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Review

The biochemical, physiological and therapeutic roles of ascorbic acid

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Ascorbic acid is an important micronutrient necessary for a significant number of metabolic reactions in humans and other primates. It is a strong reducing agent involved in reduction reaction and it is structurally related to glucose. Experimental and epidemiological studies have documented the biochemical, physiological and therapeutic roles of ascorbic acid. It is an essential water-soluble antioxidant vitamin derived from exogenous source; thus its role in human health is worthy to be considered particularly because of the current global economic trend. The biochemical, physiological and therapeutic roles of ascorbic acid is reviewed, providing further insights into the role of ascorbic acid in biological functions.

Key words: Ascorbic acid, human health, disease, biological function, therapeutic roles.

INTRODUCTION

Ascorbic acid was first isolated in 1928 by the Hungarian scientist and Nobel prize winner Szent-Gyorgyi. The search for the active ingredients in fruits and vegetables which cured scurvy, led to the isolation and identification of ascorbic acid from plants in 1932 (Burns et al., 1987; Larsen, 1997). Purified ascorbic acid is a crystalline compound with an empirical formula of $C_6H_8O_6$ and a molecular weight of 176.1. It is a keto-lactone with structural formula similar to that of carbohydrates (Levine, 1995; 1996). Chatterjee (1973, 1975) reported that insects, invertebrates and fish lack the ability to synthesize ascorbic acid. The biosynthetic capacity was found to start in the amphibians and reptiles in which the synthesis was localized in the kidney while the synthetic capacity disappeared in the guinea pig, the flying mammals, indian fruit bats, the monkeys and man. The failure of primates to synthesize ascorbic acid has been found to be due to a common defect which is the absence of the terminal enzyme in ascorbic acid biosynthesis, L-gulonolactone oxidase (Burns et al., 1987; Chatterjee, 1973; Levine, 1986). This enzyme catalyzes the oxidation of L-gulonolactone to L-2-ketogulonolactone which spontaneously isomerizes to form ascorbic acid. It is the loss of this single enzyme (the gene for the enzyme is defective) that renders ascorbic acid an essential vitamin (Levine, 1995; 1996).

It has been reported that elevated blood histamine levels occur following the injection of vaccines or toxoids in guinea pigs. Blood histamine levels are also increased by other stresses such as heat or cold, infections and by several drugs in guinea pigs and even by sleep-deprivation and by early ascorbate depletion in man. The effects of increased tissue histamine levels, including asthma, hay-fever or allergic rhinitis and nettle-rash or urticaria have been documented. It was the work of Parrot and Richet in guinea pigs which revealed that ascorbate depletion increases histamine sensitivity and reported that the dose of histamine causing death in half the animals (LD50) was reduced from 8 to 2.5 mg/kg after 15 days on ascorbic acid-deficient diet. It is now known that even sub-optimal plasma ascorbate levels are associated with increased blood histamine levels, both in guinea pigs and in man (Parrot and Richet, 1945; Clemetson, 1980; 1989; 1999). The work of Chatterjee et al. (1975) has shown that optimal blood ascorbate levels are needed for the detoxification of histamine by converting it to hydantoin-5-acetic acid and then to the simple amino acid, aspartic acid. Results from animal experimental studies can be extrapolated and to some extent give a picture of what results from human subjects could be like. However, more human studies are needed to clarify any doubt or confirm results with animal models. Nelson et al. (1981) presented experimental evidence to show that the synthesis of carnithine, which transports

long-chain fatty acids into mitochondria for beta-oxidation involves hydroxylation reactions catalysed by enzymes whose activity is enhanced by ascorbic acid. Ascorbic acid is also known to play an enhancing role in tyrosine metabolism involving the hydroxylation of β-hydroxyphenylethylamine into homogentisic acid (Windsor et al., 1975; May and Mikulecky, 1985).

