Ascorbic Acid: An Old Player with a Broad Impact on Body Physiology Including Oxidative Stress Suppression and Immunomodulation: A Review

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Abstract: Ascorbic acid is a low molecular weight antioxidant well known as anti-scorbut acting vitamin C in humans, primates and guinea pigs. This review summarizes basic data about ascorbic acid in its physiological action point of view. It is divided into biochemistry of ascorbic acid synthesis, mechanism of antioxidant action and participation in anabolism, pharmacokinetics and excretion, exogenous ascorbic acid immunomodulatory effect and participation in infectious diseases, impact on irradiation and intoxication pathogenesis, and supplementary demands. The primary intention was to consider ascorbic acid not only as an antioxidant but also as a chemical compound affecting multiple pathways with a potential beneficial impact in many diseases and processes in human body.

Keywords: Vitamin C, low molecular weight antioxidant, oxidative stress, inflammation, reactive oxygen species.

INTRODUCTION

Ascorbic acid is a simple six carbon compound L-threo-hex-2-enono-1,4-lactone with the correct IUPAC name (2R)-2-[(1S)-1,2-dihydroxyethyl]-4,5-dihydroxyfuran-3-one. From a chemical point of view, it is a sugar acid well soluble in water and poorly penetrating into lipid fraction. The solubility predicts physiological function as discussed below. Ascorbic acid is frequently used as food additive. In the European Food Safety Authority list of additives (E number codes) accepted by the Codex Alimentarius Commission, ascorbic acid and its salts are individually listed. E300 corresponds to ascorbic acid and E301 - E303 correspond to sodium, calcium and potassium salts, respectively. The last two derivatives are fatty acid esters: ascorbyl palmitate (E304) and ascorbyl stearate (E305). Ascorbic acid is one of the most important low molecular weight antioxidants (LMWA) in human body.

In the body, L-ascorbic acid acts as vitamin C. Its discovery was considered important due to the fact that vitamin C malnutrition has serious impact on human health. In 1937, the Nobel Prize in Physiology or Medicine was awarded to Albert Szent-Gyorgyi "for his discoveries in connection with the biological combustion processes, with special reference to vitamin C and the catalysis of fumaric acid".

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The name ascorbic acid is an acronym of the two Latin words: scorbutus referring to scurvy and prefix "a" indicating that there is no scurvy during the adequate compound intake. Scurvy is then a disease caused by a lack of ascorbic acid intake and was well recognized for thousands of years typically affecting sailors due to an insufficient feed [1]. While cutaneous manifestations, bleeding and gum symptoms are the most striking symptoms of scurvy [2], the disease also leads to psychical alterations. Though scurvy is not typical disease in population with an adequate access to food, the disease is still diagnosed worldwide [3, 4]. The fact that Nobel Prize was awarded to Szent-Gyorgyi for his discoveries related to the ascorbic acid clearly shows the seriousness of scorbut impact before the commercial production of ascorbic acid in the first half of 20th century was possible.

Despite the fact, that ascorbic acid was discovered long time ago and its role in the body is relatively understood, an extensive research focusing on both benefits of the compound and the role of its depletion during deterioration processes in the organism is still ongoing. In recent years, endogenous as well as exogenous ascorbic acid was investigated as a potent antioxidant with treatment potential in infectious diseases, intoxications, and shock conditions, such as ionizing radiation in animal models as well as humans. The role of ascorbic acid in the immune system is also intriguing. Sepsis, cancer and acute inflammation lead to an impaired antioxidant potential due to the depletion of ascorbic acid [5]. This review is aimed at summarization of

