ANTIOXIDANTS IN HEALTH AND DISEASES

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The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health. In fact, the discovery of the role of free radicals in chronic degenerative diseases is as important as the discovery of the role of microorganism in infections disease (Bray, 1999).

It is well known that ROS is generating spontaneously in the living cell during several metabolic pathways. These comprise components of biological electron transport systems (photosynthetic, mitochondria, microsomal), including various enzymes and biomolecules: neutrophil, xanthine oxidase, cyclooxigenase, lipooxigenase, autooxidation of catechol amines (Halliwell and Gutteridge, 1986). Althought known as very harmfull, there is at least one case however, in which organism employ radicals in a controlled way to achieve a useful purpose: the action of phagocytic cell (neutrophils and monocytes, macrophage). Free radicals are necessary in the immune system, prostaglandine biosynthesys and antibacterial cell activities. Beside, above mentioned physiological systems, ROS generation are induced by several exogenous factors such are pollution, smoke, radiation, pesticide, drug consumption...

REACTIVE OXYGEN SPECIES

Highly reactive oxygen molecules and oxygen radicals are generated from the triplet state oxygen by excitation or reduction. Beside singlet state oxygen, having two higher and lower states $^1\Delta_g$ and $^1\Sigma^+_g$, which arise mainly by photo excitation, more reactive reduced oxygen radicals are formed through univalent reduction of oxygen molecules. One electron reduction of oxygen produces superoxide $HO_2^{\cdot}$ Or $O_2^{\cdot-}$. The superoxide is degradaded into oxygen and hydrogen peroxide by disproportionation reaction.The hydrogenperoxide is fairly stable, but has week bonding energy and undergoes 1-electron reduction for form hydroxyl radical $OH^{\cdot}$ and water.

Why oxygen is toxic in biological systems?

SUPEROXYDE THEORY

Although for aerobic organism oxygen is necessary for life it has been accepted that oxygen is toxic. Explanation is in the "Superoxide theory of oxygen toxicity" which was postulated by Irwin Fridowich and Joe McCord in 1969 year, and states that oxygen is toxic because some of it is metabolized to make supeoxide radical. However it is not very reactive radical species, and it does not appear to react at significant rates with DNA, phospholipids or proteins. Much of the toxicity of superoxide is thought to be due to its conversion into more damaging species, including peroxynitrite and hydroxyl radicals. Superoxide can react with some biomolecules. Its protonated form, $HO_2^{\cdot}$, is more reactive and can oxidize polyunsaturated fatty acids, although it has not been shown to be capable of attacking membrane lipids. Superoxide dismutation produces H_2O_2 that can exert some direct toxic effect and can be a precursor of $OH^{\cdot}$. Release of iron ions from iron-sulphur cluster (by $O_2^{\cdot -}$ or $ONOO^{\cdot}$) or from ferritin (by $O_2^{\cdot -}$) and from heam proteins by H_2O_2 can provide the iron needed for Fenton chemistry. Peroxynitrite is powerfull nitrating; nitrosylating and oxidizing species under physiological conditions and can also displace redox-active cupper from ceruloplasmin (Halliwell, 1999).

Although nor supeoxide radicals and nor H_2O_2 are highly reactive species, their activity as active oxygen species come from their potential to produce extremely highly reactive $HO^{\cdot}$ radicals through the Fenton reaction (I) and Haber-Weiss reaction (II):

$\rightarrow H_2O_2 + Fe(II) \quad Fe(III) + OH^{\cdot} + OH^{-}$ (I)

$\rightarrow H_2O_2 + O_2^{\cdot -} \quad O_2 + OH^{\cdot} + OH^{-}$ (II)

OH radical is because of its extreme reactivity the main factor of so-called oxygen toxicity. It reacts with all biological materials, oxidatively by hydrogen withdrawal, double bond addition, electron transfer and radical formation, and initiates autoxidation, polymerization and fragmentation.

LIPID PEROXIDATION:

Is complex process occurring in aerobic cells and reflect the interaction between molecular oxygen and polyunsaturated fatty acids. This involves formation and propagation of lipid radicals ($L\cdot$), uptake of oxygen, rearrangement of double bonds, generation of lipid alkoxyl ($LO\cdot$), lipid peroxyl ($LOO\cdot$) radicals, lipid hydroperoxide (LOOH) as well as variety of degradation products.

