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The Multifaceted Biological Roles of Vitamin C

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Abstract

Scurvy due to severe vitamin C (ascorbic acid) deficiency among sailors was one of the earliest documented occupational diseases. This review first discussed some reduction potential-based biochemical activities of vitamin C such as assisting the post-translational collagen modification as well as acting as an antioxidant and pro-oxidant. These known activities of vitamin C were then used to address recent outcomes of vitamin C clinical trials on the prevention and treatment of non-scurvy diseases such as cardiovascular diseases and cancer. The pro-oxidant activity may contribute to the adverse effect of high-level vitamin C supplementation in clinical trials.

Keywords: Vitamin C; Collagen; Cardiovascular disease; Cancer; Cataract

Abbreviations: Gulo-/-: Gunololactone oxidase gene completely inactivated; cannot synthesize vitamin C; RDA: Recommended Dietary Allowance; SVCT: Sodium-dependent vitamin C transporter; SVCT2-/-: SVCT2 gene completely inactivated; cannot undergo inter-tissue distribution of vitamin C; UL: Tolerable Upper Intake Level

Introduction

Vitamin C (ascorbic acid) deficiency is known to cause scurvy in humans [1]. Guinea pigs need dietary vitamin C as well and thus historically serve as the only mammalian model for vitamin C studies. Most other species including commonly used animal models, rats and mice, fulfill their need of ascorbic acid by liver synthesis. In the absence of significant intake, mice maintain plasma levels of ascorbic acid at levels comparable to or higher than the maximal levels observed in healthy humans [2-5]. Furthermore, when mice face excessive vitamin C loss, a compensatory change in the endogenous synthesis of ascorbic acid has been reported [6]. These fundamental differences between species make the extrapolation of results from vitamin C studies on rats and mice to humans challenging.

In recent years, natural mutations and genetic engineering have created experimental models that facilitated the understanding of vitamin C. Of these ascorbic acid-producing species, a disruption in the endogenous synthesis (Gulo-/-) led to the vitamin C requirement. Similar to humans, symptoms of scurvy developed in Gulo-/- animals at low ascorbic acid intake [7-10]. Disrupting the gene encoding sodium-dependent vitamin C transporter 2 (SVCT2) (SVCT2-/-) prevented the inter-tissue distribution of ascorbic acid, leading to a near complete absence of ascorbic acid in the extrahepatic tissues and to perinatal lethality [11].

After much intensive studying of vitamin C since its isolation in 1928, it has become apparent that all its known biological activities originate from its chemical property as a reducing agent [12]. Biological activities of vitamin C relevant to the outcomes of recent clinical trials are first discussed. Because reducing activity is not a property unique to vitamin C, many vitamin C-dependent reactions are not vitamin C-specific although some are apparently more specific than others.

The second part of the review is on the physiological relevance of vitamin C in disease prevention and treatment as revealed in recent clinical trials. The reducing activity of vitamin C, while essential for its physiological function, has *in vivo* significance only under selected conditions. Many seemingly contradictory findings on vitamin C have been demonstrated in the cell-free or cell culture experiment. These reactions may have little biologically relevance partly because of the poor bioavailability of vitamin C at high doses. The absorption and retention of vitamin C is limited by the transporter SVCT1 in the intestine and kidney, respectively [13]. As a result, the plasma concentration of vitamin C does not rise beyond 80 μM when daily intake increases over 20 folds above the required amount of ~100 mg to 2.5 g per day [4,5]. The Recommended Dietary Allowance (RDA) is 90 mg for adult male and 75 mg for adult female. Even an intravenous injection of 0.5 g vitamin C/kg body weight (i.e., 35 g for a 154 lb individual) only transiently raised the plasma concentration to 8 mM [14].

Biochemical and Chemical Activities of Vitamin C

Post-translational modification of collagen by enzymatic reactions requiring vitamin C

Vitamin C participates in a spectrum of transition metal-catalyzed enzymatic reactions as described in most advanced nutrition textbooks. The universal property of vitamin C in these reactions is to reduce the oxidized iron (Fe^{+3}) or copper (Cu^{+2}) back to their reactive catalytic forms, Fe^{+2} or Cu^{+1}. Some roles of vitamin C as the transition metal-reducing coenzyme may not be exclusive. For example, one function of vitamin C is to assist the synthesis of catecholamines, norepinephrine and epinephrine, by the Cu^{+1}-dependent dopamine mono-oxygenase reaction. Although the adrenal gland of SVCT2-/- mice had no detectable vitamin C [11], catecholamines levels were decreased by only 50 and 20%, respectively, in these mice [15].

