PRETHODNA SAOPŠTENJA

ANTIOXIDANT AND PRO-OXIDANT EFFECT OF ASCORBIC ACID

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Free radicals attack proteins, lipids, enzymes and DNA causing pathological changes in organism. There are many mechanisms that organism uses to fight against free radicals. Ascorbic acid is one of the strongest reducers and eliminators of free radicals. It reduces stable oxygenic, azoth and thyol radicals and acts as a primary defense against water radicals in blood. When radicals are dissolved in water suspensions of erythrocytes and low density lipoproteins (LDL), ascorbic acid catches and eliminates free radicals before they arrive to the membrane and LDL molecules. Even though ascorbic acid is not capable of eliminating free radicals out of fluid medium, it acts as synergist to alpha-tocopherol in lipid section, contributes to the lessening of lipid tocoperoxil radicals, and above all, regenerates alpha-tocopherol. Ascorbic acid may act as pro-oxidant under in vitro conditions in the presence of metals; however, this effect is probably not important under in vivo conditions where metal ions, being sequestered, become second reducers.
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Introduction

There is increasing evidence that oxidative stress leads to many biochemical changes and is an important contributing factor in several human chronic diseases (1), such as atherosclerosis and cardiovascular diseases (2), mutagenesis and cancer (3), several neurodegenerative disordes, and probably the aging process per se (4). We know that lipid peroxidation products and oxidized forms of low density lipoproteins accumulate in atherosclerotic lesions, and that numerous modified DNA bases are formed under conditions of oxidative stress and are highly mutagenic. For many of these oxidatively modified biomolecules, there are repair enzymes, including numerous peroxidases that reduce lipid hydroperoxides to their corresponding alcohols and glycosylases that remove specific DNA lesions. In addition to repairing oxidatively damaged biomolecules, another layer of defense against oxydative stress and resultant damage is to prevent formation of reactive oxygen and nitrogen species or to scavenge these species before they can cause oxydative damage to biomolecules (5). Among these defenses are antioxidant enzymes, which are mostly intracellular and include several forms of superoxide dismutases and catalase.

The antioxidant enzymes are complemented by small-molecule antioxidants, some of which are derived exclusively from the diet and those are vitamins. These small-molecule antioxidants are present extracellularly and intracellularly, and include ascorbic acid, glutathione, and tocopherols (6). Intracellular concentrations of these compounds can be substantial, i.e., in the millimolar range both for ascorbate and glutathione. Alfa-tocopherol is by far the most abundant lipid-solubile antioxidant in humans, present in cellular and subcellular membranes and lipoproteins (7). The mechanisms by which these antioxidants act at the molecular and cellular level include roles in gene expression and regulation, apoptosis, and signal transduction. Thus, antioxidants are involved in fundamental metabolic and homeostatic processes. However, there are still many gaps in our knowledge of the basic mechanisms of oxidative damage and antioxidant defenses.

Free radicals possess strong reactivity; they react almost with all biological molecules, causing the damage of the whole range of cell systems and functions. In addition, they attack proteins, lipids, enzymes, nucleonic acids and, at the same time, lead to their oxidative modification. They take part in the onset of lipid per oxidation on cell membranes, which runs as catalytic process and ends with irreversible damage of both structure and function of cell membrane (5).

Aerobe organisms are protected by the whole range of defense systems. Anti- oxidative free radicals protective system includes enzyme and non-enzyme anti-oxidative components.

Preventive anti-oxidants separate per-oxides or sequester metal ions for lessening of free radicals creation, whereas anti-oxidants, which break the chain, eliminate free radicals for the purpose of attack

inhibition and/or breaking the chain of oxidation. Ascorbic acid is a strong antioxidant acting under both *in vitro* and *in vivo* conditions (8). It acts as free radical eliminator and presents the first defense line in fluid phase. Moreover, it is a very powerful reagent in reduction of oxygen, azoth and thyol radicals.

Ascorbic acid is not the only anti-oxidant that breaks the chain of oxidation. The same feature is also possessed by alpha-tocopherol, ubikvinol-10, beta-carotene, aecidium uricum, and non-conjugated bile-ruby (7,9). Acidum uricine possesses a special feature as a powerful, hydrophilic anti-oxidant (9). Wayne and associates (10) measured the capacity of anti-oxidants in humane plasma and showed that it had been 35–65% in urates, 0–24% in acrobats, 5–10% in tocopherol, 10–50% in proteins of plasma. On the other hand, Stocker and associates (9) noticed that antioxidants had reacted with peroxyl radicals in the following order; ascorbic acid, bilirubin and urates (11). Frei and associates examined the effect of ascorbic acid and noticed that it acted as the first defense line during the attack of free radicals in fluid phase.

