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REVIEW ARTICLE

Vitamin C in Disease Prevention and Cure: An Overview

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Abstract The recognition of vitamin C is associated with a history of an unrelenting search for the cause of the ancient haemorrhagic disease scurvy. Isolated in 1928, vitamin C is essential for the development and maintenance of connective tissues. It plays an important role in bone formation, wound healing and the maintenance of healthy gums. Vitamin C plays an important role in a number of metabolic functions including the activation of the B vitamin, folic acid, the conversion of cholesterol to bile acids and the conversion of the amino acid, tryptophan, to the neurotransmitter, serotonin. It is an antioxidant that protects body from free radical damage. It is used as therapeutic agent in many diseases and disorders. Vitamin C protects the immune system, reduces the severity of allergic reactions and helps to fight off infections. However the significance and beneficial effect of vitamin C in respect to human disease such as cancer, atherosclerosis, diabetes, neurodegenerative disease and metal toxicity however remains equivocal. Thus further continuous uninterrupted efforts may open new vistas to understand its significance in disease management.

Keywords Vitamin C · Atherosclerosis · Diabetes · Immunity · Cancer · Infertility · Heavy metal toxicity

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Introduction

Vitamins are essential nutrients that are required for various biochemical and physiological processes in the body. It is well known that most of the vitamins cannot be synthesized in the body and hence their supplementation in diet is essential. Vitamins are classified on the basis of their solubility as water soluble (C and B complexes) and fat soluble vitamins (A, D, E, K). Vitamin C or ascorbic acid (AA) was first isolated in 1923 by Hungarian biochemist and Nobel laureate Szent-Gyorgyi and synthesized by Howarth and Hirst [1]. It exists in reduced [ascorbate] and oxidized forms as dehydroascorbic acid which are easily inter-convertible and biologically active thus it acts as important antioxidant. Vitamin C is easily oxidized acid and destroyed by oxygen, alkali and high temperature. Most of the plant and animal species have the ability to synthesize vitamin C from glucose and galactose through uronic acid pathway but man and other primates cannot do so because of deficiency of enzyme gulonolactone oxidase [EC 1.1.3.8] required for it's biosynthesis. Deficiency of this enzyme is a result of a mutation which occurred approximately 40 million years ago [2].

The body requires vitamin C for normal physiological functions. It helps in the synthesis and metabolism of tyrosine, folic acid and tryptophan, hydroxylation of glycine, proline, lysine carnitine and catecholamine. It facilitates the conversion of cholesterol into bile acids and hence lowers blood cholesterol levels. It also increases the absorption of iron in the gut by reducing ferric to ferrous state. As an antioxidant, it protects the body from various deleterious effects of free radicals, pollutants and toxins. The therapeutic effect of vitamin C was explored by Linus Pauling however his work on therapeutic role of vitamin C in his later years generated much controversy yet he was

the first to introduce the concept of high doses of vitamin C for the treatment of various conditions from common cold to cancer [3]. Since then mega doses of vitamin C have been widely used in the treatment and prevention of a large number of disorders like diabetes, atherosclerosis, common cold, cataracts, glaucoma, macular degeneration, stroke, heart diseases, cancer and so on.

Deficiency of this vitamin is often associated with anemia, infections, bleeding gums, scurvy, poor wound healing, capillary haemorrhage, muscle degeneration, atherosclerotic plaques and neurotic disturbances. For the correction of deficiency, vitamin C is often supplemented in large doses and unlike fat soluble vitamins, toxicity is rare. Recently the role of vitamin C in infection and immunity has also been investigated. In view of the vast biological, physiological functions and therapeutic role of vitamin C, this review is an attempt to summarise various evidences in this context.

Dietary Sources of Vitamin C

Vitamin C is found in citrus fruits, green peppers, red peppers, strawberries, tomatoes, broccoli, brussels sprouts, turnip, Indian gooseberry and other leafy vegetables. The animal sources are poor in vitamin C content and the level is usually <30–40 mg/100 g. Therefore plant sources become important because of high content of vitamin C up to 5,000 mg/100 g. It's absorption in the buccal cavity is by passive diffusion however in gastrointestinal tract absorption is by active sodium dependent vitamin C transporters (SVCT) [4, 5].