At least two paths are known for the formation of lipid peroxide *in vivo*. One occurs through autooxidation of catecholamine, thiols, quinones, and others, and redox reactions of oxyhemoglobin and myoglobin, and the other from active oxygen by the action of xanthine oxidase, NADPH oxidase, and other enzymes.

ROS AND LP IN HUMAN PATHOLOGY AND DISEASES

In the case of disturbed balance between formation of free radicals and antioxidant defense, in the cell we have oxidative stress and the free radicals can play a role in the development of various diseases.

Overproductions of ROS have been implicated in the etiology of host degenerative diseases including cardiovascular diseases, diabetes, cancer, Alzheimer's disease, retinal degeneration, ishemic dementa, and other neurovegetative disorders and aging. In addition they also play a role not only in acute conditions, such as trauma, stroke, and infection, but also in physical exercise and stress.

CARDIOVASCULAR DISEASES

Heart diseases continue to be the biggest killer, responsible for about half of all death in developed countries. Understanding and potentially controlling oxidative events as they affect cardiovascular disease (CVD) therefore, has the potential to provide enormous benefits to our population in health and lifespan.

Polyunsaturated fatty acids occur as a major part of the low-density lipoproteins (LDL) in blood and oxidation of these lipids components in LDL play a role in atherosclerosis. The three most important cell types in the vessel wall: endothelial cells, smooth muscle cell and macrophage can release free radical, which affect lipid peroxidation. With acontinuated high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque the symptom of atherosclerosis. Oxidized LDL is atherogenic, and is thought to be important in the formation of atherosclerotic plaques. Furthermore oxidized LDL is cytotoxic and can directly damage endothelial cells (De Whallel et al., 1990).

CANCEROGENESIS

Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation and transformation, particularly in the past 10 years. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystem could lead to mutation, transformation and ultimately cancer (Simic, 1988). Induction of mutagenesis, the best known of the biological effect of radiation, occurs mainly through damage of DNA by the $HO\cdot$ radical and other species produced by radiolysis of water, and also by direct radiation effect on DNA. The reaction of $HO\cdot$ radicals are mainly addition to double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety resulting in chain scission of DNA. These effects can cause cell mutagenesis and carcinogenesis. Lipid peroxides are also suspected of being responsible for the activation of benzo(a)pyrene and other carcinogens, as well as for the production of some types of promoter.

FREE RADICALS AND AGING

The human body is in constant battle to keep from aging. Strong experimental evidence supports the free radical theory of aging. An increasing number of diseases and disorder, as well as aging process itself, demonstrate link ether directly or indirectly to these reactive and potentially destructive molecules. Not much is known about the mechanism of aging and what determine lifespan. Leading theories attribute these to programs written in DNA and/or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

ANTIOXIDANT DEFENSE SYSTEM

Antioxidant defense system against oxidative stress is composed of several lines, and the antioxidants are classified into four categories based on function (Noguchi et al., 2000):

1. First line of defense is the preventive antioxidants, which suppress formation of free radical (enzymes: glutathione peroxidase, catalase; selenoprotein, transferrin, ferritin, lactoferrin, carotenoids etc.)
2. Second line of defense is the radical scavenging antioxidants suppressing chain initiation and/or breaking chain propagation reactions: radical scavenging antioxidants
3. Third category: repair and de novo antioxidant (some proteolitic enzymes, repear enzymes of DNA etc)
4. A fourth line is an adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site.

Antioxidants act as: radical scavenger, hydrogen donors, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents

Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environmental to detoxify ROS. To provide maximum intracellular protection these scavengers are strategically compartmentalized thought the cell (table 1).

