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Antioxidant Vitamins, Oxidant Injuries and Diseases

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Abstract – Over the past few decades antioxidant vitamins have been shown to aid in disease prophylaxis as well as treatment. Deficiencies of these vitamins in diets have resulted in associated deficiency syndromes in both humans and animals. Since a handful of disease conditions is associated with imbalances of antioxidant enzymes such as catalase, superoxide dismutase, glutathione as well as increases in reactive oxygen species (ROS), nitrogen oxide species (NOS) and lipid per-oxidation markers such as malondialdehyde, supplementation with antioxidant vitamins has resulted in amelioration of oxidative damage and ultimately disease recession. Vitamins A, C and E together with compounds such as carotenoids have been extensively studied for their roles in disease modulation or exacerbation. However, while Vitamins C and E have been shown to have immense potentials in the alleviation of several conditions, Vitamin A and especially carotenoids had shown little or no use in conditions such as cardiovascular disease and cancer prevention. This review highlights the documented roles of these vitamins in disease prevention over the past few decades and the potentials that need to be explored further.

Keywords: Antioxidant vitamins, disease, oxidative stress, antioxidant enzymes

Introduction

An antioxidant is any substance that significantly reduces or impairs the oxidation of a substrate once present in small amounts. The term *oxidizable substrate* includes every type of molecule found *in vivo* (Halliwell, 2001; Niki, 2014). Antioxidant vitamins are readily available in food items; however, most of them are not available in sufficient quantities to meet the daily recommended amount for humans or animals. Thus, in order to avert problems associated with chronic deficiencies, supplemental doses have to be taken to meet up the shortages. Most of the classical deficiency syndromes associated with these vitamins are not seen in the western countries. However, chronic deficiencies are common and associated with a lot of complex health problems especially in the elderly (Iqbal et al., 2004; Rao & Rao, 2007).

Oxidant injuries to cells result from production of reactive oxygen species (ROS) and nitrogen oxides (NO). Reactive oxygen species such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) cause respiratory burst and damage to cells. On the other hand, cellular enzymes such as catalase, glutathione and superoxide dismutase (SOD) modulate various chemical reactions that prevent oxidant injury to the cell. For example, while catalase activates the breakdown of hydrogen peroxide into water and oxygen, SOD catalyzes the dismutation of superoxide radicals to O_2^- and H_2O_2 and glutathione plays a major role in the reduction of oxygen species formed during respiratory burst and cellular metabolism (Halliwell & Whiteman, 2004). Since various disease processes have been shown to induce oxidant injury in cells, the use of antioxidant vitamins have been equally shown to modulate such oxidant injuries and ameliorate its deleterious effects in the body (Blokhina et al., 2003; S. Zaidi & Banu, 2004; S. M. Zaidi et al., 2005). Thus, this review discusses the various antioxidant vitamins and their mechanisms of preventing certain illnesses or conditions.

Antioxidant vitamins

There are three main documented antioxidant vitamins, namely Vitamin A, Vitamin C and Vitamin E (α-tocopherol). Carotenoids (β-carotene) and selenium are also considered important since they have been shown to function synergistically with Vitamins A and E, respectively. Carotenoids are converted to Pro-Vitamin A and thus posses similar antioxidant roles in the physiological system to Vitamin A. This write-up also discusses the major antioxidant Vitamins A, C and E, as well as carotenoids and their mechanisms of action.