Vitamin C Bioavailability

Bioavailability or the effective concentration of vitamin C essentially depends on its effective absorption from intestine and renal excretion. Vitamin C, consumed either with diet or dietary supplements, is absorbed by the epithelial cells of the small intestine by SVCT1 or, subsequently diffuses into the surrounding capillaries and then the circulatory system [5–7]. Circulating AA is filtered from kidney capillary bed into the Bowman's capsule through a general filtration mechanism. AA is reabsorbed through SVCT1 transporter in proximal convoluted tubule [6]. The difference between the amount of AA filtered and reabsorbed constitutes renal excretion [8]. Together, intestinal absorption and renal excretion controls the serum level of vitamin C and thus its bioavailability. At low concentrations, most vitamin C is absorbed in the small intestine and reabsorbed from the renal tubule [9]. However, at high concentrations, SVCT1 is down regulated [10] which limits the amount of AA absorbed from the intestine and kidney [11]. This imposes a physiological restriction on the maximal effective serum vitamin C concentration (or its bioavailability) that is attainable by oral consumption [12]. This value has been determined to be 200 mmol/L [12], although "normal" physiological serum concentrations of ascorbate in healthy humans range from 60 to 100 mmol/L [13]. Vitamin C levels in circulating blood cells, such as platelets, are much higher than the plasma [13], as these cells express the SVCT2 transporter [14], which mediates intracellular ascorbate accumulation [15].

Reduced bioavailability of vitamin C is often seen in stress, alcohol intake, smoking, fever, viral illnesses, usage of antibiotics, pain killers, exposure to petroleum products or carbon monoxide, heavy metals toxicity and so on. However, the precise mechanism behind low vitamin C level in the body is not well defined. Presumably, an increased utilization of this vitamin in these conditions and/or a decreased absorption from the gut could be a cause of decreased vitamin C level [16]. In the event of high consumption, AA and its metabolites such as dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid are excreted via kidney in humans. AA is generally non-toxic but at high doses (2–6 g/day) it can cause gastrointestinal disturbances or diarrhoea [17, 18]. The side effects are generally not serious and can be easily reversed by reducing the intake of AA. Furthermore, there is no consistent and compelling data on serious health effects of vitamin C in humans [18, 19].

Biochemical Functions of Vitamin C

The biochemical functions of AA are largely dependent on the oxido-reduction properties of L-AA which is a co-factor for hydroxylation and activity of mono-oxygenase enzymes in the synthesis of collagen, carnitine and neurotransmitters [20]. AA accelerates hydroxylation reactions by maintaining the active centre of metal ions in a reduced state for optimal activity of enzymes hydroxylase and oxygenase. Thus, it is crucial in the maintenance of collagen which represents about one-third of the total body protein. In an experimental study AA has been shown to have involvement in synthesis and release of type IV collagen into the culture medium [21]. Further, it has also been reported that AA 2-phosphate, a long-acting vitamin C derivative, stimulates both cell growth and the expression of mRNA for type III collagen in human osteoblast-like MG-63 cells and in normal human osteoblasts, as well as in human bone marrow mesenchymal stem cells, but not the expression of type I collagen in these cells [22]. However, in another study Kishimoto et al. [23] have observed that AA induced the expression of type 1 and type 4 collagen and SVCT2 in

cultured human skin fibroblasts. Collagen constitutes the principal protein of skin, bones, teeth, cartilage, tendons, blood vessels, heart valves, inter vertebral discs, cornea, eye lens. AA is essential to maintain the enzyme prolyl and lysyl hydroxylase in an active form. AA deficiency results in reduced hydroxylation of proline and lysine, thus affecting collagen synthesis. AA is also an essential co-factor for hydroxylations involved in the synthesis of muscle carnitine "β-hydroxybutyric acid" [24, 25]. Carnitine is required for the transport and transfer of long chain fatty acids into mitochondria for energy production. Further, AA is also a co-factor for the enzyme dopamine-β-hydroxylase, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine [19] and hence essential for the synthesis of catecholamines. In addition, AA catalyzes other enzymatic reactions involving amidation necessary for maximal activity of hormones oxytocin, vasopressin, cholecystokinin and alpha-melanotropin [26]. It is also involved in the transformation of cholesterol to bile acids as it modulates the microsomal 7α-hydroxylation, the rate limiting reaction of cholesterol catabolism in liver [27]. Deficiency of AA affects this conversion and as a result cholesterol accumulates in the liver leading to hypercholesterolemia [28, 29], cholesterol gall stones formation etc [30, 31].

Vitamin C and Common Cold

Apart from the well accepted role of vitamin C in the prevention of scurvy, the most widely known health beneficial effect of AA is in the prevention and relief of common cold. Pauling was the first to introduce the concept of high dose of vitamin C and suggested that ingestion of 1–3 g of AA effectively prevents/ameliorates common cold [32]. The role of oral vitamin C in the prevention and treatment of cold however remains controversial despite many controlled studies [33]. A number of clinical trials with varying doses of AA showed that it does not have significant prophylactic effect, but reduces the severity and duration of symptoms of cold during the period of infection. Randomized and non-randomized trials on role of vitamin C in prevention and treatment of the common cold indicated that AA in dose of 1 g/day during severe winter months produced no beneficial effect on the incidences of common cold [34]. In both preventive and therapeutic trials, there was a consistent beneficial but generally modest therapeutic effect on the duration of cold symptoms. There was no clear indication of the relative benefits of different regimes of vitamin C doses. However, in trials that tested vitamin C after cold symptoms occurred, there was some evidence of greater benefits with larger doses than with lower doses [34, 35]. Attenuation of immunity in common cold is well known. There has been a continuous debate about the role of AA in boosting immunity during rhinitis. AA has been shown to stimulate immune system by enhancing T-cell proliferation in response to infection. These cells are capable of lysing infected targets by producing large quantities of cytokines and by helping B cells to synthesize immunoglobulins to control inflammatory reactions. Further, it has been shown that AA blocks pathways that lead to apoptosis of T-cells and thus stimulate or maintain T-cell proliferation to attack the infection. This mechanism has been proposed for the enhanced immune response observed after administration of vitamin C during rhinitis [36, 37].

