

Antioxidant treatment in chronic diseases.

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ABSTRACT

It is well established that reactive oxygen species (ROS) play an important role in the etiology of numerous diseases, such as atherosclerosis, diabetes and cancer. Among the physiological defense system of the cell, the importance of antioxidant molecules such as glutathione and vitamins is no longer matter of question. However, very recently the interest of researchers has been conveyed on antioxidant enzymes, such as the heme oxygenase/biliverdin reductase system, since it has been shown to be modulated by dietary substances (polyphenols, beta-carotene etc), to counteract oxidative damage very efficiently and finally to regulate physiologic phenomena such as aging. Although in vitro evidence has shown that antioxidant therapy is cytoprotective, the potential clinical benefit deriving from both nutritional and supplemental antioxidants is still under debate. Importantly, the misuse of some lipophylic vitamins has been shown to originate cancer rather than prevent it. Aim of this paper is to review recent progress regarding the potential benefit of dietary antioxidants in the treatment of chronic disease with a special focus on immune system and neurodegenerative disorders.

Keywords: antioxidant, free radical, oxidative stress, immunity, inflammation, cancer, neurodegeneration.

INTRODUCTION

The term “free radicals” include a family of chemical entities characterized by great reactivity due to the impaired electron in the outer orbital. To this group belong reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide as well as reactive nitrogen species (RNS) which include nitric oxide and peroxynitrite. Although different in nature, free radicals share the same mechanism to harm body’s cells and tissues: the damage on macromolecules such as proteins, DNA and lipids [1]. The alteration on membrane functions through phospholipid modifications are among the major injures created by radical species, either when considering the whole organism or specific integrated functions such as the immune response [2]. The potential therapeutic applications of antioxidants in free radical-related diseases led to the hypothesis of their use to reverse the cognitive decline associated with neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), or spongiform encephalopathies by blocking the effect of proinflammatory cytokines and the resulting oxidative damage [3-7]. However, several clinical studies demonstrated that not only malnutrition, but also the excess of certain nutrients (e.g. iron, alpha-tocopherol, beta carotene ascorbic acid) may give rise to oxidation phenomena and therefore to cellular injury [8,9]. It must therefore be taken into consideration that before of introducing antioxidant therapy into mainstream medicine will require significant advances in basic cell biology, pharmacology and clinical bioanalysis.

OXIDATIVE STRESS

The body is normally under a dynamic equilibrium between free radical generation and quenching. The physiological defense systems to counteract free radicals comprise endogenous enzymes (catalase, glutathione reductase ad superoxide dismutase) as well as endogenous

(glutathione, urate and coenzyme Q) or exogenous factors (β -carotene, vitamin C, vitamin E and selenium) [10]. All these molecules have an antioxidant effect due to their ability to transform ROS into stable and harmless compounds or by scavenging both ROS and RNS with a redox-based mechanism [10]. Very recently, a main role in the fight against oxidative stress has been assumed by enzymes such as heme oxygenase (HO) and biliverdin reductase (BVR). Heme oxygenase is a microsomal enzyme which metabolizes heme into ferrous iron, carbon monoxide and biliverdin (BV); this latter is then reduced by BVR into bilirubin (BR) a molecule endowed with strong antioxidant and antinitrosative activities [11-14]. Interestingly, all these protective factors act in concert and enhance the antioxidant defense system of the cell. When the balance between ROS/RNS and antioxidants turns in favor of the formers oxidative/nitrosative stress occurs. A classic example of an oxidation product apparently leading to disease is oxidized cholesterol in low-density lipoprotein (LDL), much more atherogenic than native LDL, and mainly involved in the pathogenesis of atherosclerosis and coronary heart disease (CHD) [15].

At the cellular level, a large body of data clearly demonstrated that ROS when produced in low amounts and in a controlled manner are physiological components of the signalling generated by cytokines, growth factors and neurotrophic peptides [16-21], but ROS may also activate apoptotic cell death [22]. Extracellularly generated ROS can diffuse through anion channels into the cytoplasm; the resulting variation in the cell's redox state leads to the modulation of a variety of transcription factors (eg. NF- κ B, AP-1), protein kinases (e.g. AKT, JNK, p38) and receptor activated MAP kinases implicated in some forms of apoptosis [16, 24,25]. Moreover, both Fas and Fas ligand (FasL) are under positive transcriptional regulation after exposure to oxidants [26]. Interestingly, Krammer and colleagues demonstrated that in vitro administration of vitamin E suppresses FasL mRNA expression and protects T cells of HIV-1 infected individuals from Fas mediated apoptosis [27]. Moreover, it was demonstrated that

administration of combinations of vitamin E and C to cultures of human umbilical vein endothelial cells (HUVEC) treated with lipopolysaccharide could prevent apoptosis by upregulation of *Bcl-2* [28].