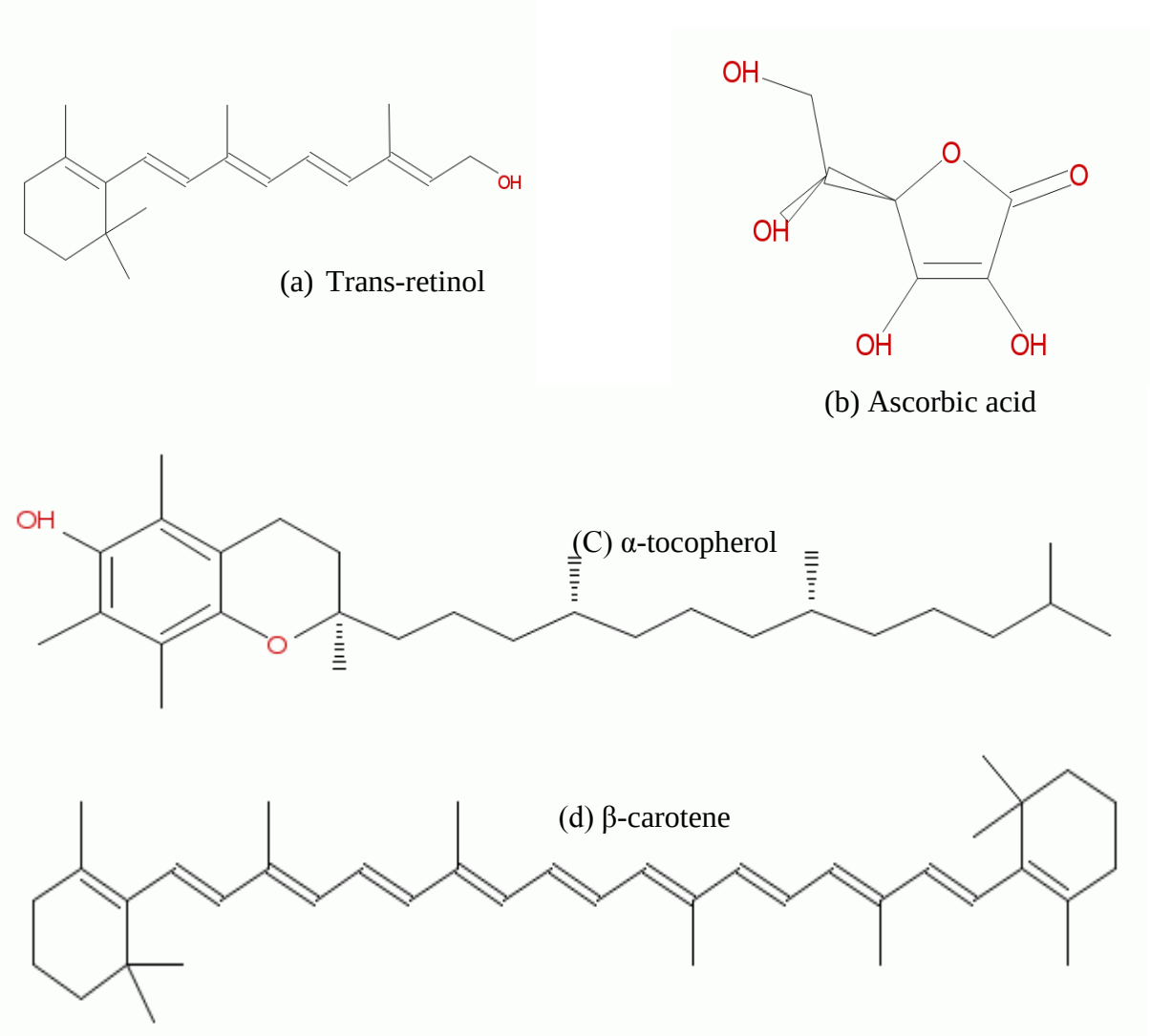

Figure 1: Chemical structures of (a) trans-retinol, (b) Ascorbic acid, (c) alpha-tocopherol and (d) β-carotene.

Mechanisms of antioxidant vitamin action

Aerobic microorganisms are shielded from ROS and NOS produced from oxidative stress by a diverse mechanism involving multiple antioxidants which have different functions and roles (Niki, 2014). While all antioxidants are either micro or macro molecules such as proteins and enzymes, they all proffer several defensive strategies against damage caused by oxidant damage (Halliwell & Whiteman, 2004). The first defensive action involves the prevention of ROS/NOS production by catalyzing the breakdown of hydroperoxidase and hydrogen peroxides to hydroperoxides and water. In the second defensive action, the antioxidants neutralize ROS/NOS before they induce cellular injury to the cells. Thirdly, damage caused to membranes and tissues are repaired by these antioxidant compounds or enzymes. Hence, antioxidants act cooperatively and synergistically in a dynamic defensive network to

cope with oxidative stress (Niki, 2014). Antioxidant defense against oxidative stress involves both enzymatic and non-enzymatic activities. While enzymatic defenses require enzymes such as glutathione peroxidase, catalase and superoxide dismutase, non-enzymatic defenses involve the presence of antioxidants such as carotenoids, lipoic acid, Vitamin C and Vitamin E. In order to minimize the effects of ROS, cells produce superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase catalyzes superoxide anions to oxygen and hydrogen peroxide, catalase then reduces the hydrogen peroxide formed to water. Ascorbate peroxidase and dehydroascobate peroxidase formed by ascorbic acid in the cell also catalyzes the breakdown of hydrogen peroxide to water (Blokhina et al., 2003). This is a very important step in the neutralization of the peroxide and in alleviating oxidative stress in the cell.