Vitamin C and Tissue Healing

It is also fairly understood that wound healing requires synthesis and accumulation of collagen and subsequent cross-linking of the fibre to give new tensile strength to the damaged tissue. An early study demonstrated that maximum tensile strength of scar tissue in guinea pig was achieved after supplementation of vitamin C [38]. Since then various studies have been carried out to evaluate the role of AA in wound repair and healing/regeneration process as it stimulates collagen synthesis. Adequate supplies of AA are necessary for normal healing process especially for post-operative patients because there is rapid utilization of AA for the synthesis of collagen at the site of wound/burns during post-operative period hence, administration of 500 mg to 1.0 g/day of AA are recommended to accelerate the healing process [39]. Of late, Jagetia et al. [40] demonstrated that AA pre-treatment was beneficial in healing of irradiated wounds and suggested a vitamin C related therapeutic strategy to accelerate wound repair in such conditions and in the cases of combined injury situation.

Vitamin C and Iron

AA is known to enhance the availability and absorption of iron from non-heme iron sources [41]. It's supplementation is found to facilitate the dietary absorption of iron. The reduction of iron by AA has been suggested to increase dietary absorption of non-heme iron [42, 43]. Vitamin C rich fruits such as goose berry has been reported to increase the bioavailability of iron from staple cereals and pulses [44]. Recent observations are of the view that vitamin C inhibits the expression of hepcidin and by affecting erythropoietin receptor in HepG2 cells and the bioavailability of iron provides protection against anaemia due to iron deficiency [45]. Darius Lane et al. [46], has considered ascorbate as a novel modulator for the classical transferrin

Fe^{+} uptake pathway, acting through intracellular reductive mechanism. It is also well known that AA acts as a pro-oxidant in invitro in the presence of redox-active iron and might contribute to the formation of hydroxyl radical, which eventually may lead to lipid, DNA or protein oxidation [47]. Thus vitamin C supplementation in individuals with high iron and or bleomycin detectable iron in some preterm infants could be deleterious due to the production of oxidatively damaged molecules [48–51]. However, Proteggente et al. [52] have observed no pro-oxidant effect of AA supplementation on DNA damage in presence or absence of iron.

Vitamin C and Fertility

Vitamin C has been used in the management of male infertility on empirical grounds, particularly in the presence of non-specific seminal infections [53]. It's presence in the seminal plasma of healthy adults in high concentration, ranging from 2.5 to 12 mg/dL, has been reported by various authors [54, 55]. However, the precise role of vitamin C in relation to male reproduction is not yet clear. Chinoy [56] stated that AA was essential for the structural and functional integrity of androgen-dependent reproductive organs. Low concentration of vitamin C showed marked degenerative changes in the testes, epididymis and vas deferens of scorbutic guinea pigs [57]. Besides degeneration of the spermatogenic epithelium, steroidogenesis and plasma testosterone level also showed a decline [58]. On the other hand, excessive intake of vitamin C has been reported to cause reproductive failure in the males [59]. However, Sapra et al. [60] could not observe any definite effect of vitamin C supplementation on Leydig cells in guinea pigs. AA as the principle antioxidant in seminal plasma of fertile men, contributes up to 65 % of its total chain breaking antioxidant capacity [61]. It's concentration in seminal plasma is almost ten times higher than plasma concentration. In various studies AA content in seminal plasma of fertile and infertile men was found to be significantly different [62, 63] and the percentage of sperm with normal morphology correlated significantly with seminal AA in both the groups [63–65]. AA deficiency may lead to an increase in oxidative damage induced by reactive oxygen species (ROS) and increased ROS was observed in the semen of 25–45 % of infertile men [66]. This is further corroborated by association of decreased AA with increase seminal plasma lipid peroxidation as observed in human trial [67]. Others have also observed oxidative stress induced deleterious effect on male fertility [68, 69]. Increased free radicals in the seminal plasma of infertile subjects by lowering the effective vitamin C levels may potentiate the deleterious effects that result in abnormal sperm parameters [70, 71]. Further studies report that supplementation of AA leads to significant reduction in ROS concentration [72, 73], sperm membrane lipid per-oxidation [73] and sperm DNA oxidation [74] and increased sperm quality [72–74]. The results of a recent animal experimental study indicated that, vitamin C improves antioxidant enzymes activity and significantly reduce MDA in testis compared with the test group [75]. Vitamin C supplementation as antioxidant in dose dependent manner in men may improve sperm quality [76]. It's supplementation also increases progesterone levels in infertile women with luteal phase defect [77].

