NATURAL ANTIOXIDANTS AND AGEING: MYTH OR PARADOX?

Bruno Nunes
Professor Auxiliar
Faculdade de Ciências da Saúde – UFP
bruno@ufp.pt

Victor Balcão
Professor Associado
Faculdade de Ciência e Tecnologia – UFP
vbalcao@ufp.pt

Resumo

Os danos oxidativos podem ser prevenidos *in vivo* através da actividade protectora de antioxidantes biológicos (enzimáticos e não-enzimáticos). A importância dos antioxidantes biológicos é tal que, na sua ausência a vida estaria continuamente sob a ameaça da oxidação. Este fenómeno interfere com a estrutura, enovelamento e função das proteínas, com a integridade dos lípidos membranares e com a estrutura do DNA; a oxidação resulta também no processo de envelhecimento, através da acumulação de danos nas estruturas subcelulares.

Palavras-chave

Antioxidantes, saúde, envelhecimento, stress oxidativo, carotenoides

Abstract

Oxidative damage can be prevented *in vivo* through the protective activity of biological antioxidants (enzymatic and non-enzymatic). The importance of the biological anti-oxidants is such that, in their absence, life would be continuously under threat from oxidation. This general phenomenon interferes with protein structure, folding and function, with the integrity of membrane lipids and also with the structure of DNA; furthermore, oxidation results in the onset of the aging process, through the accumulation of damages in subcellular structures.

Key-words

Antioxidants, health, aging, oxidative stress, carotenoids

INTRODUCTION

Oxidation is the chemical process by which a chemical compound loses electrons, usually after interaction with other chemical entities avid for additional electrons. This is the general case of several chemical species composed by oxygen atoms, named reactive oxygen species (ROS) (Jacobson, 1996). Oxygen is essential for the respiration phenomena, and acts as the final acceptor of electrons at the end of the respiratory chain, used by aerobic cells in order to obtain energy from simple sugars, mainly at the mitochondria. However, respiratory processes frequently end up in the production and ultimate release of short lived chemical intermediate species, characterized by instability and strong tendency to react with adjacent chemical structures. Additionally, other chemicals with which the organisms contact are able to cause further modifications in the cellular redox status. Several pharmaceutical drugs, which are environmental toxicants, are considered oxidative agents, since they can exert significant chemical modifications favouring the onset of oxidative damage, due to over-production of oxygen free radicals that will react with endogenous compounds present in varied tissues and organs. In general terms, oxidation is a natural process, and is responsible for physiological modifications common to living organisms, such as aging. The loss of functionality of cells and tissues, caused by the modification of compositions and structures of biological macromolecules (e.g. proteins, DNA and membrane lipids) is responsible for a continuous and inevitable physiological decay. The amount of oxidative damage varies among conditions, and may range from skin aging (e.g. loss of elasticity) to the onset of carcinogenesis. Due to all the above-mentioned reasons, the study of the oxidation mechanisms and the potential therapeutic usefulness of antioxidants is the main objective of the present review article, in order to enlighten the possible future use of antioxidants in anti-aging practice.

OXIDATIVE DAMAGE

A large number of conditions and chemical compounds have been related to the onset of oxidative stress-mediated changes that can threat cellular viability and impair normal physiological status. The first of the compounds to be related to oxidative stress is oxygen itself (Jacobson, 1996). Without the contribution of oxygen, always present during the development of respiratory processes, oxidative stress would not present the dimensions that turns it a question of on-going debate and general scientific concern. Oxygen is essential to aerobic life, but is also the main factor responsible for oxidative damage. The main intermediate chemical species responsible for oxidation are composed by oxygen, and oxidation is an intrinsic feature to life depending on oxygen. The major noticeable consequence of oxidation is the development of the aging process, with the progressive impairment of physiological functions. Ultimately, the inexorable accumulation of oxidative damage spreads to several key structures of living cells. Membranes, due to their lipoproteic composition, are prone to lipid peroxidation (Buettner, 1993). After peroxidative damage, lipids exhibit altered chemical composition, with sensible modifications in their viscosity and other physical properties. These changes are directly responsible for the loss of the membranes´ initial fluidity, which is an evident sign of the aging process (Korshunov *et al.*, 1997). Damages derived from oxidation are not confined to lipid peroxidation, but also include oxidation of proteic structures, with consequent impairment of functional integrity. This is particularly important in the case of structural proteins and enzymes, with are not able to comply with their original functions.