Vitamin A and β-carotene in disease prevention

Vitamin A is one of the most essential vitamins of the biological system. It is required for maintaining the injury of the epithelium, immune function and cellular differentiation. Retinol is the active compound that is responsible for its numerous functions in the body system. The primary storage of Vitamin A in the kidneys, liver and adipose tissues is in the form of long chain fatty esters and provitamins (carotenoids). Its antioxidant action is exhibited through its excellent radical quenching ability, thus making it more effective in hypoxic state (Harabawy & Mosleh, 2014; Palace et al., 1999). Vitamin A has not been as efficient as Vitamins C and E in regulating the levels of oxidative stress enzymes such as glutathione, catalase and superoxide dismutase in stressed cells.

In a study of chickens exposed to carbon tetrachloride, Vitamin A supplementation reduced glutathione levels in the plasma of chickens. However, while concurrent administration of Vitamin E was found to reduce plasma levels of SOD and glutathione, Vitamin A was found to attenuate this effect (Mahmoud & Hijazi, 2007). Similarly, it was earlier observed that retinol administration resulted in increased levels of SOD, catalase and glutathione peroxidase in rat sertoli cells thereby modulating oxidative enzyme activities (Dal-Pizzol et al., 2001). Vitamin A supplementation has been shown to lower the risk of coronary heart disease and ischemic heart disease. In another study, Vitamin A was observed to reduce injury and scarring following pyelonephritis (Dalirani et al., 2011). B-carotene, which is a precursor for retinol and a carotenoid has been shown to have no protective effects against various conditions. For example in a randomized trial study in 18,314 smokers and asbestos workers given 30 mg of beta-carotene and 25,000IU of Vitamin A per day, it was observed that there was no reduction in the incidence of lung cancer, and adverse effects were seen in cancer development and cardiovascular disease (Omenn et al., 1996). In another randomized trial, administration of 20mg/day of beta-carotene was reported to be associated with a slight increase in the incidence of angina pectoris in 29,133 male smokers (Rapola et al., 1996). In a related study, a metanalysis study on the effect of beta-carotene administration and lung cancer development found an association between high dose of beta-carotene and risk of cancer (Tanvetyanon & Bepler, 2008). However, in a 9.4-year randomized trial study testing the effects of supplementation of 50mg of beta-carotene given every other day to women, there was no overall effect of the supplementation on the outcomes of myocardial infarction, stroke, coronary revascularization or cardiovascular disease death (Cook et al., 2007).

Thus, it can be seen from these numerous studies that the vitamin has very little or no beneficial effect on the prevention of conditions such as cancer and cardiovascular disease. Earlier studies with carotenoids reported beneficial and protective effects of carotenoids against ischemic heart disease, stroke, cancer, aging, immunomodulation, macular degeneration, cataract and photo-protection (Mayne, 1996; Rao & Rao, 2007). However, the manner at which most of these studies were conducted such as source of carotenoids, dose and duration of exposure were variable, and in most cases the studies were irreproducible. Nevertheless, these studies have contributed to the literature of the compound (Mayne, 1996; Rao & Rao, 2007).

Vitamin C and its prophylactic role in disease

Vitamin C (ascorbic acid) is a commonly available compound that acts effectively as an antioxidant and reducing agent at physiological pH, thus neutralizing the effect of reactive oxygen species (ROS)

