The Antioxidants and Pro-Antioxidants Network: An Overview

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Abstract: Living beings have evolved over the past two billon years through adaptation, to an increasing atmospheric oxygen concentration, by both taking advantage of oxygen activating function and developing a complex control network. In these regards, potentially damaging species (reactive oxygen, nitrogen and chlorine species) arise as by-products of metabolism and also work as physiological mediators and signalling molecules. Oxidative stress may be an important factor in numerous pathological conditions, i.e. infection if micronutrients are deficient. Levels of these species are controlled by the antioxidant defence system, which is composed by antioxidants and pro-antioxidants. Several components of this system are micronutrients (e.g. vitamins C and E), are dependent upon dietary micronutrients (e.g. CuZn and Mn superoxide dismutase) or are produced by specific endogenous pathways. The antioxidant defences act, to control levels of these species, as a coordinated system where deficiencies in one component may affect the efficiency of the others. In this network some of the components act as direct antioxidants whereas others act indirectly (pro-antioxidants) either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins. Thus, entities usually not considered as antioxidants, also act efficiently counteracting damaging effects of oxidative species. In this contest, the design of new molecules that take into account synergistic interactions among different antioxidants, could be useful both to address mechanistic studies and to develop possible therapeutic agents. In this review the principal categories of antioxidants and pro-antioxidants that goes from vitamins through phyto-derivatives to minerals, are critically reviewed, with particular emphasis on structure-function considerations, together with the perspective opened, in the design of possible therapeutic agents, by the antioxidants interplay.

Key Words: Antioxidants, pro-antioxidants, structure-function relationships, drug design.

INTRODUCTION

ROS and RNS produced *in vivo* at levels that cannot be adequately counteracted by endogenous antioxidant systems can lead to the damage of lipids, proteins, carbohydrates and nucleic acids. The oxidative modification of these molecules by toxic levels of ROS and RNS represents an extreme event that can lead to deleterious consequences such as loss of cell function, this occurrence is known as "oxidative stress". More recently, however, interest has been focused on the formation of these species at sub-toxic levels, for their potential to act as biological signal molecules. Subtoxic ROS and RNS production can lead to alterations in cellular and extracellular redox state, and it is such alterations that signal changes in cell functions. By the use of a variety of cell types it has been shown that numerous cellular processes including gene expression can be regulated by subtle changes in redox balance [1]. Examples of this include the activation of certain nuclear transcription factors, and the determination of cellular fate by apoptosis or necrosis [2]. Cellular redox balance is, under normal circumstances, probably under genetic control and maintained by an array of enzymatic systems that ensure that overall reducing conditions prevail. Antioxidants are an heterogeneous family of molecules, difficult to classify by common shared structural properties. Moreover, other compounds should be also considered that does not act directly as antioxidants, but just indirectly either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins, promoting their synthesis and/or availability. We may propose for these substances the term of pro-antioxidants. Several classifications have been attempted in the past taking into account the origin (natural or synthetic), nature (enzymatic or non-enzymatic), chemical-physical properties (hydrophilic or lipophilic), structure (flavonoids, polyphenols, etc.), mechanism (preventive, chain-breaking, etc.). Because antioxidants functions are expressed through a complex network they would be better characterized by function-structure considerations. Taking this into account, several classes of antioxidant and pro-antioxidants agents may be envisaged. The coverage of all possible aspects and implications exceed the aim of this work, in this review the principal classes of antioxidant and pro-antioxidant molecules will be classified in view of structure-function considerations.

Considering the wide number and different molecules provided, directly and/or indirectly, of antioxidant effects we may consider the following classes: vitamins; fats and lipids; amino acids, peptides and proteins; plant derived antioxidants; minerals; enzymes.

Vitamins

This class of molecules exerts its antioxidant activity directly by an intrinsic free radical scavenging mechanism (i.e. vitamin C) and/or indirectly participating to the regulation and expression of enzymes (i.e. iNOS).

Retinol. Vitamin A presents both kind of antioxidant activities: it is able to scavenge ROS [3] by a direct

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1381-6128/04 $45.00+.00

mechanism and also inhibits, by its oxidized metabolite retinoic acid, NO production through inhibition of iNOS gene transcription in different tissues as vascular, cardiac and endothelial [4, 5]. 1,25-Dihydroxyvitamin D3 as well as vitamin K2 and B3 (vitamin PP, Niacin) also inhibit iNOS expression in smooth muscle and brain as well [6, 7].

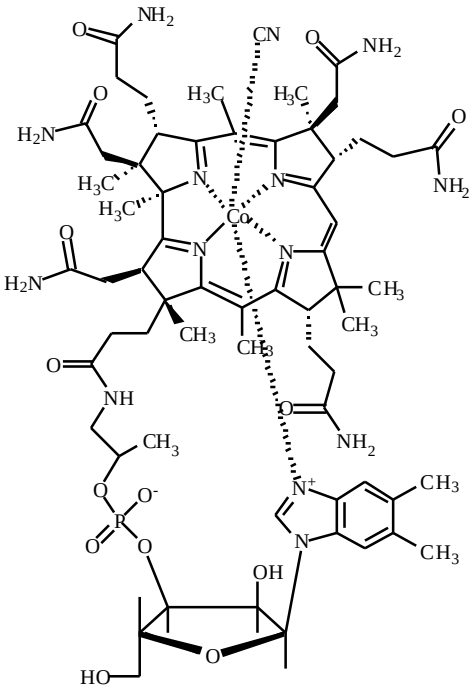
Vitamin E is the most important antioxidant in lipids. It also acts by at least two different mechanisms: directly scavenges ROS and up-regulates antioxidant enzymes such are GPX, CAT from liver, SOD, GST, GR and NAD(P)H: quinone reductase (DT-diaphorase)activities [8-11].

Vitamin C is the most important antioxidant present in the hydrophilic compartments, its activity is expressed through a variety of mechanisms, which still await to be fully clarified. In principal it acts by scavenging ROS directly, and among these species probably the most important are superoxide and peroxynitrite [12]. Secondly, it has been shown that ascorbate can recycle alpha-tocopherol, which in turn helps to prevent lipids oxidation [13]. Without ascorbate, the alpha-tocopheroxyl radical can assume a pro-oxidant role and continue or even enhance the chain reaction of lipid peroxidation [14]. The important role of ascorbic acid within the network of recycling antioxidants has been extensively investigated [15, 16] and although known since long time, the studies on this vitamin are far to be complete. For example, we have recently highlighted its possible role in the transport of drugs, which do not cross itself the blood brain barrier [17]. In these regards, it may act as a double targeting molecule, working both as a carrier and as antioxidant in neurodegenerative diseases.

Nicotinamide (Niacin, Vitamin B3) inhibits lipid peroxidation induced by photosensitization with an activity comparable to that of glutathione and superior to that of vitamins E and C. This activity is exerted through multiple mechanisms, which involves increase of GSH and GST levels and direct quenching of ROS (i.e. $^1O_2$) [18]. Moreover, as stated above, vitamin B3 is able to modulate iNOS expression.

Riboflavin and **Niacin** are components of $NADP^+$/ NADPH, NAD^+/NADH, and FAD/$FADH_2$, that play a fundamental role within the recycling antioxidant network, restoring the reducing capabilities of antioxidant molecules such is dihydrolipoate. The final acceptor of the oxidized species, the CRS CAT activity is strictly related to NADPH [19,20]. Moreover, both NADPH and FAD serves as cofactors for glutathione reductase, which produces GSH from GS-SG [21]. In addition, the production of NO, that antagonizes superoxide production, requires NADPH [22].

Homocysteine, has a potent pro-oxidative activity and might induce atherosclerosis by damaging the endothelium either directly or by altering the oxidative status. For these reasons this amino acid is considered a vascular disease risk factor. Because **vitamin B6** (pyridoxal-5'-phosphate) and **B12**, along with folates, play a fundamental role in homocysteine metabolism, by serving as cofactors for methionine synthase (B12), cystathionine synthase (B6), and cystathionase (B6), they might be considered indirect antioxidants because they are able to lower homocysteine levels and thus the associated oxidative damages [23, 24]. Moreover, vitamin B6 is a necessary co-factor for several TSS pathway enzymes, this imply that a lack of B6 limits the availability of cysteine, one of most important antioxidant amino acids, involved in GSH biosynthesis [25].



Vitamin B12

Fig. (1).

Fats and Lipids

PUFAs (omega 3 and 6) and others. In general, this class of molecules is readily oxidized by ROS; moreover, excess intakes of lipids by foods enhances the amount of ROS produced in the respiratory chain [26]. In these terms lipids are pro-oxidant species and also induce iNOS expression in many cell types. However, conflicting reports have appeared in literature on this topic. Indeed, within the class of lipids, PUFAs, and among them omega-3-PUFAs, contained in fish oil (docosahexaenoic acid, eicosapentaenoic acid, and alpha-linolenic acid), appear to behave differently, acting as inhibitors of free radical generation. This mechanism is particular and involves the increase of expression of antioxidant genes and the inhibition of iNOS expression and inducible NO synthesis [27]. This in contrast to the parent omega-6-PUFAs that appear to increase oxidative stress [28]. Very recently this statement has been revised in a comparative study on fish and olive oils. The results show that administration of fish oil rich diets in rats, increased lipid peroxidation and affected iron metabolism. On the other hand, the olive oil rich diet did not increase oxidative stress and did not alter iron metabolism. Based on these conflicting results, we may conclude that the pattern is far to be completely elucidated and that fish oil supplementation should be advised carefully [29].

Aminoacids, Peptides and Proteins

Aminoacids

Taurine, a sulphur containing ß amino acid, is the most abundant intracellular amino acid in humans, and is impli-

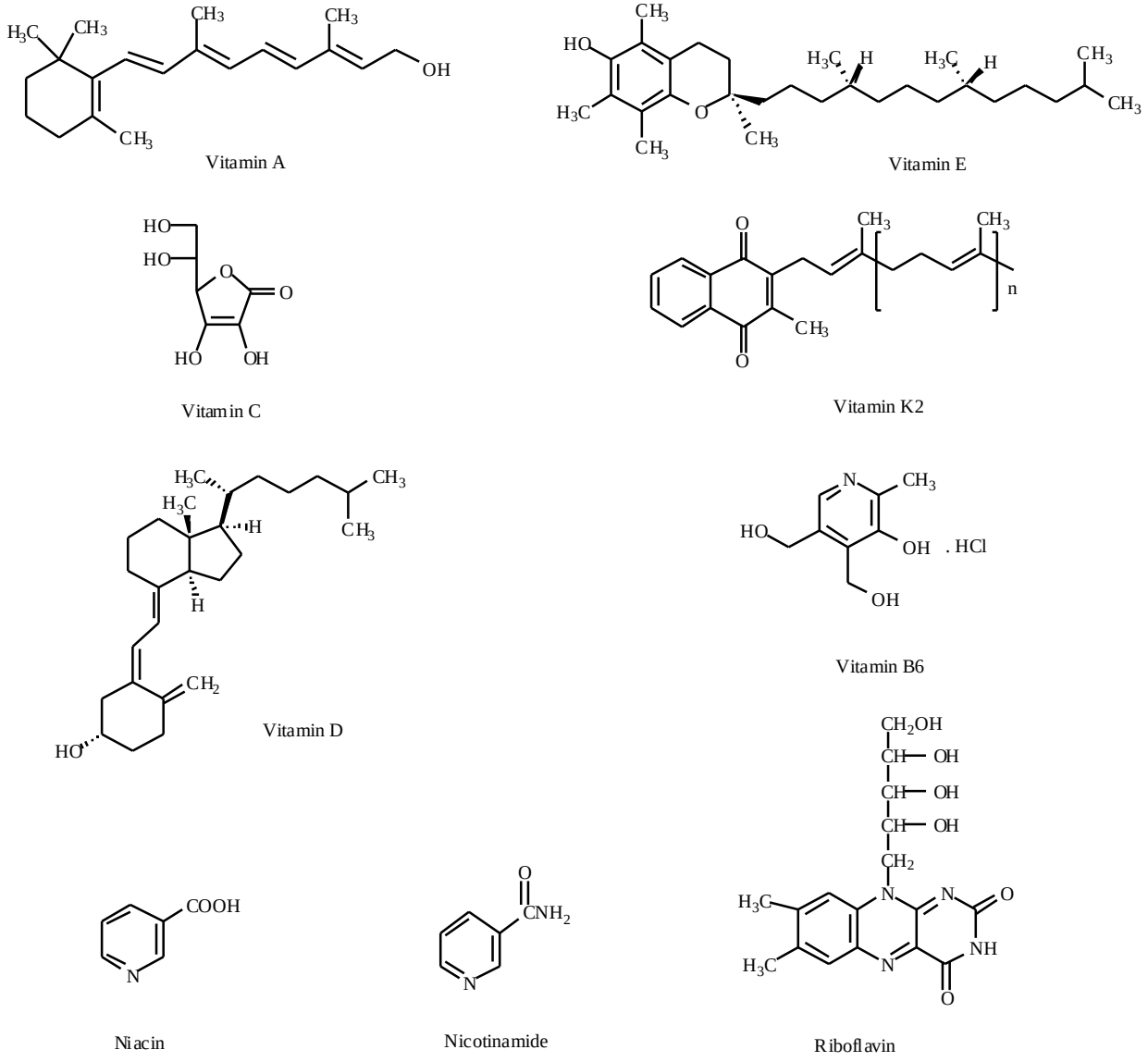


Fig. (2).