FUNCTIONS OF ASCORBIC ACID

Larsen (1997) reported that Linus Pauling, a Nobel Prize winner in 1970 advocated the use of megadoses (1-3 g/day) of ascorbic acid for the prevention of common cold and related infections. From a preliminary report, it has been found that ascorbic acid supplementation had significant protective effect against beta-haemolytic streptococcal throat infection. Ascorbic acid is said to enhance the human immune defence system and this it does by enhancing the random migration of human polymorphonuclear leucocytes to the site of infection (Burns et al., 1987; Watson, 1994; Kee-Ching, 1996; Levine, 1997). Scientists found that ascorbic acid may serve as just a shield to save a key type of immune cell from self annihilation (Levine, 1997). While it has been shown to stimulate variable serum antibody levels in human volunteers ingesting one or more grams of ascorbic acid for several days or weeks, its most striking and reproductive effects are on the production and proper functioning of the white blood cells that play important role in cell-mediated immunity. In contrast to studies on healthy human subjects, there are relatively fewer reports on the effects of ascorbic acid supplementation on T-cell responses in immuno-suppressed persons. Most significant among these is a clinical report from South Africa dealing with measles in native children who experience a high death rate from secondary infections (Watson, 1994). These children also exhibit depressed T-cell counts and abnormal T-cell ratios similar to those seen in AIDS patients. Administration of a few grams per day of ascorbic acid during the convalescent phase was shown to restore the T-cell ratio to normal levels. Also in another study, Oguntibeju et al. (2006) reported that HIV-positive/AIDS patients supplemented for three months on ascorbic acid-containing supplement showed reduced viral load and improved haematological parameters.

In a cohort study conducted by Tang et al. (1993), 25% of the volunteers with the highest intake of ascorbic acid (715 mg/day) in food and supplements combined had 0.55 of the average change of progression to AIDS and also reported that ascorbic acid was associated with increased survival but no relationship was found between ascorbic acid from supplement alone and survival. Allard et al. (1998) found evidence that people taking ascorbic acid and vitamin E showed significant reduction in viral load. Allard randomized 49 people with HIV infection to receive either vitamin E (800 IU daily) and 1000 mg ascorbic acid daily or a matched placebo. At three months, there was clear evidence of a reduction in the oxidation of body lipids and viral load of patients on supplement.

Ascorbic acid has also been demonstrated to show a direct bacteriostatic and bactericidal effect that dates back to the 1930. In one study, it was shown to be effective in the inactivation of a wide range of pathogenic bacteria such as *Staphylococcus aureus*, *Esherichia coli*, and haemolytic *Streptococcus* species. The mechanism by which ascorbic acid inactivates bacteria has been ascribed to the attack by free radicals generated as a result of the reaction of ascorbic acid and molecular oxygen in the presence of transition metals such as copper ions as deduced from *in vitro* models. A striking property of ascorbic acid is its ability to inactivate viruses and inhibit viral growth in their host by the same mechanism. Ascorbic acid has been known to be an antiviral agent and the crystalline ascorbic acid has been shown to inactivate the poliovirus (Watson, 1994).

It has been demonstrated that neutrophils destroy bacteria and viruses with a two-stage attack. First they produce oxidants that attack a bacterium's cell wall, with the neutrophil engulfing the bacterium. Research by Levine (1997) noted that neutrophils may avoid "poisoning" themselves by absorbing extra ascorbic acid which can neutralize oxidants. To test this theory, Levine (1997) collected neutrophils from people with bacterial infections and incubated the cells for one hour in two sets of dishes stocked with ascorbic acid and nutrients. One set was infected with bacteria, the other was bacteria free. Within 20 min, the neutrophils from the infected dishes had accumulated up to 30 times more ascorbic acid than those in dishes without bacteria. The key question is whether dosing up on ascorbic acid makes a person's neutrophils better bacteria killers. If so ascorbic acid could be an approach to tackling antibiotic-resistant bacteria. Although, neutrophils may behave differently in the laboratory from human system, it somehow shows the potential role of ascorbic acid in body defense mechanism. White blood cells carry ascorbic acid in the bloodstream and during scurvy reduced rate of phagocytosis have been observed and it is believed that ascorbic acid possibly plays a role in enabling the white blood cells to "recognize" invading tissues as foreign.