Vitamin C and Atherosclerosis

There are several publications on the role of vitamin C in lipid metabolism and atherogenesis with diverse observations. The significance of dietary inadequacy of vitamin C in the aetiology of dyslipidemia and atherosclerosis first became apparent from the clinical studies of Myasnikova in 1947. The study showed lowering of cholesterol level by administration of AA in the hypercholesterolemic patients [78]. Since then several authors have also persuaded similar studies. One reviewed the evidence for the role of vitamin C in bile acid synthesis [27] while others gave particular emphasis on the potential involvement of vitamin C in pathogenesis of atherosclerosis [79, 80]. There are reports indicating increase in total body cholesterol and hypercholesterolemia in acutely scorbutic guinea pigs. However, some studies could not observe any effect of vitamin C in similar animal models [81]. Das et al. [82] observed that administration of AA lowers blood cholesterol, triglycerides, lipid per-oxidation and increases HDL cholesterol. Most of the earlier studies were conducted using rabbit as an animal model for examining vitamin C deficiency. As such rabbit is not a suitable model for such studies as it can synthesize AA unlike higher primates and human beings. It is difficult to elicit vitamin C deficiency in animal models. In view of the conflicting observations based on the acutely scorbutic animal model chosen by most of the workers, Ginter et al. [83] designed a model of chronic latent vitamin C deficiency in guinea pigs. This model in contrast to others, enabled the effect of AA deficiency on lipid metabolism and atherosclerosis to be followed in long term experiments. In protracted hypovitaminosis C lasting for 10 weeks, there was a considerable accumulation of cholesterol in liver and also increased concentration in serum [83–85].

It was also reported that the deficiency of vitamin C leads to enhanced accumulation of cholesterol in thoracic aorta along with pathomorphological changes in blood vessels [83, 86, 87]. Various human trials have also

indicated vitamin C induced reduction in blood lipid levels in normal and hypercholesterolemic subjects [88, 89]. Marc and Kothari and Sharma have further observed that vitamin C administration causes significant reduction in LDL and increase in HDL [87] and there by provides protection against CAD [87, 90]. Similar observations have been given by others also [91–96]. Chronic AA deficiency in man can lead to impaired cholesterol metabolism resulting in atheromatous changes in the vascular system [87]. This is further supported by the observation that vitamin C lowers cholesterol [88] and reduces the risk of developing cardiovascular disease (CHD) [97, 98]. Numerous studies have also looked into the association between AA intake and blood lipids. A large prospective epidemiological study in Finnish men and women suggested that high intake of AA was associated with a reduced risk of death from CHD in women than in men [98]. Similarly, several other studies showed that high intake of AA in American men and women appeared to benefit only women [97, 99]. Yet, another cohort study suggested that cardiovascular mortality was reduced in both sexes by vitamin C [100]. It is likely that cholesterol lowering effect of vitamin C is affected by several factors like initial cholesterol levels, age and sex of the subjects, dose and mode of the administration. The influence of age may be important because SAA levels have been found to be lower in elderly as compared to adolescents [101, 102] and therefore elderly subjects could be more responsive to the administration of vitamin C. In UK, a study showed that the risk of stroke in those with highest intake of vitamin C was only half that of subjects with the lowest intake. No evidence is suggestive of lower rate of CHD in those with high vitamin C intake [103]. A recent meta-analysis study on the role of AA and antioxidant vitamins also showed no evidence of significant benefit in prevention of CHD [104]. Thus, no conclusive evidence is available on the possible protective effect of AA supplementation on CHD.