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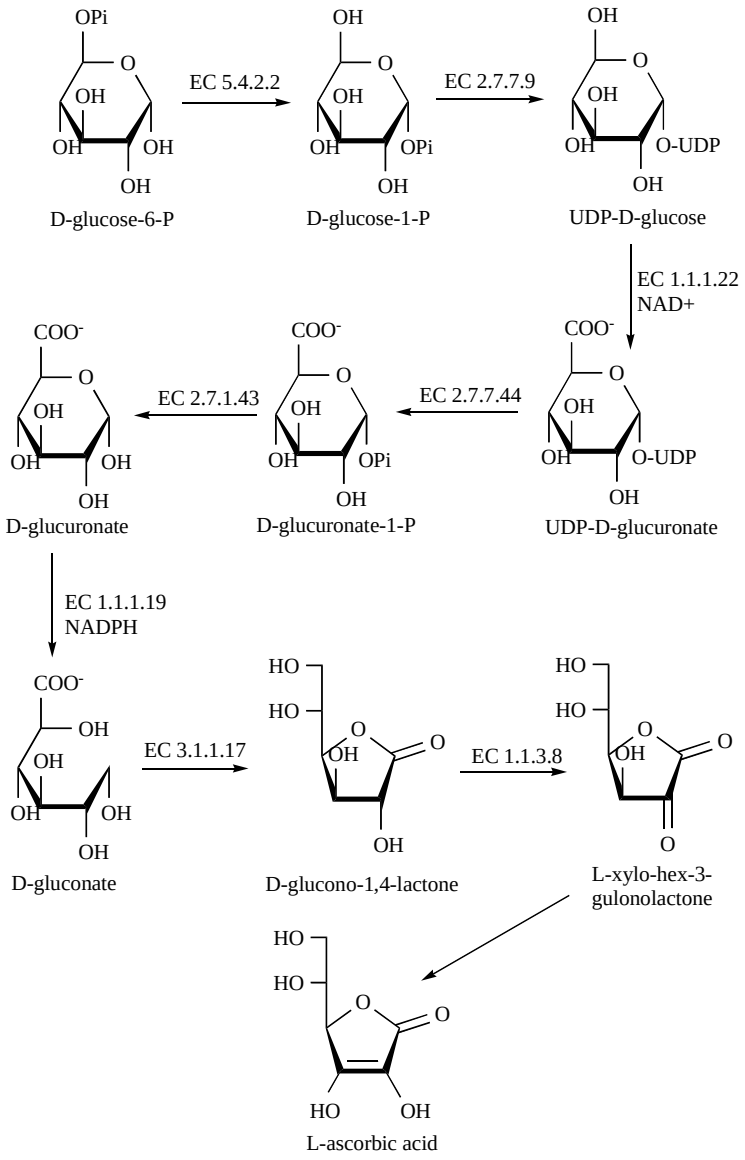

Fig. (1). The glucuronate pathway of L-ascorbic acid synthesis. Phosphate group is indicated as P in names and Pi in structures.

known facts about ascorbic acid in the above-mentioned areas and outlines expected trends for the future.

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF ASCORBIC ACID SYNTHESIS

The major way of L-ascorbic acid production in animals is based on glucurunate pathway [6]. The biosynthesis recognized in plants is quite different to the animals; it can be synthesized through D-fructose-6-phosphate into L-gulose-1-phosphate, L-galactose-1-phosphate or D-galacturonate [7]. Beside the pathway reported by Valpuesta and Botella [7], the older pathway was described for plants by Smirnoff *et al*. [8]. It consists from intermediates GDP-D-mannose, L-galactose, and L-galactono-1,4-lactone. Though fungi can comprise metabolism of ascorbic acid like plants, yeast constitutes D-erythroascorbic acid instead of ascorbic acid. The D-erythroascorbic acid undergo to D-erythroascorbyl radical quenched by NADH-cytochrome B_5 reductase [9].

Glucuronate pathway is depicted in the Fig. (**1**). In the first step, D-glucose-6-phosphate is isomerized into glucose-1-phophate by phosphoglucomutase (EC 5.4.2.2; Mg^{2+} cofactor). D-glucose-1-phophate is then conjugated into UDP-D-glucose by UDP-glucose pyrophosphorylase (EC 2.7.7.9; Co^{2+} cofactor) and oxidized by NAD dependent UDP-glucose dehydrogenase (UDP-glucose-6-dehydrogenase; EC 1.1.1.22; NAD^{+} cofactor) into UDP-D-glucuronate. UDP is split off the sugar moiety by glucuronate-1-phosphate uridylyltransferase (EC 2.7.7.44) forming D-glucuronate-1-phosphate. Phosphate group in position one is removed by glucuronokinase (EC 2.7.1.43; Mg^{2+} cofactor) and D-glucuronate is reduced into D-gluconate (L-gulonic acid) by NADP dependent glucuronate reductase (EC 1.1.1.19; NADPH cofactor). D-glucono-1,4-lactone (synonymous L-gulono-1,4-lactone) is formed by gluconolactonase (EC 3.1.1.17; cofactor Zn^{2+}, Mn^{2+} or Mg^{2+}) with simultaneous releasing of water. The final enzyme catalyzed step is carried out by monooxigenase L-