cated in numerous biological and physiological functions and is found in high concentrations in tissues containing catecholamines [30]. Taurine has been suggested to have cytoprotective actions via a number of different mechanisms. In healthy individuals the diet is the usual source of taurine; although in the presence of vitamin B6 it is also synthesised from methionine and cysteine. Taurine has a unique chemical structure that implies important physiological functions: bile acid conjugation and cholestasis prevention, antiarrhythmic/ inotropic/chronotropic effects, central nervous system neuromodulation, retinal development and function, endocrine/ metabolic effects and antioxidant/anti-inflammatory properties. Taurine is a unique amino acid with antioxidant and osmolytic properties, and its capability to inhibit oxidative damage to DNA, through inhibition of quinone formation, has been recently highlighted [31]. Sulfur containing amino acids, such as taurine, could also serve to reduce cellular damage associated with both NO and metal-stimulated catecholamine oxidation [32]. **Glutamine**, the preferred fuel for the gut, liver, and immune cells, also has an important role because it serves as a precursor for antioxidants [33]. Glutamine and glutamate with proline, histidine, arginine and ornithine, comprise 25% of the dietary amino acid intake and constitute the "glutamate family" of amino acids, which are disposed of through conversion to glutamate. It is a component of the antioxidant glutathione and of the polyglutamated folic acid [34].

L-Arginine is a semiessential amino acid with a terminal guanidinium group, that serves as natural substrate for the synthesis of NO by different NOS. The mechanism by which L-arginine exerts its protective effects is still unclear. L-arginine may act as direct antioxidant by scavenging oxygen-derived free radicals [35]. The complex reaction involves the transfer of electrons from NADPH, via the flavins FAD and FMN in the carboxy-terminal reductase domain, to the haem in the amino-terminal oxygenase domain, where the

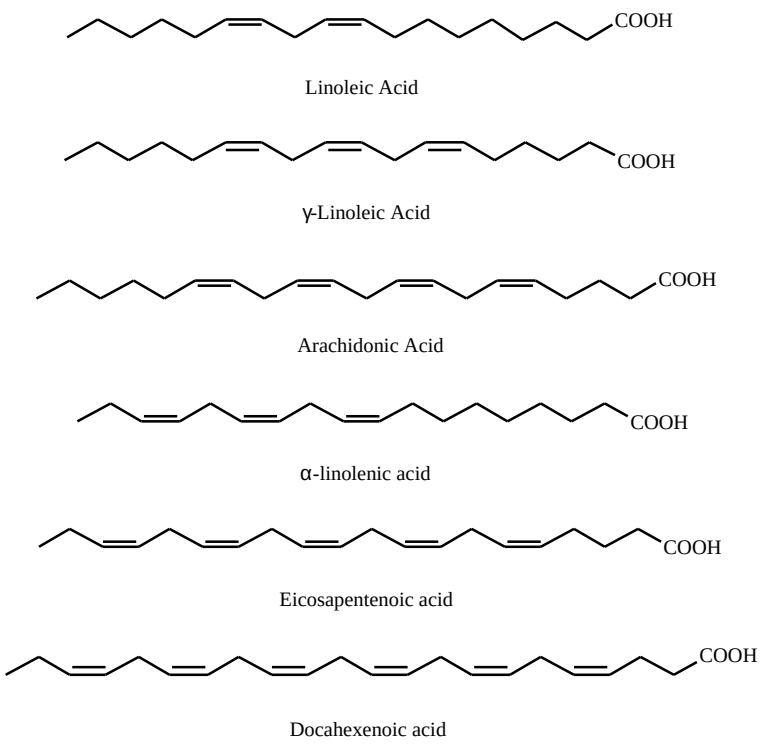


Fig. (3).

substrate L-arginine is oxidised to L-citrulline and NO. The haem is essential for dimerisation as well as NO production. The pteridine/tetrahydrobiopterin is a key feature of NOS, affecting dimerisation and electron transfer, although its full role in catalysis remains to be determined. NOS can also catalyse superoxide anion production, depending on substrate and cofactor availability. There are three main isoforms of the enzyme, named neuronal NOS, inducible NOS (iNOS), and endothelial NOS (eNOS), which differ in their dependence on Ca^{++}, as well as in their expression and activities. These unique features give rise to the distinct subcellular localisations and mechanistic features, which are responsible for the physiological and pathophysiological roles of each isoform [36].

Histidine, can prevent LDL modification, both because it may act as a singlet oxygen scavenger, but also because it may complex Cu^{++} ions and thus prevent lipid peroxidation [37,38]. Based on this rational**,** zinc-histidine complex has been recently proposed as synergistic approach to ìmprove zinc absorption, and contribute to the antioxidant status of plasma, providing antioxidant properties against LDL oxidation (as well as other patho-physiological processes) through transition metal-chelating mechanisms [39]. Being the component of the dipeptide carnosine, the behaviour of these two antioxidants has been also compared. Histidine was more effective at inhibiting copper-promoted formation of carbonyls on bovine serum albumin than carnosine, but carnosine was more effective in inhibiting copper-induced ascorbic acid oxidation than histidine. However, neither carnosine nor histidine resulted capable to inhibit 2,2'-azobis (2-amidinopropane)dihydrochloride-promoted oxidation of LDL; this result seems to indicate that their main antioxidant mechanism is through copper chelation [40].

Glycine, as antioxidant, is involved in kidney protection from massive injury induced by ischemia-reperfusion, protects renal antioxidant enzymes and Na^{+}-K^{+}ATPase, normalises malondialdehyde, and nitric oxide levels. Data obtained on hypoxia/reperfusion injuries suggests a protective role of glycine against ROS. The mechanism proposed involves the regulation of antioxidant enzymes such are SOD, GPX, CAT. As well as the NO production by iNOS [41].

Thiols. Biothiols are quite efficient antioxidants able to react with free radicals and thus protecting the cells against their damages. In such reactions, thiols undergo one-electron oxidation with the formation of thiyl radicals. Because of these properties, thiols as attracted consistent attention. On the contrary, the reactivity of the corresponding thiyl radicals ($RS^{\bullet}$), formed simultaneously in these reactions, have been often underestimated. Indeed, protective and repairing efficacy of thiols depends not only on their capacity to detoxify free radicals, but also on chemical character and reactivity of the formed thiyl radical. Furthermore, quick and efficient removal of RS radical leads to a disturbance in balanced state of antioxidant reaction, which effectively increases repairing capacity. Dangerous thiyl radicals, which can cause peroxidative injury, should immediately undergo regenerative reduction to thiols. Under physiological conditions, thiyl radicals can react with thiolate anion yielding disulfide radical anion ($RSSR^{\cdot-}$) as an intermediate and finally disulfides and superoxide radical anion ($O_2^{\cdot-}$), which is next inactivated in the reaction catalysed by SOD. Thiyl radicals can also be reduced to thiols by reacting with ascorbate with the formation of low-activity ascorbyl radical that subsequently enters disproportiation reaction [42].

Thiol groups are central to most, if not all, redox-sensitive cell signalling mechanisms. Oxidation of thiol groups is a reversible process that represents a sensitive redox-regulated functional switch. Additionally, increasing evidence suggests that thiol groups located on various molecules act as redox sensitive switches thereby providing a common trigger for a variety of ROS and RNS mediated signalling events. Particular attention has been paid to the importance of thiols and thiol-containing molecules in these processes [43]. While reversible **cysteine** oxidation and reduction is part of well-established signalling systems, the oxidation and the enzymatically catalysed reduction of **methionine** is just emerging as a novel molecular mechanism for cellular regulation. Methionine sulfoxide reductase, which reduces methionine sulfoxide to methionine in a thioredoxin-dependent manner, is therefore an enzyme important for the repair of age- or degenerative disease-related protein modifications. It is also a potential missing link, in the post-translational modification cycle, involved in the specific oxidation and reduction of methionine residues in cellular signalling proteins, which may give rise to activity-dependent plastic changes in cellular excitability [44].

N-Acetyl Cysteine (NAC) is another known precursor for glutathione synthesis that has been shown to act on redox balance and to be capable of significantly improving the antioxidant potential by elevating reduced GSH levels. Antioxidants such as NAC have been used as tools for investigating the role of ROS in numerous biological and pathological processes. NAC inhibits activation of c-Jun N-terminal kinase, p38 MAP kinase; modulate redox-sensitive activating protein-1 and nuclear factor kappa B transcription factor activities, thus regulating expression of numerous genes. NAC can also prevent apoptosis and promote cell survival by activating extracellular signal-regulated kinase pathway, a concept useful for treating certain degenerative diseases [45]. NAC directly modifies the activity of several proteins by its reducing activity [46]. A comprehensive survey of the literature highlights the role of antioxidant agents in counteracting the unfavorable effects of ROS and oxidative stress in cancer progression and particularly in the onset of tissue wasting and cancer-related anorexia/cachexia syndrome (CACS) [47]. Recent results confirm a favorable effect of antioxidant agents, such as ALA and NAC, on several important T-cell functions *in vitro* in advanced-stage cancer patients, which may potentially translate into a more effective control of tumour cell growth and prevention/ reversal of CACS also *in vivo* [48]. Moreover, very recently NAC was shown to inhibit TNF-alpha mediated phosphorylation of p65 (ser536) in vascular endothelial cells. This is an interesting indication that natural antioxidants may serve as potent Nuclear Factor kappa B (NF-kappaB) inhibitors in vascular endothelial cells, yet acting through unique and divergent pathways [49]. **S-Adenosyl-L-methionine** (SAMe) is a precursor of cysteine, one of the 3 amino acids composing glutathione, and exerts many key functions in the liver. SAMe is particularly important in opposing the toxicity of free oxygen radicals generated by various pathogens, including alcohol, which cause oxidative stress largely by the induction of cytochrome P4502E1, and its metabolite acetaldehyde [50].

Peptides

Carnosine is a beta-alanyl-L-histidine dipeptide found in skeletal muscle and nervous tissue, is a physiological dipeptide, which can delay ageing and rejuvenate senescent cultured human fibroblasts. The anti-oxidant, free radical- and metal ion-scavenging activities of carnosine alone,

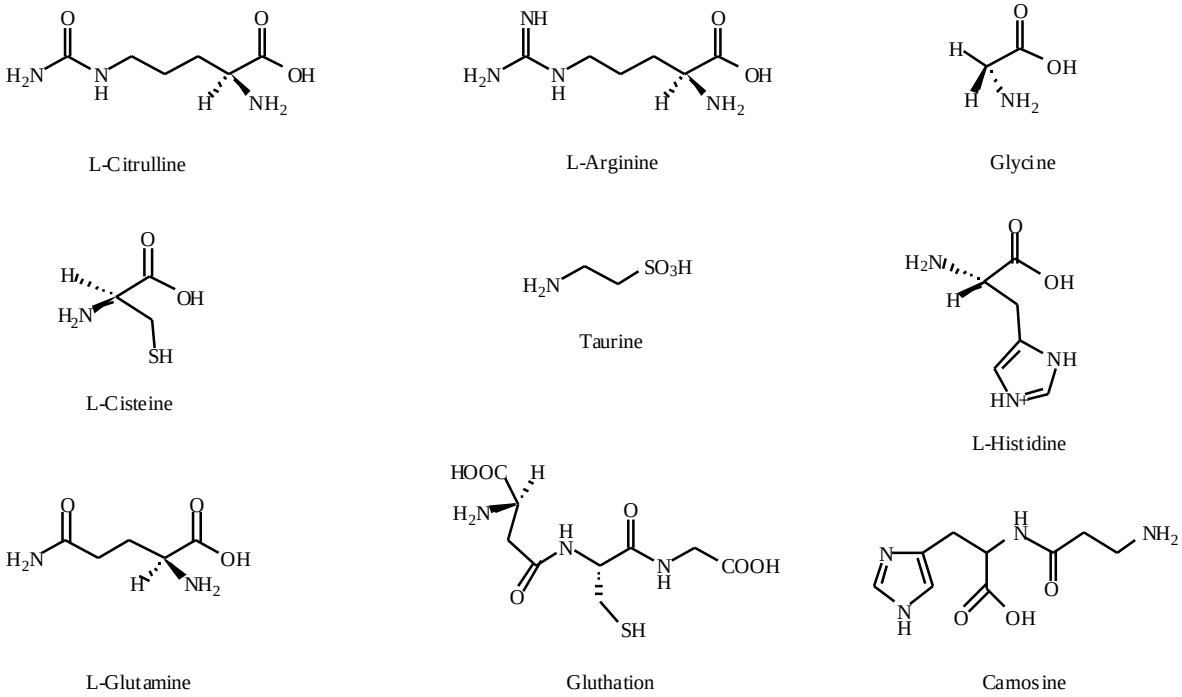


Fig. (4).

cannot adequately explain these effects. Indeed, carnosine is also able to reacts with small carbonyl compounds (aldehydes and ketones) and protects macromolecules against their cross-linking actions [51, 52]. This process is termed 'carnosinylation', and because ageing is associated with accumulation of carbonyl groups on proteins, carnosine act by at least two different mechanism (i.e. antioxidant and carnosinylation) as an anti-ageing molecule. This reaction has been proposed to occur in cultured fibroblasts and *in vivo.* In these studies carnosine was able to suppress diabetes-associated increase in blood pressure in fructose-fed rats, an observation consistent with carnosine's anti-glycating actions [53].

