsules of immunocompetent mice. Ascorbate added to the diet had no effect, but, when incorporated into the drinking water, it was effective. A stereoisomer of ascorbic acid, D-isoascorbic acid, which has only 5% the antiscorbutic potency of L-ascorbic acid, had similar antitumor activity. It appears, therefore, that in this system, the antitumor activity of ascorbic acid is due not to its metabolism as a vitamin but to its chemical properties.

Adjuvant and Toxicity-Reducing Therapeutic Applications

Dr Paul Okunieff (Harvard Medical School, Cambridge, Mass) described the radioprotective effects of ascorbic acid on skin and bone marrow in mice with established fibrosarcomas. In nonirradiated animals, ascorbic acid did not modify or prevent tumor growth. When it was given immediately preceding radiation, there was a significant reduction in the toxic effects of radiation in both skin and bone marrow, but the tumor

was not protected, resulting in a therapeutic gain of 1.25. This protective effect did not seem to be solely a result of hypoxia secondary to the high dose of ascorbic acid. Ascorbic acid also reduced the radiosensitizing potency of misonidazole in normal tissue.

Dr Gary Meadows (Washington State University, Pullman) described the effects of ascorbic acid on transplanted B16 melanoma in mice. Ascorbate added to the drinking water of female B6D2F$_1$ mice inhibited subcutaneous B16 melanoma growth, enhanced levodopa methylester chemotherapy, and, singly or in combination, increased survival in tumor-bearing mice. The tumors were smaller and less invasive, whereas metastatic lesions tended to be encapsulated. Dehydroascorbate was not effective, increasing tumor growth and decreasing survival. Although ascorbate alone has some antitumor activity against B16 melanoma, ascorbate primarily served as an adjuvant to levodopa methylester treatment and augmented the antimetastatic activity of a tyrosine phenylalanine-restricted diet.

In studies that may have therapeutic implications, observations on hypovitaminosis C in patients treated with interleukin-2 (IL-2) and lymphokine-activated killer (LAK) cells were reported by Dr Stuart Marcus (Lederle Laboratories, Pearl River, NY). During the first phase of treatment (IL-2 alone), plasma levels of ascorbic acid dropped by more than 80%. These levels were undetectable in 12 of 15 patients after the third treatment phase (IL-2 plus LAK cells). Excessive renal excretion was not the reason for the loss. The finding seemed specific for vitamin C, as blood levels of pantothenate and vitamin E remained normal. In some patients, the cytotoxic activity of lymphocytes cultured in the presence of IL-2 was stimulated by ascorbic acid. Dr Marcus suggested that a controlled study is needed to ascertain the effects of IL-2 on ascorbate requirements.

Dr Hiroshi Kan Shimpo (Fujita Health University, Toyoake, Japan) described a significant reduction in doxorubicin-induced toxicity and a prolongation of survival in animals receiving ascorbic acid and its derivatives. Experimentally, ascorbic acid prevented the elevation of lipid peroxide levels found in the heart following administration of doxorubicin. In addition, it significantly reduced the cardiac changes seen by electron microscopy. Dr Shimpo proposed that ascorbate should be prospectively tried clinically in patients treated with doxorubicin.

Dr Morimitsu Nishikimi (Institute of Applied Biochemistry, Mitake, Japan) described the evolutionary basis for ascorbate deficiency in humans. The inability of humans to synthesize vitamin C results from a lack of gulonolactone oxidase, which catalyzes the last step of ascorbic acid biosynthesis. This enzyme deficiency, which is found in all humans, is the most prevalent genetic disorder. The human gulonolactone oxidase gene has rapidly accumulated mutations after ceasing to be active and now exists as a pseudogene within the human genome. The enzyme loss, or original mutation, occurred 70 million years ago in primates, before the divergence of the Old World and the New World monkeys.