gulonolactone oxidase (EC 1.1.3.8; GULO) providing L-xylo-hex-3-gulonolactone (L-xylo-hex-2-ulono-1,4-lactone) and hydrogen peroxide. L-xylo-hex-3-gulonolactone spontaneously undergo into L-ascorbic acid without any action catalyzed by enzymes.

Microsomally located GULO is crucial in ascorbic acid synthesis. It was recognized early during the investigation of ascorbic acid synthesis pathway that cold-blooded vertebrates synthesize ascorbic acid in kidneys. During evolution, ascorbic acid synthesis was transferred into liver of warm blooded vertebrates in order to provide higher production capacity [10]. It should be emphasized that GULO is not specific only for evolutionary higher organisms. Beside animals and plants, some prokaryotes such as *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Bacillus anthracis* bear closely related orthologs of GULO [11]. Mutations in GULO gene lead to the inability to produce endogenous ascorbic acid and, as a result, ascorbic acid as vitamin C has to be supplemented from diet. Humans, hominids, bats and guinea pigs are typical GULO deficient organisms [12, 13]. As described by Nishikimi *et al*. [14], ancestor of guinea pigs lost GULO approximately 20 million years ago. The gene homologue of the rat GULO in guinea pigs shows several modifications. While the gene for GULO in rat consists of 12 exons, the exons I and V were found deleted and exon VI highly mutated in guinea pigs. Moreover, the GULO gene homologue in guinea pigs contains three stop codons [14]. Danish pigs also bear mutant GULO pseudogene, where deletions of 77 bp in exon VIII and deletions in two introns lead to a frame shift [15]. The human nonfunctional gene for GULO was extensively studied and several mutations and two stop codons were found [16]. Only homologues of exons VII, IX, X and XII out of the original 12 exons were identified boxed together on chromosome 8. In addition, residual exon VIII and short sequences with similarities to exon XI were also found in human genome [12].

Humans and hominoids ancestors lost ability to express the functional GULO approximatelly between 55 and 35 millions years ago in Eocene [17]. Investigation of GULO pseudogene in hominoids is an intriguing way for the evolutionary biology to study relations of humans to the individual monkey species. The GULO pseudogene in humanoids accumulates mutations throughout phylogenesis without evolution pressure to discriminate mutant individuals. Ohta and Nishikimi [18] studied nucleotide substitutions in GULO pseudogene in human, chimpanzee, orangutan and macaque and confirmed familiarity known from the taxonomy. Various theories about the reason for the loss of GULO gene have been put forward, including a lack of ascorbic acid need or accelerated evolution due to the lower antioxidant capacity [17].

PHYSIOLOGICAL EFFECTS OF ASCORBIC ACID

Ascorbic acid acts as a physiological antioxidant. It is well soluble in water and poorly soluble in fat due to the polarity of the molecule. Ascorbic acid is a weak acid dissociating one proton, which results in ascorbate formation at physiological pH due to keto - enol tautomerism. The mechanism of proton dissociation from ascorbic acid molecule is depicted in Fig. (**2**). The enol double bound resonates resulting in asymmetrical lay-out of charge and, eventually, the enolate is formed [19].

