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Effects of Vitamin C on health: a review of evidence

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1. ABSTRACT

Vitamin C is an essential dietary nutrient for the biosynthesis of collagen and a co-factor in the biosynthesis of catecholamines, L-carnitine, cholesterol, amino acids, and some peptide hormones. The lack of vitamin C causes scurvy, a pathological condition leading to blood vessel fragility and connective tissue damage due to failure in producing collagen, and, finally, to death as result of a general collapse. Vitamin C is potentially involved also in cancer and cardiovascular diseases prevention. In addition, vitamin C effects on nervous system and chronically ill patients have been also documented. This review attempts to summarize recent and well established advances in vitamin C research and its clinical implications. Since vitamin C has the potential to counteract inflammation and subsequent oxidative damage that play a major role in the initiation and progression of several chronic and acute diseases, it represents a practical tool to administer for the early prevention of these pathologic conditions.

2. INTRODUCTION

Vitamin C, or ascorbic acid, is an essential dietary nutrient for a variety of biological functions. Under physiological conditions, it is fundamental in the biosynthesis of collagen through facilitating the hydroxylation of proline and lysine residues, thus allowing proper intracellular folding of pro-collagen for export and deposition as mature collagen (1)**.** Vitamin C serves in humans also as a co-factor in several important hydroxylation reactions, such as the biosynthesis of catecholamines (through the conversion of dopamine to norepinephrine), L-carnitine, cholesterol, amino acids, and some peptide hormones (2)**.**

The growing understanding of mechanisms of vitamin C on human health led to calls for continuous updated reappraisals regarding the dietary requirements for this nutrient. Given the potential involvement of vitamin C in cancer and cardiovascular diseases (CVD), as well as its

effects on nervous system and chronically ill patients, the aim of this review is to address the potential effects of vitamin C at both experimental and clinical stages focusing on recent evidences supporting a potential role for vitamin C in degenerative diseases prevention.

3. VITAMIN C IN HUMANS: ADSORPTION, DEFICIENCY, EXCESS

Though most animals are able to endogenously synthesize large quantities of vitamin C, humans do not have the capability to synthesize vitamin C due to a series of mutations of the gene encoding gulonolactone oxidase which catalyses the last enzymatic step in ascorbate synthesis (3, 4). However, the requirement for vitamin C is satisfied by natural sources and vitamin C supplements existing in the ordinary diet. The lack of vitamin C causes scurvy, a pathological condition leading to blood vessel fragility, connective tissue damage, fatigue, and, finally, death. In addition to poor dietary intake of vitamin C, alcoholism (5), elderly age, socioeconomic deprivation (6), mental illness (7), malabsorption disorders, kidney failure, hemodialysis (8), and peritoneal dialysis (9) have been identified as risk factors for low vitamin C endogenous levels and developing clinical symptoms of scurvy (10-12). Intake of 10 mg per day of vitamin C is appropriate to prevent scurvy. This amount results in plasma concentrations of vitamin below 10 μM, already higher than that necessary to prevent scurvy (13). However, the current recommended dietary allowance (RDA) for vitamin C for adult men and women, is set at 75 mg/day for women and 90 mg/day for men (14).

The adsorption of vitamin C from the dietary sources depends on the facilitated diffusion and a saturable-substrate transport mechanism involving the ascorbate-specific transporters, which saturation and low expression (induced by substrate downregulation) control the effective serum vitamin C concentration. The facilitated diffusion is mediated by the facilitative glucose transporters (GLUT) whereas the active transport depends on the sodium vitamin C transporters (SVCT). The gradient-driven transport mediates the absorption of oxidized form of vitamin C, dehydroascorbic acid (DHA), in an energy-independent manner especially in osteoblast (15), muscle (16), and retinal cells (17), where the GLUT transporters are predominantly expressed. DHA and glucose share the same GLUT transporters leading to a competitive inhibition particularly secondary to pathologies that alter serum glucose levels and attenuate the bioavailability of vitamin C, for instance under hyperglycemic conditions caused by diabetes (18-20).

SVCT transporters, present in humans in 2 isoforms (SVCT1 and SVCT2), actively transfer ascorbate directly into the cell. SVCT1 is subject to substrate feedback inhibition by ascorbate and its expression is attenuated by high concentrations of vitamin C *in vitro* (21) and by oral ingestion (22). SVCT2 is sensitive to the changes in intracellular ascorbate levels (23), which may play a regulatory role in maintaining ascorbate homeostasis inside the cell (22). Furthermore, age-related decline in SVCT1 expression in rat liver cells has been observed (24), explaining why elderly individuals require higher levels of vitamin C (25). On the contrary, unlike SVCT1, SVCT2 levels were not observed to decline with age, perhaps as a result of low abundance of this transporter in the liver (24).

