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Mechanisms of the suppression of free radical overproduction by antioxidants

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1. ABSTRACT

In accordance with the mechanism of suppression of free radical overproduction in biological systems all antioxidants can be divided into two main groups: chain-breaking antioxidants and preventive antioxidants. Chain-breaking antioxidants, often referred to as free radical scavengers, protect against oxidative stress as a result of scavenging initial, peroxyl and rarely alkyl radicals. Preventive antioxidants act as chelators of transition metals, inhibitors of enzymatic systems responsible for the generation of reactive oxygen species (ROS) or reduce hydrogen peroxides and organic hydroperoxides and can prevent an appearance of initiating radical and frustrate a free radical chain reaction from ever setting in motion. Biological and health effects of any given antioxidant depends on numerous factors, such as the chemical reactivity toward radicals or another target related to oxidative stress, absorption and distribution in body tissue. Understanding specific mechanisms by which antioxidants may affect pathogenesis of inflammatory and cardio-vascular diseases, neurological disorders and cancer might create a wealth of potential for the treatment and prevention of human diseases.

2. INTRODUCTION

The term antioxidant initially referred to a molecule that prevented the consumption of oxygen, in other words, a molecule capable of counteracting the oxidation of other molecules by oxygen. From the late 19th century, antioxidants were widely adopted in various industrial fields, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines. Consequently extensive studies of antioxidants have been carried out in different areas of chemistry. However among them we can specify only a few biochemical studies devoted mostly to the application of antioxidants in preventing oxidative fat rancidity in food products (1). the situation was cardinally changed at the middle of 20th century. Antioxidant properties of vitamins A, C, and E were established by this time and this led to the recognition of the vital role of antioxidants in aerobic organisms (2, 3). Since then tremendous number of scientific publications related to various aspects of biological significance of antioxidants has been accumulating every year. Certainly, the definition of "antioxidant" currently takes on a new meaning: "substances that may protect cells against the effects of free

Table 1. Molecular mechanisms underlying chain-breaking and preventive effects of antioxidants

Basic mechanisms	Specific mechanisms	Potential antioxidants
Chain-breaking mechanisms	Inhibition of free radical chain reactions as result scavenging of chain initiating radicals	Vitamin C and E, plants polyphenols, hydroquinones, thiols, superoxide dismutase
	Inhibition of free radical chain reactions as result of peroxyl radical scavenging	Vitamin E, BHT, BHA, plants polyphenols
	Inhibition of free radical chain reactions as result of alkyl radical scavenging	Quinones, nitroxyl radicals
Preventive mechanisms	Binding (chelating) transition metals	Phenanthroline, DFO, EDTA, plants polyphenols
	Inhibition of enzymatic systems responsible for free radical generation.	Plants polyphenols, vitamin E
	Reduction of hydrogen peroxides and organic hydroperoxides	Catalase, glutathione peroxidases, thioredoxin peroxidase, glutathione S- transferases
	Enzymatic hydrolysis of ester bonds to remove peroxidized fatty acids from lipids	Phospholipase A_2

radicals and oxidative stress". Free radicals are chemically active atoms or molecular fragments that have an unpaired electron. Free radicals containing oxygen, known as reactive oxygen species (ROS), are the most biologically significant free radicals. Besides oxygen-centered radicals: superoxide ($O_2^{\bullet -}$) hydroxyl radical, ($^{\bullet}OH$), peroxyl ($ROO^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals; ROS include nitric oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$) and non-radical molecules, derivatives of oxygen, such as peroxynitrite ($ONOO^-$ + ONOOH) the singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). Oxidative stress occurs when the production of free radicals is beyond the protective capability of the antioxidant defenses.

3. CLASSIFICATION OF ANTIOXIDANT MECHANISMS

All effects of antioxidant in biological systems can be classified in two main groups:

- Chain-breaking effects. After initiating radical has arisen it reacts with diverse neighboring molecules to form a second radical and thus initiate harmful chain reactions. Antioxidants block the process of oxidation by neutralizing or scavenging free radicals. Consequently antioxidants operating in this manner often referred to as free radical scavengers. As a result of scavenging free radicals, the antioxidants themselves become oxidized. That is why there is a constant need to replenish our antioxidant resources.
- Preventive effects. Antioxidants can preclude an appearance of initiating radical and frustrate a free radical chain reaction from ever setting in motion.

