Antioxidants and Healthy Aging

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Abstract

Although the free radical theory of aging is widely accepted among scientists, the possibility of using antioxidants to delay the aging processes seems to encounter considerable skeptism among clinicians. This may be due, at least in part, to lack of knowledge about the basic chemistry and biological behavior of oxidative stress, antioxidants, and the complex interactions between them. However, one cannot ignore the explosive growth of information concerning the mechanisms underlying the processes of aging, their consequences, and the use of antioxidants in suppressing such effects. In order to provide patients with the most accurate information regarding the use of antioxidant supplementation in their diet, it is important to obtain basic data regarding oxidative stress and antioxidants. This article explores the role of oxidative stress in the aging phenomena, recent evidence supporting supplementation of antioxidants for aged people, the ability of antioxidants to prevent or retard cancer and atherosclerosis (the major causes of mortality in the aged population), and the ability of antioxidant supplementation to delay age-dependent deterioration of cognitive function. Based on the data presented, we conclude that current knowledge provides insufficient and inconclusive support for antioxidant supplementation as a means of delaying aging processes, despite the encouraging results obtained in many studies.

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What is a free radical? Who is an antioxidant?

A free radical is defined as a species capable of independent existence, which contains one or more unpaired electrons. In respiring cells, there appears to be a leakage of electrons from the mitochondrial electron transport chain, to eventually yield a variety of such free radicals and active oxygen derivatives that are collectively called reactive oxygen species. Such species include, among others, superoxide anion, hydroxyl radical, and nitric oxide radical. However, free radicals may also arise from exogenous origin, such as those associated with environmental stresses [1]. ROS are capable of causing damage to macromolecules leading to lipid peroxidation, oxidation of amino acid side chains (especially cysteine), formation of protein-protein cross-links, and oxidation of polypeptide backbones, resulting in protein fragmentation, DNA damage and DNA strand breaks. High doses of ROS, which may be generated during chronic and acute inflammatory diseases, or from environmental stresses, are usually cytotoxic. It should be noted that reactive oxygen species have been implicated in the physiologic basis of the aging process and in the mechanisms underlying the damage induced by numerous human diseases, such as malignant diseases, Diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, human immunodeficiency virus infection, ischemia/reperfusion injury and others [2].

ROS = reactive oxygen species

The cells cope with excess of ROS by the employment of antioxidants. In the strictest definition, antioxidant is any substrate that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [1]. Biological systems contain various antioxidant systems, which can be divided into antioxidant enzymes (superoxide dismutases, catalases, glutathione peroxidases, etc.) and non-enzymatic, low molecular mass antioxidants, which can be divided again into diet-derived (ascorbic acid, α-tocopherol, carotenoids, etc.) and *in vivo* synthesized (glutathione, uric acid, bilirubin, etc.). Disturbances in the prooxidant-antioxidant balance (redox status) in favor of the former constitute oxidative stress and may lead to the above-described oxidative damage.

The antioxidant network concept

Most of the biological antioxidants are potent reductants with a redox potential capable of reducing the ultimate oxidizing molecule – the oxygen atom. As shown in Figure 1, when vitamin E reduces the ROO (lipid peroxide), it itself becomes a radical. This radical may be decomposed further or alternatively can be reduced by a stronger reducing agent, such as ascorbic acid (vitamin C), which will result in regeneration of the reduced form of vitamin E (α-tocopherol). However, vitamin C, upon reducing the vitamin E radical, becomes oxidized as dehydroascorbate is formed. Then a stronger reducing molecule such as glutathione can reduce the oxidized form of vitamin C. While performing this, GSH is oxidized into GSSG, the oxidized form of glutathione, which is reduced back to GSH by the most potent reducing biological molecule beside hydrogen – the nicotinamide dinucleotide. This recycling of vitamins C and E has been termed the "network action of vitamins." Evidence for the existence of this phenomenon has accumulated in several biological systems. For example Reinhold et al. [3] observed it in red blood cell membranes. It was also observed in other systems, such as low density lipoproteins [4], ischemia-reperfusion of the heart [5], and plasma exposed to cigarette smoke [6].