Oxidation is a serious threat to the cellular genetic content, since oxidative damage to DNA is also an effect to consider.

FREE RADICALS AND ANTIOXIDANTS

The oxygen we breathe is consumed in the mitochondria of our cells to produce the energy required to run all of the chemical reactions that maintain our metabolism and sustain life. Free radicals are atoms or molecules with a solitary unpaired electron in the outer shell of the atom. Free radicals containing oxygen are called ROS, or Reactive Oxygen Species, and they are a natural and integral part of the respiration process and mitochondrial oxidation of ATP into energy. Free radicals tend always to chemically react with other molecules such that an electron could be "stolen" from the attacked molecule in order to pair the radical's unpaired electron. This tendency to react and "steal" an electron is the cause for the non-selective attack of free radicals on several important biological molecules. Most radicals are highly reactive and thus unstable. Being so reactive and non-selective, free radicals usually react easily with the membrane lipids, proteins, and nucleic acid, causing damage to the cell and interfering with its normal functioning and reproduction (Halliwell, 1994). The most important ROS (Korshunov *et al*., 1997; Shigenaga *et al.* 1994) are manifested as superoxide anion (a molecule of oxygen with an extra electron), hydrogen peroxide, and the highly reactive (and consequently toxic) hydroxyl radical. Superoxide anion for example, reacts with membrane lipids, proteins, and nucleic acids, causing cumulative and irreversible damage to the cells that sustain vital tissues and organs. Environmental and other external factors such as alcohol abuse, smoking, radiation, air and water pollution, and overexposure to sunlight can also speed the formation of free radicals. On the other hand, free radicals and specifically ROS, play a crucial role in several important biological functions such as immunity, cell and DNA repair, and combating the inflammation process. In other words, free radicals are a natural result of metabolism and a constant companion of life. Free radicals in biological systems are normally kept in check by natural antioxidant defense mechanisms. Natural antioxidants are biological substances that neutralize and "disarm" free radicals before they can damage cells and tissues. As we age, however, this process apparently becomes less efficient (Lenaz *et al*., 2002). Free radical oxidative damage to cell membranes, genetic material, and proteins increases, resulting in the dysfunction or death of cells and tissues, and eventually entire organs. Antioxidants are substances in the body that work against free radicals and disarm them before irreversible damage to cells and tissues is caused (Halliwell *et al.* 1995). Antioxidants' role is to react with free radicals, thus diverting their attack on vital cellular components. In other words, antioxidants are "sacrifice molecules" that prevent and minimize free radicals' attacks on cells and tissues. Antioxidants form new free radicals when attacked and oxidized by ROS, but have the chemical capability to disperse and spread the charge of the odd electron over the specialized molecular structure of the antioxidant, thus achieving its stabilization. Antioxidants have the capability of quenching the ROS and stabilizing themselves by releasing the extra energy as heat or dispersing the extra charge over the molecule's atoms and bonds (Aruoma, 1994). There are two major types of antioxidants present in our body and capable of working together to protect the cells and tissues, and they act in concert in cell differentiation, immune system, and normal DNA repair: (i) water-soluble antioxidants (such as Vitamin C) protect the aqueous parts of the cells, and the hydrophilic regions and surfaces of molecules, for example the bloodstream; (ii) hydrophobic or oil-soluble antioxidants (such as Vitamin E or astaxanthin) protect the non-aqueous

components of cells and the hydrophobic regions and surfaces of molecules, for example, cell membranes.