Increased attention is being paid to involvement of low density lipoprotein (LDL) in atherogenesis. There are reports indicating that lipid peroxidation and oxidative modification of LDL are implicated in development of atherosclerosis [105]. Vitamin C provides protection against oxidative changes in LDL in different types of oxidative stress including metal induced oxidative stress [106]. Addition of iron to plasma devoid of AA resulted in lipid peroxidation, whereas endogenous and exogenous AA was found to inhibit the lipid oxidation in iron-over loaded human plasma [107]. In an invitro study, when AA was added to human serum supplemented with Cu^{2+}, antioxidant activity were observed rather than pro-oxidant effects [108]. AA is known as important antioxidant that scavenges free radicals and thus primarily prevents the oxidation of LDL in aqueous medium [109]. In addition, invitro studies have also shown that AA strongly inhibits LDL oxidation by vascular endothelial cells at physiological concentrations [110–112]. An important factor that initiates atherosclerosis is the adhesion of leukocytes to the endothelium. Invivo studies have shown that AA inhibits leukocyte-endothelial cell interactions induced by cigarette smoke [113, 114] or oxidized LDL [115]. Further, lipophilic derivatives of AA showed protective effect on lipid-peroxide induced endothelial injury [116]. In endothelial cells, AA prevented atherogenic modification of mildly oxidized LDL [110] and preserved α-tocopherol in both cells and LDL [117]. Although AA may not reverse established atherosclerosis, it can prevent the endothelial dysfunction that is the earliest sign of many vascular inflammatory conditions. AA is responsible for increased endothelial cell proliferation and also checks tumour necrosis factor (TNF) alpha induced endothelial cell growth inhibition. Vitamin C as antioxidant helps in endothelial cell proliferation and also correlated with expression of collagen IV in endothelial cells. Study has also shown that when proliferating endothelial cells were treated with AA, increased retinoblastoma protein (Rb) phosphorylation was observed with decreased level of p53 as compared to untreated cells. Furthermore, the addition of AA to TNF-alpha-treated proliferating endothelial cells blocked both the inhibition of retinoblastoma protein phosphorylation and enhanced p53 expression induced by TNF-alpha and TNF-alpha-induced apoptosis [117].

A number of studies were carried out in humans to determine the protective effect of AA supplementation (500–100 mg/day) on invivo and exvivo lipid peroxidation in healthy individuals and smokers with inconclusive findings. AA supplementation showed reduction or no change in lipid peroxidation products [111, 118–122]. There are reports where vitamin C has been found to decrease LDL peroxidation even in passive smokers [123]. In this context, it is important to note that during exvivo LDL oxidation, AA is removed at initial stage of LDL isolation from plasma therefore, no change in exvivo appears [124]. May and Li, examined the role of vitamin C in oxidation of LDL which causes endothelial dysfunction, an important early manifestation of atherosclerosis. They observed that up-regulation of endothelial cell SVCT2 expression and function may help to maintain intracellular ascorbate during oxLDL-induced oxidative stress, and that ascorbate in turn can prevent this effect [125]. Overall, both invitro and invivo experiments showed that AA protects isolated LDL and plasma lipid peroxidation induced by various radicals or oxidants generating systems. However, there are reports in experimental animals that large doses of exogenous iron and AA promote the release of iron from iron binding proteins and also enhance invitro

lipid peroxidation in serum. This finding supports the hypothesis that high intake of iron along with AA could increase invivo lipid peroxidation of LDL and therefore could increase risk of atherosclerosis [126]. However, Chen et al. [127], demonstrated that ascorbic acts as an anti-oxidant towards lipids even in presence of iron load invivo systems. Vitamin C also helps in prevention of atherosclerosis by strengthening the artery walls through its participation in the synthesis of collagen and by preventing the undesirable adhesion of white blood cells to damaged arteries [128–132].

Vitamin C and Cancer

The notion that vitamin C may have a preventive role in cancer was first proposed in 1949. It was demonstrated by Cameron et al. [133–135], that high-dose vitamin C improved the survival of patients with terminal cancer. However, the first documented study in which vitamin C was administered to cancer patients was carried out in the 1970s, by Pauling and Cameron. They gave 10 g (10,000 mg) of vitamin C per day to 100 terminally ill cancer patients and compared their outcome with 1,000 cancer patients who were given conventional therapy. It was observed that 10.3 % cancer patients receiving vitamin C survived while all patients on conventional therapy without vitamin C died [134]. Other studies have also confirmed these findings. Murata and Morishige showed in a study conducted on Japanese patients with uterus cancer receiving 5–30 g of vitamin C that these patients survived six times longer than those on vitamin C <4 g per day. When comparison was made between those supplemented with or without vitamin C, survival rate was 15 % higher in those supplemented with vitamin C [136]. The overwhelming evidence supports that a high intake of vitamin C is linked with a low risk for cancer of oesophagus, oral cavity, stomach, pancreas, cervix, rectum and breast [137, 138] and also non-hormonal cancers [139]. One of the most important amenable determinants of cancer risk is diet. Several research panels and committees have independently concluded that high fruit and vegetable intake reduces the risk of different types of cancer [140, 141] and mortality rate was also found to be inversely related to vitamin C intake [142, 143]. However a study involving 34,000 post-menopausal women, reported no such association between the intake of vitamins A, C and E and a reduced risk of developing breast cancer [144]. Intravenous vitamin C has also been reported to have beneficial effect in advanced cancer [145]. Several mechanisms proposed indicating involvement of vitamin C in the treatment and prevention of cancer are: enhancing the immune system; stimulating the formation of collagen; preventing metastasis (spreading) by inhibiting enzymes; preventing viruses that can cause cancer; correction of vitamin C deficiency which is often associated with cancer patients; wound healing in cancer patients after surgery; enhancing the effectiveness of chemotherapy; reducing the toxicity of chemotherapy; preventing free radical damages and neutralising some carcinogens [146].