Ascorbic acid acts as antioxidant in aquaeous conditions in plasma and cytoplasm and undergoes transformation to dehydroascorbic acid form in the presence of oxidants. Mechanism of ascorbic acid redox action is shown in Fig. (**3**). Ascorbic acid crosses the cytoplasmic membrane by sodium-dependent vitamin C transporters (SVCT) 1 and 2 into cells, where it works as a scavenger for reactive oxygen species (ROS). The individual SVCT transporters show differences in expression in different cell lineages. Thus, while SVCT1 is extensively expressed in cells of the small intestine, liver, and kidney [20], SVCT2 is expressed by many different cells (e.g. epithelium [21], in central [22] and peripheral nervous system [23]). Compared to the ascorbic acid, dehydroascorbic acid can cross barriers by glucose transporters GLUT [24].

Ascorbic acid is present in body at quite high levels, but these levels can vary due to various conditions. Thus, human semen plasma contains ascorbic acid at concentrations of 300 - 500 μM , but the semen plasma from non fertile men was shown to contain the acid at the bottom limit of the concentration range and the same was shown for smoking non fertile men [25]. While cells can accumulate ascorbic acid up to the concentration of 4 mM, plasma levels are typically in the range of 40 - 120 μM [26]. Ascorbic acid levels also show differences by gender. Thus, while female plasma contains ascorbic acid at the average concentration of 59 μM, male plasma contains approximately only 80% (48 μM) of the female levels [27]. However, it is stil poorly understood whether the differences in ascorbic acid levels are due to different dietary customs or different physiological regulations within male and female body.

Despite extensive research, the complex effects of ascorbic acid on oxidative stress are still unclear. Although the direct scavenging of ROS is commonly considered as the major pathway, other mechanisms of action are likely. Lipophilic vitamin E (from the family of fat soluble

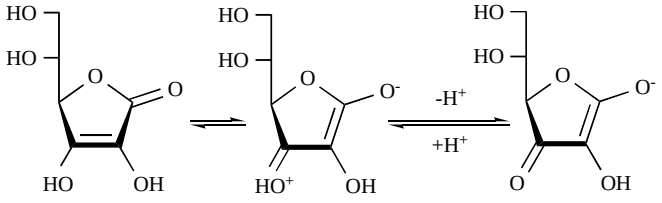

Fig. (2). Dissociation of proton from ascorbic acid molecule.

tocopherols) protects membranes prom lipid peroxidation by undergoing a transformation to the radical form once it captures free ROS in membrane. Ascorbic acid is able to reduce the radical form of vitamin E and, by this mechanism, vitamin E becomes reactivated [28]. Link between ascorbic acid and vitamin E was reported by many scientists. Li *et al*. [29] found higher level of reduced α-tocopherol in neuronal cells undergoing stress, when ascorbic acid was supplemented. On the other hand, May *et al*. [30] proved, that ascorbic acid was not able to protect mitochondrial α-tocopherol from oxidation, but also showed mitochondrial ability to convert dehydroascorbic acid into ascorbic and transfer it into cytosol. Clearly further studies are needed to improve our understanding of vitamin E / ascorbic acid relations *in vivo*.

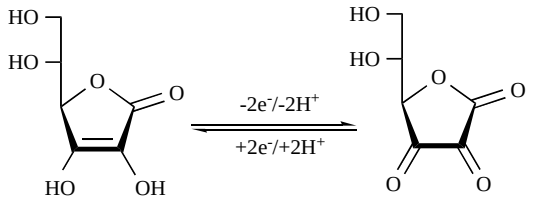

Fig. (3). Oxidation of ascorbic acid into dehydroascorbic acid.