Numerous molecular mechanisms underlying chain-breaking and preventive effects that have been found by now are summarized in Table 1.

4. CHAIN-BREAKING MECHANISMS

4.1. Inhibition of free radical chain reactions as result of scavenging chain initiating radicals

By now numerous antioxidants were found to inhibit free radical chain autoxidation of various substances by scavenging initial free radicals. For example adrenaline (epinephrine) and related catecholamines: norepinephrine and dopamine are easily involved in autoxidation processes. Superoxide was found to be a possible chain initiating radical (Eqn. 1) and an obligatory propagating intermediate (Eqn. 2, 3) (4-6).

$$RH_3^- + O_2^{\bullet -} + 2H^+ \rightarrow RH_3^{\bullet} + H_2O_2 \quad (1)$$

$$RH_3^{\bullet} + O_2 \rightarrow RH_2 + O_2^{\bullet -} + H^+ \quad (2)$$

$$RH_2 + O_2^{\bullet -} + H \rightarrow RH^{\bullet} + H_2O_2 \quad (3)$$

Superoxide dismutase and ascorbic acid (vitamin C) strongly inhibit this mechanism (4).

It has been shown that the addition of asbestos fibers to rat peritoneal macrophages enhances the production of superoxide and hydrogen peroxide (7-9). This finding provided an insight into the overproduction of ROS by NADPH-oxidase in response to the «frustrated» phagocytosis of mineral particles is the main cause of asbestos cytotoxicity, and is the initial trigger event in pathogenesis of asbestos related diseases as a whole. Flavonoids containing a catechol structure in the B-ring such as quercetin, rutin, taxifolin, epicatechin gallate and epigallocatechin gallate were effective in protecting phagocytic cells against injury caused by asbestos (10-12) and the protective efficacy correlates quite closely with the rate constants of the reaction of these flavonoids with superoxide (Figure 1).

Almost fifty years ago Butler postulated the hemolytic fission of the carbon-chlorine bond leading to the formation of a free radical in the liver endoplasmatic reticulum (Eqn. 4) as a possible mechanism of the hepatotoxicity of chlorinated methanes, particularly carbon tetrachloride (13).

$$CCl_4 + e^- \rightarrow CCl_4^{-\bullet} \rightarrow {}^{\bullet}CCl_3 + Cl^- \quad (4)$$

The trichloromethyl free radical ($^{\bullet}CCl_3$) was eventually identified by spin trapping both *in vitro* and *in vivo* (14). The trichloromethyl radical reacts very rapidly with oxygen to yield a highly reactive trichloromethylperoxy radical ($CCl_3O_2^{\bullet}$) (14). The carbon tetrachloride-derived free radicals can bind irreversibly to hepatic proteins and lipids (mainly

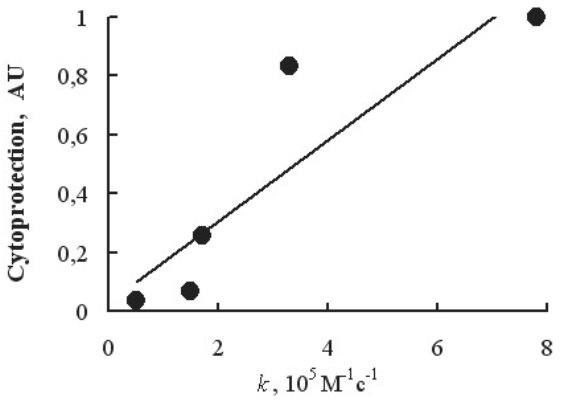

Figure 1. Analysis of correlations between cytoprotective effect, expressed in arbitrary units and antiradical properties of flavonoids. Data from (12). The correlation coefficient is 0.89 ± 0.24; $p = 0.042$.