Table 1. Important enzymatic and non enzymatic physiological antioxidants

Enzymatic antioxidants	location	properties
Superoxide dismutase (SOD)	Mitochondria, cytosol	Dismutase superoxide radicals
Glutathione peroxidase (GSH))	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxide
Catalase (CAT)	Mitochondria and cytosol	Removes hydrogen peroxide
Nonenzymatic antioxidants	location	properties
Vitamin C	Aques phase of cell	Acts as free radical scavenger and recycles vitamin E
Vitamin E	Cell membrane	Major chain-breaking antioxidant in cell membrane
Uric acid	Product of purine metabolism	Scavenger of OH radicals
Glutathione	Nonprotein thiol in cell	Serves multiple roles in the cellular antioxidant defense
-lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
carotenoids	Lipid soluble antioxidants, located in membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
bilirubin	Product of heme metabolism in blood	Extracellular antioxidant
ubiquinones	mitochondria	Reduced form are efficient antioxidants
Metals ions sequestration: transferrin, ferritin, lactoferrin,		Chelating of metals ions, responsible for Fenton reactions
Nitric oxide		Free radical scavenger, inhibitor of LP

NATURAL AND DIET-DERIVED ANTIOXIDANTS

Live forms living in the Earth's atmosphere must be equipped with systems to deal with the action of oxygen in living matter. Plants are especially susceptible to damage by active oxygen (exposed to radiation UV light) this is why plants developed numerous antioxidant defense systems that results in certain numbers of very potent antioxidants. Beside plants many of microbial and animal products as well as fermented products, seaweeds, protein hydrolisates were found to be powerful antioxidants. Daily foods contain a wide variety of free radicals scavenging molecules, thus vegetables, fruit, tea, wine are product rich in natural antioxidant compounds such. Among numerious antioxidants following plant secondary products are of particular interest (Larson, 1988):

1. Plant phenolics: phenylproponaoids, coumarines, flavonoids
2. polyphenolic: tannins, proanthocyanidins
3. nytogen containing compounds; alkaloids, nonproteins aminoacids, isotiocyanate, indoles
4. phytosterols
5. carotenoids
6. chlorophyl derivatives

Plant phenolic, particularly flavonoids, tannins and phenylpropanoids are of particular interest. Much of the interest in the bioactivity of plant phenolic has been spurred by the dietary anomaly referred as the "*French paradox*" the apparent compatibility of a high fat diet with allow incidence of coronary atherosclerosis (Renaud&Lorgeril, 1992). Many so-called secondary products can act as potent bio-antimutagens. Cheng et al., (1989) showed antimutagen action of green tea extract, for which epigallocatecin gallate seems to be most responsible. Therefore, there is currently a strong interest in the study of natural compounds with free radical scavenger capacity and their role in human health and nutrition.

Dietary antioxidants may contribute to the decrease of cardiovascular disease by reduction of free radical formation as well as oxidative stress in general, by protection of LDL oxidation and platelet aggregation and by inhibiting synthesis of proinflamatory cytokines (Kushi, 1996).

Epidemiological studies have shown that a higher intake of these compounds is associated with lower risk of mortality from cancer and coronary heart disease.

The use of spices has been valued from prehistorically times not soley because of their flavor, but also because of their food-preserving power. Numbers of studies have been done on their antioxidant activity as well as their antiseptic activity (Chipault et al., 1952; Madsen et al., 1997; Mimica-Dukic, 2001, Mimica-Dukic & Bozin, 2002, Bozin et al., 2002). Several compounds from spicy and aromatic plants are confirmed to posse's strong antioxidant activity. Thus, phenolic diterpenoids: carnosol, rosmanol, carnosoic acid from sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.), in thyme (*Thymus vulgars* L.) dimers of thymol and flavonoids, flavonoids in oregano *(Origanum vulgare* L.) and pepper (*Piper nigrum* L.) were reported as strong antioxidative compounds. In our recent study we found that essential oils, as volatile compounds of many aromatic plants, besides their well known antimicrobial activity posses also significant antioxidant properties. As a strong free radical scavenger monoterpene ketones (thujone, menthone, carvone) and hydrocarbons are confirmed (Mimica-Dukic, 2001, Mimica-Dukic & Bozin, 2002, Bozin et al., 2002).

Therefore many aromatic and spicy plants as well as their essential oils, could serve not only as a flavor agent but also as a safe food antioxidant and supplement in preventing deterioration of foodstuff products. Consumption of food produced with natural essential oil or aromatic plant extracts are expecting to prevent the risk of many free radicals mediated diseases.

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