Recently a number of experimental studies have observed that different types of cancer cells either do not grow at high vitamin C concentration or it leads to tumour shrinkage [147, 148]. Further recent experimental studies have also found that ascorbate supplementation hinders metastasis, tumour growth and inflammatory cytokine secretion as well as enhanced encapsulation of tumours in Gulo KO mice [149, 150]. Reports have shown that intravenous injection increases vitamin C concentration more than 70 times in relation to oral administration and effectiveness of treatment is linked to vitamin C concentration [12, 145]. Thus controversy is because of mode, dose and duration of administration.

Newly available pharmacokinetic data improved the understanding of the regulation of vitamin C transport, and the growing evidence on the therapeutic efficacy of vitamin C. This has stimulated interest to reassess the feasibility of using vitamin C in the prevention and treatment of cancer. Though different in their methodologies, most recent studies on vitamin C and cancer have two central themes:(1) the effects of high-dose AA on the development and progression of tumours; and (2) the mechanisms of action that may contribute to the anti-cancer effect [144]. Research has also refocused on the implications and applicability of high i.v. dose of vitamin C in cancer therapy. In contrast to normal physiological concentration of AA (0.1 mmol/L) pharmacological concentrations of AA (0.3–20 mmol/L) selectively targets and kills tumour cells in invitro. This tumour-killing phenomenon is attributable to the pro-oxidant property of vitamin C, which, at high concentration mediates the production of hydrogen peroxide thus provides a potential mechanism of action for the anti-tumour effect of vitamin C and it's implication as a pro-drug in cancer treatment [145, 147]. However, it is difficult to assess the precise contribution of vitamin C in the clinical outcome, as subjects under examination simultaneously receive different therapeutic treatments [151]. Therefore, the therapeutic value of high-dose vitamin C administration in cancer progression or remission is not unequivocally supported but i.v. administration of vitamin C in high doses improves the health-related quality of life even at the advanced stage of the disease [152].

Vitamin C and Diabetes

Diabetes is becoming a pandemic and numbers are expected to rise to 366 million (4.4 % of the global

population) by 2030 [153]. In diabetic patients, long-term damage, dysfunction, and failure of different organs, especially the eyes (diabetic retinopathy), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart (myocardial infarction), and blood vessels (atherosclerosis) are related to uncontrolled hyperglycaemia [154–156]. Hyperglycaemia induces oxidative stress [157] primarily by ROS [18]. There is convincing experimental and clinical evidence that the generation of ROS increases in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress [158]. Vitamin C has been associated with decreased risk of developing diabetes mellitus (DM). In Norfolk Prospective Study the association between fruit and vegetable intake and plasma levels of vitamin C and risk of type 2 DM was established [159]. During 12-years of follow-up, 735 incident cases of diabetes were identified among nearly 21,000 participants. A significant inverse association was found between plasma levels of vitamin C and risk of diabetes (odds ratio = 0.38, 95 % confidence interval: 0.28–0.52) [159]. This is further supported by a study with longer follow up of 23 years which reported that antioxidants induced risk reduction of type 2 diabetes [160] and vitamin C level was found to be significantly lower in both insulin dependent and non dependent diabetes [161, 162]. Vitamin C reduces fasting and postprandial oxidative stress [163]. Sharma et al. have observed reduced vitamin C levels in diabetic subjects. They further reported that vitamin C level is also associated with various components of metabolic syndrome and with the increment in component there is a sharp reduction in vitamin C level [164]. In recent experimental studies it has been found that Vitamin C and E supplementation relieves oxidative stress in the blood and tissues of diabetic aged rats by modulating the antioxidant system and lipid profile [165, 166].

Diabetes is associated with various micro vascular and macro vascular complications. Hyperglycaemia in diabetes is responsible for micro vascular ROS generation which causes endothelial dysfunction [167] and vitamin C blocks acute hyperglycaemic impairment of endothelial function in diabetic subjects [168]. One of the most important micro vascular complications is diabetic nephropathy. According to statistical prediction, out of 30 million patients with diabetes in India, diabetic nephropathy is expected to develop in 6.66 million [169]. Qin et al. [170] reported that vitamin C supplementation significantly decreased podocyte injury in diabetic rats. Perhaps AA protects podocyte by increasing antioxidative capacity and ameliorating the renal oxidative stress [171]. The role of vitamin C in diabetic retinopathy has also been reported in various studies. Vitamin C and E supplementation reduces neovascularization, prevent the inhibition of retinal glutathione reductase, glutathione peroxidase and superoxide dismutase activities; hence vitamin C and E prevent oxidative stress induced retinopathy [172–174]. Neuropathy is also one of the micro vascular complications often manifested in uncontrolled DM. Some studies report that the role of vitamin C in diabetic neuropathy is not as well pronounced as other antioxidants [175]. Some suggest that the AA levels are significantly low in diabetic polyneuropathy patients [176]. Role of vitamin C and other dietary antioxidants have been reviewed by several authors with controversial findings [177].