Ascorbic acid (vitamin C) and the tripeptide thiol, **gamma-glutamyl cysteinyl glycine** (GSH) are the major low molecular weight soluble antioxidants in plant cells. The pathway of glutathione biosynthesis is similar in animals and plants while that of ascorbate biosynthesis differs considerably between the two kingdoms. The mechanisms of thiol metabolism and chemistry have particular relevance to both cellular defences against toxicant exposure and to redox signalling. The major pathways for GSH metabolism in defence of the cell are reduction of hydroperoxides by GPX and some peroxiredoxins, which yield GSSG, and conjugation reactions catalysed by glutathione-S-transferases. GSSG can be reduced to GSH by glutathione reductase, but glutathione conjugates are excreted from cells. The exoenzyme GGT removes the glutamate from extracellular GSH, producing cysteinyl-glycine from which a dipeptidase then generates cysteine, an amino acid often limiting for de novo GSH synthesis. Synthesis of GSH from the constituent amino acids occurs in two regulated, enzymatically catalysed steps. The signalling pathways leading to activation of the transcription factors that regulate these genes are a current area of intense investigation [54]. GSH also participates in redox signalling through the removal of H_2O_2, which has the properties of a second messenger, and by reversing the formation of sulfenic acid, a moiety formed by reaction of critical cysteine residues in signalling proteins with H_2O_2 [55]. Moreover, the concentrations of ascorbate and glutathione are greatly modified in response to a variety of environmental triggers, particularly those that cause increased oxidative stress. It is essential that the signals and associated signal transduction pathways that trigger enhanced antioxidant accumulation are elucidated, as these offer an important alternative means of achieving greater nutritional value in edible plant organs [56]. GSH, which has one cysteine, and the small protein thioredoxin, which has two cysteines in its active site, often have complementary, if not overlapping roles in cytoprotection. [57]. The role of GSH as a substrate for GPX, to give GSSG, represents the predominant mechanism for reduction of H_2O_2 and lipid hydroperoxides, [58] thus although it does not react directly with hydroperoxides, it works effectively as an antioxidant.

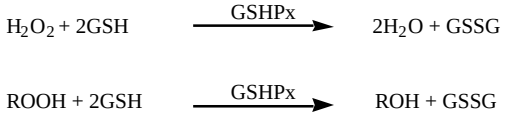


Fig. (5).

Another role for GSH in antioxidant defense depends upon its ability to react with carbon radicals, acting in a concerted manner with superoxide dismutase to prevent oxidative damages [59].

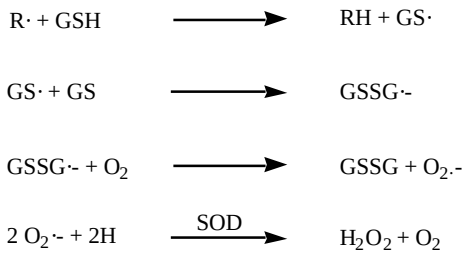


Fig. (6).

Proteins

Generally speaking, insufficient protein intake may induce indirect effects such as zinc deficiency with direct effects on the bioavailability of Cu, Zn-SOD [60]. Proteins such albumin and intracellular metallothionein, are zinc carriers. Thus proteins paucity may induce oxidative stress due to lack of antioxidant proteins.

Albumin. Albumin is the most abundant protein in the circulation, and can function as an antioxidant. It has been recently demonstrated that *in vitro* glycoxidation of human serum albumin induced a marked loss of antioxidant activity of this molecule in the regards of copper-mediated oxidation of LDL, which may be caused by the generation of superoxide [61].

Thioredoxin. Almost all forms of ROS oxidize methionine residues of proteins to a mixture of the R- and S-isomers of methionine sulfoxide. Because organisms contain MSRs, which can catalyse the thioredoxin-dependent reduction of the sulfoxides back to methionine, it was proposed that the cyclic oxidation/reduction of methionine residues might serve as antioxidant system to scavenge ROS, and also to facilitate the regulation of critical enzyme activities [62].

Lactoferrin. Lactoferrin is an iron binding protein involved in a large spectrum of biological actions including antimicrobial actions. Lactoferrin plays a central role in ferrokinetics: it binds free iron with great affinity limiting the amount of ions available for microorganism's metabolism. Lactoferrin is also involved in the modulation of immune system and recent studies indicate that lactoferrin directly modulates both production and function of neutrophils and monocytes. Lipid auto-oxidation in milk is affected by a complex interplay of pro- and anti-oxidants. Several of these compounds are also important nutrients in the human diet and may have other physiological effects in the gastrointestinal tract and other tissues. Lactoferrin has an important role by binding pro-oxidative iron ions [63].

Transferrin. Iron transport occurs by the well-known (Tf)-receptor system and by a second as yet uncharacterized system [64]. The iron carrier protein Tf plays a prominent antioxidant role in the lower respiratory tract and is present

at elevated concentrations in lung epithelial lining fluid relative to plasma [65].

Bilirubin. Bilirubin was long considered a useless metabolite of heme catabolism, responsible for the clinical manifestation of jaundice, and potentially toxic in high doses, particularly in neonates. However, in the last 10 years, *in vitro* and *in vivo* studies, have demonstrated that bilirubin exhibits potent anti-oxidant properties preventing the oxidative damage triggered by a wide range of oxidant-related stimuli [66]. This suggests a beneficial and physiological role for bilirubin in cytoprotection against short and long-lasting oxidant-mediated cell injury [67]. Its role is probably that of a physiological antioxidant present in human extracellular fluids. However, other studies showed that bilirubin in the presence of the transition metal ion Cu(II) causes strand cleavage in DNA through generation of ROS, particularly the hydroxyl radical [68]. Thus bilirubin possesses both antioxidant and prooxidant properties. In order to understand the chemical basis of the different biological properties of bilirubin, structure-activity relationships of bilirubin and its precursor biliverdin have been investigated. The bilirubin is more active both as an antioxidant and oxidative DNA cleaving agent as well, thus possessing antioxidant and toxic properties at the same time. Further, it appear that the structural features of the bilirubin molecule which are important for its prooxidant action, are also those that exert antioxidant activity.

Ceruloplasmin. Ceruloplasmin is a ferroxidase that oxidizes toxic ferrous iron to nontoxic ferric form. Recent results indicate that ceruloplasmin plays an important role in maintaining iron homeostasis in the CNS and in protecting the CNS from iron-mediated free radical injury [69]. Therefore, the antioxidant effects of ceruloplasmin could have important implications for various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur.

Plants Derived Products

Phenols. Tocopherols and tocotrienols are essential components of biological membranes where they have both antioxidant and non-antioxidant functions. There are four tocopherol and tocotrienol isomers (α−, β−, γ−, δ−) which structurally consist of a chroman head group and a phytyl side chain giving vitamin E compounds amphipathic character. Relative antioxidant activity of the tocopherol isomers *in vivo* is α>β>γ>δ, which is due to the methylation pattern and the amount of methyl groups present in the phenolic ring of the polar head structure. Hence, α−tocopherol, with its three methyl substituents, has the highest antioxidant activity among tocopherols. Vitamin E is a chain-breaking antioxidant, i.e. it is able to repair oxidizing radicals directly, preventing the chain propagation step during lipid autoxidation. It reacts with alkoxy radicals ($LO^{\cdot}$), lipid peroxyl radicals ($LOO^{\cdot}$) and with alkyl radicals ($L^{\cdot}$), derived from PUFA oxidation. The reaction between vitamin E and lipid radical occurs in the membrane-water interphase where vitamin E donates an hydrogen ion to lipid radical with consequent tocopheroxyl radical ($TO^{\cdot}$) formation. Regeneration of the tocopherol back to its reduced form can be achieved by vitamin C (ascorbate), reduced glutathione or coenzyme Q. In addition, tocopherols act as chemical scavengers of oxygen radicals, especially singlet oxygen (via irreversible oxidation of tocopherol), and as physical deactivators of singlet oxygen by charge transfer mechanism. TOH formation sustains pro-oxidant action of tocopherol. At high concentration tocopherols act as pro-oxidant synergist with transition metal ions, lipid peroxides or other oxidising agents [70]. In addition to antioxidant functions vitamin E has several non-antioxidant functions in membranes. Tocopherols have been suggested to stabilize membrane structures. Earlier studies have shown that α-tocopherol modulates membrane fluidity in a similar manner to cholesterol, and also membrane permeability to small ions and molecules.

Indeed, the above discussed comparative scale of antioxidant activity should be taken with caution, discrepancy in relative antioxidant effectiveness are found in the respect of the target of activity. Thus, γ-tocopherol was found to be more potent than α-tocopherol in its interaction with reactive nitrogen oxide species [71]. Also, attention should be paid, when considering results obtained by tocopherols coming from different origin (i.e. natural or synthetic, optically pure or racemic). Moreover, antioxidant activity of tocotrienols *vs*. tocopherols is far less studied, α−tocotrienol is proven to be a better antioxidant than α−tocopherol in a membrane environment [72]. Higher antioxidant activity was observed with tocotrienol against lipid peroxidation in rat liver microsomes than with α-tocopherol [73]. Similarly, palm tocotrienol complex in rat brain mitochondria, showed a stronger effect of γ-tocotrienol. Reasons for that may be ascribed to different mechanisms including: i) a more uniform distribution in the membrane lipid bilayer, ii) a more efficient interaction of the chromanyl ring with lipid radicals, and iii) a higher recycling efficiency from chromanoxyl radicals [74, 75]. This data, taken together may suggest important clinical implications for tocotrienols.

Polyphenols: Polyphenols comprise a wide number of natural substances of plant origin. Almost all of them exhibit a marked antioxidant activity. Typical examples in order of increased complexity are hydroxy stilbenes such as resveratrol, an antioxidant in grapes wine and Polygonum cuspidatum, [76] oligomeric catechol structures based on caffeic acid moieties found in several Lamiatae plants (rosmarinic acid, salvianolic acids, yunnaneic acids, etc.), the large group of flavonoids, monomeric and oligomeric flavan-3-ols [derivatives of (+)-catechin or (-)-epicatechin, also known as proanthocyanidins or condensed tannins], or gallo- and ellagitannins (hydrolyzable tannins) [77].