Generally, high doses of vitamin C can be toxic (26). Excess ascorbate is normally excreted harmlessly in the urine, but the excess of formation of oxalate can accumulate in various organs in patients with renal failure or renal insufficiency (such as kidney transplanted patients) and in patients undergoing dialysis (27, 28). Administration of high doses of vitamin C is contraindicated for patients with oxalate kidney stones or hyperoxaluria (due to the incapacity of eliminating oxalate) and in patients with a deficiency in glucose-6-phosphate dehydrogenase (due to the occurring of intravascular haemolysis) (26, 29).

4. MECHANISM OF ACTIONS OF VITAMIN C

4.1. Collagen synthesis

Vitamin C is required for collagen synthesis by acting as a cofactor for non-heme iron α-ketoglutarate-dependent dioxygenases such as prolyl 4-hydroxylase. Vitamin C stimulates all types of collagen synthesis by donating electrons required for hydroxylation of proline and lysine in procollagen by specific hydroxylase enzymes (30). In the catalytic cycle, the co-substrate, α-ketoglutarate, undergoes oxidative decarboxylation to form succinate and a highly reactive iron-oxo (Fe+4) species. In the absence of a substrate molecule, the enzyme becomes uncoupled and then ascorbate reduces oxo-iron back to Fe+2, restoring the enzyme's activity. Coordination of ascorbate with enzyme-bound iron would provide the necessary electrons in uncoupled reaction cycles to reactivate the enzyme, consistent with the observation that the role of ascorbate is to keep the non-heme iron in the catalytically active, reduced state (31). Collagen synthesis is required for maintaining normal vascular function but also for tumor angiogenesis (32, 33).

4.2. Regulation of hypoxia-inducible factor 1α

Ascorbate has been shown to assist prolyl and lysyl hydroxylases in the hydroxylation of hypoxia-inducible factor 1α (HIF-1α), a transcription factor responsible for the cellular response to low oxygen conditions through activation of genes controlling several cellular transduction pathways by regulating growth and apoptosis, cell migration, energy metabolism, angiogenesis, vasomotor regulation, extracellular matrix and barrier functions, and transport of metal ions and glucose (34, 35). Under normoxic conditions, the HIF-1α subunit is targeted for degradation by HIF-specific prolyl hydroxylases. Under hypoxic conditions, such as those existing in fast growing tumors, HIF-1α hydroxylation is repressed with the result that HIF-dependent gene transcription increases, thus promoting angiogenesis and tumor growth. Because HIF-1α prolyl hydroxylase is stimulated by ascorbic acid, low vitamin C levels would reduce HIF-1α hydroxylation and thus stabilize HIF-1α, thereby promoting HIF-dependent gene transcription and tumor growth (36).

4.3. Antioxidant action

In all of its known functions, vitamin C functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body (37). At physiological concentrations, vitamin C is a potent free radical scavenger in the plasma, protecting cells against oxidative damage caused by ROS (38-41). The antioxidant property of ascorbic acid is attributed to its ability to reduce potentially damaging ROS, forming, instead, resonance-stabilized and relatively stable ascorbate free radical (AFR) serving as a one-electron donor (42). The AFR is reduced back to ascorbate within cells by NADH- and NADPH-dependent reductases that have a high affinity for the low concentrations of the radical generated (43, 44). If the AFR significantly accumulates in areas not accessible to these enzymes, or if its concentration exceeds their capacity, two molecules of the AFR reactor dismutate to form one molecule each of ascorbate and DHA (45).

This mechanism might explain a number of cytoprotective functions of vitamin C, including prevention of DNA mutation induced by oxidation (46-49), protection of lipids against peroxidative damage (50, 51), and repair of oxidized amino acid residues to maintain protein integrity (50, 52, 53). Since oxidative stress is involved in the pathogenesis of many morbid conditions, vitamin C (frequently administered in combination with other antioxidants) have been often used to prevent or treat several diseases due to its antioxidant properties (26, 54) .

4.4. Pro-oxidant action

Vitamin C, under certain conditions such as low concentrations and/or in the presence of free transition metals such as copper and iron, may function as a pro-oxidant (55). Metal ions are indeed reduced by ascorbate and, in turn, may react with hydrogen peroxide leading to the formation of highly reactive and damaging hydroxyl radicals (56). The pro-oxidant activity of vitamin C leads to the formation of ROS (57) or glycated proteins (58). On the other hand, *in vitro* model suggested that certain pro-oxidant effects of ascorbate such as the capacity to promote protein thiol oxidation in rat liver microsomes (59) can also be advantageous.