There is increasing epidemiological evidence that increased intakes of ascorbic acid may help reduce the risk of diseases associated with increased oxidative stress. Few ascorbic acid supplementation trials have shown a trend towards sparing tissue vitamin E and a study of premature infants reported an increase in plasma vitamin E levels following ascorbic acid supplementation (Wen et al., 1997; Jacob et al., 1997). The ability of ascorbic acid to readily donate electrons, endows it with the chemical capacity to reduce oxidizing substances or free radicals. Each molecule of ascorbic acid has two redox-active hydrogen atoms in its molecular structure that carries two high-energy electrons, distinct functions that make it a premier antioxidant in the biological system. At ordinary concentrations, at which it

is normally present, ascorbic acid functions as an electron-carrier similar to other non-enzymatic radical scavengers such as vitamin E, beta-carotene, glutathione and the thiol-containing amino acids (Frei, 1994; Watson, 1994; Sies and Stahl, 1995). In an *in vitro* study, analyzing the antioxidant activities of various components of human blood plasma, it was shown that ascorbic acid is the most effective antioxidant that completely protected plasma lipids from oxidative damage caused by free and peroxyl radicals (Frei, 1994; Uddin and Ahmad, 1995; Kontush, 1996; Malins, 1996; Schwartz, 1996). Research has shown that ascor-bic acid could protect vitamin A from being oxidized by reducing potential oxidizers. If vitamin A is oxidized, it would become biologically inactive and incapable of per-forming its biological and physiological functions (Larsen, 1997). This possibly explains the role of ascorbic acid in anti-carcinogenesis and degenerative aspects of ageing.

Nyyssonen et al. (1997) followed 1605 randomly-selected men aged 42-60 for eight and half years, 13.2% of the men who had low blood levels of ascorbic acid (< 2 mg/l) had a heart attack during this period. Only 3.8% with normal ascorbic acid levels had one. The researchers concluded that low ascorbic acid levels increased the likelihood of having a heart attack 3.5 times. Wise (2001) found a clear dose-response rate between high levels of ascorbic acid and a reduced rate of heart disease. Measurement of plasma ascorbic acid concentration in 19496 men and women aged 45-79 years and followed up for 4 years showed an increase in plasma ascorbic acid concentration that was strongly and independently associated with a reduction in mortality from cardiovascular disease and ischaemic heart dis-ease. The risk of death among individuals with the highest ascorbic acid concentrations was about half the risk compared with individuals with the lowest ascorbic acid concentrations.

Another notable role of ascorbic acid is in drug metabolism. In view of the effect of diet on the metabolism and toxicology of drugs, Parke (1979) reported that guinea pigs that were ascorbate deficient exhibited lower levels of hepatic cytochrome P_{450} and reduced drug metabolizing capacity, and abnormality which was corrected by treating animals with ascorbic acid. It was also reported that in the presence of D-aminolevulinic acid (a haem precursor) and ascorbic acid, the recovery of the drug metabolizing capacity was very rapid, suggesting that ascorbic acid participates in haem synthesis and that it probably exerts its influence on cytochrome P_{450} via the haem cycle. It is presumed that in man too, ascorbic acid deficiency can lead to the loss of drug metabolizing capacity and consequently to enhance drug action and toxicity (Levine, 1996).

Research reveals that ascorbic acid activates the catabolism of cholesterol into simpler components for the eventual synthesis of steroid hormones. During scurvy, elevated levels of cholesterol can easily be measured in the blood due to an accumulation of non-catabolized cholesterol. The role of ascorbic acid in cholesterol metabolism has been studied (Woodhouse and Khaw, 1994; Gale, 1995; Sahyoun, 1996). Leggot (1986) found that the ingestion of one gram of ascorbic acid by humans who have been in the state of chronic latent ascorbic acid deficiency did have hypo-cholesterol effect. He also reported that this effect was dependent on the initial concentration of plasma cholesterol; the higher the initial cholesterol, the greater the hypo-cholesterolemic effect of the vitamin. Oguntibeju and Fafunso (2002) also reported the beneficial effect of ascorbic acid and consumption of fruits on cholesterol level and said that eating fruits rich in ascorbic acid may have beneficial effect in type-1 diabetes. Another study reported that ascorbic acid may improve glycemic control, lowering both fasting plasma glucose level and glycosylated haemoglobin (Eriksson et al., 1997). In addition, it has been suggested that antioxidant treatment using high-dose ascorbic acid may help prevent renal injury in type-1 diabetic rats. It is said that ascorbic acid modulates insulin action and this probably explains its action in lowering plasma glucose and lipids particularly cholesterol and triglyceride. This poses a challenge for further research especially in human subjects.