Polyphenols possess the ideal chemical structure for free radical scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. Antioxidative properties of polyphenols arise from i) their high reactivity as hydrogen or electron donors, ii) from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and iii) from their ability to chelate transition metal ions (termination of the Fenton reaction) [78]. Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes. These changes could

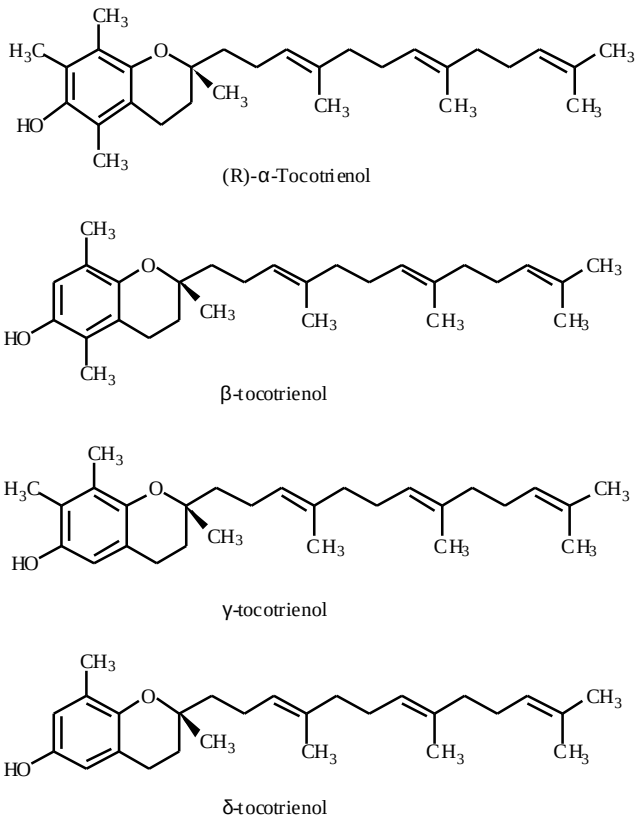


Fig. (7).

sterically hinder diffusion of free radicals and restrict peroxidative reactions. Moreover, it has been shown recently, that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells [79]. Polyphenols are reducing agents, and together with other dietary reducing agents, such as vitamin C, vitamin E and carotenoids, protect tissues against oxidative stress and associated pathologies such as cancers, coronary heart disease and inflammation [80]. These dietary phytophenolics have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species including $O_2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, and $^1O_2^{\cdot}$, but they can also act as pro-oxidant in some conditions. The ESR signals of phenoxyl radicals are eliminated by monodehydroascorbate radical (MDA) reductase, suggesting that phenoxyl radicals, like the ascorbate radical, are enzymatically recycled to parent phenolics [81]. Thus, phenolics in plant cells can form an antioxidant system equivalent to that of ascorbate. In contrast to their antioxidant activity, phytophenolics also have the potential to act as pro-oxidants under certain conditions. For example, flavonoids and dihydroxycinnamic acids can impair DNA functions via the production of radicals in the presence of Cu and O_2. Phenoxyl radicals can also initiate lipid peroxidation. Recently, Al, Zn, Ca, Mg and Cd have been found to stimulate phenoxyl radical-induced lipid peroxidation [82].

Flavonoids. Flavonoids are constituents of fruits, vegetables, and plant-derived beverages, as well as components in herbal-containing dietary supplements, with established *in vitro* antioxidant properties and potential cardioprotective effects. Several compounds belong to flavonoid family, such are flavones (apigenin, luteolin, kaempferol, quercetin, myricetin and rutin), isoflavonoids (genistein, daidzein, biochanin A, and genistin), flavanones (taxifolin, naringenin and naringin) and a flavanol (catechin). The antioxidant and prooxidant activities of flavonoids, belonging to several classes, have been studied in detail to establish their structure-activity relationships against different oxidants [83]. Activity behaviour is particularly related to hydroxyl groups present on the molecule scaffold. Special attention has been paid to the flavonoids quercetin (flavone), taxifolin (flavanone) and catechin (flavanol), which possess different basic structures but the same hydroxylation pattern (3,5,7,3'4'-OH). It was found that these three flavonoids exhibited comparable antioxidant activities against different oxidants leading to the conclusion that the presence of ortho-catechol group (3',4'-OH) in the B-ring is determinant for a high antioxidant activity. The flavone kaempferol (3,5,7,4'-OH), however, in spite of bearing no catechol group, also presents a high antioxidant activity against some oxidants. This fact can be attributed to the presence of both 2,3-double bond and the 3-hydroxyl group, meaning that the basic structure of flavonoids becomes important when the antioxidant activity of B-ring is small [84].

Biochanin A. Biochanin A belongs to the isoflavone class of flavonoids. It is also classified as a phytoestrogen since it is a plant-derived nonsteroidal compound that posseses estrogen-like biological activity. Biochanin A has been found to have weak estrogenic activity. The polyphenolic structures of flavonoids and isoflavonoids give them the ability to either scavenge free radicals and chelate transition metals, a basis for their potent antioxidant abilities. Another possible contribution to their antioxidant activities derives from their ability to stabilize membranes by decreasing membrane fluidity. As stated above, localization of flavonoids and isoflavonoids into the membrane and the resulting restrictions on fluidity of membrane components could sterically hinder diffusion of free radicals and thereby decrease the kinetics of free radical reactions [85].

Glucosinolates. This class of molecules is a large group of sulfur-containing glucosides (β-thioglucoside *N*-hydroxysulfates) and isothiocyanates, widely distributed in Cruciferous vegetable (syn. Brassicaceae). This latter is comprised of familiar foods of the species *Brassica oleracea* (e.g. cabbage, broccoli, cauliflower, Brussels sprouts, kohlrabi and kale) as well as >350 other genera that include a variety of food plants (e.g. arugula, radish, daikon, watercress, horseradish and wasabi). A growing number of studies confirms the chemopreventive activity of cruciferous vegetables, due to the high glucosinolates content [86]. They induce phase-2 detoxication enzymes, increase antioxidant status, and protect against chemically induced cancer, at least in animals. Glucosinolates are hydrolyzed by myrosinase (an enzyme found in plants and bowel microflora) to form isothiocyanates. *In vivo*, isothiocyanates are conjugated with glutathione and then sequentially metabolized to mercapturic acids. These metabolites are collectively designated dithiocarbamate [87, 88]. The cancer preventive effects of cruciferous vegetables could be related to protection from mutagenic oxidative DNA damage [89].

Carotenoids. Dietary carotenoids are thought to provide health benefits, particularly in decreasing the risk of certain

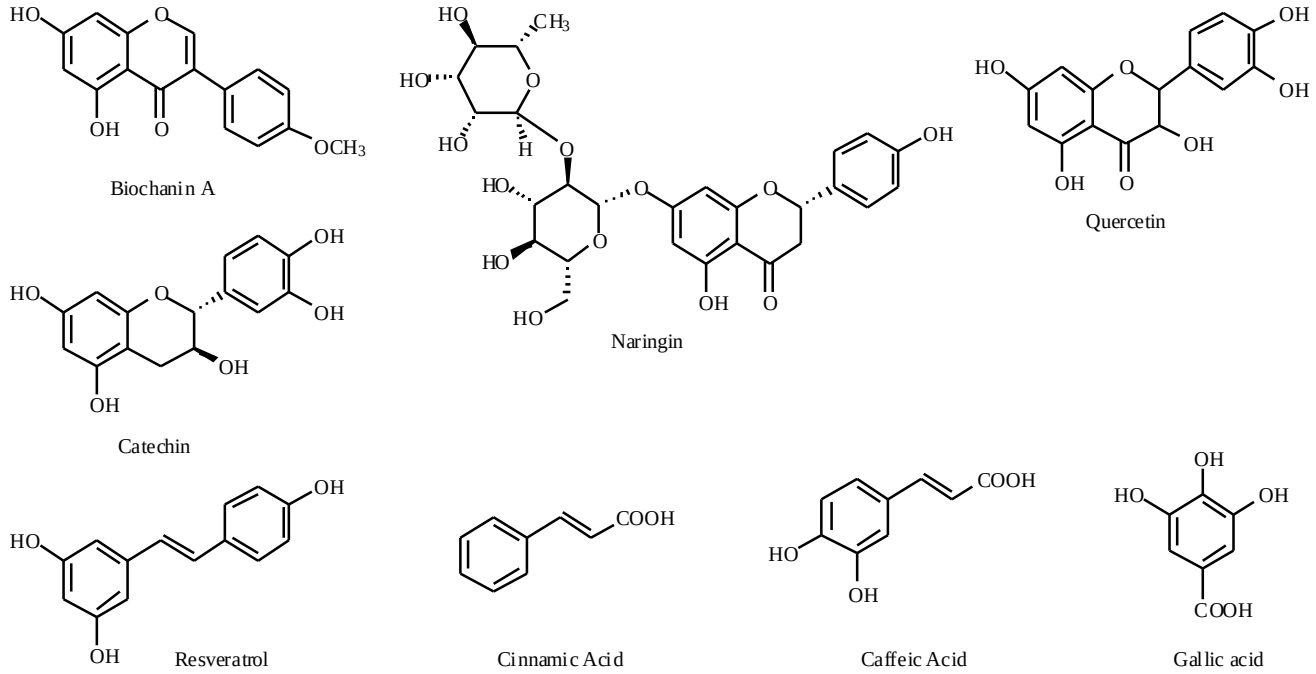


Fig. (8).

cancers and eye diseases. The carotenoids that have been most studied in this regards are β-carotene, lycopene, lutein, and zeaxanthin. The earliest role established for β-carotene in animals was as a vitamin A precursor, a role it shares with other pro-vitamin A carotenoids. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidants, because carotenoids are excellent scavengers of singlet oxygen and respectable scavengers for other reactive oxygen species [90]. The ability of dietary carotenoids such as β-carotene and lycopene to act as antioxidants in biological systems is dependent upon a number of factors. While the structure of carotenoids, especially the conjugated double bond system, gives rise to many of the fundamental properties of these molecules, it also affects how these molecules are incorporated into biological membranes. This implies that *in vivo* behaviour may differ significantly from the results observed *in vitro*. Moreover, interaction with other antioxidants, particularly vitamins E and C, greatly improve the effectiveness of carotenoids as antioxidants [91]. On the other hand, carotenoids, may lose their antioxidant activity at high concentrations or at high partial pressures of oxygen and this behaviour has been related with the low probability of pro-oxidants effects *in vivo* [92]. Increasing data supports pro-oxidants properties of carotenoids as well as possible implications in human health [93, 94]. The antioxidant activity of carotenoids is exerted through the reaction with different ROS ($CCl_3O_2^{\cdot}$, $RSO_2^{\cdot}$, $NO_2^{\cdot}$), which produce via electron transfer, the radical cation of the carotenoid. Reaction with arylperoxyl radicals, instead produce, by hydrogen atom transfer, the neutral carotene radical. The interaction of carotenoids and carotenoid radicals with other antioxidants is of importance with respect to anti- and possibly pro-oxidative reactions of carotenoids. Indeed, in polar environments the vitamin E radical cation is deprotonated ($TOH^{\cdot +}$ --> $TO^{\cdot}$ + H^+) and $TO^{\cdot}$ does not react with carotenoids, whereas in nonpolar environments such as hexane, $TOH^{\cdot +}$ is converted to TOH by hydrocarbon carotenoids. However, the nature of the reaction between the tocopherol and various carotenoids shows a marked variation depending on the specific tocopherol homologue. Similarly, the radical cations of the carotenoids all react with vitamin C restoring the parent carotenoids [95]. Increasing studies, are continuing to deepen the knowledge on carotenoids interactions. Other aspects regards the less investigated polar carotenoids, lutein and zeaxanthin, that constitute the macular pigment were they exert a recently disclosed role in protecting the eye from the blue light [96]. Moreover, antineoplastic activity of carotenoids have been also related to their antioxidant properties, [97] although the formation of retinoids from diverse carotenoids may also account for their action. Dietary intakes of tomatoes and tomato products containing lycopene have been shown to be associated with decreased risk of chronic diseases such as cancer and cardiovascular diseases in numerous studies [98]. Serum and tissue lycopene levels have also been inversely related to the risk of lung and prostate cancers, in this regards, lycopene can trap singlet oxygen and reduce mutagenesis in the Ames test [99]. It has to be considered that, most of the studies have been conducted with either tomato products or lycopene extracted from tomatoes that also contain other carotenoids in various proportions. Therefore, the results cannot be attributed to the solely effects of lycopene [100]. Food sources of these compounds include a variety of fruits and vegetables, although the primary sources of lycopene are tomato and tomato products, but these carotenoids have now become very popular and also available in supplement form [101].