We next discuss the effects of vitamin C in preventing or treating chronic and acute pathologic conditions due to all its properties listed above.

5. ANTI-CARCINOGENIC EFFECTS OF VITAMIN C

Since the second half of ‘90s, a growing body of literature aimed at demonstrating that vitamin C may reduce the incidence of most malignancies in humans (60). Indeed, high-dose of intravenous vitamin C has been found to increase the average survival of advanced cancer patients and for a small group of responders, survival was increased to up to 20 times longer than that of controls (61-63). Other researchers reported benefits consisting of increased survival, improved well-being and reduced pain (64, 65). The anti-inflammatory action of ascorbic acid in cellular ambient is evident in a number of cytoprotective functions under physiological conditions, including prevention of DNA mutation induced by oxidation (39-41, 46-49). Since DNA mutation is likely a major contributor to the age-related development of cancer, attenuation of oxidation-induced mutations by vitamin C may be considered as a potential anti-cancer mechanism (66). Plasma vitamin C at normal to high physiological concentrations (60–100 μmol/L) neutralizes potentially mutagenic ROS thus decreasing oxidative stress-induced DNA damage (46-49). Moreover, *in vivo* studies confirmed that consumption of vitamin C-rich foods is inversely related to the level of oxidative DNA damage (67-70).

Vitamin C may also function as cancer cells killer due to its pro-oxidant capacity (56). The tumor cell-killing action is dependent upon ascorbate incubation time and extracellular ascorbate concentration (71). The effective concentration of vitamin C required to mediate cancer killing can be easier achieved by intravenous injection than by *per os* ingestion (71, 72). Regarding the modality of cytotoxicity to cancer cells, it remains an unsolved issue. Among the possible mechanisms, stimulatory effects on apoptotic pathways (73-75), accelerated pro-oxidant damage that cannot be repaired by tumor cells, and increased oxidation of ascorbate to the unstable metabolite DHA, which in turn can be toxic, have been hypothesized. The killing of cancer cells is dependent on extracellular H2O2 formation with the ascorbate radical as an intermediate. The H2O2 formed from pharmacological ascorbate concentrations diffuses into cells (76) and tumor cells are killed by exposure to H2O2 in less than minutes (77-81). The H2O2 within the cells may cause breaks in DNA and mitochondria and the mitochondria in some cancer cells may have increased sensitivity to H2O2 (79, 81-83).

Among other mechanisms of anti-cancer action of vitamin C, it has been earlier hypothesized a possible role of ascorbic acid in increasing collagen synthesis (84) and inhibiting hyaluronidase (85). These mechanisms are supposed to prevent cancer spread by increasing extracellular matrix, thus walling in tumors (86-88).

In contrast with these results, other studies have reported no effects after using vitamine C as a therapeutic drug (89, 90). Another randomized, placebo-controlled clinical study in which a high dose of vitamin C was given orally to advanced cancer patients led to inconsistent results, ultimately casting doubt over the effectiveness of vitamin C in treating cancer (90). Due to the controversy of results on the vitamin C-cancer correlation and lack of validated mechanistic basis for its therapeutic action, further research is needed to determine the feasibility of using vitamin C in clinical treatment or prevention of cancer.

6. VITAMIN C AND CARDIOVASCULAR DISEASES

Reactive oxygen species (ROS) are highly reactive molecules that derive mainly from the mitochondrial electron transport chain and that are

necessary for sever normal cellular functions, ranging from their role as signaling molecules to the more unexpected role in inducing certain cancers. However most studies have linked the excessive generation of ROS, so-called oxidative stress, to disease states, such as cancer, insulin resistance, diabetes mellitus, cardiovascular diseases, atherosclerosis, and aging (39-40, 91-94) and superoxide is the most biologically relevant radical in vasculature, as it is naturally produced by most vascular cells (95). Vitamin C provides collagen synthesis, hence allowing proper folding into the triple helical collagen molecule that is then secreted to form the extracellular matrix, or to form part of the basement membrane with regard to type IV collagen (33). By contrast, lack of ascorbate results in friable vessels and especially capillaries that are more prone to rupture, creating the typical petechial hemorrhages and ecchymoses observed in scurvy and in the cerebral cortex of SVCT2 knockout mice (96).

Vitamin C has been found to prevent apoptosis by blocking the activity of inflammatory cytokines and oxidized LDL both in cultured endothelial cells (97-99) and patients with congestive heart failure in which treatment with vitamin C decreased release of microparticles derived from endothelial cells (98).