OXIDATIVE STRESS AND AGING

Free radicals are produced in the mitochondria during normal cell processes involving oxygen (Cadenas and Davies, 2000). Free radicals are highly reactive oxidizing substances that easily and rapidly react with lipid membranes, fats, proteins, enzymes, and DNA, thus interfering with normal cells function and reproduction. The attack of free radicals on the various cells' components is called oxidative damage, and its resulting substances accumulate in every living cell. While natural antioxidants reduce the damage to our cells' membranes, DNA, and proteins, oxidative stress describes the level of oxidative damage in a cell or tissue caused by the attack of reactive oxygen species (ROS), and it is a direct result of an improper balance between the formation and destruction of free radicals. Oxidative stress in cells is believed to be one of the major components of age-related disorders such as cancer, strokes, age-related macular degeneration (ARMD), osteoarthritis, and cardiovascular and neurodegradative diseases. By damaging cells and tissues, oxidative stress appears to contribute to the general decline in the optimum body functions, a process known as aging. Aging could be defined as a progressive deterioration of cells (Finkel and Holbrook, 2000), tissues, and organs, as this process is accelerated with increased age. Free radicals produced by our cells that escape the antioxidant defense are the basis for the free radical theory of aging, since as we age, this "cleaning process" becomes less and less efficient. This aging theory (Reiter, 1995), first proposed in the 1950s by pioneer anti-aging researcher Dr. Denham Harman, stated that a very small fraction of the free radicals generated during normal metabolism in the mitochondria "escape" the antioxidant defense barrier and oxidize important cellular structures, thereby impairing cells' activity. The effect of this process is cumulative and is exhibited by an age-associated decrease in the overall health and dynamic state of the organism, eventually culminating in death. The free radical theory, which is known as the mitochondrial theory of aging, has four key elements: (i) the mitochondria use the most oxygen in the production of energy, and therefore are believed to be the major source of free radical production; (ii) the mitochondria are more susceptible to damage by free radicals because of their proximity to the components involved in free radicals production; (iii) the genetic code of the mitochondria is unique in that unlike nuclear DNA, it lacks substances (histones) to protect it from free radical attack; and (iv) The machinery specialized for repair of the mitochondrial DNA damaged after free radical assault is much less efficient than its nuclear counterpart (Sastre *et al.*, 2000). The free radical or mitochondrial theory of aging has received increasing recognition during the past 20 years. A basic tenet of this theory is that the fundamental changes associated with the aging process are the cumulative result of the reactive oxygen species (ROS), which are normal by-products of aerobic life. The gradual loss of energy with age is paralleled by a decrease in the number of mitochondria per cell, as well as the health and energy-producing efficiency of those remaining mitochondria.

ENZYMATIC ANTI-OXIDANT DEFENCE MECHANISMS

Among natural defences against oxidative stress, enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase are of special importance. Superoxide dismutase is responsible for the detoxification of the short-lived, highly reactive oxygen

species superoxide anion. Glutathione peroxidase exerts its protective role by acting as scavenger for high concentrations of hydrogen peroxide; during this process, glutathione is oxidized and loses its protective capability (Kaçmaz *et al.* 1999). Glutathione reductase is the enzymatic species responsible for the reversion of the oxidized form of glutathione, thus leading to the formation of two molecules of glutathione. The obtainance of molecular glutathione, reaction mediated by the enzyme glutathione reductase, allows the performance of the protective effect of glutathione. Glutathione is tripeptidic structure, composed by residues of glutamic acid, cisteine and glycine, arranged to form a larger structure, often designated as γ-glutamylcisteinilglycine. This specific compound exhibits an extraordinary natural detoxification role, since it is highly electrophilic and reacts promptly with free radicals, namely reactive oxygen species such as the superoxide anion and the hydroxyl radical. It thus acts as a strong scavenger of intermediate reactive species, preventing the establishment of permanent oxidative damage in biological structures adjacent to specific sites where ROS are formed. The proportion of the intracellular pools of reduced and oxidised glutathione is extremely important for the evaluation of the redox overall status of the cells. Low levels of reduced glutathione (usually represented as GSH) are usually signs of oxidative aggression. The amount of oxidised glutathione (GSSG) is thus the result of the direct oxidation of the reduced glutathione, which acted previously as a scavenger agent for the ROS. The before mentioned enzymatic forms are of special importance among the anti-oxidant intracellular arsenal, since they limit, impair and prevent the oxidative damage consequent to the overproduction of the superoxide anion. The complementary roles attributed to the enzymes and to the presence of glutathione works as a potent factor to take into account for the decreased physiological oxidative stress observed in the majority of the organisms. Other enzymes are also of great interest, such as the ones related to the detoxification potential of the studied organisms; glutathione-S-transferases (GSTs) are a group of widely distributed enzymes that catalyse the conjugation of xenobiotics with glutathione. Furthermore, this conjugation is followed by transfer of the glutamate by c-glutamyltranspeptidase, by loss of glycine through cysteinyl glycinase, and finally by acetylation of the cysteine amino group. The toxicological importance of the conjugation process is very high, since the removal of reactive electrophiles allows the protection of vital nucleophilic groups in macromolecules such as proteins and nucleic acids. Besides the production of free radicals (e.g. superoxide anion and hydroxyl radical), the hyperproduction of hydrogen peroxide may also be a subject of concern that needs to be considered in oxidative stress cenarios. The hyperprodution of hydrogen peroxide is favoured by the hepatic expression of hydrogen peroxide-generating peroxisomal fatty acyl-CoA oxidase, and is followed by a compensatory mechanism of disproportionate increase in the activity of hydrogen peroxide-degrading enzymes, such as catalase. The toxic effects of hydrogen peroxide are consequent to: a) the mentioned overproduction and to b) a simultaneous decrease in the activity of glutathione peroxidase (Yeldandi *et al.*, 2000).