$^{\bullet}CCl_3$) and can initiate a process of autocatalytic lipid peroxidation by attacking the methylene bridges of unsaturated fatty acid side chains of microsomal lipids (CCl3O2•) necrosis leading to necrosis or steatosis (15, 16). It was found that CCl3O2• reacts rapidly with polyunsaturated fatty acids and much more rapidly with various antioxidants: ascorbic acid, beta-carotene, alpha-tocopherol and promethazine. The values of second order rate constants were found to be in the range 106 M-1s-1 - 109 M-1s-1 (17). •CCl3 reacts with various substrates more slowly than CCl3O2• (values of second order rate constants less than 105 M-1s-1 (17), besides the rate of covalent binding of •CCl3 with lipids and proteins does not differ significantly from the rate of the reaction with antioxidants (alpha-tocopherol and promethazine). Therefore alpha-tocopherol and promethazine inhibit CCl3O2• induced lipid peroxidation both *in vitro* and *in vivo* but, do not significantly influence the reaction of covalent binding of $^{\bullet}CCl_3$ to hepatic constituents (18). Among 1, 2-benzoquinones were found compounds that effectively inhibited lipid peroxidation without substantial influence on the covalent binding. One of these compounds, MBQ (4-[4-N-sodium-N-(5-ethyl-1-thia-3,4-diazol-2-yl)sulfophenylamino]-5-methoxy-1,2-benzoquinone) was used as a tool for investigating mechanisms responsible for the hepatotoxic effects of carbon tetrachloride *in vivo*. Pretreatment of rats with MBQ before carbon tetrachloride intoxication inhibited lipid peroxidation but did not prevent cytochrome P-450 destruction, decrease of hydroxylase activity, and the loss of the capability to bioactivate carbon tetrachloride in rat liver microsomes (19, 20).

Myeloperoxidase (MPO) is a key enzyme involved in the generation of highly reactive species such as hypochlorous acid, that are crucial ones for protection against the attack by foreign microorganisms. Recently, it has been found that in the presence of hydrogen peroxide, MPO can oxidize nitrite (NO_2^-) to nitrogen dioxide radical ($^{\bullet}NO_2$). $^{\bullet}NO_2$ can nitrate tyrosine and other aromatic compounds and promotes lipid peroxidation in low-density lipoprotein (LDL) (21, 22). From these observations it was concluded that MPO might be involved in atherogenesis by forming reactive nitrogen intermediates. It was found that oxidation of LDL by MPO in the presence of physiological concentration of nitrite was inhibited by micromolar concentrations of flavonoids (Table 2), presumably by scavenging initial $^{\bullet}NO_2$ (23). The antioxidant action of flavonoids varies considerably and depends on the structure of aromatic backbone and the type, number and position of functional groups. There are three functional groups (Figure 2) that are mainly attributed to the scavenging potential of flavonoids: the *o*-dihydroxy structure of the B ring, the C2-C3 double bond, and 3-hydroxyl in the C ring. The catechol arrangement in the B-ring plays the key role in preventing oxidative modification of LDL by MPO while the 3-OH group and 2, 3-double bond in the C ring appear to be of minor importance.

4.2. Inhibition of free radical chain reactions as the result of scavenging of peroxyl radicals

Vitamin E, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and many flavonoids can effectively scavenge peroxyl radicals. As a chemical class, these compounds are phenols or polyphenols chemicals that can react with peroxide radicals by hydrogen atom abstraction forming hydroperoxides (Eqn. 5) and terminating free radical chain oxidation (Eqn. 6 and 7) (24).

$$ROO^{\bullet} + PhOH \rightarrow ROOH + PhO^{\bullet} \quad (5)$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \quad (6)$$

$$R^{\bullet} + O\text{-}O \rightarrow ROO^{\bullet} \quad (7)$$

Where $ROO^{\bullet}$, PhOH, ROOH, $R^{\bullet}$, RH are peroxyl radical, antioxidant, peroxide, alkyl radical and unoxidized substrate respectively.

Generally, antioxidant efficiency depends on numerous factors (25) and the most important are listed below:

- the chemical reactivity toward radicals,
- localization of antioxidant,
- concentration and mobility in the microenvironment,
- fate of antioxidant-derived radical,
- interaction with other antioxidants,
- absorption, distribution, retention, metabolism, and safety.