Regulation of Enzyme Function

Dr Beverly Peterkofsky (NIH) indicated that the concept that many clinical manifestations of vitamin C deficiency result from a failure of the body to convert proline to hydroxyproline may be incorrect. New results suggest that the regulation of collagen synthesis in nonrepairing tissues in scurvy is independent of the action of ascorbate in proline hydroxylation and is similar to the effects of fasting. The in vivo defects in collagen synthesis could be transmitted to human fibroblasts and other cells by means of culturing these cells with ascorbate in sera from guinea pigs that have fasted or are scorbutic. Decreased synthesis of collagen was caused by an inhibitor in the sera whose activity was reversed by an insulinlike growth factor (IGF-I). The inhibitor appears to be an IGF-binding protein that inhibits the binding of IGF to its receptor and thus could inhibit IGF-I-dependent functions, such as collagen synthesis. Induction of these circulating binding proteins in vitamin C deficiency may be responsible for the in vivo inhibition of collagen synthesis in scurvy. During the discussion, it was mentioned that these results suggest a role for IGF-I in the excessive production of collagen that occurs in desmoplastic tumors.

Dr Mario Chojkier (University of California, San Diego) reported that ascorbic acid stimulates collagen gene expression through pro-oxidation. Lipid peroxidation and reactive aldehydes, necessary for collagen gene expression, are induced by ascorbate in the presence of a transition metal. Vitamin E inhibits gene expression presumably by preventing lipid peroxidation. Acetaldehyde, which also stimulates collagen transcription, is a major metabolite of ethanol, and it is well known that collagen is produced in the livers of alcoholics.

Another biologic function—related to dopamine hydroxylation—was described by Dr Emanuel Diliberto, Jr (Wellcome Laboratories, Research Triangle Park, NC). In addition to its role as a cofactor in catecholamine synthesis, ascorbic acid may function as a neuromodulator. In response to various secretagogues, chromaffin cells release ascorbic acid from both intravesicular and extravesicular compartments. Stress, in addition to its known effect on adrenal cortical ascorbic acid, causes depletion of adrenomedullary ascorbic acid, suggesting another role for vitamin C as an extracellular mediator.

Dr Patrick Fleming (Georgetown University, Washington, DC) discussed the role of cytochrome b561 in mediating electron transfer from cytoplasmic ascorbic acid to intravesicular semidehydroascorbic acid. The apparent transmembrane structure of this protein suggests that the heme is near the cytoplasmic side of the membrane, adjacent to a cationic ascorbate-binding site. Electron transfer through the protein may facilitate reduction of semidehydroascorbate within the catecholamine vesicle.

Dr Charles Rebouche (University of Iowa, Iowa City) described the relation of ascorbate to carnitine biosynthesis. L-Carnitine is required for the entry of long-chain fatty acids into mitochondria, where they are used for energy production. Synthesis of L-carnitine requires ascorbate as a cofactor. In scorbutic guinea pigs, carnitine concentrations are decreased, possibly accounting for the fatigue and lassitude that accompany scurvy. These observations might also be relevant to cancer cachexia.

Dr Betty Eipper (The Johns Hopkins University, Baltimore, Md) reviewed research on peptidylglycine α-amidating mono-

oxygenase, an ascorbate-dependent enzyme essential for the α-amidation of neuroendocrine peptides. At the carboxyl terminus of many of the bioactive peptides, such as gastrin, releasing hormones, bombesin, substance P, and melanocyte-stimulating hormone, is an α-amidated amino acid, which is essential for biologic activity. Dr Eipper provided evidence that ascorbate may be involved in the production of α-amidated peptides with paracrine and autocrine activity.

Nonenzymatic Mechanisms of Action

As shown by Dr Miriam Salpeter (Cornell University, Ithaca, NY) through biochemical studies and electron-microscopic autoradiography, ascorbic acid stimulates acetylcholine-receptor synthesis. In some cells, the stimulatory effect on messenger RNA correlates with an increase in the number of surface acetylcholine receptors.