Although ascorbic acid is considered to be an antioxidant and is therefore included in many drug formulations, it can also cause serious oxidative damage. For example ferrum is reduced by ascorbic acid when it appears as free ferric cation in biological fluids. Probably for this reason, transferrin as ferric cation carrier was evolutionary formed. In the next step of the reduction reaction, the newly created ferrous cation reacts with physiologically presented hydrogen peroxide molecule to re-create ferrous cation and hydroxyl anion with hydroxyl radical. The re-oxidation of ferrous cation in presence of hydrogen peroxide is called Fenton reaction, since it was originally described by Henry John Horstman Fenton *in vitro* [31]. Hydroxyl radical created by Fenton reaction is quite strong ROS apparently attacking multiple biological targets. Voss *et al.* [32] have shown Ca^{2+} - ATPase of the sarcoplasmic reticulum (SERCA) to be one and described sarcoplasmic reticulum oxidation by hydrogen peroxide generated in Fe^{2+}/hydrogen peroxide/ascorbic acid system. Trolox, water soluble derivate of vitamin E, and thiol containing LMWA pyridoindole stobadine were found effective to partially protect sarcoplasmic reticulum, but they failed to protect SERCA. These facts underline the importance of ascorbic acid pro-oxidative effect. An increased level of extracellular hydrogen peroxide (up 200 μM) following the intravenous administration of ascorbic acid 500 mg/kg in mice was reported by Chen *et al.* [33]. Contrary to that study, Colpo *et al.* [34, 35] did not find significantly increased markers of oxidative stress in healthy volunteers after intake of 2 g of ascorbic acid and Fe^{2+}. However, the experiments done by Colpo *et al.* were performed using a lower dose of ascorbic acid than the one by Chen *et al*. and therefore the data may actually suggest a dose dependent effect.

Ascorbic acid does not only act as LMWA but it is also involved in anabolic pathways. It participates in formation of collagen [36] and, for this reason, it is frequently included in cosmetic preparations [37]. Amino acids proline and lysine are hydroxylated due to ascorbic acid, which is well documented by ascorbic acid deficient rats, where abnormalities of proline and lysine hydroxylation, a lower molecular weight of expressed collagen and a decrease of bone quality occurs [38]. Beside collagen, ascorbic acid also participates in the synthesis of other necessary compounds in humans, where the biosynthesis of carnitine can be one example [39]. In addition, ascorbic acid is involved in the metabolism and regulation of many systems including immunity [40-42], where its effect is strictly dose dependent and not always easily interpretable.

The data summarized above thus show the ambivalent effects that ascorbic acid exerts on physiological processes. It can be considered as antioxidant, pro-oxidant, anabolic precursor, and immunity modulator depending on the dose and other circumstances. This wide range of specific effects of ascorbic acid, on the other hand, provides a promising potential in individuals suffering from various illnesses as discussed in the following text.

ASCORBIC ACID PHARMACOKINETICS

To provide ascorbic acid to the tissues in humans, ascorbate must be ingested and absorbed in the gastrointestinal tract. Ascorbic acid is typically present in fruits and vegetables. The majority (about 80 - 90 %) of the ascorbic acid obtained from the food is in the reduced form [43, 44] and the rest is obtained as dehydroascorbic acid.

Previously published studies determined that ascorbic acid is absorbed in the intestine by a Na^+-dependent active transport system [45, 46]. The majority of dehydroascorbic acid is immediately reduced to ascorbic acid upon crossing the intestinal wall. The intestinal micro flora is also capable to reduce dehydroascorbic acid to ascorbic acid or hydrolyse it to diketo glukuronic acid [47]. The highest level of absorption of ascorbic acid was found to occur in the proximal intestine [48]. Marked differences in ascorbic acid absorption were described among individuals and the possibility of regulation of ascorbic acid transport from the intestine is still discussed. It was reported that application of high dose of ascorbic acid leads to alterations in absorption of this antioxidant [49, 50].

Normal blood concentrations of ascorbic acid are in the range of 5 – 90 μM, but it varies highly among living subjects. [49]. Dehydroascorbic acid is present in a low concentration that corresponds to approximately 2 % of the total level of ascorbic acid [51, 52]. Some reports proposed a relationship between the intake of ascorbic acid from the intestine and its plasma level, but the relationship is difficult to interpret as ascorbic acid is accumulated and recycled in the body [53-55] and a strict control of the diet of tested individuals is necessary. Other study has shown, that there is a dose dependent sigmoid saturating kinetics between dose (in range 30 and 100 mg/day) of ascorbic acid and its plasma concentrations [56]. The approximate half-time of ascorbic acid in plasma was shown to be approximately 7-14 days [50], but this result is probably affected by initial plasma concentrations.