The chemical reactivity toward radicals, which contributes mainly to antioxidant efficiency, may be expressed in terms of the ratio of the rate constant of scavenging lipid peroxyl radicals by antioxidant (k_5) to that of hydrogen atom abstraction from polyunsaturated lipids by peroxyl radicals (k_6). Mathematically it is written as: k_5/k_6. The phenoxyl radicals ($PhO^{\bullet}$) produced as a result of reaction 5 are rather stable intermediates and normally cannot be involved in the following free radical chain oxidations (24, 26). However, the fate of antioxidant-derived radical is important in determining antioxidant capacity (25). Phenoxyl radicals as a rule recombine with peroxyl radicals (Eqn. 8). In addition, phenoxyl radicals may be reduced by ascorbic acid (vitamin C) (25).

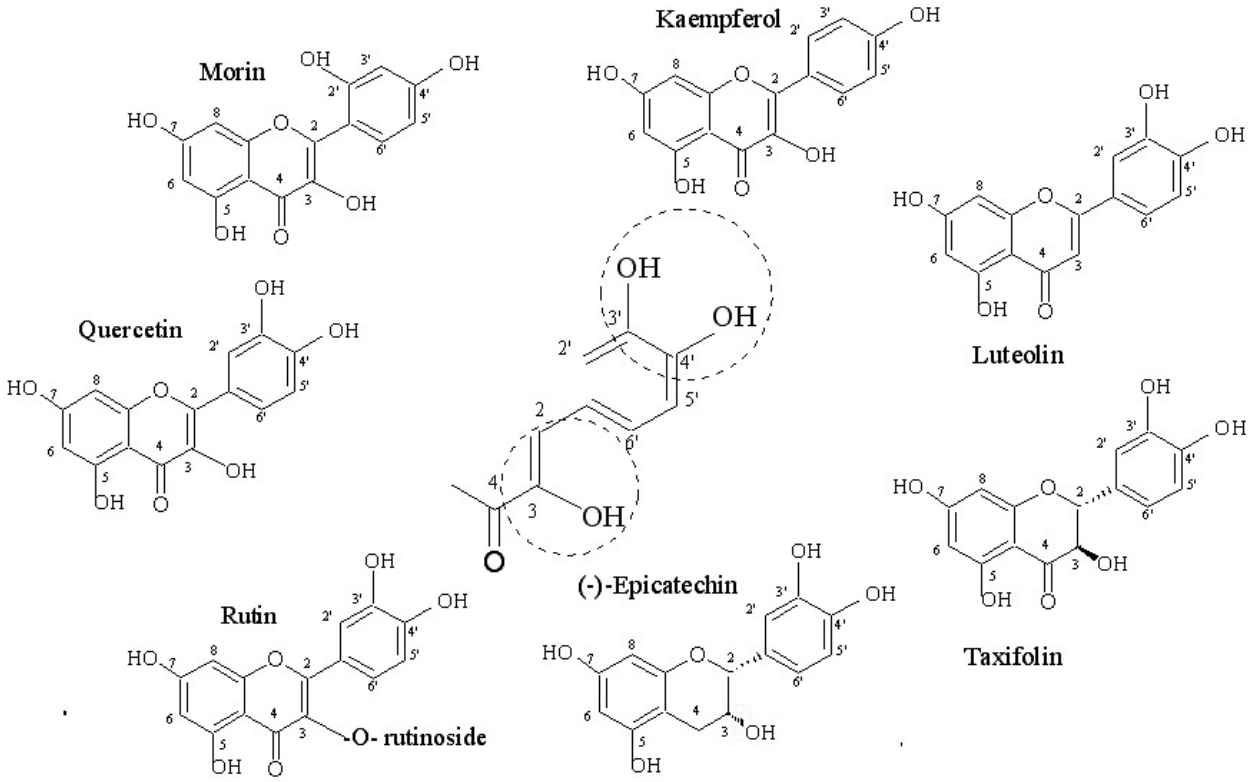

Figure 2. The chemical structure of selected flavonoids and functional groups attributed mainly to their scavenging potential

$ROO^{\bullet} + PhO^{\bullet} \rightarrow$ molecular products (8)