Vitamin C and Immunity

Vitamin C affects several components of the human immune system. Vitamin C appears to play a role in a number of neutrophil functions including increased chemotaxis, increased particulate ingestion, enhanced lysozyme-mediated non-oxidative killing, protection against the toxic effects of superoxide anion radical, inhibition of the halide-peroxide-myeloperoxidase system without a pronounced bactericidal effect, and stimulation of the hexose monophosphate shunt [178].

The role of vitamin C seems to be more pronounced in cell mediated response instead of humoral immunity as the T-cell hyporesponsiveness was observed to be reversed in Crohn's disease patients on oral supplementation of vitamin C. In the same study no effect was observed on humoral immunity [179]. Another study supports the fact that vitamin C acts with other micronutrients synergistically and enhances skin barrier function as well as protective activities of immune cells but its role in antibody protection is not as pronounced [180]. On the contrary animal studies support the role of supplementation of vitamin C in humoral immunity as it increases serum levels of antibodies [181] and C1q complement proteins [182] in guinea pigs, which cannot synthesize vitamin C like humans and hence depend on dietary supplementation. Vitamin C along with other micronutrients help in reverting potential damage caused by free radicals at cellular level and modulates immune cell functions through regulation of redox-sensitive transcription factors and affects production of cytokines and prostaglandins. Adequate intake of vitamins C along with other vitamins and micronutrients like B_6, folate, B_{12}, E, selenium, zinc, copper, and iron supports a Th1 cytokine-mediated immune response [183, 184] with sufficient production of proinflammatory cytokines, which maintain an effective immune response. Supplementation with these micronutrients reverses the Th2 cell-mediated immune response to Th1 cytokine-regulated response with enhanced innate immunity [183].

Vitamin C inhibits the excessive activation of the immune system to prevent tissue damage. It also supports antibacterial activity, stimulates natural killer (NK) cells

and differentiation of Th0 subset into Th1 subset [184, 185]. In addition, vitamin C also modulates synthesis of proinflammatory cytokines, or expression of adhesive molecules [185].

Mikirova et al. [186] have demonstrated that intravenous vitamin C treatment reduces pro-inflammatory cytokines IL-1α, IL-2, IL-8, TNF-α, chemokine eotaxin and CRP in cancer patients. Several studies have shown that the modulation of inflammation by intravenous vitamin C correlated with decrease in tumour marker levels [186–188]. Studies conducted on human subjects reported that that plasma vitamin C and dietary intakes of vitamin C are inversely associated with some markers of the acute phase response and haemostasis that have been associated with greater risk of CVD and non-vascular disease. Plasma vitamin C, fruit intake, and dietary vitamin C intake were significantly and inversely associated with mean concentrations of C-reactive protein, an acute phase reactant, and tissue plasminogen activator antigen, a marker of endothelial dysfunction, even after adjustment for confounders. The findings suggest that vitamin C has anti-inflammatory effects and is associated with lower endothelial dysfunction in men with no history of CHD or diabetes [189–196]. Vitamin C concentrations in the plasma and leukocytes rapidly decline during infections and stress. Supplementation of vitamin C has been shown to improve components of the human immune system such as antimicrobial and NK cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity as discussed above. Vitamin C contributes in maintenance of the redox integrity of cells and thereby protects them against ROS generated during the respiratory burst and in the inflammatory response [197].

Thus, vitamin C has diverse role as an antioxidant protecting the immune cells against intracellular ROS production during inflammatory response, acting as an enzymatic cofactor and maintaining tissue integrity and plays a crucial role in formation of skin, epithelial and endothelial barriers [185]. Of late vitamin C supplementation has been found to be beneficial in various inflammatory conditions.

Vitamin C and Heavy Metal Toxicity

Metals including iron, copper, chromium, and vanadium undergo redox cycling, while cadmium, mercury, and nickel, as well as lead deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of ROS as superoxide ion, hydrogen peroxide, and hydroxyl radical. As a consequence, enhanced lipid peroxidation, DNA damage, and altered calcium and sulfhydryl homeostasis occur [198]. Various experimental studies report the beneficial effect of vitamin C against heavy metal toxicity. Lead is considered as one of the common environmental poison in which protective role of vitamin C is extensively studied. A recent experimental study based on histopathological examination revealed the diminution of detrimental effects of chronic lead intoxication on liver, kidneys, brain and testes [199]. In another study lead induced electrophysiological changes were inhibited in rat colon by AA administration [200]. The beneficial effect of AA on lead concentrations in human studies is however inconclusive. A large survey comprising of 19,578 participants (6–90 years) without prior history of lead poisoning reported that blood levels of AA are inversely related to plasma AA and recent dietary intake had no influence on blood levels. This study surmises that there may be a protective relationship between AA and lead [201].