Several lines of evidence do support an indispensable role for vitamin C in maintaining good-quality collagen. Vitamin C assists the post-translational modification of collagen by reducing iron in the participating enzymes, lysyl hydroxylase and prolyl hydroxylase [16]. Modified/mature collagen undergoes important protein-protein

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interaction yielding a unique structure that is essential for its functions [17,18]. Genetic knockouts of lysyl hydroxylase or prolyl hydroxylase both led to embryonic death [17,19]. Although vitamin C-independent proline hydroxylation of collagen has been reported [20], complete collagen maturation cannot be achieved in vitamin C deficiency. Km is the substrate concentration that yields the half-maximal velocity in an enzyme-mediated reaction. While prolyl hydroxylase has a lower affinity for vitamin C with Km of 100 μM, lysyl hydroxylase has a Km of 5 μM, which is within the physiological range expected for the prevention of scurvy [21].

Many symptoms of scurvy can be explained by having poor-quality collagen. The evolutionarily conserved and wide-ranged importance of collagen family has been reviewed [18]. Collagen is the major protein from fibroblasts, osteoblasts and chondrocytes [18]. A key symptoms of scurvy is microvascular bleeding. In the absence of vitamin C in most tissues, SVCT2-/- mice died perinatally from intraparenchymal brain hemorrhage [11,22], a feature also found in mice and human with collagen, Col4a1, mutation [23-36]. In addition, aortic wall damage due to structural defects was observed in Gulo-/- mice fed a low vitamin C diet [27]. Because collage is the main protein in the bone [28], skeletal defects were also found in scurvy as expected [29] and in vitamin C-dependent animal models with low vitamin C intake [7,8,10,30]. Furthermore, high vitamin C intake has been found to be associated with lower bone loss in older adults [31,32]. Skin was shown to be the most sensitive tissue to vitamin C deficiency in an animal study [33], which may relate to the function of collagen-producing fibroblasts. Hyperkeratosis and poor wound healing are also seen in scurvy. Interestingly, higher vitamin C intake has been linked to a better appearance of aging skin based on the data from NHANES [34].

Vitamin C as an antioxidant

Although vitamin C is a popular antioxidant and its ability to directly neutralize free radicals can be predicted from the reduction potential [12], the only well-established antioxidant role of vitamin C is its reduction of oxidized vitamin E. Fat-soluble vitamin E helps to maintain cell membrane integrity by scavenging free radicals and thus breaking the oxidation chain reaction of unsaturated fatty acids in the phospholipid bilayer [35]. Vitamin E deficiency, although rare, manifested as hemolytic anemia caused by damaged red blood cellular membrane [36]. Vitamin C is known to reduce the vitamin E radical formed as a result of the scavenging activity back to the functional form of vitamin E. This indirect antioxidant role of vitamin C has been shown in cell membrane preparations [37] as well as in vitamin C-deficient tissues [38].

Because this antioxidant activity of vitamin C depends on the chemical reduction potential, it is likely not a property unique to vitamin C. Other dietary phytochemicals may also be able to recycle vitamin E [39]. Clinically, the symptoms of scurvy do not include the oxidative stress-induced hemolytic anemia as observed in vitamin E deficiency. In addition, the lipid antioxidant activity of vitamin E overlaps with that of endogenous antioxidant system such as membrane-associated glutathione peroxidase [40], illustrating the presence of evolutionarily conserved redundant antioxidant systems.

Chemical reduction by vitamin C could lead to the generation of toxic by-products under specific conditions. A cell-free experiment showed that vitamin C at 0.1 to 2 mM can react with lipid hydroperoxides in the absence of transition metals [41]. This *in vitro* reaction produced DNA- and histone-reactive compounds [41,42]. Because the *in vivo* relevance of this cell-free reaction was never demonstrated, the significance of this observed vitamin C toxicity was not clear. However, this observation illustrated the promiscuous nature of the chemical reactivity of vitamin C and is relevant to the discussion later on the potential toxicity from long-term high dose supplementation in Part 2.3.

Vitamin C as a toxic pro-oxidant

The transition metal-dependent toxic pro-oxidant activity of vitamin C is also a result of its reduction potential. In the absence of enzymes, vitamin C reduces free Fe^{+3} and Cu^{+2} to the reactive Fe^{+2} and Cu^{+1}, which then participate in the radical-producing Fenton reaction [43]. Except among individuals with genetic abnormalities in iron metabolism [44], the pro-oxidant activity was not a concern at the physiological level of vitamin C (<0.1 mM) because transition metals are well-shielded by specific binding proteins. Redundant endogenous antioxidant systems are also expected to neutralize limited amount of radicals.

Nevertheless, this pro-oxidant activity, because it can be easily demonstrated at high concentrations of vitamin C (1-20 mM), has led to numerous and still ongoing cell culture-based studies reporting that vitamin C kills cancer cells [45-47]. Vitamin C at mM concentration in the transition metal-containing culture medium can dose-dependently increase the level of free radicals [14,48], which affect cellular processes [49,50] and eventually lead to cell death. The EC50, a concentration of vitamin C that is needed to kill 50% of cancer cells, ranges from 1 to >20 mM for different cancer cell lines [45,47]. A few studies also used the pro-oxidant activity to demonstrate a partial efficacy of vitamin C in cancer treatment using mice with tumor xenografts as the model. Invariably, vitamin C was injected into ascorbic acid-producing mice repeatedly at extremely high concentrations, for example, 4 g/kg body weight (equal to 280 g for a 154 lb person) [47]. Orally ingested vitamin C did not prevent or treat cancer in rats [51]. The relevance of applying extremely high dose of vitamin C for partial cancer control is questionable when a wide variety of effective chemotherapeutic agents are available now.