One of the most effective natural chain-breaking antioxidant is apparently alpha-tocopherol. This methyl derivative of tocol is usually referred to as vitamin E, however, some other plant tocol derivatives such as beta-tocopherol, gamma-tocopherol, delta-tocopherol also possess vitamin E activity (27). These compounds vary in the number and position of methyl groups in aromatics ring (Figure 3). Antiradical activity of tocopherols decreases in the following order: alpha, beta, gamma, delta, tocol (24, 28). The values of rate constant of scavenging lipid peroxyl radicals by alpha-tocopherol given by various sources varied from $1,5 \cdot 10^5$ M^{-1} s^{-1} to $3,2 \cdot 10^6$ M^{-1} s^{-1} (24, 28, 29). The values of rate constant of scavenging lipid peroxyl radicals for beta-, gamma- and delta-tocopherols are $1,3 \cdot 10^6$ M^{-1} s^{-1}; $1,4 \cdot 10^6$ M^{-1} s^{-1} and $0,44 \cdot 10^6$ M^{-1} s^{-1} correspondingly (24). However, above-mentioned rate constants were determined under non-physiological conditions in homogeneous solution of nonpolar solvents. In heterogeneous (membrane) systems the antioxidant activity of tocopherols as well as other phenolic antioxidants was found to be reduced by more than 2 orders of magnitude compared to that in homogeneous solutions. For example, the rate constant of scavenging peroxyl radicals by alpha-tocopherol is about 500 times less in the membranes than in chlorobenzene (30). There are several causes that affect the efficiency of scavenging peroxyl radicals by lipophilic antioxidant in heterogeneous (membrane) systems. Firstly, lipid peroxyl radicals as rather hydrophilic compounds, which can partially escape scavenging by antioxidants going from lipid environment to aqueous phase (30). Secondly, the lateral and especially vertical mobility of tocopherols within membranes and lipoproteins is very low. This is due to the long phytyl side chain. The side chain substitute is important for incorporation and retaining antioxidant molecular in membrane. However, it was found that the longer side chain, the lesser is the mobility of antioxidants within membranes and lipoproteins and the lesser is the peroxyl radicals scavenging efficacy (31).

4.3. Inhibition of free radical chain reactions as result of scavenging alkyl radicals

Alkyl radicals are produced as intermediates in free radical chain reaction (Eqn. 6). Lipid alkyl radicals, in particular, are involved in lipid peroxidation. Normally oxygen reacts with lipid alkyl radicals (Eqn. 7) with a nearly diffusion-controlled, bimolecular rate constant (32) greatly exceeding any competing $R^{\bullet}$ scavengers. However, at low oxygen tensions e.g. under conditions of ischemia quinines and nitroxyl radicals may react with alkyl radicals and significantly attenuate oxidative modifications in biomolecules and membranes (33). In addition, it has been mentioned that lipid oxidation catalyzed by lipoxygenase, prostaglandin endoperoxide synthase, and cytochrome P450 involves formation of enzyme-bound radical intermediates, including lipid alkyl ($L^{\bullet}$) radical species. In turn $L^{\bullet}$ react with $^{\bullet}NO$ at diffusion-limited rates. Thus, reaction of $^{\bullet}NO$ with enzyme-bound lipid radicals will modulate rates of formation of eicosanoids playing critical

Table 2. The antioxidant effectiveness of flavonoids against oxidation of LDL by MPO in the presence of physiological of nitrite.

Flavonoids	IC_{50} values (μM)
Quercetin	2.2
Rutin	3.0
Taxifolin	3.8
Epicatechin	4.4
Luteolin	4.5
Morin	11.1
Kaempferol	>20

Data from (23)

Table 3. IC_{50} values (μM) for the inhibition of superoxide-driven reduction of nitroblue tetrazolium by certain flavonoid metal complexes and corresponding ligands.