Arsenic toxicity is essentially associated with lipid peroxidation and oxidative stress. Arsenic in drinking water may even cause chromosomal aberration leading to molecular disorders [202]. Arsenic exposure during gestation and lactation leads to significantly increased lipid peroxidation in the rat brain which was reversed by supplementation of vitamin C, E and Zn [203]. In another study arsenic induced normocytic and normochromic anemia as well as a significant increase in hemolysis, TBARS production, catalase activity, hyperlipidemia, and impairment in renal functions in mice pups during gestation and lactation which was partially reverted by the administration of AA [204]. Arsenic induced hepatotoxicity has also been reported by recent experimental studies which have suggested that vitamin C supplementation improves mitochondrial structure and function along with restriction of apoptosis due to caspase-3 inhibition in arsenic trioxide exposed rat liver. The overall report is of view that vitamin C and vitamin C rich fruits such as goose berry provides protection against metal induced hepatotoxicity [205, 206].

Cadmium is an extremely toxic metal commonly found in industrial workplaces similar to lead and arsenic also causes lipid peroxidative changes in various tissues. An experimental study discussed protective role of vitamin C supplementation in lung and brain of rat exposed to excessive cadmium [207]. Vitamin C also reverted haematological changes in mercury and cadmium exposed Wistar rats [208]. Vitamin C was also observed to be protective against concomitant exposure to heavy metal and radiation in another experimental study [209].

Vitamin C and Neurodegenerative Disorders

Schizophrenia is one of the major neurological disorders associated with great deal of morbidity and economic burden. It is a multifactorial disease and hence has a poor outcome in spite of the best available treatments. It is worth

mentioning that simple water soluble vitamin C, adequately present in fruits and vegetables had drawn attention of the psychiatrists almost seven decades ago for the treatment of schizophrenia. A study conducted on 12 schizophrenics showed that urinary excretion of vitamin C was significantly lower than healthy controls and intravenous injection of large dose of vitamin C produced improvement in mental condition in 75 % of patients [210]. In another study it was observed that vitamin C level was significantly low in plasma and urine of schizophrenics as compared to normal controls. Administration of vitamin C improved plasma vitamin C level and thus concluded that schizophrenic patients require higher levels of vitamin C than the suggested optimal AA requirement for healthy individuals [211]. Several investigators have implicated role of increased free radical generation in pathogenesis of schizophrenia. Alteration in the optimum activities of antioxidant enzymes [212–214] and related parameters of lipid peroxidation [215, 216] in blood have been detected in schizophrenics. Brain contains large amount of unsaturated fatty acids, catecholamines and monoamines, which are the target molecules for lipid peroxidation [217, 218]. Brain is rich in iron containing compounds and thus it is an easy target for lipid peroxidation through the formation of hydroxyl radicals. Monoamine and catecholamine oxidation also produces superoxide anions in the brain [219]. AA, an antioxidant vitamin, plays an important role in protecting free radical-induced damage in the brain. Dadheech et al. [220] reported the antioxidant deficit in schizophrenics and it was associated with increased MDA level in blood which is a marker for lipid peroxidation. Vitamin C is present in dopamine dominant areas at high concentrations in the brain tissue as compared to other organs [221, 222]. Recently, Arvindakshan et al. [223] reported reduction in brief psychiatric rating scale (BPRS) and positive and negative syndrome scale score after supplementation with omega-3 fatty acids, vitamin C, and vitamin E. A decrease in the levels of tocopherol, total AA and reduced glutathione was found in schizophrenics compared to normal controls. Further a significant rise in oxidative stress and decreased antioxidant status was observed in the chronic stage of schizophrenia as compared to those in acute condition. A significant rise in dehydroascorbic acid with concomitant fall in reduced AA suggests scavenging action of AA and its utilization with increased oxidative stress as indicated by high blood malondialdehyde levels. Leucocyte AA, a better index of AA status was also found to be reduced in schizophrenics, suggesting depletion of body stores of AA and the condition worsened with advancing age [224].

Very few studies have examined the effect of vitamin C with typical antipsychotics in the treatment of schizophrenia. Oral supplementation of vitamin C with antipsychotic reverses AA levels, reduces oxidative stress, and improves BPRS score, hence both the drugs in combination can be used in the treatment of schizophrenia [225]. The findings of another study suggest that antioxidant supplement therapy as an adjuvant therapy is useful in patients with stress-induced psychiatric disorders [226]. There are also reports advocating beneficial effect of vitamin C in neurodegenerative disorders including Alzheimer's disease. Overall there is large body of evidence supporting that maintaining healthy vitamin C level can have a protective function against age related cognitive decline but avoiding vitamin C deficiency is likely to be more beneficial than taking supplements on top of normal healthy diet [227].

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