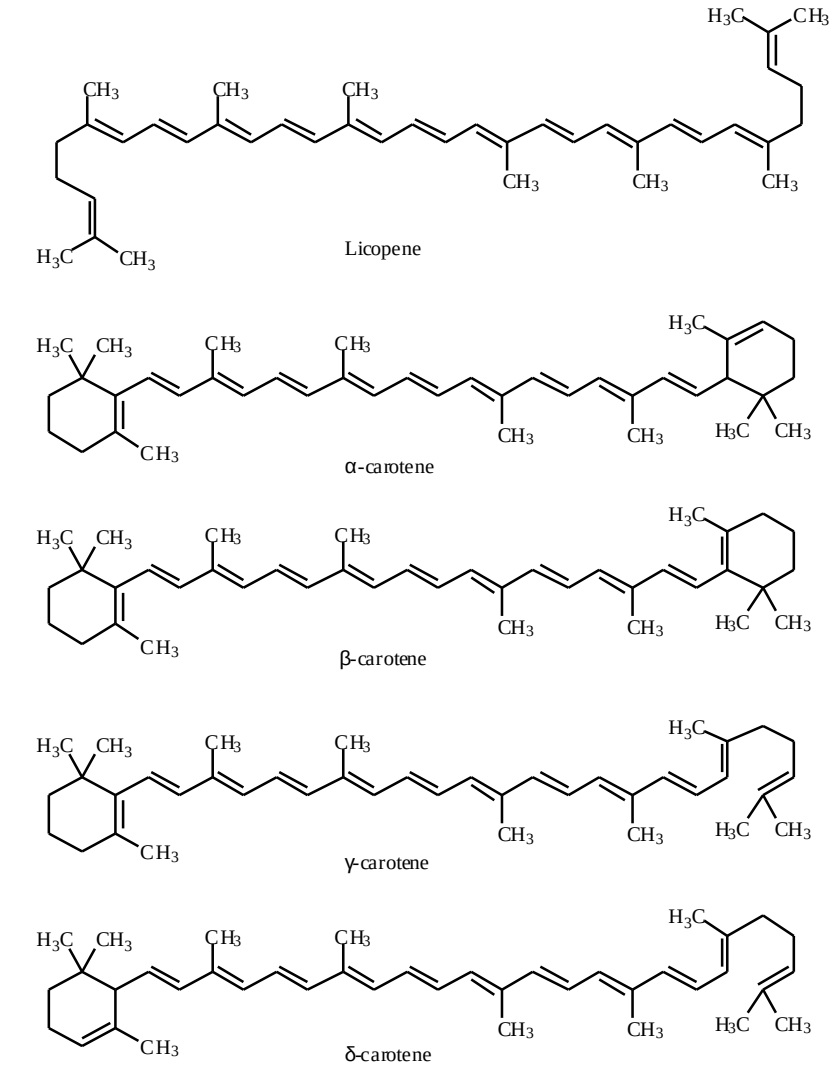

Licopene
α-carotene
β-carotene
γ-carotene
δ-carotene

Fig. (9).

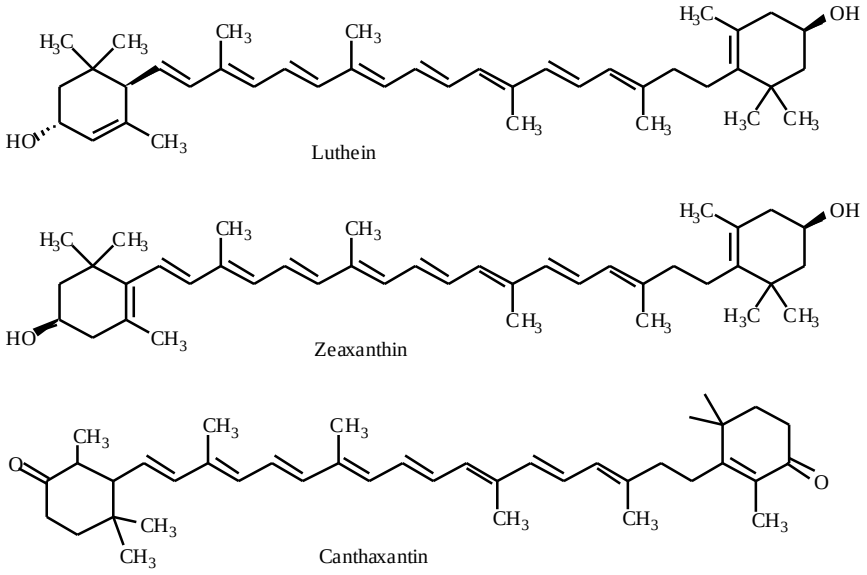

Luthein
Zeaxanthin
Canthaxantin

Fig. (10).

Phytic acid. Phytic acid (myo-inositol hexaphosphate**)** has been extensively studied in animals and is being promoted as an anti-cancer agent in health food stores. It is naturally found in legumes, wheat bran, and soy foods. It is believed to be the active ingredient that gives these substances their cancer fighting abilities [102]. Its cancer chemopreventive activity is thought to be related to its ability in inhibiting the generation of reactive oxygen species from H_2O_2 by chelating metals [103].

Allicin. Various preparations of garlic, mainly aged garlic extract (AGE), have been shown to have promising antioxidant potential. However, the presence of more than one compound in garlic, with apparently opposite biological effects, has added to the complexity of the subject. The organosulfur compounds, responsible, at least in part for the antioxidant activity of AGE are S-allyl-L-cysteine and S-allylmercapto-L-cysteine [104]. Raw garlic homogenate has been reported to exert antioxidant potential but higher doses have been shown to be toxic to the heart, liver and kidney [105]. In view of its strong antioxidant properties, garlic, has been suggested, as a "panacea", for the reduction of risks of several pathological events such are: cardiovascular disease, increased platelet aggregation, thrombus formation, cancer and diseases associated with cerebral aging, arthritis, cataract formation. Moreover it has been reported to rejuvenate skin, improve blood circulation and energy levels [106].

Minerals

The role of minerals in enzyme functions has been studied extensively in therapy, nutrition and biochemistry [107]. For example, magnesium is a cofactor for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, two pentose-cycle enzymes catalyzing the production of NADPH from $NADP^+$. Thus, a deficiency of dietary magnesium reduces glutathione reductase activity and results in radical-induced protein oxidation (indicated by the generation of protein carbonyls) and marked lesions in tissues (e.g. skeletal muscle, brain, and kidney). Iron is the most abundant trace element in the body, and almost all iron occurs bound to proteins. Free iron concentrations are particularly low for two reasons: i) Fe^{3+} is not water soluble, and ii) Fe^{2+} participates in the generation of free radicals. Thus, an increase in extracellular or intracellular iron concentrations, which can result from dietary protein deficiency, dietary iron loading, low concentrations of iron-binding proteins, or cell injury, promotes ROS production, lipid peroxidation, and oxidative stress. Increasing the extracellular concentration of non-heme iron also enhances iNOS protein expression and inducible NO synthesis in many cell types, including cultured proximal tubule cells and macrophages, further exacerbating oxidative damage via peroxynitrite generation.

Zinc. Zinc is present in all organs, tissues, and fluids of the body. Zinc binds to a number of biologic molecules and influences their conformation, stability and activity. Zinc serves as a catalyst for enzymes responsible for DNA replication, gene transcription, and RNA and protein synthesis. Therefore, the ability of zinc to retard oxidative processes has been recognized since many years. Although the evidence for the antioxidant properties of zinc is compelling, the mechanisms are still unclear. In general, the mechanism of antioxidation can be divided into acute and chronic effects [108]. Chronic effects involve exposure of an organism to zinc on a long-term basis, resulting in the production of other antioxidant molecules, such as the metallothioneins. In these terms zinc act as a precursor (pro-antioxidant). Thus, zinc paucity results in lack of defences against some kind of oxidative stress. The acute effects regards either i) protection of protein sulfhydryls or ii) reduction of $OH^{\cdot}$ formation from H_2O_2 through the antagonism of redox-active transition metals, such as iron and copper. Zn^{++} may induce metallothionein synthesis, forming a zinc-thiolate moiety that functions as a preferred sacrificial site for oxidant attacks, preserving skin and its components [109, 110]. Different mechanisms have been proposed for protein sulfhydryl groups protection, among them: i) binding of zinc to the sulfhydryl, ii) steric hindrance by in close proximity to the sulfhydryl group on the protein or iii) a conformational change from binding to some other site on the protein. From these data it appears that much needs to be done to fully elucidate antioxidant properties of this mineral. It is likely that from these researches new antioxidant functions and possibly new applications for zinc will rise up.

Iron. It is essential for aerobic life and is required for the biosynthesis of a variety of iron-containing proteins and for DNA synthesis. Iron is utilized as a catalyst at the active site of numerous enzymes involved in oxygen metabolism and also within proteins involved in oxygen and electron transport, and storage. It can exist in a variety of redox states, but in biological systems is mainly restricted to the ferrous (Fe^{II}), ferric (Fe^{III}), and ferryl (Fe^{IV}) states. The living beings has developed strategies to control, by specific enzymes, negative iron effects and to transport it in a non reactive form binded to specific proteins. On the other hand, low molecular mass (LMrFe) iron form, behave differently. Indeed, LMrFe may act both as a pro-oxidant, leading to the formation of ROS or act as a redox signal molecule [111]. In view of its capability to drive one-electron reactions, iron is the principal responsible in the production and metabolism of free radicals in biological systems. In these regards, ferrous iron (Fe^{++}) can catalyse the decomposition of peroxides to hydroxyl radical from hydrogen peroxide or alkoxyl radicals. Moreover, oxygen is reduced to superoxide radical by Fe^{++}, in turn this latter is promptly restored to the Fe^{+++} form by different reducing agents (i.e. vitamin C) resulting in the production of superoxide anion from molecular oxygen, which can finally dismutate to give oxygen and hydrogen peroxide. Thus, the participation of iron is essential in the production of hydroxyl radical and in the propagation of free radical reactions by decomposing peroxides [112].

Copper. It is a transition metal, essential for life, capable of undergoing one-electron oxidation-reduction conversions. It has several important roles in the body, apparently related to maintenance of immune function, bone health, arterial compliance, haemostasis and protection against oxidative and inflammatory damage [113]. Copper attends to important catalytic functions in a number of enzymes such as Cu, Zn-superoxide dismutase, cytochrome oxidase, and ceruloplasmin. In these enzymatic reactions, copper tightly binds to proteins such that redox activity of the resulting chelate formed is strictly regulated. Copper and zinc, and manganese

are indispensable metals for the activities of Cu, Zn-SOD and Mn-SOD, respectively. Therefore, dietary deficiencies of these minerals markedly decrease tissue Cu,Zn-SOD and Mn-SOD activities and result in peroxidative damage and mitochondrial dysfunction. A deficiency of copper or zinc in rats also enhances cytochrome P-450 activity in microsomes of liver and lung, stimulates ROS generation, and increases intestinal iNOS expression. Such effects render the animal more susceptible to lipid peroxidation and gastrointestinal infection. However, the beneficial redox properties of copper may also result in a pro-oxidant activity: it can catalyse production of free radical intermediates from molecular oxygen, particularly when is released from proteins [114]. Indeed, when the copper-binding domains are altered the redox activity of copper is enhanced and cell damage and death are observed. Thus it is not surprisingly that the delivery of copper is rigorously controlled in cells and biological fluids. Similarly to iron, which is bound to ferritin or transferring, copper is transported and inactivated by specific binding proteins. In plasma, ceruloplasmin and albumin are the two major proteins (along with transcuprein) responsible for binding and transport of copper and prevention of its detrimental redox activity [115]. Taken together these data suggest copper as an essential element, with a complex pattern of utilization, to be taken in high consideration in any issue related to nutrition and supplementation as well.

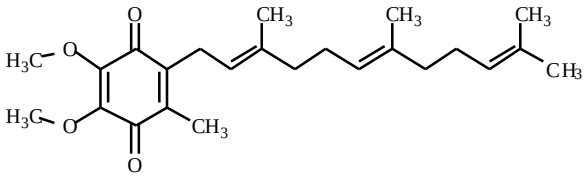
Selenium. It is essential for the functionality of the immune system in both animals and humans [116]. The antioxidant effects of selenium appear to be mediated through the GPX [117] that removes potentially damaging lipid hydroperoxides and hydrogen peroxide. Since the discovery of glutathione peroxidase as a selenium-dependent enzyme, selenium has been identified as an essential cofactor for selenoprotein P and other selenoproteins. At least five of these peroxidases have now been identified as operating in different cell and tissue compartments. Thus, selenium can act as an antioxidant in the extracellular space, the cell cytosol, in association with cell membranes preserving structure integrity [118]. The Keshen disease may be taken as a paradigm of the essential role of selenium in promoting and preserving human health. The name derives from the village in China were the effects of selenium deficiency were firstly discovered [119]. However, excess of selenium intake (selenosis), for example with diet or supplementation, may be also very harmful, leading to loss of hair and nails, skin and nervous system lesions and death [120].

Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. A growing number of studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules, therefore the toxicities associated with these metals might be due to oxidative tissue damage [121]. Redox-active metals, such as iron, copper and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury and others deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes. Either redox-active or redox-inactive metals may cause an increase in production of ROS such as $HO^{\cdot}$, $O_2^{\cdot -}$ or H_2O_2, thus leading to oxidative stress that can be partially responsible for the toxic effects of heavy metals. Although these data suggest a possible role for antioxidants in counteracting and preventing some of the dangerous aspect of heavy metals, the biochemical basis for metal-induced oxidative stress needs to be further investigated prior to suggest a general use of antioxidants in heavy metals related diseases [122]. Several studies are currently underway to determine the effect of antioxidant supplementation following heavy metal exposure.

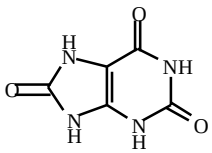
Enzymes

Antioxidant defence mechanisms are present to counteract both metabolic and environmental sources of ROS. Antioxidant defence mechanisms include small non-enzymatic molecules and enzymes. Antioxidant enzymes have been well conserved from bacteria to humans. Antioxidant enzymes include the families of SOD, CAT, GPX, glutathione S-transferase, and thioredoxin. Each family has isoenzymes that are distinguished primarily by their distribution. For instance, the three mammalian SODs are cytosolic (SOD1), mitochondrial (SOD2) [123], extracellular (SOD3), and the two thioredoxins are cytosolic (Trx1) and mitochondrial (Trx2) [124]. Superoxide dismutase (SOD) converts $O_2^{\cdot -}$ into H_2O_2, which is then rapidly reduced by catalase and/or glutathione peroxidase to H_2O and O_2. The rapid methabolism of $O_2^{\cdot -}$ and H_2O_2 is critical because competing mechanism may lead to the generation of more active and toxic radical species [125]. Peroxiredoxins (Prxs) are enzymes lacking prosthetic groups that catalyse the reduction of H_2O_2 and organic hydroperoxides [126]. They are ubiquitous proteins found in organisms ranging from bacteria to humans. In mammalian cells, they typically constitute 0.1 to 0.8% of the total soluble protein of the cell. In particular, PrxII is the second most abundant protein in mouse red blood cells. The abundance of Prxs partly compensates for their moderate catalytic proficiency. For example, other antioxidant enzymes such as catalase and glutathione peroxidase reduce peroxide at rates that are one to three orders of magnitude greater than the peroxide reduction rate of the peroxiredoxins. In eukaryotic cells, the Prxs are both antioxidants and regulators of H_2O_2-mediated signaling. Importantly, human PrxII has been implicated in several disease states including cancer and neurodegenerative disorders [127]. Antioxidant enzyme may be induced by different stimulus [128]. For example, very recently, extracts of Urtica dioica L. have been found able to induce GST, DTD, SOD and CAT activity in the forestomach and SOD and CAT activity in the lung [129].

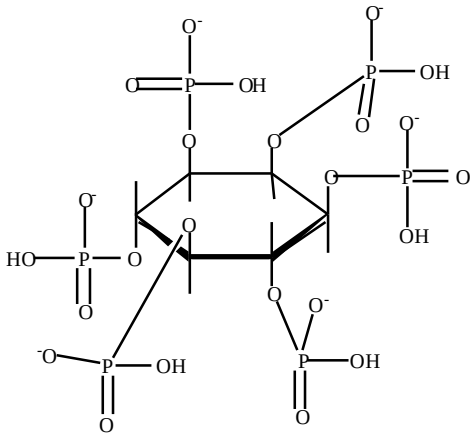
Coenzyme Q. Ubiquinone is a biological compound that is widely distributed in plants, animals, and in most microorganisms. It is present in all tissues associated with biomembranes. However, its biological function is not clear so far. In accordance with the chemistry of redox-cycling ubiquinone, one may assume that this compound acts both as an electron carrier and proton translocator. In mitochondria, coenzyme Q is involved in energy-linked redox processes. Due to its lipophilicity ubiquinone interacts with dehydrogenases and shuttles a pair of two single electrons to cytochromes by diffusion [130]. Coenzyme Q is known to play an important role as a mobile redox proton carrier in the energy-transducing membranes of mitochondria and chloroplasts. The reduced form of Coenzyme Q, ubiquinol,



Ubiquinone



Uric acid



Inositol Esaphosphate

Fig. (11).

has been shown to act as an antioxidant against free radical-mediated oxidations in membranes and lipoproteins [131].

Uric acid. Uric acid is one of the most important antioxidants in plasma. Urate (the soluble form of uric acid in the blood) can scavenge superoxide, hydroxyl radical, and singlet oxygen and can chelate transition metals. Peroxynitrite is a particularly toxic product formed by the reaction of superoxide anion with nitric oxide that can injure cells by nitrosylating the tyrosine residues (nitrotyrosine formation) of proteins. Uric acid can also block this reaction [132]. Recently, Hink *et al*. [133] reported that uric acid may also prevent the degradation of SOD3, an enzyme critical in maintaining endothelial and vascular function. The removal of $O_2^{\cdot-}$ by SOD3 prevents the reaction and inactivation by $O_2^{\cdot-}$ of the important endothelial vasodilator, nitric oxide (NO). SOD3, by removing $O_2^{\cdot-}$, therefore helps to maintain NO levels and maintain endothelial function. Normally, SOD3 is inactivated in the presence of H_2O_2, suggesting a feedback inactivation of the enzyme. However, uric acid blocks SOD inactivation by H_2O_2 by regenerating SOD3 with the production of an urate radical. This latter radical, although potentially pro-oxidant, has been found to be markedly less reactive [134] than classic oxidants and can be rapidly regenerated back to urate in the presence of ascorbate.

What is Behind the Corner?

Several diseases have been recognized as directly related to oxidative damages. In few cases the oxidative activity is as central pathophysiological feature (i.e. amyotrophic lateral sclerosis, ALS), in many other cases the oxidative damage is part or determining factor in the cellular disfunctions and thus symptoms (i.e. neurodegeneration, cataract, diabetes) [135]. Thus, in contrast to the current receptor-based pharmacological approaches, the idea of an antioxidant therapy that could work as a general protection against damages induced by oxidized species is particularly attractive [136].

Moreover, during the last decade, the concept of health promotion has become a legitimate part of health care. In the attempt to counteract the oxidative stress damages, the strategy of implementing the diet with antioxidants, especially deriving from natural sources, is becoming more and more convincing [137]. Therefore, antioxidants have become very well recognized nutraceutical ingredients. Although the great number of positive reports, several problems still remain to be solved before such interventions may become standard tools in the treatment of major diseases and health promotion.

1) First of all free radical pathways appear as very complex to be regulated and much need to be done in terms of basic studies in order to highlight the mechanisms at the base of their action. This also involves possible toxic effects, related to impairment of such pathways. As an example, it has been very recently reported that lipid mediators generated by oxidative pathways play essential roles in vascular disease but also in homeostasis, activating signal transduction pathways that control a variety of cellular functions [138, 139]. Several other evidences are also reported in literature [140].

2) The problem of stability: as higher the activity as higher will be instability and reactivity with oxidized and free radical species. This aspect is also related to possible toxic effects due to antioxidants over-reactivity: some of them may become pro-oxidant in certain circumstances [141].

3) Free radical species are highly diffusible entities, involved in radical-chain reactions and with variable half lives which ranges from nanoseconds to minutes and hours. Thus, it is difficult to imagine that the antioxidant will be present at the exact moment and place where the oxidative damage will occur.

4) Antioxidant effects may be acute or chronic, resulting either from antioxidant or pro-antioxidant activities. Moreover, most antioxidants works in a synergistic manner, with a recycling kind of mechanism: thus α-tocopherol works because restored, after quenching of peroxyl radicals, by ascorbate, which is itself regenerated by the dihydrolipoate-lipoate couple that is finally restored by the NAD-NADH system [142, 143].

5) These latter issues are also complicated by distribution problems: it is difficult to imagine that supplementation by ascorbate and α-tocopherol, one hydrophilic and the other lipophilic, will reach the same biological compartment at the same time, to quench the free-radicals to work synergistically.

These considerations point to the fact that more efforts should be directed toward the development of synthetic compounds, able to overcome the above stated problems but endowed with the versatility of their natural counterparts. Newer molecules could be useful both to address mechanistic studies and to develop possible therapeutic agents.

The Centaur Tactic

In Greek mythology, the centaurs were the half-man, half-horse creature, descendants of Centaurus, a son of the music god Apollo. Much like the centaur, the satyr combined the qualities of the hoofed with the human. Our recently developed strategy for the discovery of novel antioxidants, can be illustrated on the base of these mythological considerations. Indeed, we have obtained molecular combinations of antioxidants, designed in the aim to improve the pharmacology, bioavailability and stability of the parent compounds. Increasing evidences support the idea that a combination of antioxidants may offer a better overall protection against oxidative stress than that exerted by individual antioxidants. In these regards, the existence of cooperative interactions between carotenoids and tocopherols, tocopherol and ascorbic acid, in biological systems, has been reported [144, 145]. In view of such potential interactions, we have recently designed novel entities, deriving from the conjugation of antioxidant cooperative moieties, through stable chemical bonds. In particular, we have recently proposed molecular combinations of the pharmacophores of synergistic antioxidants, i.e vitamin E with vitamin C or carotenoids, as a tool to improve the antioxidant activity by concomitant scavenging of lipoperoxylradicals (preferred by α-tocopherol) and ROS (preferred by vitamin C) [146, 91]. Following the same approach, we have more recently explored molecular combinations between vitamin C and polyphenols, also obtaining a consistent improvement in the antioxidant activity [147]. Other very recent results of this strategy also indicate potential usefulness against degenerative diseases based on oxidative damages [148, 149]. We have very recently reported on molecular combination between idebenone and other cooperative antioxidant entities, obtaining an increase of activity in the respect of the parent compounds [150].

Finally, the molecular combination strategy was afterwards positively considered by us, as a new approach for CNS drug targeting. We have recently investigated the conjugation of ascorbic acid with neurotropic drugs, as a possible means to improve the entry of such CNS drugs, that difficulty cross the blood brain barrier. [17] Further studies and results are currently being submitted for publication. Taking this into account, modification of antioxidants can represent a possibility for exponentially increase their antioxidant activity, and in view of our results, we think the centaur tactic as an important tool in the i) study the mechanism of interaction of cooperative antioxidants; ii) evaluation of new approaches to potential therapeutic agents in chronic diseases in which a free radical damage is involved; iii) investigation of new endogenous carriers to improve the drug bioavailabity of the transported drugs; iv) improvement of stability of antioxidant compounds.

ABBREVIATIONS

AGE = Aged garlic extract

ALA = Alpha-lipoic acid

ALS = Amyotrophic lateral sclerosis

CAT = Catalase

CNS = Central nervous system

GGT = Gamma-glutamyltranspeptidase

ESR = Electron spin resonance

4-HNE = 4-Hydroxynonenal

MSRs = Methionine sulfoxide reductases

ROS = Reactive oxygen species

RNS = Reactive nitrogen species

CRS = Cellular reduction systems

GSH = Glutathione

GPX = Glutathione peroxidase

GS-SG = Glutathione disulfide

GST = Glutathione S-transferase,

GR = Glutathione reductase

$NADP^+$/ NADPH = Nicotinamide adenine dinucleotide phosphate

NAD^+/ NADH = Nicotinamide adenine dinucleotide

NO = Nitric oxide

NOS = NO synthases

FAD/ $FADH_2$ = Flavin adenine dinucleotide

FMN = Flavin mononucleotide

MAP = Mitogen activated protein

Prxs = Peroxiredoxins

PUFAs = Poly unsaturated fatty acids

TNF = Tumour necrosis factor

TSS = Trans-sulfuration

NF-kappaB = Nuclear Factor kappa B

SAMe = S-Adenosyl-L-methionine

Tf = Transferrin

REFERENCES

References 151-153 are related articles recently published in Current Pharmaceutical Design.

[1] Packer L, Valacchi G. Antioxidants and the response of skin to oxidative stress: vitamin E as a key indicator. Skin Pharmacol Appl Skin Physiol. 2002; 15: 282-90.

[2] Matsuo Y, Hirota K, Nakamura H, Yodoi J. Redox Regulation by Thioredoxin and its Related Molecules. Drug News Perspect. 2002; 15: 575-80.

[3] Keys SA, Zimmerman WF. Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Res 1999; 68: 693-702.

[4] Hirokawa K, Oshaughnessy KM, Ramrankha P, Wilkins MR. Inhibition of nitric oxide synthesis in vascular smooth muscle by retinoids. Br J Pharmacol 1994; 113: 1448-54.