in the body. This vitamin is required in many other processes such as collagen synthesis, synthesis of L-carnitine and conversion of dopamine to nor-epinephrine. Most animals can synthesize ascorbic acid physiologically, but humans, primates and hamsters cannot synthesize this vitamin because of the deficiency of gulonolactone oxidase. Hence, Vitamin C is unable to meet their daily requirements and they need daily supplementation in order to avert deficiency syndromes such as scurvy, spontaneous bleeding and joint and muscle pains (Iqbal et al., 2004; Y. Li & Schellhorn, 2007). As a non-enzymatic antioxidant defense system, ascorbic acid plays a crucial role in the regulation of hydrogen peroxide levels in oxidative stress. It has been shown to work concurrently with glutathione and glutathione transferase, thus its presence may boost cellular glutathione levels. Since it does not have a direct role in lipid peroxidation, it may not significantly alter the level of malondialdehyde in oxidative stress (Padayatty et al., 2003). In addition, Vitamin C was found to regulate the levels of SOD, catalase, GST, GSH and MDA in immobilized stressed rats (S. M. Zaidi et al., 2005). This shows the potential of regulating increased level of MDA and decreased levels of SOD, catalase, GST and GSH by this vitamin. However, since different factors such as infection, injury and toxicity inflict oxidative stress via different mechanisms/paths, the amelioration of elevated enzyme levels reported has been conflicting (Padayatty et al., 2003).

Moreover, Vitamin C via its antioxidant properties have been shown to prevent or alleviate a lot of conditions including viral diseases. It has been found to alleviate hypoxia-reperfusion induced apoptosis via the release of Cytochrome C and the activation of Caspases 9 and 3 in human endothelial cells (Montecinos et al., 2007). In cancer cells and normal endothelial cells, it was found to alleviate oxidative stress by the uptake of cysteine (Park, 2013). Decreased lung pathology in mice and prevention/suppression of flu symptoms in man were also observed following ascorbic acid administration (Gorton & Jarvis, 1999; W. Li et al., 2006). Vitamin C has also been shown to arrest cancer cell development via both its antioxidant and pro oxidant effects (Y. Li & Schellhorn, 2007). The supplementation of Vitamin C in exercise-stressed rats was shown to cause reduction in the levels of previously elevated enzymes such as Copper SOD, magnesium SOD and glutathione (Ryan et al., 2010).

The *in vitro* and *in vivo* anti-viral effects of Vitamin C have been studied in a number of viral infections. In general, Vitamin C exhibits either a direct effect on the virus by inhibiting its replication or an indirect one by modulating ROs and NOS production by decreasing bioavailability of superoxide dismutase (SOD), inhibition of SOD mediated NO activation and by preserving the normal enzymatic activity of NOS (Brinkevich et al., 2012). *In vitro* viral inactivation from Vitamin C administration has been reported in the rabies virus (Madhusudana et al., 2004), cytomegalo virus (Cinatl et al., 1995), influenza virus (Cheng et al., 2012), herpes virus and paramyxo virus (White et al., 1986), and herpes simplex virus (Betanzos-Cabrera et al., 2004), just to mention a few.

The *in vivo* actions of Viatamin C have been reported in HIV, where ROS contributing to viral replication via the activation of NFK-B was attributed to changes in ascorbic acid, Vitamin E, carotenoids, selenium, SOD and glutathione. Overall concentrations were observed to decline in plasma despite an unchanged redox status during the infection course (Baker & Wood, 1992). Ascorbic acid has also been shown to increase levels of IL2, $CD4^+$, $CD8^+$, IgM^+ cells and anti IgG antibodies in vaccinated chickens challenged with infectious bursal disease virus (Wu et al., 2000). Decreased lung pathology in mice and prevention/suppression of flu symptoms in man were also observed following ascorbic acid administration (Gorton & Jarvis, 1999; W. Li et al., 2006).

Vitamin E (α-tocopherol) and disease prevention

There are eight isomers in the Vitamin E family namely α-, β-, γ-, and δ-tocopherols and tocotrienols (4 Tocopherols and 4 Tocotrienols). Several studies have been conducted on the pharmacological uses of these isomers since almost a century ago (1922), when the vitamin was first discovered. Vitamin E is a scavenger for active free radicals through the transportation of hydrogen atom to produce Vitamin E and radical free product. In some situations, Vitamin E may scavenge free radicals through a mechanism of electron transfer to yield a Vitamin E cation radical, which subsequently undergoes fast de-protonation to produce a Vitamin E radical. In other scenarios, Vitamin E scavenges lipid peroxyl