ANTIOXIDANTS, THE IMMUNE SYSTEM AND RELATED DISORDERS

The physiological function of protection against external pathogens carried out by the immune system is by itself a source of ROS since activated leukocytes, such as neutrophils, produce free radicals [29]. Moreover, during the inflammatory process, activation of phagocytes through the interaction of proinflammatory mediators, or bacterial products, with specific receptors on the leukocyte plasma membrane results in the assembly of the multicomponent flavoprotein NADPH oxidase which catalyzes the production of large quantities of the superoxide anion radical (O_2^-) [30]. In addition to classical reactive oxygen metabolites, activated neutrophils and monocytes secrete the hemoprotein myeloperoxidase (MPO) into the extracellular space, where it catalyzes the oxidation of Cl^- by H_2O_2 to yield hypochlorous acid (HClO) [31]. HClO is a non-specific oxidizing and chlorinating agent that reacts rapidly with a variety of biological compounds, such as sulphhydryls, polyunsaturated fatty acids, DNA, pyridine nucleotides, aliphatic and aromatic amino acids and nitrogen-containing compounds [32-34]. Moreover, apart from the direct toxic effects, neutrophil-derived oxidants may promote tissue injury indirectly by altering the protease/antiprotease equilibrium that normally exists within the intestinal interstitium. The oxidative inactivation of important protease inhibitors, coupled to the oxidant-mediated activation of latent proteases, creates a favorable environment for neutrophils that allows degradation of the interstitial matrix through elastases, collagenases and gelatinases, as well as injury to epithelial cells [35,36]. However, immune cells not only produce ROS necessary for the microbicidal activity, but they are also sensitive to external ROS due to their

high polyunsaturated fatty acids (PUFA) content. Immune cells are atypical, as compared with other somatic cells, in that they contain high levels of antioxidant vitamins, presumably providing protection against lipid peroxidation and immunosuppression both of which are well known risks posed by high PUFA content [37]. The reactivity of immune cells to exogenous ROS has been shown to be age-dependent. In fact, lymphocytes from elderly individuals appear to be more sensitive to exposure to hydrogen peroxide than those from young adults [38]. Moreover, it has been demonstrated that vitamin E supplementation to healthy elderly patients produced an increase in antibody titer to both hepatitis B and tetanus vaccine [39], thus enhancing T-cell mediated functions. Thus, maintaining adequate antioxidant status may provide a useful approach in attenuating the cellular injury and dysfunction observed in some inflammatory/autoimmune disorders [40,41].

Autoimmunity has been for decades considered the result of a breakdown in self-tolerance. At the present state of the art, it is known that autoimmunity is a physiological process [42]. This phenomenon becomes pathological when the number of autoreactive cells, and particularly the avidity of their receptors for autoantigens, increases [42]. Triggering of the disease usually depends both on the increase in immunogenicity of the target cell, which may be secondary to a viral infection (Chediak-Higashi syndrome and Griscelli syndrome by EBV), and on the individual's own capacity to recognize the autoantigens (HLA, or T cell repertoire in Familial hemophagocytic lymphohistiocytosis [FHL]) [43]. Moreover, apart from the genetic defects that may predispose to autoimmune diseases, one must take into account the environmental factors that are implicated in the development of such pathologies. Among them, an important role is played by xenobiotics such as chemicals, drugs and metals [44]. Iron, aluminum, manganese readily cross the blood brain barrier via specific or non-specific carriers, and contribute to the nervous tissue damage [45,46]. The toxic effects of metals are mediated

through free radical formation, or enzyme inhibition [47-51]. In addition, metals may act as immunosuppressants (cytostatically), or as immunoadjuvants (through non-specific activation of the immune response) [52,53]. Several mechanisms are proposed on how metals act within the immune system and induce autoimmunity. Patients suffering from scleroderma develop autoantigens with metal-binding sites. After metal binding, free radical species are generated which fragment auto-antigens thereby exposing cryptic epitopes, which may then trigger autoimmunity [54-55]. Taken together, these findings underlie the importance of exogenous factors in the pathogenesis of autoimmunity; nevertheless, all these elements are not sufficient to provoke *chronic* autoimmune diseases such as MS, myasthenia gravis, IDDM or Hashimoto's thyroiditis and the passage to chronic disease is usually secondary to a defect in immunoregulation.