The control of ascorbic acid plasma level is mediated by intestinal absorption, tissue transport and also by renal excretion. In the kidney, the ascorbic acid is filtered through the glomeruli and reabsorbed in the proximal tubule by the ascorbic acid transporter SVCT1 [27]. The sigmoid saturating kinetics with upper range of ascorbate level in plasma mentioned above may be then also explained by the renal excretion. The transport through the proximal tubule is, similarly to the intestinal absorption, a Na^+-dependent process [57, 58].

Tubular reabsorption is influenced by the concentration of ascorbic acid in the tubule. Maximal tubular reabsorption was shopwn to be 1.5 mg/ml of glomerular filtrate [59]. It is unclear, however, whether other mechanisms of regulation of ascorbic acid reabsorption exist and whether there is a mechanism of an active secretion of ascorbic acid into the renal tubules.

THE IMMUNOMODULATORY IMPACT OF ASCORBIC ACID

The effects of ascorbic acid in the regulatory mechanisms of the immune system are not well understood . The fact that ascorbic acid could influence immune system was recognized in 1970s. The potentiating effect on production of complement compartments and some isotypes of immunoglobulins was recognized by, for example Prinz *et al.* [60]. Subsequently many clinical tests confirmed anti-inflammatory effects of ascorbic acid in human volunteers as well as animal models. For example ascorbic acid admistration in asthmatic patients suppresses symptoms during asthma attacks and therefore vitamin C enriched food is considered as supportive therapy [61]. T lymphocytes accumulate preferably dehydroascorbic acid rather than ascorbic acid by glucose transporters GLUT 1 and 3. The GLUT 1 and 3 are up regulated in activated T lymphocytes and ascorbic acid is accumulated in the cells at higher concentrations [40]. High doses of ascorbic acid stimulated T_{H1} response to antigen challenge in mice and decreased level of IgG and IgE [41].

The complex immunomodulatory effects of ascorbic acid as well as the other low molecular weight antioxidant seem to be different in individual cell lineages. Moreover, excessive intake of ascorbic acid could suppress pathways related to TNFα, NF-κB, protein kinase C, and p38 MAPK as the endogenous ROS are exhausted [62]. Ascorbic acid application after a stimulation of the immune system by bacterial lipopolysaccharide was shown to cause multiple shifts in assayed cytokines. A decrease in the activated monocyte population led to decreases in IL-6 and TNF-α [62]. In addition, it induced a reduction of lymphocytes producing IL-2, but monocytes producing IL-1 and IL-8 and lymphocytes producing TNF- α and IFN-γ were not affected [63]. In a separate experiment, Chang *et al*. [64] proposed that ascorbic acid supplementation leads to production of T_{H1} instead of T_{H2} lymphocytes. Juice with high concentration of ascorbic acid down regulated an expression of inter-cellular adhesion molecules-1 in human keratinocyte cells NCTC 2544 [65]. Despite good modulatory effect of ascorbic acid on cell lines, clinical tests on volunteers provide typically less significant data and ascorbic acid impact is not well described [66]. The mechanism of ascorbic acid action should be elucidated further in future experiments. However, its anti-inflammatory and pro-adaptive immunity effects are clearly documented by the above-mentioned studies. Challenge of the immune system by ascorbic acid administration was also tested during infectious diseases, but the conclusions are ambiguous. Similarly, the reasons for the depletion of low molecular weight antioxidants including ascorbic acid during sustained infectious disease are still unclear. For example HIV-1 positive patients have significantly reduced plasma antioxidant levels and increased markers of membrane oxidation such as malondialdehyde [67]. Overall impact of ascorbic acid on immune system and some basic facts are summarized in Table **1**.