radicals and lipid hydroperoxide resulting in the formation of Vitamin E radicals (Niki, 2014). Although there are conflicting reports on its mode of action as an anti-oxidant, some believe that its major function is that of peroxyl radical scavenger, which is important for maintaining the integrity of membranes of cells (Traber & Atkinson, 2007). However, others believe its action are due to its antioxidant properties (Brigelius-Flohé & Davies, 2007). Since it has been shown that the most abundant and readily available form of Vitamin E is α-tocopherol, most of the studies were conducted using this isomer (Paul et al., 2012; Traber & Atkinson, 2007). Interestingly, other researchers argued that tocotrienols and other tocopherol isomers (δ and λ) are better than α-tocopherol especially in preventing cancer (Yang et al., 2012). As a potent lipid peroxyl radical, tocopherol has been reported to reduce the level of lipid peroxidation associated with oxidative stress in highly lipogenic tissues like the brain. Thus it was found to reduce the level of MDA in the tissues and alleviate tissue damage. Additionally, it was found to reduce the level of SOD, GST, catalase and glutathione (S. Zaidi & Banu, 2004). Vitamin E was also found to restore decreased SOD, catalase, GST, GSH and increased MDA, ALT and AST in rats after immobilization (S. M. Zaidi et al., 2005). In a related study, the vitamin was found to decrease SOD and GPx activities that were increased following restraint-induced stress (S. M. Zaidi et al., 2005).

Moreover, Vitamin E has been reported to reduce the incidence of cancer, aging, restoration of blood flow following ischemia and reperfusion injury, arthritis, cataract and platelet hyper-aggregability (Packer, 1991). Several human and animal trial studies have shown the beneficial roles of Vitamin E in the prevention of a number of diseases and conditions. However, some findings are contradictory in the sense that different research groups propose different findings. An example is in the case of prostate cancer; while some found it to have a preventive role (Klein et al., 2011; Virtamo et al., 2014), others found it to predispose more to the development of cancer (Albanes et al., 2014; Lippman et al., 2009). However, Yang et al. (2014) explained that the vitamin can have a double role in cancer prevention or potentiation due to the absence of other isomers in current therapeutic forms. In other studies, Vitamin E administration was found to reduce cardiovascular conditions and related deaths in women by 7% and 24%, respectively (Lee et al., 2005). However in a recent study, the administration of vitamin E did not show any beneficial effect in the prevention of cardiovascular disease in human participants (Myung et al., 2013). Other reported uses of the vitamin include the prevention of pro inflammatory state in calves and the enhancement of passive maternal antibody transfer (Krueger et al., 2014); prevention of vascular degeneration through reduction in homocysteine and cholesterol levels in rats (Kirac et al., 2013); reduction in inflammatory stressor; IL2, C-reactive protein and vascular endothelial growth factor which lead to atherosclerosis due to heavy and chronic alcohol consumption (Shirpoor et al., 2013); protection against contrast induced acute kidney injury in chronic kidney disease patients (Tasanarong et al., 2013); the reduction in renal scarring secondary to pyelonephritis in children (Sobouti et al., 2013); the reduction in the incidence of angina pectoris in male smokers (Rapola et al., 1996), and the prevention of mono sodium glutamate induced renal toxicity in rats, among many other preventive functions (Paul et al., 2012).

Synergistic actions of antioxidant vitamins in prophylaxis

Several antioxidant vitamins have been shown to have synergistic effects when combined together. For instance, Vitamins A, C and E prevented the gastro-esophageal disease, Barret's esophagus and esophageal carcinoma (Lukic et al., 2012), while other observed a low incidence of in heavy metal toxicity following the administration of a combination of Vitamins A, C, E and selenium in fish, as compared to administration of a single vitamin (Harabawy & Mosleh, 2014). In a 9.4-year randomized trial study testing the effects of a combination of 600IU of Vitamin E and 500mg of Vitamin C on women, there were fewer incidences of stroke in participants taking a combination of the two vitamins as compared to those taking either of the two (Cook et al., 2007). These few examples show the compatibility these vitamins have in the biological system and they may act better together than alone, in some instances.

Conclusion

Antioxidant vitamins play important roles in the biological system as they counteract the oxidative stress and imbalances in the system produced by excess ROS and NOS production. Vitamins E and C

are most effective in ameliorating decreased levels of SOD, GSH, GPx and catalase, while they were also found to modulate increased levels of MDA. Vitamin A has so far been shown to be poor in modulating these enzyme alterations. Therefore, the safety of prolonged usage of this vitamin in humans and other animals still needs to be evaluated as several reports have shown them to be either inefficient or even increase mortalities in certain trail cases (Bjelakovic et al., 2012).

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Author Contribution
All authors contributed equally to this work and have read and approved the final manuscript.

Conflict of Interest
None to declare

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