GSH = glutathione

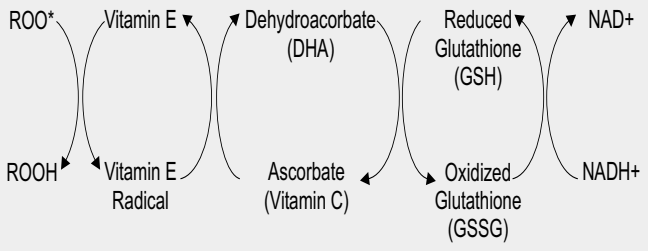

Figure 1. The Antioxidant Redox Chain.

The free radical theory of aging

The free radical theory of aging was introduced in 1956 by Harman [7], who suggested that normal aging is a consequence of random deleterious damage to tissues by free radicals. The idea that aging may be the result of accumulated damage to proteins and DNA led many investigators to examine the hypothesis that the efficacy of antioxidant defense systems decreases with time. However, on the one hand, studies that examined the levels of catalase, glutathione peroxidase and superoxide dismutase provided inconsistent results. On the other hand, comparisons have shown that it is possible to identify long and short-lived individuals of the same fly populations by measuring the levels of antioxidant activity and free radical damage at the midpoint of their life [8].

The most convincing evidence supporting the role of free radicals in the process of aging comes from the field of nutrition. Indeed, the most reliable way of extending both mean and maximum life span in laboratory animals remains caloric restriction: that is, the reduction of food intake without loss of essential nutrients and minerals. First experiments in caloric restriction were conducted in the 1930s, and experiments with higher mammals and primates are on the way. There is considerable evidence to show that dietary restriction might be acting to reduce the formation of free radicals. For example, it has been shown that age-related reductions in catalase, glutathione peroxidase and glutathione reductase are partially prevented by caloric restriction, and this is also accompanied by lower levels of superoxide and hydroxyl radicals, along with decreased lipid peroxidation of liver mitochondrial and microsomal membranes [9].

Antioxidant administration in aging

Since free radicals are believed to play major roles in the aging processes, it was hypothesized that antioxidant administration should extend life span. This was first suggested by Harman [7], and since then, many antioxidants have been examined for their ability to prolong life span in many species. Although most of these studies have shown a beneficial effect of dietary supplements, with the average increase of life span being 15–20%, it has also become clear that antioxidant administration (unlike caloric restriction) does not lead to extension of maximum life span, while dietary antioxidants could enable more animals to reach the end stage of their natural life span but not beyond. These results may be attributed to the fact that antioxidants help protect against disease rather than aging. Some reasons for this failure of antioxidants may lie in the decreased absorption of high doses of antioxidants with age, or the down-regulation of cellular enzymes such as SOD by the reduced environment. In addition, although some antioxidants are believed to be able to reach the interior of the cell, it does not necessarily mean that they are located at the sites of free radical production, such as mitochondria. Substances that have been reported to increase the maximum life span of animals include the monoamine oxidase B inhibitor, L-deprenyl and PBN (N-tert-butyl-α-phenyl nitrone), which neutralizes free radicals, and was able to reduce age-dependent protein oxidation and improve a radial arm maze task that tested cognitive function and memory [10]. However, other beneficial effects of the antioxidants should not be overlooked: animals treated with antioxidants tend to have lower body weights, accumulate less lipofuscin, have fewer tumors, and in some studies appeared to suffer less from autoimmune diseases.