Several classes of regulatory T cells, such as Th2, CD25+ and natural killer (NK) T cells, are implied in autoimmune pathologies. In an animal model of a Th2-dominated autoimmune syndrome, the administration of the antioxidant N-acetyl-cysteine (NAC) induced a decrease in mast-cell expression of both IgE and IL-4 [56]. Of major interest is the discovery of the therapeutic potential of a new benzoquinone-containing product derived from wheat germ fermentation. This latter has been shown to have immune restorative properties because it affects the Th1/Th2 network by inhibiting the Th2 response [57]. Another beneficial effect of this molecule is its anti-metastatic activity in various human malignancies and Jurkat leukemia cell line [58]. Intriguingly, the combined treatment with wheat germ and vitamin C profoundly inhibited metastasis formation in various tumor models of different origin (Lewis lung carcinoma, B16 melanoma and human colon carcinoma xenografts [HCR25]) [59]. On the contrary, wheat germ had no toxicity on peripheral blood leukocytes (PBLs) at doses that affected tumor cells. The crude powder extract of fermented wheat germ inhibits nucleic acid ribose

synthesis primarily through the non-oxidative steps of the pentose cycle [58]. Curiously, another quinone compound, carnosic acid quinone, like wheat germ, recovers potent antioxidant activity upon standing [60].

Keeping in mind the importance of oxidative stress in the regulation/dysregulation of immune system, the use of antioxidants in such diseases has been reasonably proposed. Rheumatoid arthritis (RA) is a classic example of autoimmune disease. Joint inflammation in rheumatoid arthritis (RA) is characterized by invasion of T cells in the synovial space and proliferation of activated macrophages and fibroblasts in the synovial intima [61]. Therefore, in the rheumatic joint there is an increased activity of fibroblasts and leucocytes which produce ROS [62,63]. Very recently, antioxidants have been successfully used as adjuvant therapy in RA [64,65]. Although the results obtained with RA seemed to be very promising, the indiscriminate use of antioxidants in autoimmune disorders is not recommended. In fact, autoimmune lymphoproliferative syndrome (ALPS), MS, type 1 diabetes and multiple autoimmune syndrome, have been linked to a decrease in Fas functionality [66] and, as discussed previously, antioxidants may up-regulate Fas and FasL in vitro. Increasing evidence provides support that oxidative stress and apoptosis are closely linked physiological phenomena and are implicated in diseases including autoimmune diseases. Therefore molecules that target both apoptosis-related signal transduction and oxidative stress, like antioxidants, are likely to result in the improvement of these pathologies.

A novel possible approach to modulate immune system thus preventing autoimmunity or transplant rejection is the activation of cytoprotective and antioxidant enzymes such as HO-1. Heme oxygenase-1, the inducible isoform of HO, is a key protein in the cell stress response and its up-regulation is a common event during pro-inflammatory conditions [11,67-70]. Recent papers clearly demonstrated that regulatory T cells overexpress HO-1 and release CO under pro-

oxidant conditions. Carbon monoxide may inhibit the proliferation of effector T cells, thus reducing the immune response and prevent autoimmunity and/or graft reaction [71,72]. Dietary antioxidants, in particular polyphenols, has been shown to increase HO-1 expression in different in vitro systems [3,73,74] and the potential use of this natural substances to regulate immune response should be carefully addressed.

ANTIOXIDANTS, CANCER AND NEURODEGENERATIVE DISORDERS

It is well known that the dietary ingestion of fruits, vegetables, herbs, or their phytochemical constituents aid in cancer prevention [75-77]. It is believed that the antioxidant properties of such foods protect cells from ROS-mediated DNA damage that can result in mutation and subsequent carcinogenesis. ROS-induced DNA damage can take many forms, ranging from specifically oxidized purine and pyrimidine bases, to DNA lesions such as strand breaks, sister chromatid exchanges (SCEs), and the formation of micronuclei [78]. However, the equation “antioxidant = benefit” is not always true. In vivo experiments demonstrated that retinol increases both the humoral and the cell-mediated immune response and could enhance immune surveillance against tumorigenesis [79-81]. Retinol may influence the immune response by quenching free radicals, which could lower the level of immunosuppressing lipid peroxides, alter arachidonic acid metabolism etc. [80,82]. Interestingly, the association of high fruit and vegetables consumption and decreased risk of lung cancer in healthy individuals has been shown and a combination of β -carotene, vitamin E and selenium reduced stomach cancer mortality in China [83,84]. Conversely, supplemental β -carotene alone or in combination with retinol or vitamin E did not have any effect on cancer risk or increased the development of lung cancer in smokers [85-87]. The reason why β -carotene may exert dual activity, namely antioxidant or pro-

carcinogenic has been debated for long time. The first hypothesis is that at high concentrations, β -carotene stimulates free radical production whereas at lower concentrations β -carotene exerts antioxidant activity [88]. Furthermore, in the presence of cigarette smoke-derived free radicals β -carotene is cleaved into many derivatives which are very unstable and may trigger further oxidation [89,90]. A recent corollary to this theory is the evidence that β -carotene alone or in combination with cigarette smoke condensate repressed HO-1 expression both in rat fibroblasts and human lung cancer cells [91]. The reduced expression of HO-1 accounted for a reduced production of CO and BR both of which have a marked antiproliferative effects [91-95]. Vitamin E has also been shown to act at the immune system level; in fact, supplementation with this vitamin can increase production of antibodies and enhance cell-mediated immunity in both experimental animals and in humans [96].

Neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) are triggered, at least in part, by oxidative and nitrosative stress and sustained by inflammatory cytokine production [11,68,97-99]. Similarly, autoimmunity mainly contribute to the pathogenesis of MS, characterized by central and peripheral loss of nerve myelin [100,101]. Although the specific sources of the damaging ROS and the affected target structures differ between the neuronal pathologies, the following general features can be defined. Increased levels of oxidation-altered metabolites are found in post-mortem tissues in many of the neurodegenerative diseases listed above [102-108]. An oxidative stress response and compensatory defense reactions can be seen in the affected neural cells; further, disturbances of the mitochondrial metabolism are observed, which may account for an increased leakage of ROS originating from the respiratory chain [11,68,99,109]. However, in addition to the direct induction of oxidative stress, metabolic disorders underlying

every single disease can also indirectly generate an oxidative microenvironment, for example via the induction of a local immune response [110,111]. On this basis, antioxidant and antiinflammatory drugs, such as polyphenols and non-steroidal antiinflammatory drugs (NSAIDs), have been proposed in the treatment of different neurodegenerative diseases [112-114]. However, both polyphenols and NSAIDs gave rise to some problems when used in clinical setting. Due to their scarce bioavailability, only a negligible amount of polyphenols reaches brain tissue and the concentration achieved are much lower than those efficacious in vitro [3]. As far as NSAIDs, *ad hoc* designed clinical trials with a large number of patients, clearly demonstrated that these drugs do not have any significant effect in slowing cognitive decline in patients suffering from mild-to-moderate AD [115,116]. Similar disappointing results have been obtained in the treatment of ALS, a systemic motor neuron disease that affects corticospinal and corticobulbar tracts, ventral horn motor neurons and motor cranial nerve nuclei [117,118]. Approximately 10% of cases are familial and have been linked to point mutation in the gene encoding for Cu/Zn superoxide dismutase (SOD) (for an updated review see [119]). Mice transgenic for mutated SOD1 develop symptoms and pathologies similar to those in human ALS. Mutant SOD1 toxicity is mediated by damage to mitochondria in motor neurons, and this may trigger the functional decline of motor neurons and the onset of ALS in mice [120]. Unfortunately, although the role played by free radical to the pathogenesis of ALS has been demonstrated, antioxidants did not have any effect to prevent or slow down its progression. Desnuelle et al., clearly demonstrated that alpha-tocopherol, given together with riluzole, did not affect the survival and motor functions in ALS patients respect to the group treated with riluzole alone [121]. A new compound, AEOL-10150 (Aeolus), structurally related to the SOD catalytic site, is under phase I clinical investigation, but further clinical trials will be necessary to evaluate the real efficacy of this compound for the therapy of ALS [122,123].

CONCLUSIONS

The field of antioxidants is moving rapidly. About 20 years ago the hypothesis that diet might have a substantial influence on the development of some pathologies, such as cancer, has been raised by many scientists. In this light, during the last decade, efforts have been made to analyze the effects of plant food and synthetic antioxidants on the development and prevention of chronic diseases. Nowadays, antioxidants are used on a large scale to try to obtain and preserve optimal health. While there is no doubt that the correct balance between endogenous and exogenous antioxidant capacity is essential to life, the curative power of antioxidants has often been overestimated. In fact, according to the popular idea “if one is good two is better”, antioxidants are taken in excess very often and the risk to originate diseases instead of prevent them is strong. It is noteworthy to underline that as for all drugs, antioxidants may give important side effects if used incorrectly or in combination with other drugs. Vitamin A, E and β -carotene for instance, have been shown to have pro-oxidant effects at higher doses or under certain conditions [38]. Another point of criticism is the possibility to take experimental results “from the bench to the bedside”. In fact, although the promising results obtained by in vitro experiments, the use of antioxidants in the treatment of human disease states has not been as successful as might have been envisaged due to intrinsic pharmacokinetic or pharmacodynamic limitations.

In conclusion, the correct use of antioxidants may be useful to prevent free radical-related disorders. However, the repair of existing critical structural damage may be beyond the possibilities of antioxidants and therefore they may not be considered to be useful in therapeutic clinical applications, where their limits and eventual side effects must be better understood.

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