[5] Grosjean S, Devaux Y, Seguin C, et al. Retinoic acid attenuates inducible nitric oxide synthase (NOS2) activation in cultured rat cardiac myocites and microvascular endothelial cells. J Mol Cell Cardiol 2001; 33: 933-45.

[6] Sano M, Fujita H, Morita I, Uematsu H, Murota S. Vitamin K-2 (menatetrenone) induces iNOS in bovine vascular smooth muscle cells: no relationship between nitric oxide production and carboxylation. J Nutr Sci Vitam 1999; 45: 711-23.

[7] Garcion E, Nataf S, Berod A, Darcy F, Brachet P. 1,25-Dihydroxyvitamin D-3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. Brain Res Mol Brain Res 1997; 45: 255-67.

[8] Masaki H, Okano Y, Ochiai Y, Obayashi K, Akamatsu H, Sakurai H. Alpha-tocopherol increases the intracellular glutathione level in HaCaT keratinocytes. Free Radic Res 2002; 36: 705-9.

[9] Naziroglu M, Kokcam I, Yilmaz S. Beneficial Effects of Intraperitoneally Administered Alpha-Tocopheryl Acetate on the Levels of Lipid Peroxide and Activity of Glutathione Peroxidase and Superoxide Dismutase in Skin, Blood and Liver of Thermally Injured Guinea Pigs. Skin Pharmacol Appl Skin Physiol 2003; 16: 36-45.

[10] Calfee-Mason KG, Spear BT, Glauert HP. Vitamin E inhibits hepatic NF-kappaB activation in rats administered the hepatic tumor promoter, phenobarbital. J Nutr 2002; 132: 3178-85.

[11] Tauler P, Aguilo A, Fuentespina E, Tur JA, Pons A. Diet supplementation with vitamin E, vitamin C and beta-carotene cocktail enhances basal neutrophil antioxidant enzymes in athletes. Pflugers Arch 2002; 443: 791-7.

[12] May JM. How does ascorbic acid prevent endothelial dysfunction? Free Radic Biol Med 2000; 28: 1421-9.

[13] Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997; 337: 408-416.

[14] Doba T, Burton GW, Ingold KU. Antioxidant and co-antioxidant activity of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, upon peroxidation of aqueous multilamellar phospholipid liposomes. Biochim Biophys Acta 1985; 835: 298-303.

[15] Guo Q, Packer L. Ascorbate-dependent recycling of the vitamin E homologue Trolox by dihydrolipoate and glutathione in murine skin homogenates. Free Radic Biol Med 2000; 29: 368-74.

[16] Thiele JJ, Schroeter C, Hsieh SN, Podda M, Packer L. The antioxidant network of the stratum corneum. Curr Probl Dermatol 2001; 29: 26-42.

[17] Manfredini S, Pavan B, Vertuani S, Scaglianti M, Compagnone D, Biondi C, et al. Design, synthesis and activity of ascorbic acid prodrugs of nipecotic, kynurenic and diclophenamic acids, liable to increase neurotropic activity. J Med Chem 2002; 45: 559-62.

[18] Kamat JP, Devasagayam TP. Methylene blue plus light-induced lipid peroxidation in rat liver microsomes: inhibition by nicotinamide (vitamin B3) and other antioxidants. Chem Biol Interact 1996; 99: 1-16.

[19] Cossins E, Lee R, Packer L. ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations. Biochem Mol Biol Int 1998; 45: 583-97.

[20] Zamocky M, Koller F. Understanding the structure and function of catalases: clues from molecular evolution and in vitro mutagenesis. Prog Biophys Mol Biol 1999; 72: 19-66.

[21] Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal 2000; 2: 811-20.

[22] Wu G, Haynes TE, Li H, Yan W, Meininger CJ. Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. Biochem J 2001; 353: 245-52.

[23] Mennen LI, de Courcy GP, Guilland JC, Ducros V, Bertrais S, Nicolas JP, et al. Homocysteine, cardiovascular disease risk factors, and habitual diet in the French Supplementation with Antioxidant Vitamins and Minerals Study. Am J Clin Nutr 2002; 76: 1279-89.

[24] Earnest C, Cooper KH, Marks A, Mitchell TL. Efficacy of a complex multivitamin supplement. Nutrition 2002; 18: 738-42.

[25] Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology 2002; 180: 33-44.

[26] Dobarganes C, Marquez-Ruiz G. Oxidized fats in foods. Curr Opin Clin Nutr Metab Care 2003; 6: 157-63.

[27] Komatsu W, Ishihara K, Murata M, Saito H, Shinohara K. Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-gamma plus lipopolysaccharide-stimulated murine macrophages by inhibiting the oxidative stress. Free Radic Biol Med. 2003; 34: 1006-16.

[28] Yun-Zhong Fang, Sheng Yang, Guoyao Wu. Free Radicals, Antioxidants and Nutrition. Nutrition 2002; 18: 872-9.

[29] Miret S, Saiz MP, Mitjavila MT. Effects of fish oil- and olive oil-rich diets on iron metabolism and oxidative stress in the rat. Br J Nutr 2003; 89: 11-8.

[30] Lourenco R, Camilo ME. Taurine: a conditionally essential amino acid in humans? An overview in health and disease. Nutr Hosp 2002; 17: 62-70.

[31] Messina SA, Dawson R Jr. Attenuation of oxidative damage to DNA by taurine and taurine analogs. Adv Exp Med Biol 2000; 483: 355-67.

[32] Biasetti M, Dawson R Jr. Effects of sulfur containing amino acids on iron and nitric oxide stimulated catecholamine oxidation. Amino Acids 2002; 22: 351-68.

[33] Boelens PG, Houdijk AP, de Thouars HN, Teerlink T, van Engeland MI, Haarman HJ, et al. Plasma taurine concentrations increase after enteral glutamine supplementation in trauma patients and stressed rats. Am J Clin Nutr 2003; 77: 250-6.

[34] Tapiero H, Mathe G, Couvreur P, Tew KD. II. Glutamine and glutamate. Biomed Pharmacother 2002; 56: 446-57.

[35] Lass A, Suessenbacher A, Wolkart G, Mayer B, Brunner F. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. Mol Pharmacol 2002; 61: 1081-8.

[36] Andrew PJ, Mayer B. Enzymatic function of nitric oxide synthases. Cardiovasc Res 1999; 43: 521-31.

[37] Patterson RA, Leake DS. Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less acidic pH. FEBS Lett 1998; 434: 317-21.

[38] van Hinsbergh WM, Havekes L, Kempen HJM. Role of endothelial cells and their products in the modification of low-density lipoproteins. Biochim Biophys Acta 1986; 878: 49-64.

[39] Yeomans VC, Rechner AR, Rice-Evans CA. The mechanism of action of zinc-histidine complex (Curazink) as an antioxidant. Free Radic Res 2002; 36: 717-8.

[40] Decker EA, Ivanov V, Zhu BZ, Frei B. Inhibition of low-density lipoprotein oxidation by carnosine histidine. J Agric Food Chem 2001; 4: 511-6.

[41] Matilla B, Mauriz JL, Culebras JM, Gonzalez-Gallego J, Gonzalez P. Glycine: a cell-protecting anti-oxidant nutrient. Nutr Hosp 2002; 17: 2-9.

[42] Wlodek L. Beneficial and harmful effects of thiols. Pol J Pharmacol 2002; 54: 215-23.

[43] Moran LK, Gutteridge JM, Quinlan GJ. Thiols in cellular redox signalling and control. Curr Med Chem 2001; 8: 763-72.
[44] Hoshi T, Heinemann S. Regulation of cell function by methionine oxidation and reduction. J Physiol 2001; 53: 1-11.
[45] Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci 2003; 60: 6-20.
[46] Behr J, Maier K, Degenkolb B, Krombach F, Vogelmeier C. Antioxidative and clinical effects of high-dose N-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. Amer. J. Respir. Crit. Care Med 1997; 156: 1897-901.
[47] Mantovani G, Maccio A, Lai P, Massa E, Ghiani M, Santona MC. Cytokine activity in cancer-related anorexia/cachexia: role of megestrol acetate and medroxyprogesterone acetate. Semin Oncol. 1998; 25: 45-52.
[48] Mantovani G, Maccio A, Melis G, Mura L, Massa E, Mudu MC. Restoration of functional defects in peripheral blood mononuclear cells isolated from cancer patients by thiol antioxidants alpha-lipoic acid and N-acetyl cysteine. Int J Cancer 2000; 86: 842-7.
[49] Schubert SY, Neeman I, Resnick N. A novel mechanism for the inhibition of NF-kappaB activation in vascular endothelial cells by natural antioxidants. FASEB J 2002; 16: 1931-3.
[50] Lieber CS. S-adenosyl-L-methionine: its role in the treatment of liver disorders. Am J Clin Nutr 2002; 76: 1183S-7S.
[51] Hipkiss AR, Brownson C. A possible new role for the anti-ageing peptide carnosine. Cell Mol Life Sci. 2000; 57: 747-53.
[52] Aldini G, Carini M, Beretta G, Bradamante S, Facino RM. Carnosine is a quencher of 4-hydroxy-nonenal: through what mechanism of reaction? Biochem Biophys Res Commun. 2002; 298: 699-706.
[53] Hipkiss AR, Brownson C, Carrier MJ. Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. Mech Ageing Dev 2001; 122: 431-45.
[54] Dickinson DA, Iles KE, Watanabe N, Iwamoto T, Zhang H, Krzywanski DM, et al. 4-hydroxynonenal induces glutamate cysteine ligase through JNK in HBE1 cells. Free Radic Biol Med. 2002; 33: 974-87.
[55] Dickinson DA, Forman HJ. Glutathione in defense and signaling: lessons from a small thiol. Ann N Y Acad Sci 2002; 973: 488-504.
[56] Foyer CH. Prospects for enhancement of the soluble antioxidants, ascorbate and glutathione. Biofactors 2001; 15: 75-8.
[57] Dale A. Dickinson and Henry Jay Forman Cellular glutathione and thiols metabolism. Biochem Pharmacol 2002; 64: 1019-26.
[58] Cohen G, Hochstein P. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. Biochemistry 1963; 2: 1420-28.
[59] Winterbourn CC. Superoxide as an intracellular radical sink. Free Radic Biol Med 1993; 14: 85-90.
[60] Machilin LJ, Bandito A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987; 1: 441-52.
[61] Sakata N, Moh A, Takebayashi S. Contribution of superoxide to reduced antioxidant activity of glycoxidative serum albumin. Heart Vessels 2002; 17: 22-9.
[62] Stadtman ER, Moskovitz J, Berlett BS, Levine RL.Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism. Mol Cell Biochem 2002; 234-235: 3-9.
[63] Lindmark-Mansson H, Akesson B. Antioxidative factors in milk. Br J Nutr 2000; 84: 103-10.
[64] Kaplan J. Mechanisms of cellular iron acquisition: another iron in the fire. Cell 2002; 11: 603-6.
[65] Widera A, Beloussow K, Kim KJ, Crandall ED, Shen WC. Phenotype-dependent synthesis of transferrin receptor in rat alveolar epithelial cell monolayers. Cell Tissue Res. 2003; 312: 313-8.
[66] Tomaro ML, Batlle AM. Bilirubin: its role in cytoprotection against oxidative stress. Int J Biochem Cell Biol 2002 ; 34: 216-20.
[67] McGeary RP, Szyczew AJ, Toth I. Biological properties and therapeutic potential of bilirubin. Mini Rev Med Chem 2003; 3: 253-6.
[68] Asad SF, Singh S, Ahmad A, Khan NU, Hadi SM. Prooxidant and antioxidant activities of bilirubin and its metabolic precursor biliverdin: a structure-activity study. Chem Biol Interact 2001; 137: 59-74.
[69] Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. J Neurosci 2002; 22: 6578-86.
[70] Yoshida Y, Niki E, Noguchi N. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chem Phys Lipids 2003 Mar; 123: 63-75.
[71] Cooney RV, Franke AA, Harwood PJ, Hatch-Pigott V, Custer LJ, Mordan LJ. Gamma-tocopherol detoxification of nitrogen dioxide: Superiority to alpha-tocopherol. Proc Natl Acad Sci USA 1993; 90: 1771-75
[72] Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. Journal of Nutrition 2001: 131; 369S-373S.
[73] Serbinova E, Kagan V, Han D, Packer L. Free radical recycling and intramembrane mobility in the antioxidant properties of α-tocopherol and α-tocotrienol. Free Radic Biol Med 1991; 10: 263-75.
[74] Fuchs J, Weber S, Podda M, Groth N, Herrling T, Packer L, et al. HPLC analysis of vitamin E isoforms in human epidermis: correlation with minimal erythema dose and free radical scavenging activity. Free Radic Biol Med. 2003; 34: 330-6.
[75] Weber SU, Thiele JJ, Han N, Luu C, Valacchi G, Weber S, et al. Topical alpha-tocotrienol supplementation inhibits lipid peroxidation but fails to mitigate increased transepidermal water loss after benzoyl peroxide treatment of human skin. Free Radic Biol Med 2003; 34: 170-6.
[76] Brandolini V, Maietti A, Tedeschi P, Durini E, Vertuani S, Manfredini S. Capillary Electrophoresis Determination, Synthesis and Stability of Resveratrol and related 3-O-beta-D-Glucopyranosides J Agr Food Chem 2002; 50: 7407-11.
[77] Bors W, Michel C. Chemistry of the antioxidant effect of polyphenols. Ann N Y Acad Sci 2002; 957: 57-69.
[78] Shafiee M, Carbonneau MA, d'Huart JB, Descomps B, Leger CL. Synergistic antioxidative properties of phenolics from natural origin toward low-density lipoproteins depend on the oxidation system. J Med Food. 2002; 5: 69-78.
[79] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot (Lond) 2003; 91: 179-94.
[80] Tapiero H, Tew KD, Ba GN, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 2002; 56: 200-7.
[81] Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 2002; 177: 67-80.
[82] Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 2002; 177: 67-80.
[83] Mira L, Fernandez MT, Santos M, Rocha R, Florencio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res 2002; 36: 1199-208.
[84] Silva MM, Santos MR, Caroco G, Rocha R, Justino G, Mira L. Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radic Res 2002; 36: 1219-27.
[85] Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 2000; 373: 102-9.
[86] Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001; 56: 5-51.
[87] Shapiro TA, Fahey JW, Wade KL, Stephenson KK, Talalay P. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. Cancer Epidemiol Biomarkers Prev 2001; 10: 501-8.
[88] Johnson IT. Glucosinolates: bioavailability and importance to health. Int J Vitam Nutr Res 2002; 72: 26-31.
[89] Deng XS, Tuo J, Poulsen HE, Loft S. Prevention of oxidative DNA damage in rats by brussels sprouts. Free Radic Res 1998; 28: 323-33.
[90] Handelman GJ. The evolving role of carotenoids in human biochemistry. Nutrition 2001; 17: 818-22.
[91] Palozza P, Piccioni E, Avanzi L, Vertuani S, Calviello G, Manfredini S. Design, synthesis, and antioxidant activity of FeAOX-6, a novel agent deriving from a molecular combination of