Ascorbic acid eliminates oxygen radicals quickly, and prevents oxidative processes. However, hydrophilic and ascorbic acid cannot eliminate lipophilic radicals in lipid region of membranes and in lipoproteins as well. It is also the case with other hydrophilic radicals, such as aecidium uricum. Nevertheless, unlike aecidium uricum, ascorbic acid can act as synergist with tocopherol (13).

Ascorbic acid can prevent oxidation of lipids when radicals are generated in lipid region and when oxidation takes place in lipid domain. Tocopherol, located in lipid region, can of course prevent oxidation; in addition, tocopherol is dissipated during its elimination of lipid peroxide radicals. Along with the addition of ascorbic acid to this system, oxidation was prevented for a longer period of time, just as when tocopherol was highly present. The speed of tocopherol wasting was considerably decreased by ascorbic acid addition, while tocopherol started decreasing fast after full dissipation of ascorbic acid. The speed of ascorbic acid decrease in tocopherol absence was rather slow; yet, in tocopherol presence it was dissipated fast and even faster than tocopherol (14).

Ascorbic spectroscopy proved that tocopheroxil radical had been reduced by ascorbic acid and that it generated tocopherol. Such synergistic inhibition of lipid per- oxidation by ascorbic acid comes into being in the system of heterogenic membrane (15), which was experimentally confirmed as well, both in membranes and in micelle system (16).

These results indicate that ascorbic acid acts as a synergist with tocopherol and functions as antioxidant even when oxidation takes place in lipid domain. Such an interaction between ascorbic acid and tocopherol occurs in membrane and lipoproteins, where ascorbic acid and tocopherol are in their fluid phase, that is, in lipid layer. Obviously, ascorbic acid cannot reach peroxide radicals in phospholipids two-layered membrane. However, it must be capable of reacting with tocopheroxil radical, which then must be located on the surface of membranes or close to them. These findings point that tocopherol is located on the outer surface of lipoproteins.

Cysteine and glutathione are also capable of reducing tocopheroxil radical and regenerating tocopherol (17). However, ascorbic acid is much more active and efficient than cysteine and glutathione in the reduction of peroxyl radicals (18). Acidum uricum doesn't effectively reduce tocopheroxil radical. The results of the research showed that the efficiency in regeneration of tocopherol by ascorbic acid decreased along with penetration of peroxyl radicals deep into the membrane. When ascorbic acid reduces tocopheroxile radical, it renders monodehydroascorbat which is further oxidized until it becomes dehydroascorbic (19). There are systems of enzymes *in vivo* reducing dehydroascorbate into ascorbate (20,21). This full cycle can function *in vivo* in order to maintain the vital anti-oxidative defensive system.

It has been observed under certain circumstances that ascorbic acid acts as a pro-oxidant rather (22) than as an antioxidant (23). In fact, the iron-ascorbate mixture has been used as an initiating system in in vitro experiment. However, in vitro studies of oxidative DNA damage indicate that vitamin C acts as an antioxidant unless added or endogenous metal ions are present (24). This result is expected to be based on the known pro-oxidant role of vitamin C in the presence of metal ions *in vitro* (25). In contras, *in vitro* studies of lipid peroxidation in plasma and LDL overwhelmingly demonstrate an antioxidant role for vitamin C, even in the presence of added metal ions. The *in vitro* data on protein oxidation suggest that ascorbate cannot inhibit thiol oxidation or protein carbonyl formation in biological fluids (26).

A vast majority of the animal studies reviewed show that vitamin C acts as an antioxidant *in vivo* and *ex vivo* toward both lipids and proteins, with and without oxidative challenge. There are, however, insufficient animal studies of DNA oxidation to draw conclusions. An important point to note about studies in animals that can synthesize vitamin C, such as rats, is that the results may not reflect the situation in humans. Supplementation of these animals with vitamin C may even reduce endogenous levels of ascorbate (27).

Does vitamin C act as a pro-oxidant under physiological condition?