Iron and ascorbate seem to go hand in hand. Dr Kenneth Bridges (Brigham and Women's Hospital, Boston, Mass) described how ascorbic acid retards ferritin degradation. Although iron is essential to cell metabolism, it can promote free-radical damage to membranes and lipids. Excess intracellular iron is stored in the ferritin molecule until needed. Breakdown of ferritin, occurring in cytoplasmic lysosomes, releases iron, which damages membranes and lipids. This breakdown is slowed by ascorbic acid, which retards the autophagic uptake of ferritin into lysosomes. Ascorbate, however, does not retard the synthesis of ferritin. As a result, the ferritin content of cells treated with ascorbate increases, leading to an increase of free iron in the cytoplasm. This free iron can then cause damage to the intracellular membranes and lipids through the formation of hydroxyl radicals. In patients with iron overload, the consumption of excessive amounts of ascorbate has produced toxic effects, rarely manifested by cardiac failure and death.

Dr Irving Shapiro (University of Pennsylvania, School of Dental Medicine, Philadelphia) described the effect of ascorbic acid on the maturation of cartilage along the epiphyseal growth plate. Ascorbate is needed for the expression and posttranslational modification of type X collagen, which is unique to cartilage. Ascorbate also stimulates alkaline phosphatase activity, which is required for mineralization. In chondrocytes, the vitamin also changes oxidative activity, leading to a marked decrease in anaerobic glycolysis, and modulates mitochondrial oxidative phosphorylation activities. The role of ascorbate in the maturation of chondrosarcomas has not been explored.

Human Requirements

Dr R. Jacob (United States Department of Agriculture) studied the effects of intake of low levels of vitamin C on human immune functions and oxidant defense by feeding a controlled diet deficient in ascorbic acid to eight healthy men. No ascorbate-related changes in cell-mediated immune functions and antioxidant enzyme activities of blood were found. The ratio of levels of oxidized glutathione and reduced glutathione in plasma increased and erythrocyte nicotinamide-adenine dinucleotide phosphate levels decreased during 60 days of ascorbate depletion. Concurrently, levels of fecal mutagens (fecapentaenes) and an oxidatively modified DNA base (8-hydroxy-2-deoxy guanosine) in sperm DNA increased in most subjects. The findings suggest that an intake of low levels of vitamin C may result in increased oxidative stress and possibly damage, even in the absence of overt scorbutic symptoms.

Dr Mark Levine (NIH) discussed the fact that dietary requirements for ascorbic acid and other vitamins are based on preventing deficiency diseases. No method for measuring optimal intake, however, has been developed. To determine optimal requirements, Dr Levine applied the principles of reaction kinetics for the first time to vitamin-dependent reactions in situ. The technique, known as in situ kinetics, has been validated by means of norepinephrine biosynthesis, a vitamin C-dependent reaction. In situ kinetics were also described as being used in the study of human neutrophils and fibroblasts. The data indicate that in situ kinetics is a new and promising approach to solving the problem of establishing optimal vitamin C requirements.

Cancer Prevention

Dr Gladys Block (NCI) summarized the current epidemiologic data on the role of ascorbic acid in cancer prevention. Of 46 reports on epidemiologic studies, 33 described significant protective effects on cancer mortality or incidence. Strong epidemiologic evidence indicates that vitamin C, or other components in fruit, is protective against cancers of the esophagus, larynx, oral cavity, and pancreas. Evidence also exists for a protective effect against cancers of the stomach, rectum, lung, breast, and uterine cervix. Factors other than vitamin C may confer additional protection.

Conclusion

The conference conveyed clearly that vitamin C has multiple complex effects on a variety of biologic activities, perhaps more widespread than those of any other nutrient. Some of these effects are the result of the interaction of vitamin C and enzymes, whereas others are independent of enzymatic function. Many of these biologic effects appear to be related to the chemical properties of vitamin C and not to its role as a vitamin. What seems to be needed is a unifying principle, or hypothesis, that can explain the seemingly unrelated effects of vitamin C. The studies presented in this conference should be systematically confirmed in other laboratories. Any role for ascorbate in the prevention and treatment of cancer will be established only through additional scientific studies and increased knowledge of its biologic actions.