Polyphenols	Free ligands	Complexes with metals (1:1)		
		Cu^{2+}	Fe^{2+}	Fe^{3+}
Rutin	9.0	0.50	2.7	2.5
Taxifolin	1.9	0.48	0.6	0.55
Luteolin	14.2	0.80	2.5	2.5
(–)-Epicatechin	1.3	0.32	0.3	0.3

Data from (52)

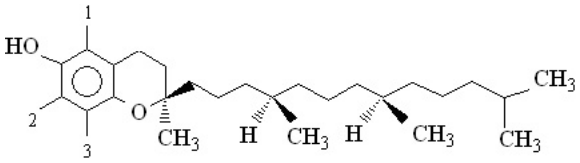

Figure 3. The chemical structure of tocopherols alpha-tocopherol - (1 – CH_3, 2 – CH_3, 3 – CH_3); beta-tocopherol - (1 – CH_3, 2 – H, 3 – CH_3); gamma-tocopherol - (1 – H, 2 – CH_3, 3 – CH_3); delta-tocopherol - (1 – H, 2 – H, 3 – CH_3); tocol - (1 – H, 2 – H, 3 – H).

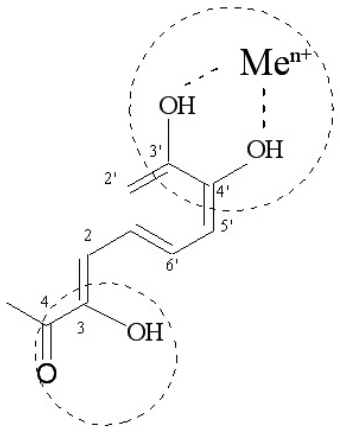

Figure 4. The principal binding sites for metal ions in the flavonoid molecule

signaling roles in the regulation of vascular cell function and inflammatory responses (34). Competition of antioxidants with •NO for the scavenging of alkyl radicals may be beneficial to cells.

5. PREVENTIVE MECHANISMS

5.1. Binding (chelating) transition metals

It is well known that interaction between hydrogen peroxide and ferrous or cuprous ions lead to the generation of hydroxyl radical (•OH):

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + {}^{-}OH + Fe^{3+} \quad (9)$$

This reaction (Eqn. 9) known as the Fenton reaction for more than 100 years is generally accepted as the initial step of the pathway leading to oxidative injury *in vivo*. For instance, the role of hydroxyl radicals produced through Fenton chemistry in iron and copper toxicity was well established (35, 36).

Although the total content of body iron in adult humans is near 4 g, or approximately 50 mg/kg in men and 40 mg/kg in women, the pool of free iron that is available to catalyze cleavage of H_2O_2 to hydroxyl anion and hydroxyl radical is, under normal conditions, extremely limited. The increased iron level observed in brain tissue following a variety of cerebral insults such as ischemia (37, 38), Parkinson's disease (39, 40) and Friedreich's ataxia (41). Normally, the pool of free copper even much smaller than that of free iron. There is indication that intracellular copper is limited to less than one free ion per cell (42). However, extremely hepatic and neuronal copper overload was found in patients with Wilson's disease.

As transition metal ions play a vital role in the initiation of free radical processes via Fenton reaction, the binding (chelation) of metal ions is widely considered as important preventive mechanism of antioxidant activity. Proteins, carbohydrates, and phenolic compounds that have carboxyl, hydroxyl, sulfate, phosphate, and amino groups can bind metal ions to form appropriate complexes. In such a complex the redox potentials of the couple Fe^{2+}/Fe^{3+} will depend on the ligands and, as a result, the bound metal ion may be more or less effective in Fenton reaction. For instance, it was found that complexes of iron with certain low-molecular-weight ligands such as citrate-anion, ethylenediaminetetraacetic acid (EDTA) or nucleotides catalyze cleavage of H_2O_2 more effectively than free metal ions. Among nucleotides the most effective Fenton catalysts are guanosine triphosphate and adenosine triphosphate. At the same time, complexes of iron with deferoxamine (DFO) destroy hydrogen peroxide more slowly than free metal ions (43-46). Many biological and health effects of DFO linked to the ability to convert Fenton's active catalyst into inert form were revealed *in vitro* and *in vivo* by now. For example DFO significantly attenuated neuronal toxicity of amyloid-β (47). Walker and Shah demonstrated that the DFO lessen the GM-induced reduction in glomerular filtration rate and the severity of the tubular damage (48).