Patients receiving chemotherapy and suffering from *Helicobacter pylori* infection had significantly increased eradication time when challenged with ascorbic acid or vitamin E [68]. Postoperative administration of ascorbic acid to septic patients had a significant antiapoptotic effect on

Table 1. Summarization of Ascorbic Acid Effect on Immune System

Intervention	Mechanism / dose	reference
Recommended minimal daily intake	100-120 mg	[89]
Recommended daily intake	200-300 mg	[88]
Risk of scurvy	Plasmatic level under 4 μM (30 - 60 mg per a day)	[49]
Plasma saturation and excretion	Plasma saturation at 80 μM (400-500 mg per a day)	[88]
High exogenous doses	Stimulation of T_{H1}, decrease of IgG and IgE	[41]
High exogenous doses	Suppression of TNFα NF-κB, protein kinase C, and p38 MAPK	[62]
High exogenous doses	Reduction of lymphocytes producing IL-2	[63]
Interleukin 2 therapy	Risk of ascorbic acid depletion	[5]
Depletion of endogenous level	During AIDS, lower ascorbic acid level is associated with higher progression of disease, a dose 750 mg per a day suggested for HIV positive	[90, 92]

peripheral blood neutrophils [69]. Dahl and Degre [70] and others described the antiviral activity of ascorbic acid. The antiviral effect is not probably based on antioxidant potency, as the dehydroascorbic acid is more potent antiviral agent than ascorbic acid [71]. Supplementation of food by some natural products containing ascorbic acid leads to inhibitory effect on replication of influenza virus and isolated parts of the tested natural products including ascorbic acid and green tea extracts had similar antiviral effect [72]. Application of ascorbic acid and vitamin E and B supplements is recommended for pharmacological purposes. Webb and Villamor reported and discussed these supplements as convenient pharmacological tools for modulation of immunity including alteration of innate immune cells, lymphocyte proliferation, and the delayed-type hypersensitivity [73].

RADIO-PROTECTIVE AND ANTITOXIC EFFECTS

There is a direct effect that both some toxins and ionizing radiation exert to cause the oxidative stress [74]. The ionizing radiation affects the organic matter both by direct interactions and by the radiolysis of H_2O, that generates ROS capable of causing an indirect damage. It is generally believed that the ratio of direct and indirect effects is directly proportional to linear energy transfer (LET) of ionizing radiation. Ascorbic acid does not seem to affect the direct effect. Narra *et al*. [75] examined the potential of ascorbic acid to protect the radiosensitive spermatogonial cells in mouse testes against the effects of chronic irradiation by radionuclides incorporated into tissue and observed no radioprotective effect when ^{210}Po, an emitter of high-LET alpha-particles, was administered. On the other hand, antioxidants generally modify indirect effects of ionizing radiation, which means that ascorbic acid can scavenge ROS and detoxify them to radicals that are less or not genotoxic [76]. Additionally, ascorbic acid may possess other radioprotective features specific to the nature of protected biomolecules. Pretreatment with antioxidants inhibits ROS accumulation and receptor activation [77]. ROS scavenging is also responsible for the inhibitory mechanism exerted by ascorbic acid on cellular senescence, which is mediated by p38 MAPK phosphorylation in human fibroblasts [78, 79]. However, it is not clear whether the signals activating p38 come from biological membranes or whether p38 is phosphorylated in response to the DNA damage [80, 81]. The effect of ascorbic acid, similarly to the other LMWA, is probably more complex than has been expected. Some investigators have also discussed the possibility that ascorbic acid can be involved in the regulation of gene expression [82].

Similarly to ionizing radiation, the protective effect of ascorbic acid against the processes induced by toxic compounds can be found wherever the toxicity mechanism is based on a generation of ROS. Bera et. al. [83] found that ascorbic acid acted strongly cytoprotective against the effects of sodium arsenite, which caused lipid peroxidation and increased production of nitric oxide in rat hepatocytes,. Similar protective effects of ascorbic acid on rat hepatocytes were observed during intoxication with organophosphorus pesticide malathion [84]. Unfortunately, the beneficial impact of ascorbic acid is limited. Iron, copper, chromium, vanadium and cobalt undergo redox-cycling reactions forming toxic radicals. Ascorbate is able to maintain these metals in the reactive reduced valence. Especially the reduction of ferric to ferrous cation by ascorbate with subsequent oxidation of ferrous cation by hydrogen peroxide leads to the generation of hydroxyl radical and hydroxyl anion, which both have a substantial damaging effect on biological macromolecules [85, 86] (see Fenton reaction above).