Results of a randomized, double-blind, placebo-controlled study conducted on subjects with documented coronary artery disease have shown that long term oral ascorbate supplements do have persistent effects on endothelial-dependent flow-mediated brachial artery dilation (100). A possible mechanism of action has been thought to depend on the effect of vitamin C on nitric oxide (NO) synthase. Indeed, vitamin C enhances the NO synthase activity by maintaining tetrahydrobiopterin, an essential co-factor for the enzyme, in its reduced and active form (101-103), normally inhibited by ROS that oxidize and thus deplete the co-factor. By increasing NO production, vitamin C may indirectly protect the vascular endothelium due to its actions, namely smooth muscle cell relaxation, downstream vasodilatation, and inhibition the effects of pro-inflammatory cytokines and adhesion molecules important in atherosclerosis (104-107). Moreover, due to its antioxidant properties, vitamin C directly reduces nitrite by releasing NO from nitrosothiols, and scavenges superoxide, although relatively high ascorbate concentrations (>100 μM) are required to prevent the reaction of superoxide with NO (108).

The role of ascorbate in preventing uncontrolled vascular smooth muscle cells (VSMC) proliferation and dedifferentiation after acute arterial injury have been investigated in studies of coronary restenosis in pigs (109, 110) and in humans after angioplasty showing larger luminal diameters in subjects receiving oral vitamin C supplements compared to matched controls who did not receive ascorbate (111). The mechanism of action is still unclear, since vitamin C has been shown to paradoxically provide collagen synthesis, necessary for VSMC migration and proliferation (112, 113) and to prevent VSMC dedifferentiation (114, 115). A possible explanation of the protective role of vitamin C may depend on its role on protecting VSMCs (116) and mature human macrophages (117) from apoptosis and necrosis due to injury by oxidized LDL (118). Oxidative modification of LDL by ROS, such as superoxide and hydroxyl radicals, also initiates a sequence of atherogenic events in the sub-endothelial space. Physiological concentrations of ascorbic acid *in vitro* attenuate oxidative modification of LDL induced by transition metals (119, 120), homocysteine (121), and myeloperoxidase-derived HOCl (122, 123), as well as those naturally produced by human vascular endothelial cells (124). The mechanisms responsible for these actions include the ascorbate capacity of quenching aqueous ROS and reactive nitrogen species (RNS), decreasing their bioavailability in the plasma, and of reducing the affinity of LDL-bound apolipoprotein B protein for transition metal ions, enhancing the resistance of LDL to metal ion-dependent oxidation (125).

Macrophages take up modified LDL to become the foam cells and also mediate the inflammatory response that accompanies atherosclerosis (126). In recent studies performed on mouse peritoneal macrophages it has been found that ascorbate loading to intracellular concentrations of 3-10 mM prevented oxidative stress induced by latex beads (127) and stimulated several functions such as adherence, chemotaxis, phagocytosis, and superoxide production (128). Results regarding such effects of vitamin C have not been uniformly observed and controversy is ongoing between studies assessing that ascorbate inhibits macrophage function by decreasing uptake and degradation of oxidized human LDL (129-131) and others in which such effect has not been observed (81, 132), maybe due to different *in vitro* conditions (133, 134).

Regarding the hypothesis that ascorbate is required for synthesis of the collagenous framework of atherosclerotic plaques, a study performed on apolipoprotein E (ApoE) knockout mice revealed no effect of ascorbate diet on either plaque size or lipid content. However, plaque collagen content was found to be decreased in animals on marginal ascorbate diet, thus demonstrating that it plays a role on stability of atherosclerotic plaques becoming capable of rupture with associated thrombosis and infarction (135). These findings, in light of the several benefits of ascorbate on endothelial cell proliferation, function, and viability, make it plausible that increased plasma and cell ascorbate concentration might have a preventive effect on potential endothelial dysfunction.

Recently, several studies observed a decrease in plasma vitamin C levels in both type I and type II diabetes, and the effects of vitamin C administered in different ways, in addition to various combinations of different anti-diabetic drugs and other antioxidants, have been assessed (136-142). However, at present, no comprehensive agreement regarding its therapeutic effectiveness for these conditions has been reached.