Being effective chelators of transition metal ions flavonoids may also prevent generation of primary oxygen radicals and the following chain oxidation (49). Chelation potency of flavonoids is mainly related to a catechol moiety in the B ring (Figure 4) (50) while redox behavior of

ligands in complexes depends on the presence of the 3-hydroxy group in their structure (51). The beneficial effect of binding transition metal ions by flavonoids is not limited only to the influence on Fenton chemistry. The notable feature of resulting metal complexes of certain flavonoids is that their scavenging potencies toward superoxide are significantly higher than those of the parent flavonoids (Table 3). The high antiradical efficacy of the flavonoid complexes is due to the ability of chelated metal ion to operate as a superoxide scavenger with dismutating activity, in accord with the following reactions:

$$Me^{(n+1)+} + O_2^{\bullet -} \rightarrow Me^{n+} + O_2 \quad (10)$$

$$Me^{n+} + O_2^{\bullet -} + 2H^+ \rightarrow Me^{(n+1)+} + H_2O_2 \quad (11)$$

Apparently, the flavonoid molecules are able to react with metal ions directly in blood stream or tissues. The beneficial consequences of this fact would be both the inhibition of Fenton reaction and the formation of complexes possessing strong antiradical efficacy towards ROS.

5.2. Inhibition of enzymatic systems responsible for free radical generation

Certain oxidoreductases frequently referred to as prooxidant enzymes may be responsible for intracellular and tissue free radical overproduction. Among them may be mentioned cyclo-oxygenase and lipoxygenase family members (5-lipoxygenase, 8-lipoxygenase, 12/15-lipoxygenases), myeloperoxidase, inducible nitric oxide synthase, NADPH-oxidase and xanthine oxidase (XO). A constantly growing list of experimental and clinical evidences supports a crucial role of prooxidant enzymes in inflammatory and cardio-vascular diseases, neurological disorders and cancer. Therapeutic effect of many drugs is due to their ability to inhibit prooxidant enzymes as a result of drug-protein interaction. Drugs may behave as competitive inhibitors reversibly blocking the substrate-binding site or may react with catalytically important unit inactivating the enzyme. For example, the interaction of quercetin with lipoxygenase results in flavonoid co-oxidation to protocatechuic acid that in turn disturbs hydrogen-bonding network of the active site of enzyme, and finally leads to the loss of specific activity (53).

A recent study has revealed that intake of certain flavonoids in humans caused beneficial for the cardiovascular system changes in the ratio of plasma eicosanoid metabolites (54) and this phenomenon is probably attributed to the direct inhibition of 5-lipoxygenase by flavonoids (55). It was found that a combination of iron-chelating and iron ion-reducing properties appears to be required for selective 5-lipoxygenase inhibition by phenolic compounds (56). Carnosol, vitamin E and trolox were also found to be the 5-lipoxygenase inhibitors of varying potency, and all were less active as the cyclo-oxygenase inhibitors (56). Flavonoids also inhibited human platelet 12-lipoxygenase and the 15-lipoxygenase-1 from rabbit reticulocytes (57). The inhibition of the latter enzyme is of particular interest, since it belongs to a lipoxygenase sub-family (reticulocytetype 12/15-lipoxygenases) the members of which are catalysts of enzymatic lipid peroxidation as expressed by their capability of dioxygenating not only free arachidonic and linoleic acids, but also phospholipids, cholesterol esters and even complex biological structures such as biomembranes and plasma lipoproteins (58).

An increase in xanthine oxidase activity as a result of proteolytic cleavage of xanthine dehydrogenase has been proposed to play a key role in triggering oxidative stress and postischemic reperfusion injury (59, 60). It has been demonstrated that this enzyme and its substrates are present and give rise to a burst of free radical generation upon postischemic reperfusion in the isolated rat heart, as well as in bovine and human endothelial cells. In these experiments XO inhibitors allopurinol and oxypurinol inhibited radical generation and attenuate postischemic reperfusion injury. Exposure to cigarette smoke was shown to initiate an increase in apoptosis in the rat gastric mucosa that was accompanied by an increase in XO activity. The proapoptotic effect of cigarette smoke was blocked by pretreatment with allopurinol (61).