- *Atherosclerotic vascular disease*

In developed countries, atherosclerotic disease is the major cause of death in the elderly population. It has been noted in numerous studies that a healthy lifestyle, including balanced diet and regular exercise, may exert beneficial effects on the development of cardiovascular disease. Several antioxidants, such as polyphenols in red wine, and lycopene, have been proposed to delay the progression of this disease. Many early studies have demonstrated that oxidation of LDL particles may play a crucial role in the formation of the atherosclerotic disease, since this oxidized LDL is inserted into macrophages, leading to the creation of foam cells. However, only in the late 1980s was it shown *in vitro* that LDL is resistant to oxidation by $Cu2^{+}$ until α-tocopherol (vitamin E) is consumed [11]. Vitamin E is fat-soluble, and hence will be present mainly in lipids and membranes. The richest natural sources of vitamin E are vegetable oils, nuts and whole grains. Many different clinical trials have shown the ability of vitamin E, alone or in combination with the antioxidant vitamin C, to delay the progression of the atherosclerotic disease and its outcomes [12]. However, those findings are inconsistent with the fact that vitamin E is present in relatively large amounts in the atherosclerotic lesions, together with oxidized lipids. Moreover, recent research has demonstrated that antioxidant supplementation did not result in decreased mortality and morbidity due to reduced atherogenesis [13]. One explanation for this discrepancy is that in some circumstances, such as low levels of free radicals and the absence of co-antioxidants, vitamin E can promote, rather than terminate, lipid peroxidation in LDL particles, acting as a prooxidant rather than an antioxidant.

- *Cancer*

While the exact role of free radicals in carcinogenesis and tumor progression is still under investigation, evidence arising from clinical trials has demonstrated the ability of some antioxidants to decrease the incidence of some types of cancers. Vitamin E, for example, has been shown in some trials to reduce the incidence of breast, lung and colon cancers, but the most significant result has been with prostate cancer. For example: in the ATBC trial, in which the participants were all male smokers, α-tocopherol supplementation decreased prostate cancer incidence and morality [14]. This protection seems to be limited mainly to smokers. Many studies have evaluated the relationship between intake of carotenoids and cancer. Carotenoids are a class of plant-derived compounds. All of the more than 600 known carotenoids are antioxidants, including lycopene, which is present in high quantities in tomatoes. Vitamin C, a potent water-soluble antioxidant, which serves also as a co-factor in

SOD = superoxide dismutase

LDL = low density lipoprotein

hydroxylation reactions and is obtained mainly from fruits and vegetables, was shown to decrease the appearance of oral, esophagus and stomach cancers [15]. Indeed, reports from two recent prospective studies showed increased total cancer mortality among men (but not women) with lower serum vitamin C levels [16].

- *Cognitive function*

The brain is known to be vulnerable to oxidative stress due to its high oxygen use and the relatively low antioxidant levels in brain tissue, which also contains high levels of polyunsaturated fatty acids and thus is more sensitive to oxidative insults. The first correlation between antioxidant and memory performance in the aged was demonstrated by Perrig et al. [17], who showed that plasma levels of vitamin C and β-carotene were significant predictors of memory performance, mainly semantic memory, as higher plasma levels of those antioxidants had a protective effect against deterioration of the cognitive function. Another interesting result in this trial was that subjects who reported daily antioxidant supplementation showed neither higher plasma antioxidant levels nor better memory performance [17]. Those results suggested the beneficial effects of fruits and vegetables on cognitive function in the aged. Indeed, it was previously reported that feeding aged rats with extracts of strawberry, spinach or blueberry was effective in reversing age-related neuronal and behavioral deficits, and a recent study of the Third National Health and Nutrition Examination Survey (NHANES III) reported that 7% of elderly Americans suffer from poor memory, with low plasma levels of vitamin E (per plasma cholesterol level) as a risk factor [18].