7. THE ROLE OF VITAMIN C IN CRITICALLY ILL PATIENTS

Vitamin C concentrations in plasma and leukocytes have been reported to be commonly subnormal

in critically ill patients (143), inversely correlating with multiple organs failure (144) and directly with survival (145). Since sepsis is associated with increased production of ROS and peroxynitrite that deplete antioxidant molecules and oxidize proteins and lipids, potential therapeutic implication of vitamin C in the treatment of various infections has been studied for a long time. Indeed, enteral administration of vitamin C and other antioxidants in patients with sepsis has been shown to affect faster recovery (146) whereas parenteral administration decreased morbidity and mortality (147-149). *In vitro* and in animal experimental sepsis vitamin C prevented hypotension and edema in LPS-injected animals (150-152) and improved capillary blood flow, arteriolar responsiveness, arterial blood pressure, liver function, and survival (153-158). A possible mechanism of such effects may depend on the role of ascorbate in both inhibiting apoptosis in endothelial cells and stimulating their proliferation, thus preventing the loss of barrier function in sepsis condition (97-99, 159). Moreover, vitamin C improves arteriolar responsiveness to vasoconstrictors (norepinephrine, angiotensin, vasopressin) (160, 161) and prevents inhibition of endothelium-dependent vasodilation responses to acetylcholine (162, 163) in human subjects who have inflammatory disease or have been injected with LPS, thus preventing hypotension in sepsis and, consequently, edema**.** Another action of ascorbate on endothelial permeability may involve its scavenging action on superoxide and inhibition of nitric oxide and peroxynitrite formation, as well as its property of reducing the oxidation products formed by reaction of peroxynitrite with cell proteins (164). These actions of ascorbate may account for its effectiveness in preventing edema in critically ill patients and experimental models**.**

8. VITAMIN C EFFECTS ON NERVOUS SYSTEM

Several effects produced by ascorbate have been explored on nervous system (165). Vitamin C can in fact efflux from various types of cells (166, 167), including neurons (168), because of its hydrophilic nature and negative charge at physiologic pH. Vitamin C appears to be allowed to enter into several brain cell lines, improving neurotransmission (169) and leading to a number of effects on behaviors such as learning, memory and locomotion. Experimental animal models have been shown that intraperitoneal administration of ascorbate reversed memory deficits in mice (170, 171) whereas oral administration, in conjunction with vitamin E, improved performance on a passive avoidance task in 15 months mice but not in 3-month old mice or when ascorbate was administered alone (172). In addiction, ascorbate treatments either intraperitoneally for 14 days or orally for 30 days improved both acquisition and retention in this passive avoidance task (173), contrasting an earlier study in which five days of acute pre-test ascorbate dosing led to poorer performance (174).

Oral intake of vitamin C has been shown to reduce the fear response in Japanese quail chicks tested in a less stressful light-dark emergence paradigm (175). Moreover, long-term low levels of dietary ascorbate did not lead to impairments in learning and memory or anxiety in knockout mice unable to synthesize their own vitamin C (176). However, due to lack of agreement between results within these experiments and lack of correlation between different dosing regimens used and a clear pattern of results, it's hard to identify the exact mechanism through which vitamin C influence memory, although it appears reasonable to consider it a mediator especially of stress-related learning.

Regarding neurodegenerative diseases, a positive relationships has been shown between ascorbate supplement use and reduced incidence of Alzheimer's disease (177, 178) that is known to be caused by a combination of genetic and lifestyle factors and in part by oxidative stress (179), although these beneficial results are not universal (180, 181). Orally administered ascorbate protected the CA1 area of the hippocampus in rats against oxidative stress and cytokine release induced by injection of fibrillar β-amyloid (182). It also protected SH-SY5Y neuroblastoma cells from β-amyloid induced apoptosis (183).

Finally, it has been observed that intake of ascorbate as a pharmacological agent may be of benefit in protecting against Parkinson's disease improving the bioavailability of levodopa (184) although population studies revealed no effects of ascorbate intake in preventing the development of the disease (185).

9. VITAMIN C IN OCULAR DISEASES

The role of vitamin C in preventing ocular diseases has been evaluated, demonstrating that the development of cataract is influenced by ascorbate (186) and that a combination of ascorbate with other antioxidant vitamins and minerals slows down the progression of advanced age-related macular degeneration and loss of visual acuity in people with signs of this disease (187, 188). The effectiveness of vitamin C as a treatment of diabetic retinopathy has also been examined, but further studies are required to prove that it has a significant impact on its progress (189, 190).

10. CONCLUSIONS

This review attempts to summarize recent and well established advances in vitamin C research and its clinical implications. Since vitamin C has the potential to counteract inflammation and subsequent oxidative damage that play a major role in the initiation and progression of several chronic and acute diseases, it represents a practical tool to administer in humans for the early prevention of such pathologic conditions. However, many of such well-known beneficial effects of vitamin C intake are still only understood at the phenomenological level and further research is needed to explore the precise effects of ascorbate in physiological systems and in the pathology of diseases at the molecular level. A better understanding of the mechanisms of its action is of major importance in order to define novel potential therapeutic implications regarding vitamin C.

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