Antioxidant biomedicine: from free radical chemistry to systems biology mechanisms

V. K. Koltover

Institute of Problems of Chemical Physics of the Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 (496) 522 3507. E-mail: koltover@icp.ac.ru

Natural and synthetic antioxidants are widely used in modern medicine. Some of them proved to be efficient geroprotectors, *i.e.*, they extend the life span of laboratory animals when added to food or drinking water on a regular basis. *In vitro*, antioxidants inhibit free-radical chain oxidation reactions, resulting in oxidation of fatty acids, edible fats, *etc*. However, their efficiency as scavengers of oxygen free radicals in cells and tissues is negligible as compared with natural antioxidant enzymes. *In vivo*, antioxidants decrease the concentration of free radicals but they are not only and not so much direct inhibitors of free radical processes as physiologically active compounds that prevent the formation of oxygen radicals and free radical oxidation. For example, synthetic antioxidant dibunol (BHT) prevents the generation of the $O_2^{\cdot -}$ radical as a by-product of mitochondrial electron transport, while flavonoids exert a preventive antioxidant action by inducing the expression of the antioxidant enzymes, superoxide dismutase and catalase. The endocrine system and regulatory transcription and translation factors play a significant role in the antioxidant therapy. Therefore, elucidating the molecular mechanisms of the "antioxidant prophylaxis" is a task of the molecular systems biology.

Key words: antioxidants, bioantioxidants, free radicals, superoxide radical, oxygen, biosystems reliability, bioreliability.

Introduction

The vigorous research of free radical processes and of the role of antioxidants in biology and medicine started in the mid-20th century. This was stimulated by awarding the Nobel Prize to C. Hinshelwood and N. N. Semenov for the studies of free radical mechanisms of chain radical reactions in 1956. By the mid-1950s, it was already known that free radicals, specifically the hydroxyl radical ($OH^{\cdot}$), arise in water under the action of ionizing radiation and that toxic products of free radical-induced lipid oxidation, *viz.*, peroxides derived unsaturated fatty acids, appear in animal tissues on exposure to ionizing radiation.[1,2] In 1954, the ESR signals of free radicals were detected in animal tissues and in yeast cells.[3] At the same time, a free radical theory of aging was put forward, and inhibitors of free radical chain oxidation (antioxidants) were proposed for prolongation of life of humans and animals.[4,5]* At the same years, it was proved experimentally at the Institute of Chemical Physics of the USSR Academy of Sciences that 2,6-di-*tert*-butyl-4-methylphenol (called ionol or dibunol in Russian literature and butylated hydroxytoluene, BHT, in English-language literature) and some other antioxidants retard the development of leucosis in mice.[6]

Currently, the mechanism of action of antioxidants in living systems (*in vivo*) does not seem as unambiguous as half a century ago. This review presents data according to which diverse physiological, in particular, therapeutic effects of synthetic and natural antioxidants (bioantioxidants) are due not so much to direct radical trapping as to the ability of antioxidants to provide preventive protection from free radicals and their chemically reactive products.

Bioantioxidants as inhibitors of free radical processes

In chemistry, antioxidants are substances capable of terminating branching chain oxidation. In modern chemistry, these are mainly derivatives of secondary aromatic amines and phenols and organic phosphites and sulfides.[7] In modern biomedical literature, antioxidants represent a broad class of phenolic compounds, both synthetic and natural, whose valence-saturated molecules containing an

* The peroxide theory of biological oxidation was proposed in 1897 by Russian chemist A. N. Bach, who worked at that time in Paris.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 37—43, January, 2010.