In spite of the existence of the aforementioned physiological mechanisms of control of ROS, oxidative damage is sometimes likely to occur, due to extensive production/reduced protective capacity of the organism. In the case of free radical attack of biological structures, some compounds, such as malondialdehyde (MDA), may be formed, as a consequence of degradation of initial products. The extent of lipid peroxidation can be measured through the evaluation of the levels of thiobarbituric acid reactive substances (TBARS), which are mostly comprised by MDA.

CAROTENOIDS: NATURAL ANTIOXIDANTS

Carotenoids are a class of fat-soluble, natural pigments, responsible for the colors of many plants, animals, fish, fruits, vegetables, flowers, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process, or converting sunlight into chemical energy. Beta- and alpha-carotene are the compounds responsible for the orange color of carrots, lycopene for the red color of tomatoes (Mangels *et al.*, 1993), and astaxanthin imparts the pink hue associated with lobsters, shrimps, crawfish and salmon, and also the recognizable orange-pink color of flamingos' legs and feathers. One can find more than 600 carotenoids from natural sources, which can be divided into two major groups: hydrocarbon carotenoids (e.g. lycopene and beta-carotene) and their oxygenated derivatives, called xanthophylls, which contain hydroxyl or keto groups (e.g. astaxanthin, lutein, and zeaxanthin) (Di Mascio *et al.*, 1989; Ong and Packer, 1992; Rao and Agarwal, 1999). Carotenoids play a crucial role in maintaining the human health, being essential to human vision by acting as biological antioxidants that protects cells, DNA, cell membrane lipids, and proteins, from the damaging effects of free radicals, as stated above. Astaxanthin, a carotenoid naturally found in shrimp, lobster, salmon and other seafoods, is the most abundant carotenoid in the marine world, and exhibits potent antioxidant properties. The most important and abundant carotenoids are composed of a 40-carbon polyene skeleton chain, terminated by six carbon rings with or without keto or hydroxyl groups. Such specific structure of alternating single and double bonds in the polyene skeleton of carotenoid compounds is mainly responsible for the absorption of excess energy from other molecules by charge resonance, while the nature of the specific end groups on carotenoids are responsible for their polarity and hence for their solubility in both hydrophobic and hydrophilic (aqueous) environments (Britton, 1995). These properties are critical factors that might account for the amazing antioxidant properties of carotenoids (Shahidi, 1997). Because carotenoids are mainly fat-soluble (hydrophobic), and because the blood is essentially hydrophilic, they are transported in the circulating blood stream bounded to lipoproteins. The predominant carotenoid compounds found in human tissues are beta-carotene, alpha-carotene, lycopene, lutein, zeaxanthin, astaxanthin, and beta-cryptoxanthin, and the amounts in which they can be found depends primarily on the dietary intake (Mathews-Roth, 1990). As mentioned before, carotenoids are very powerful antioxidants, protecting the cells of the human body from the damage caused by free radicals (Di Mascio *et al.*, 1991). In this respect, astaxanthin possesses an anti-oxidant power ca. 500 times more potent than that of beta-carotene. Numerous clinical and epidemiological studies have shown that increased consumption of foods rich in carotenoids is associated with a marked decreased risk of development of some degenerative diseases, as well as with improved immune system function (Ho *et al.*, 1994). More recently, the protective effects of carotenoids against serious health(-threatening) disorders such as cancer, heart disease and degenerative eye disease have also been recognized (Saljoughian, 2002), and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the body immune response system (Bendich, 1989). In this respect, several researchers have also demonstrated that carotenoids possess the ability to stimulate cell-to-cell communication, suggesting that poor communication between cells might be one of the main causes of unchecked division of cells, a condition that eventually leads to onset of cancer. By promoting proper communication between cells, carotenoids may play an important role in the prevention of cancer- and age-related diseases, including age-related macular degeneration (Snodderly, 1995), cataracts, and other health conditions linked to oxidative or free radical damage (Nishino, 1998). Carotenoids are important to human nutrition as a source of vitamin A (e.g., from beta-carotene) and as a prevention