the chromanyl and polyisoprenyl moieties. Free Radic Biol Med 2002; 33: 1724-35.
[92] Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 2001; 385: 20-7.
[93] Palozza P. Prooxidant actions of carotenoids in biologic systems. Nutr Rev 1998; 56: 257-65.
[94] Palozza P. Evidences of prooxidant effects of carotenoids in vitro and in vivo: implications in health and disease. In: Kinsky N, Mayne S, Sies H Eds. Carotenoids in health and disease. N.Y. Marcell Dekker Inc. 2003, in press.
[95] Mortensen A, Skibsted LH, Truscott TG. The interaction of dietary carotenoids with radical species. Arch Biochem Biophys 2001; 385:13-9.
[96] Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. Invest Ophthalmol Vis Sci 2003; 44: 2461-5.
[97] Epstein KR. The role of carotenoids on the risk of lung cancer. Semin Oncol 2003; 30: 86-93.
[98] Oh WK, Small EJ. Complementary and alternative therapies in prostate cancer. Semin Oncol 2002; 29: 575-84.
[99] Heber D, Lu QY. Overview of mechanisms of action of lycopene. Exp Biol Med 2002; 227: 920-3.
[100] Rao AV. Lycopene, tomatoes, and the prevention of coronary heart disease. Exp Biol Med 2002; 227: 908-13.
[101] Johnson EJ. The role of carotenoids in human health. Nutr Clin Care 2002; 5: 56-65.
[102] Fox CH, Eberl M. Phytic acid (IP6), novel broad spectrum anti-neoplastic agent: a systematic review. Complement Ther Med 2002; 10: 229-34.
[103] Midorikawa K, Murata M, Oikawa S, Hiraku Y, Kawanishi S. Protective effect of phytic acid on oxidative DNA damage with reference to cancer chemoprevention. Biochem Biophys Res Commun. 2001-2; 288: 552-7.
[104] Ryu K, Ide N, Matsuura H, Itakura Y. N alpha-(1-deoxy-D-fructos-1-yl)-L-arginine, an antioxidant compound identified in aged garlic extract. J Nutr 2001; 131(3s): 972S-6S.
[105] Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. Phytother Res 2003; 17: 97-106.
[106] Rahman K. Garlic and aging: new insights into an old remedy. Ageing Res Rev 2003; 2: 39-56.
[107] Ames BN, Wakimoto P. Are vitamin and mineral deficiencies a major cancer risk? Nat Rev Cancer 2002; 2: 694-704.
[108] Powell SR. The antioxidant properties of zinc. J Nutr 2000; 130 (5S Suppl): 1447S-54S.
[109] Rostan EF, DeBuys HV, Madey DL, Pinnell SR. Evidence supporting zinc as an important antioxidant for skin. Int J Dermatol 2002; 41: 606-11.
[110] Onderci M, Sahin N, Sahin K, Kilic N. Antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins, and some minerals under a low ambient temperature. Biol Trace Elem Res. 2003; 92: 139-50.
[111] Quinlan GJ, Evans TW, Gutteridge JM. Iron and the redox status of the lungs. Free Radic Biol Med 2002; 33: 1306-13.
[112] Fraga CG, Oteiza PI. Iron toxicity and antioxidant nutrients. Toxicology 2002; 180: 23-32.
[113] O'Connor JM, Bonham MP, Turley E, McKeown A, McKelvey-Martin VJ, Gilmore WS, et al. Copper supplementation has no effect on markers of DNA damage and liver function in healthy adults (FOODCUE Project). Ann Nutr Metab 2003; 47: 201-6.
[114] Videla LA, Fernandez V, Tapia G, Varela P. Oxidative stress-mediated hepatotoxicity of iron and copper: role of Kupffer cells. Biometals 2003; 16: 103-11.
[115] Gryzunov YA, Arroyo A, Vigne JL, Zhao Q, Tyurin VA, Hubel CA. Binding of fatty acids facilitates oxidation of cysteine-34 and converts copper-albumin complexes from antioxidants to prooxidants. Arch Biochem Biophys 2003; 413: 53-66.
[116] Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. J Nutr 2003; 133: 1457S-9S.
[117] Arthur JR. The glutathione peroxidases. Cell Mol Life Sci 2000; 57: 1825-35.
[118] May SW. Selenium-based pharmacological agents: an update. Expert Opin Investig Drugs 2002; 11: 1261-9.
[119] Yang GQ, Wang SZ, Zhou RH, Sun SZ. Endemic selenium intoxication of humans in China. Am J Clin Nutr 1983; 37: 872.
[120] Tinggi U. Essentiality and toxicity of selenium and its status in Australia: a review. Toxicol Lett 2003; 137: 103-10.
[121] Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. J Nutr 2003; 133: 1700S-08S.
[122] Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. 2001; 1: 529-39.
[123] Maier CM, Chan PH. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. Neuroscientist 2002; 8: 323-34.
[124] Bowler RP, Crapo JD. Oxidative stress in airways: is there a role for extracellular superoxide dismutase? Am J Respir Crit Care Med 2002; 166: S38-43.
[125] Pong K. Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics. Expert Opin Biol Ther 2003; 3: 127-39.
[126] Wood ZA, Schroder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 2003; 28: 32-40.
[127] Georgiou G, Masip L. Biochemistry. An overoxidation journey with a return ticket. Science 2003; 300: 592-4.
[128] Kitani K, Minami C, Yamamoto T, Maruyama W, Kanai S, Ivy GO, et al. Do antioxidant strategies work against aging and age-associated disorders? Propargylamines: a possible antioxidant strategy. Ann NY Acad Sci 2001; 928: 248-60.
[129] Ozen T, Korkmaz H. Modulatory effect of Urtica dioica L. (Urticaceae) leaf extract on biotransformation enzyme systems, antioxidant enzymes, lactate dehydrogenase and lipid peroxidation in mice. Phytomedicine 2003; 10: 405-15.
[130] Nohl H, Gille L, Staniek K. The biochemical, pathophysiological, and medical aspects of ubiquinone function. Ann NY Acad Sci 1998; 854: 394-409.
[131] Niki E. Mechanisms and dynamics of antioxidant action of ubiquinol. Mol Aspects Med 1997; 18 Suppl: S63-70.
[132] Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, et al. Is there a pathogenetic role for uric Acid in hypertension and cardiovascular and renal disease? Hypertension. Epub 2003; 41: 1183-90.
[133] Hink HU, Santanam N, Dikalov S, McCann L, Nguyen AD, Parthasarathy S, et al. Peroxidase properties of extracellular superoxide dismutase: role of uric acid in modulating in vivo activity. Arterioscler Thromb Vasc Biol 2002; 22: 1402–8.
[134] Ostdal H, Davies MJ, Andersen HJ. Reaction between protein radicals and other biomolecules. Free Radic Biol Med 2002; 33: 201-9.
[135] Rustin P. The use of antioxidants in Friederich's ataxia treatment. Expert Opin Investig Drugs 2003; 12: 569-75.
[136] Maxwell SRJ. Prospects for the use of antioxidant therapies. Drugs 1995; 49: 345-61.
[137] Elsayed NM. Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. Nutrition 2001; 17: 828-34.
[138] O'Donnell VB. Free radicals and lipid signaling in endothelial cells**.** Antioxid Redox Signal 2003; 5: 195-203.
[139] Maulik N, Das DK. Redox signaling in vascular angiogenesis. Free Radic Biol Med 2002; 33(8): 1047-60.
[140] Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 2001; 11: 173-86.
[141] Palozza P, Serini S, Di Nicuolo F, Calviello G. Mitogenic and apoptotic signaling by carotenoids: involvement of a redox mechanism. IUBMB Life 2001; 52: 77-81.
[142] Guo Q, Packer L. Ascorbate-dependent recycling of the vitamin E homologue Trolox by dihydrolipoate and glutathione in murine skin homogenates. Free Radic Biol Med 2000; 29: 368-74.
[143] Chan AC, Chow CK, Chiu D. Interaction of antioxidants and their implication in genetic anemia. Proc Soc Exp Biol Med 1999; 222: 274-82.
[144] Bohm F, Edge R, Mcgarvey DJ, Truscott TG. ß-carotene with vitamins E and C offers synergistic cell protection against NOx. FEBS Lett 1998; 436: 387-89.
[145] Stahl W, Heinrich U, Jungmann H, Sies H, Tronnier H. Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. Am J Clin Nutr 2000; 71: 795-8.
[146] Manfredini S, Vertuani S, Manfredi B, Rossoni G, Calviello G, Palaozza P. Novel antioxidant agents deriving from molecular combinations of vitamins A and E analogues: 3,4-dihydroxy-5(R,S)-(6-hydroxy-2,5,7,8 tetramethylchroman-2(R,S)-yl-methyl)-

[1,3]dioxolan-4(S)-yl]-5H-furan-2-one and 3-O-octadecyl derivatives. Bioorg Med Chem 2000; 8: 2791-801.

[147] Ninfali P, Biagiotti E, Bacchiocca M, Avanzi L, Vertuani S, Manfredini S. Capacità antiossidante di prodotti agroalimentari e di antiossidanti naturali o di sintesi. Industrie Alimentari 2002; 412, 251-67.

[148] Geromel V, Darin N, Chretien D, Benit P, DeLonlay P, Rotig A, Munnich A, Rustin P. Coenzyme Q(10) and idebenone in the therapy of respiratory chain diseases: rationale and comparative benefits. Mol Genet Metab 2002; 77: 21-30.

[149] MJ. Antioxidants and Friedreich's ataxia. Lancet 1999; 354: 1300-1.

[150] Manfredini S, Vertuani S, Avanzi L, Durini E, Palozza P, Piccioni E, et al. Design, synthesis and activity of idebenone analogues. 12th European Meeting on Fat Solubile Vitamins 2003, Rieti, Italy Manuscript submitted.

[151] Abraham NG. Therapeutic applications of human heme oxygenase gene transfer and gene therapy. Curr Pharm Design 2003; 9(30): 2513-24.

[152] Pag U. and Sahl HG. Multiple activities in lantibiotics--models for the design of novel antibiotics? Curr Pharm Design 2002; 8(9): 815-33.

[153] Ploug M. Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. Curr Pharm Design 2003; 9(19): 1499-528.