Recently it has been shown that human umbilical vein endothelial cells (HUVEC) stimulated by the physiological mediator angiotensin II, generate and release $O_2^{\bullet -}$ predominantly via an NADPH oxidase pathway and overproduction of $O_2^{\bullet -}$ evokes oxidative stress in HUVEC. Dietary polyphenols and their metabolites may contribute to the control of NADPH oxidase activity, thus lowering $O_2^{\bullet -}$ generation, which, in turn, leads to elevation of the steady-state level of $^{\bullet}NO$ in the cells (62, 63).

5.3. Reduction of hydrogen peroxides and organic hydroperoxides

All aerobic organisms are well equipped with enzymes that can effectively reduce hydrogen peroxides and organic hydroperoxides. Catalase effectively decomposes hydrogen peroxide (Eqn. 12) (about 44000 molecules of H_2O_2 per sec).

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (12)$$

However, the value of Michaelis-Menton constant for hydrogen peroxide decomposition by catalase is rather high; therefore, the maximum of catalase activity exists only under elevated levels of H_2O_2, predominantly in peroxisomes (64-66), whereas in other subcellular locations a major H_2O_2, scavenger is glutathione peroxidase (Eqn. 13) (64-67).

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O \quad (13)$$

Mills was a pioneer of glutathione peroxidase research discovering that GSH-dependent enzyme protected hemoglobin from oxidative breakdown by hydrogen peroxide (70). Glutathione peroxidase possesses a wide range of substrate specificity and catalyzes two electrons reduction of various peroxides, including hydroperoxides of free polyunsaturated fatty acids (PUFA) (Eqn. 14) (71).

$$ROOH + 2GSH \rightarrow GSSG + ROH + H_2O \quad (14)$$

Later in addition to "classical" glutathione peroxidase, Ursini and co-workers have found one more selenoenzyme that effectively reduced hydroperoxides of phospholipids (72). In addition to glutathione-dependent protective system (glutathione, glutathione reductase, and glutathione peroxidase), cellular peroxides level is controlled by thioredoxin system (thioredoxin, thioredoxin reductase, and thioredoxin peroxidase) (73, 74).

Several years ago it was were postulated that low–molecular weight compounds included redox effective metal ions (iron or copper) and specific $^{\bullet}$OH-inactivating ligands could be potential catalase-like anti-inflammatory drugs (75). Late, the complex of cupric ion with 3-methoxyanthranilate (76) and complexes of verbascoside with ferrous and cupric ions (77) have been shown to meet the above criteria.

6. CONCLUSIONS AND PERSPECTIVES

Intensive experimental and epidemiological investigations have led to a significant advance in the elucidation of various aspects of the beneficial and harmful health effects of antioxidants, including molecular mechanisms of the suppression of free radical overproduction. Scientific achievements in this field mainly contributed to the formation of the widespread scientific and public opinion that dietary supplements of plant origin or antioxidant rich diet may reduce the risk of coronary heart disease and provide protection against inflammatory, neurological disorders and cancer. One much-discussed (though controversial) example of the preventive effects of antioxidants is the 'French paradox'. However, serious barriers exist on the way from a non-medical preventive strategy to the introduction of antioxidant therapies into clinical medicine. Results of only a few of randomized clinical trials with vitamin E and other antioxidants or antioxidant combinations were completely successful while the majority of them have been rather equivocal. Perhaps a major problem of antioxidant therapies is the lack of specificity. The potential benefits of antioxidant intervention may be missed as a result of perturbation the body's native redox status; moreover, unpredictable, side effects are common. In addition, the existing approach for the quantification of the redox state of patients is poorly suited for clinical application and we cannot currently take into consideration individual antioxidant/prooxidant balance and cannot monitor a patient's response to the treatment. Therefore, it is not possible to determine which individuals might benefit from which anti-oxidant therapy. To overcome these barriers it is necessary to improve existing treatment protocols in relation to the specificity of antioxidant therapy and develop facile, and accurate techniques for analysis of body's redox status applicable to the routine clinical use.

7. ACKNOWLEDGMENT

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Abbreviations: BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole; EDTA: ethylenediaminetetraacetic acid; DFO: deferoxamine; HUVEC: human umbilical vein endothelial cells; LDL: low-density lipoprotein; MPO: myeloperoxidase; ROS: reactive oxygen species; XO: xanthine oxidase

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