Ascorbic acid is a potent water soluble antioxidant capable of scavenging/neutralizing an array of reactive oxygen species (hydroxyl, alkoxyl, peroxyl, superoxide anion, hydoperoxyl radicalas) and reactive nitrogen radicals such as nitrogen dioxide, nitroxide, peroxynitrite at very low concentrations (24). Ascorbic acid can regenerate other antioxidans such as alfa-tocopheroxyl, urate and beta-carotene radical cation from their radical species (28). Thus, ascorbic acid acts as co-antioxidant for alfa-tocopherol by converting alfa-tocopheroxyl radical to alfa-tocopherol and helps to prevent the alfa-tocopheroxyl radical mediated peroxidations reactions (29).

In biological systems, iron is not freely available, but it is bound to proteins like transferrin, hemoglobin and feritin. Mobilization of iron from these bio-

molecules may be required before it can catalyze lipid peroxidation. Further, the concentration of free metal ions *in vitro* is thought to be very low as iron and other metals are sequestered by various metal binding protein (28). Another factor that may affect pro-oxidant vs antioxidant property of ascorbic acid is it concentrtion. The in *vitro* date suggest that at low concentration ascorbic acid act as a pro-oxidant, but as an antioxidant at higher levels (30). Moreover, a recent report demonstrated that large doses of exogenous iron (200 mg) and ascorbic acid (75 mg) promote the release of iron from iron binding proteins and also enhance *in vitro* lipid peroxidation in serum of guinea pigs. This finding supports the hypothesis that high intake of iron along with ascorbic acid could increase *in vivo* lipid peroxidation of LDL and therefore could increase risk of atheroscerosis (31). However, another study demonstrated that in iron overloaded plasma, ascorbic acid acts as an antioxidant and prevent oxidative demage to lipids in vivo (11, 32).

There are analyzed data from in vitro and in vivo studies in which specific biomarkers of oxidative DNA, lipid, and protein damage were measured. Of the 44 *in vitro* studies, 38 showed a reduction in markers of oxidative DNA, lipid, and protein damage, 14 showed no change and only 6 showed an icrease in oxidative damage after supplementation with vitamin C. Several of the studies showed a combination of effects depending on the study systems or experimental design. Even in the presence of iron, vitamin C predominantly reduced in vivo oxidative damage, despite its well known pro-oxidant properties *in vitro* in buffer systems containing iron. In more complex and physiologically relevant in vitro systems, such as isolated or cultured cells and biological fluids an antioxidant role, or no effect of vitamin C, predominanted over a pro-oxidant role (33). Studies that report a pro-oxidant role for vitamin C need to be evaluated carefully as to their choice of biomarkers, methodology, study system, and experimental design to rule out any oxidation artifacts. It is hoped that these four important considerations will be taken into account in all future studies of the role of vitamin C in oxidative demage.

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ANTIOKSIDATIVNI I PROOKSIDATIVNI EFEKTI ASKORBINSKE KISELINE

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Slobodni radikali napadaju proteine, lipide, enzime i DNK izazivajući patološke promene u organizmu. Organizam se, protiv slobodnih radikala, bori mnogim mehanizmima. Askorbinska kiselina je jedan od najjačih reduktora i čistača slobodnih radikala. Ona redukuje stabilne kiseoničke, azotne i thyol radikale i deluje kao primarna odbrana od vodenih radikala u krvi. Kada se radikali stvore u vodenim suspenzijama eritrocita ili lipoproteinana niske gustine (LDL), askorbinska kiselina hvata i čisti slobodne radikale pre nego što oni stignu do membrana i LDL molekula. Mada askorbinska kiselina ne može da očisti tečnu sredinu od lipidnih radikala, u lipidnom odseku, ona deluje kao sinergist alfa-tokoferolu i doprinosi smanjenju lipidnih tokoferoksil radikala uz istovremenu regeneraciju alfa-tokoferola. Askorbinska kiselina može da deluje kao pro-oksidans u *in vitro* uslovima u prisustvu metala, ali ovaj efekat nije verovatno značajan u *in vivo* uslovima gde se metalni joni sekvestriraju i postaju drugi reduktanti. *Acta Medica Medianae 2005; 44(1): 65–68.*

Ključne reči: *askorbinska kiselina, vitamin E, slobodni radikali, lipidna peroksidacija*