DIETARY RECOMMENDATIONS

Humans can obtain ascorbic acid only exogenously in their diet. Alimentary acquired ascorbic acid acts as vitamin C. An exact control of vitamin C concentrations is mediated by the tissue transport, absorption and excretion. Intestinal absorption of vitamin C at steady-state is inversely related to its dose, i.e. the higher the alimentary dose is, the less absorption occurs. Concentrations of vitamin C in tissues are higher than concentrations observed in plasma. Accumulated vitamin C in plasma and tissues also serves as a reservoir [49]. Infections and various diseases may also affect the absorption, tissue distribution and excretion of vitamin C as well as the other low molecular weight antioxidants [87].

Ascorbic acid is mainly found in fruits and vegetables. The rich sources of asorbic acid include cantaloupe, grapefruit, kiwi, mango, orange, papaya, strawberries, watermelon, asparagus, broccoli, cabbage, cauliflower, potatoes, tomatoes and other [88]. United States Department of Agriculture and national Cancer Institute guidelines recommend the ingestion of at least five fruits and vegetables daily. If these recommendations are followed, the amount of vitamin C ingested is estimated to be in the 200-300 mg range. At this dose, steady state plasma concentrations are about 70 μM. Plasma is completely saturated at doses of 400 mg daily and higher, producing a steady-state plasma concentration of approximately 80 μM. At doses of 500 mg and above, the entire absorbed dose is excreted. In the US, the currently recommended minimal ascorbic acid dose is 100-120 mg per a day for adults [89]. At plasma concentrations less than 4 μM, symptoms of scurvy may occur. A dose of about 10 mg/day of vitamin C is sufficient to prevent scurvy in adults. At plasma concentrations below 20 μM, fatigue may occur. It corresponds to oral intake of 30-60 mg of vitamin C per a day [49].

The metabolism and utilization of vitamin C is affected by various diseases. Thus since the reabsorption and excretion od vitamin C by kidneys play a key part in the control of vitamin C concentrations in plasma and tissues, it is not surprising that this control is lost in patients with end stage renal disease [49].

The production of reactive oxygen and nitrogen species increases during infectious diseases, when the immune system is activated to eliminate pathogenic organisms. Chronic infections, such as HIV infection that can be considered as an example, place a long-term strain on antioxidant defences, which may increase dietary antioxidant requirements. Low plasma concentration of vitamin C has been shown to be associated with a greater risk of

progression to AIDS in HIV-infected subjects [90]. Plasma vitamin C concentration may be decreased by the chronic immune activation during HIV infection even if the dietary intake is at a level considered to be adequate for healthy persons [91], because the utilization of Vitamin C is increased by HIV infection. It has been shown, that in HIV-infected patients there is an incrase of oxidative stress in tissues associated with significantly lower plasma antioxidant micronutrient concentrations (such as vitamin C) when compared to the HIV- seronegative controls. The addition of antioxidant vitamins led to an inhibition of HIV replication and observational studies have suggested that supplementing vitamin C in doses over 750 mg per day may have beneficial effects on HIV-disease progression and risk of development of AIDS [92]. It should be noted, however, that there are fears that vitamin C may have prooxidant or mutagenic affects and the potential toxicity of vitamin C needs further investigation [49].

CONCLUSIONS

Tha data summarized in this review indicate that ascorbic acid, due to its effects and diversity of regulated pathways, is suitable for use in various fields of medicine including immunology, toxicology, radiobiology and others . Although ascorbic acid is not a miracle drug and can even induce some adverse effects, the potential of ascorbic acid in medicinal chemistry is quite high. Significant advantages of ascorbic acid include low cost, minimal or no toxicity and simple application. Ascorbic acid is not perspective to be used as an isolated mode of treatment, but it can be co-applied as an adjuvant to regulate immunity, gene expression and other important physiological processes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of MO0FVZ0000501 and MO0FVZ0000604 from the Ministry of Defence of the Czech Republic, and from P304-10-11 from the Grant Agency of Czech Republic.

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Received: April 29, 2011 Revised: July 16, 2011 Accepted: August 12, 2011