Vitamin C in the Prevention and Treatment of Non-Scurvy Diseases in Human Trials

Cardiovascular diseases

Based on the essential role of vitamin C in collagen maturation and the role of collagen in the vascular system as discussed in Part 1.1, it is not surprising that various epidemiological studies, although not all, have found a lower risk for cardiovascular diseases among individuals with higher vitamin C intake [52,53]. However, none of the clinical trials of vitamin C supplementation has demonstrated a protective effect of vitamin C for cardiovascular diseases. In fact, no antioxidant vitamin supplementation has been shown to affect the risk of cardiovascular diseases [54-56].

The difference in the outcomes of two types of human studies can be explained by the differences in the range of intake examined, and the specificity of vitamin C-mediated reactions. Because epidemiological studies mostly examined the level of consumption ranging from deficiency to an amount slightly above the RDA, it analyzed a range of intake that could impact the plasma concentration of vitamin C and thus collagen maturation. The supplemental trial, in contrast, led to vitamin C intake at levels much higher than the RDA, a range of intake that should not impact the collagen maturation. The hypothetical benefit of additional vitamin C as an antioxidant may not be seen in the supplement trial because of several possible factors: a limitation in the

bioavailability that have been discussed in the Introduction; a limited importance of vitamin C in the overall antioxidant capacity of the body as discussed in Part 1.2; and a potential toxicity at long-term high dose that may diminish any benefits of vitamin C supplementation [57-59].

Cancers

Clinical trials on cancer prevention, similar to those on the prevention of cardiovascular diseases, have not found significant benefit of vitamin C supplementation [60,61]. A recent review of trials in the past 30 years using vitamin C for cancer treatment also has not concluded its efficacy [62]. These results are expected based on the high non-physiological concentrations of vitamin C needed to abolish cancer growth in cell culture and rodent studies discussed in Part 1.3.

While some forms of collagens have been linked to the inhibition of tumor growth [63], epidemiological studies examining the range of vitamin C intake that can affect the collagen maturation found either a small reduction or no change in cancer risk at higher dietary vitamin C intake [64-66]. One possible explanation is that the extracellular matrix is composed of various collagen family members and the net contribution of good-quality collagens may not be growth inhibitory. For example, physiological levels of vitamin C, compared to vitamin C depletion, have been shown to promote the cancer growth in cell and animal studies [59,67]. Additionally, because vitamin C is mainly found in vegetables and fruits, the limited cancer-preventing epidemiological observation of vitamin C could be due to an overall healthier diet [68].

Health concerns on long-term vitamin C supplementation

A concern of long-term supplementation is toxicity. The small elevation in plasma concentration of vitamin C after supplementation may not affect collagen maturation but the chemical activity of vitamin C can increase dose-dependently. Although the Tolerable Upper Intake Level (UL) of vitamin C is set at 2 g/day for adults, the safety issue associated with long-term vitamin C supplementation at a dose lower than UL has emerged after various clinical trials drew to an end in recent years.

No apparent vitamin C supplement-related adverse effects were reported in the long-term cardiovascular disease or cancer control trials, which mostly used a vitamin C dose of 500 mg/day. Because epidemiological studies have found a link between higher vitamin C intake and a reduced risk of age-related cataract [69], clinical trials for cataract prevention have also been carried out. While vitamin C at 500 mg/day had no beneficial or harmful effect on the risk for cataract [70], a small increase in the risk for age-related cataract was observed after 8 years or longer of 1 g/day vitamin C supplementation [71,72]. It is possible that at a dose much greater than the requirement, the pro-oxidant activity of vitamin C dominates. In a trial of single 1 g supplementation of vitamin C, a significant increase in the plasma level of ascorbate radical was observed [73]. A chemical interaction between vitamin C and lens protein was also proposed previously [74], which was consistent to the chemical reactivity of vitamin C discussed in Part 1.2.

Conclusions

The reducing activity of vitamin C has led to its diverse roles in human health. Adequate daily intake of vitamin C is important for the prevention of scurvy and other abnormalities similarly relating to the function of collagen. The contrasting antioxidant and pro-oxidant activities of vitamin C, although at times suggested disease prevention and treatment opportunities by supplementation, are unlikely to be significant among healthy population. Furthermore, long-term clinical trials observed adverse effect at >500 mg vitamin C/day. The potential long-term toxicity of vitamin C at the dose lower than the current UL of 2.0 g/day needs to be further evaluated.

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