An adequate intake of ascorbic acid is highly protective against stroke and heart attack. A research work showed that people who supplement their diets with more than 700 mg/day of ascorbic acid have a 62% lower risk of dying from heart disease than those people with a daily intake of 60 mg/day or less (Sahyoun, 1996). Supplementation with 2 g/day of ascorbic acid was found to reduce adhesion of monocytes (white blood cells) to the lining of blood vessels and thereby reducing the risk of atherosclerosis (Heitzer, 1996; Weber, 1996; Lehr, 1995). Ascorbic acid supplementation (2 g/day) also effectively reverses the vasomotor dysfunction often found in patients with atherosclerosis (Levine, 1996). One research carried out in Japan showed that restenosis (reclosing of opened arteries) after angioplasty can be significantly reduced by supplementation with ascorbic acid (500 mg/day) (Tomoda, 1996).

Supplementing with ascorbic acid has been found to significantly lower the risk of cataracts and glaucoma and other work has shown that open angle glaucoma can be reversed by supplementing with large doses of vitamin C (Hankinson, 1992; Boyd, 1995). Ascorbic acid supplementation (1000 mg/day) was found to significantly decrease the risk of developing pressure sores in surgical patients (Taylor, 1974).

Decreased maternal ascorbic acid has been associated with premature/low birth weight, preclampsia, anaemia and premature rupture of the foetal membrane (Therieko and Ettes, 1981; Awoyelu et al., 2004). Nelson and Tagfer (1971) suggested that deficiency of ascorbic acid might have teratogenic effect. Although these authors did not highlight the mechanism of action of ascorbic acid that may perhaps lead to afore-mentioned abnormalities as a result ascorbic acid deficiency, their views and findings agree with that of other scientists on the importance of

ascorbic acid in health.

Ascorbic acid is a cofactor in the biosynthesis of carnitine- a molecule required for the oxidation of fatty acids. A reduction in the ability to oxidize fat may contribute to the reported inverse relationship between ascorbic acid status and adiposity. To examine this possibility, Johnston et al. (2006) conducted a preliminary trial to evaluate the impact of ascorbic acid status on fat oxidation during sub-maximal exercise in a study on marginal ascorbic acid status in which fat energy expenditure was determined in individuals with marginal (n = 15) or adequate (n = 7) ascorbic acid status during a sub-maximal, 60-min treadmill test. Eight of the subjects with marginal ascorbic acid status completed an 8-week double-blind, placebo-controlled, depletion-repletion trial with sub-maximal exercise testing. The results showed that individuals with marginal ascorbic acid status oxidized 25% less fat per kg body weight during the treadmill test as compared to individuals with adequate ascorbic acid status. Fat oxidation during exercise was inversely related to fatigue ($r = -0.611$, $p = 0.009$). Repletion of ascorbic acid in ascorbic acid-depleted subjects (500 mg ascorbic acid/day) raised fat energy expenditure during exercise 4-fold as compared to depleted control subjects ($p = 0.011$). This study concluded that low ascorbic acid status is associated with reduced fat oxidation during sub-maximal exercise. Low ascorbic acid status may partially explain the inverse relationship between ascorbic acid status and adiposity and why some individuals are unsuccessful in their weight loss attempts.

Ascorbic acid has been indicated to play an active role in blood clotting as witnessed by increased coagulation time during scurvy. For some reason, not fully understood, there is sharply reduced blood platelet aggregation during times of scurvy with the resulting defective release of certain platelet factors necessary for normal clotting. The simple addition of ascorbic acid to platelet-rich blood taken from experimental animals suffering from acute scurvy has no effect on aggregation and the release of platelet-factors which suggests that there is a deep metabolic disruption within the platelets which will not simply return to normal after replenishing with ascorbic acid. An interesting observation is that platelets from scorbutic experimental animals tend to lose ascorbic acid into the blood plasma at a higher rate than control groups during the process of aggregation, prior to clot formation (Byshevskii et al., 1992; Tousoulis et al., 2003; Tousoulis et al., 2007).