Among the many factors believed to contribute to the etiology and progression of Alzheimer's disease is the involvement of oxidative stress. Antioxidant activity of neuronal tissue is known to be reduced with age. Amyloid β, found in senile plaques of the disease, is able to form radicals such as superoxides to induce membrane lipid peroxidation and neuronal death. Indeed, administration of vitamin E and propylgallate (synthetic antioxidant) can reduce amyloid toxicity in cell culture [19]. Sano and colleagues [20] have examined the ability of selegiline, a drug with antioxidant properties, and vitamin E, to inhibit the progression of the disease. Selegiline was able to delay primary outcome (death, institutionalization, loss of ability to perform basic activities of daily living, or severe dementia) by 655 days, and vitamin E delayed it by 670 days [20].

Conclusions

A growing body of evidence suggests that oxidative stress, along with reduction of antioxidant activity, may play important roles in the processes of aging. Although some experimental trials, mostly in animals, have shown that the provision of antioxidants may be beneficial in the aging processes, many clinical trials have not been as conclusive and have raised the question about the significance of antioxidant supplementation to elderly humans. Nevertheless, it seems likely that higher plasma levels of several antioxidants may be useful in delaying some aspects of the aging process and age-dependent morbidities. However, there is no clear evidence that antioxidant supplementation may serve this purpose, and it seems more likely that consumption of food containing high levels of dietary antioxidants, in combination with a balanced diet and physical exercise, may exert more beneficial effects on the health of the aged population.

References

1. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd edn. London: Oxford University Press, 1999:22–5.
2. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95.
3. Reinhold HS, Calvo JW, van der Berg AP. Development of blood vessel-related radiation damage in the fimbria of the central nervous system. *Int J Radiat Oncol Biol Phys* 1990;18:37–42.
4. Kagan VE, Serbinova EA, Forte T, Scita G, Packer L. Recycling of vitamin E In human low density lipoproteins. *J Lipid Res* 1992;33:385–97.
5. Haramaki N, Stewart DB, Aggarwal S, Ikeda H, Reznick AZ, Packer L. Networking antioxidants in the isolated rat heart are selectively depleted by ischemia-reperfusion. *Free Radic Biol Med* 1998;25:329–39.
6. Reznick AZ, Han D, Packer L. Cigarette smoke induced oxidation of human plasma proteins, lipid, and antioxidants: selective protection by biothiols dihydrolipoic acid and glutathione. *Redox Rep* 1997;3:169–74.
7. Harman D. Free radical involvement in aging. *Drug Aging* 1993;3:60.
8. Sohal RS. The free radical theory of aging: a critique. In: Rothstein M, ed. Review of Blogical Research in Aging. Vol. 3. New York: Alan R Liss, 1987:431–49.
9. Lee DW, Yu BP. Modulation of free radicals and superoxide dismutases by age and dietary restriction. *Aging* 1990;2:357–62.
10. Butterfield DA, Howard BJ, Yatin S, Allen KL, Karney JM. Free radical oxidation of brain proteins in accelerated senescence and its modulation by N-tert-butyl-alpha-phenylnitrone. *Proc Natl Acad Sci USA* 1997;94(2):674–8.
11. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoproteins. *Free Radic Res Commun* 1989;6:67–75.
12. Halliwell B. The antioxidant paradox. *Lancet* 2000;355(9210):1179–80.
13. Fang JC, Kinlay S, Baltrame J, et al. Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet* 2002;359(9312):1108–13.
14. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers: the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N Engl J Med* 1994;330:1029–35.
15. Byers T, Guerrero N. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *Am J Clin Nutr* 1995;62:S1385–92.
16. Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. *Lancet* 2001;357(9257):657–63.
17. Perrig WJ, Perrig P, Stavelin HB. The relation between antioxidants and memory performance in the old and very old. *J Am Geriatr Soc* 1997;45:718–24.
18. Perkins A, Hendrie HC, Callahan CM, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 1999; 150(1):37–44.
19. Behl C, Davis J, Cole GM, Shubert D. Vitamin E protects nerve cells from amyloid protein toxicity. *Biochem Biophys Res Commun* 1992;186:253–64.
20. Sano M, Ernesto C, Thomas RC, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med* 1997;336(17):1216–22.

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