agent for cancer and heart disease (e.g., lycopene) (Saljoughian, 2002; Hercberg, 2005). These compounds generally contain a conjugated polyene structure that absorbs light in an efficient fashion, and so they act as light-harvesting complexes, together with proteins, in the photosynthetic process and, most especially, they play a major role in protection against destructive photooxidation (Christophersen *et al.*, 1991). Without carotenoids, photosynthesis in an oxygenic atmosphere would be virtually impossible (Di Mascio *et al.*, 1990). Carotenoids are also important precursors of many natural flavor and fragrance products extracted from flowers and other plants, and are also widely used in the nutraceutical and cosmeceutical industry as antioxidants and to add color to foods and beverages.

SOURCES OF CAROTENOIDS

As already mentioned, carotenoids are a class of natural pigments that is widespread and it has been demonstrated that they occur in all the three domains of life, i.e. in the eubacteria, the archaea and in the eucarya. For humans, the most important source for carotenoids are plants, where the brilliant colors of the carotenoids are often masked by chlorophyll, e.g. in green leaves. The carotenoids are responsible for the colors of many fruits (e.g. pineapple, citrus fruits, tomatoes, paprika, rose hips) and flowers (e.g. Narcissus), as well as the colors of many birds (e.g. flamingo, ibis, canary), insects (lady bird), and marine animals (e.g. crustaceans, salmon), and a wide variety of invertebrate animals, where complexation with proteins may modify their colour to blue, green or purple (Ong and Tee, 1992). The total carotenoid production in nature has been estimated to be ca. 100'000'000 tons a year. Recently, it has been demonstrated by the analysis of serum and human breast milk that up to 50 different dietary carotenoids originating from fruits and vegetables may be absorbed and metabolized by human beings (Pfander, 1992). Astaxanthin is the most abundant carotenoid pigment found in aquatic animals and in the marine world (Miki, 1991), and is responsible for the pigmentation of seafoods such as wild salmon, trout, red sea bream, lobster, and shrimp, but no less important, it plays a crucial role in the animals' systems, such as protection against oxidation and UV light, immune response, and eye health (Kobayashi *et al.*, 1997). Recent scientific findings have indicated that astaxanthin is a very powerful antioxidant and can serve as a potent free-radical scavenger (Palozza and Krinski, 1992; Kurashige *et al.*, 1990); it also plays an important role in immunological defense (Terao, 1989). Astaxanthin is capable of crossing the blood-brain barrier in mammals, a unique and important property in the realm of antioxidants. This characteristic allows astaxanthin to extend its superior antioxidant activity to the central nervous system, which, being rich in unsaturated fatty acids is highly susceptible to oxidative damage by ROS. The efficacy of astaxanthin in limiting the damage produced by ROS-induced oxidative stress and improving health parameters in the tissues and the body was demonstrated in a series of in-vitro experiments, in pre-clinical studies and in human models.

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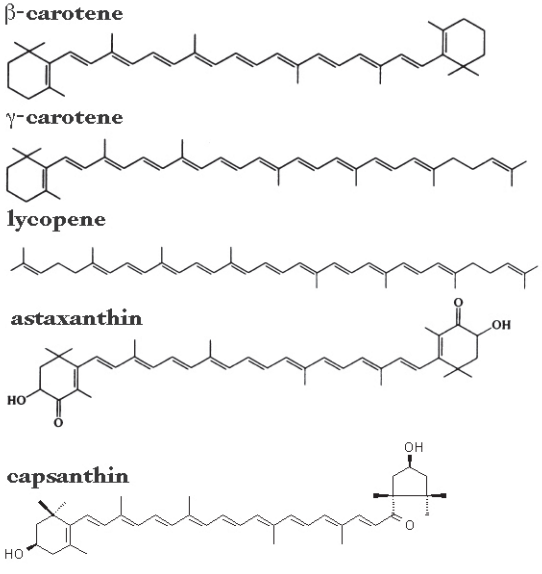

Figure 1 - Carotenoids.