One of the most outstanding effects of ascorbic acid deficiency is that of impaired collagen synthesis. Collagen is the major protein component of connective tissue. As much as 60% of the total body protein in mammals is collagen. It comprises most of the organic matter of skin, tendons, bones and teeth. Collagen contains two amino acids, hydroxyproline and hydroxylysine, which occur only rarely if at all in other animal proteins. Hydroxylation occurs after proline and lysine have been added into the peptide chain and serves to chemically cement together these amino acid building blocks by bringing about the coupled oxidation of proline and lysine with the incorporation of oxygen into one of two molecules. An enzyme called hydroxylase catalyzes this process of hydroxylation. In order for hydroxylase to properly perform its function, it needs ascorbic acid as cofactor. Although the exact role of ascorbic acid is not fully defined in this mechanism, it is believed that ascorbic acid serves to maintain the ferrous ion in its ferrous state, and in times of ascorbic acid deficiency, this will lead to an under-hydroxylized collagen. Under-hydroxylized collagen is not stable and tends to break down into unassociated components and the eventual accumulation of the unassociated components within the cell is thought to cause a feedback inhibition of further collagen synthesis. Because this process of hydroxylation occurs when new collagen is synthesized, previously formed collagen is unaffected by scurvy. This may explain why young growing cavies are much more susceptible to scurvy than an adult cavy (Ivanoy et al., 1997; Ashino et al., 2003; Stuart et al., 2005).

SOURCES AND DAILY INTAKE OF ASCORBIC ACID

Ascorbic acid is widely distributed in both plants and animals, occurring as both ascorbic acid and dehydroascorbic acid. Fruits, vegetables, liver and kidney are generally the best sources; only small amounts are found in muscle meat. Plants synthesize L-ascorbic acid from carbohydrates. Most seeds do not contain ascorbic acid but start to synthesize it upon sprouting. Some plants are believed to accumulate high levels of ascorbic acid (for example, fresh tea leaf, berries and guava). For practical reasons, citrus fruits are good daily sources of ascorbic acid, as they are generally eaten raw and are therefore not subjected to cooking procedures that can destroy ascorbic acid. The ascorbic acid contents of most foods decrease dramatically during storage due to the aggregate effects of several processes by which the vitamin can be destroyed. Ascorbic acid is susceptible to oxidation and converted to dehydroascorbic acid that is irreversibly degraded further by hydrolytic opening of the lactone ring. These reactions occur in the presence of oxygen, metal ions such as ferrous ion, heat, conditions of neutral to alkaline pH and also enhanced by exposure to oxidases in plant tissues. Therefore, losses of ascorbic acid can occur during storage and are more significantly during cooking. Losses in cooking are usually greater with such methods as boiling, as the stability of ascorbic acid is much less in aqueous solution. Alternatively, quick-heating methods can protect food ascorbic acid by inactivating oxidases (Moser and Bendich, 1991).

Linus Pauling (1970) recommended ascorbic acid intakes of 1000 mg/day or more. The official RDA is 60 mg/day (100 mg/day for smokers). It is vital to note that the RDA is not based on what is required for optimum health.

The RDA is based on the ascorbic acid content of the average diet. Many experts have now realized that the RDA of 60 mg/day is rather low to provide for optimum health and protection against diseases and degenerative aspects of ageing. A team of medical researchers at the National Institutes of Health in the USA in a study designed to determine the ascorbic acid requirements of healthy, young men, found that a minimum intake of 1000 mg/day was required to completely saturate the blood plasma with ascorbic acid. They also found that ascorbic acid should be taken in divided doses throughout the day as urinary excretion increases rapidly when individual doses exceed 500 mg. The researchers conclude that the RDA should be raised to 200 mg/day; consequently, the National Academy of Sciences, USA revises its current recommendations from 100 to 200 mg/day.

CONCLUSION AND RECOMMENDATION

It is very clear from the roles of ascorbic acid as enumerated above, that ascorbic acid plays significant roles in biological functions. This is true despite the fact that the biochemical action of ascorbic acid is still not fully understood. Besides, ascorbic acid is an essential vitamin that is derived only from exogenous sources and makes its role in human health of significant importance. Further research is urgently needed on the metabolism of the ascorbic acid. There is also a need to reach a consensus on the recommended daily allowances for individual country of the globe. Research is needed to develop and test algorithms for the use of ascorbic acid in the management of certain diseases. Studies are needed to determine the efficacy and safety of ascorbic acid supplementation in different disease conditions.

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