1066-5285/10/5901-0037

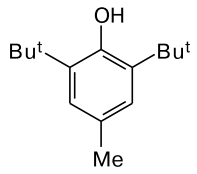
active hydrogen atom (InH) react with an active free radical $R^{\cdot}$ or $RO_2^{\cdot}$ that runs the oxidative chain to give radical ($In^{\cdot}$) of the antioxidant:

$$R^{\cdot} + InH \rightarrow RH + In^{\cdot},$$

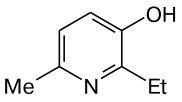
$$RO_2^{\cdot} + InH \rightarrow ROOH + In^{\cdot}.$$

The relatively unreactive free radical $In^{\cdot}$ thus formed cannot participate in chain propagation reactions and is destroyed upon collision with another radical (or the vessel wall).[7,8]

The most common antioxidants are phenolic derivatives in which the OH group is shielded (sterically hindered phenols). For example, in the mid-20th century, the antioxidant BHT found wide use as stabilizer for industrial oils and edible fats.[9]



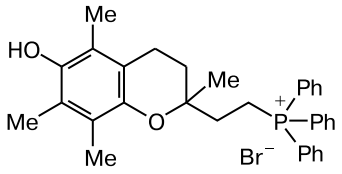
BHT



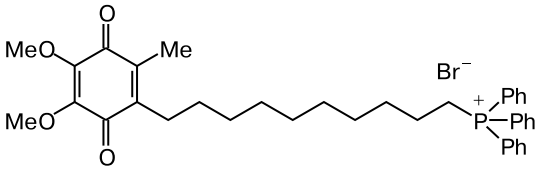
Unlike BHT, antioxidants based on alkyl-substituted hydroxypyridine are water-soluble. 2-Ethyl-3-hydroxy-6-methylpyridine hydrochloride and succinate were synthesized at the Institute of Chemical Physics of the USSR Academy of Sciences.[8]

The molecules of some antioxidants, in particular, vitamin E and ubiquinone derivatives, MitoVit E and Mito-Q, contain in addition to the hydrophobic antioxidant group, also a lipophilic cationic group (see Ref. 10). The mitochondria-targeted plastoquinone compounds with rhodamine and a triphenylphosphonium as cations (SkQ) were also synthesized.[11]

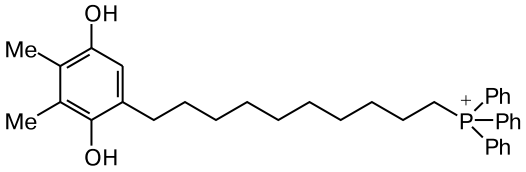
Owing to the electric charge of the cation, these molecules can use the mitochrondrial transmembrane potential to pass through the mitochrondrial membranes and, therefore, they get into mitochrondria, *i.e.*, just those organelles that generate the highest amount of reactive oxygen species.[10,11]



Mito Vit-E

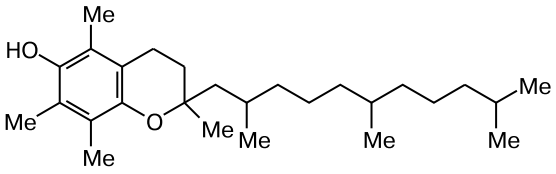


Mito-Q

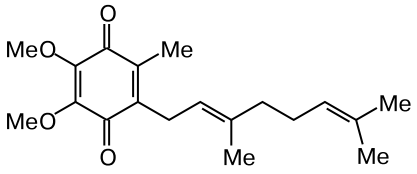


SkQ1

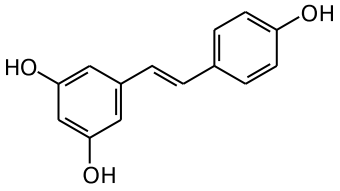
Currently, natural antioxidants include not less than twenty compounds starting from "classical" α-tocopherol (vitamin E) and ascorbic acid (vitamin C). Most of these are substituted phenols or polyphenol compounds, which, owing to their hydroxyl groups, are capable of inhibiting free-radical chain oxidation reactions in model systems (*in vitro*), for example, oxidation of linolenic acid.[9–17] Among antioxidants, melatonin and other human and animal hormones and even amino acids and peptides are mentioned.[18] Most of functionally substituted phenols and polyphenols are of plant origin. These are flavonoids, in



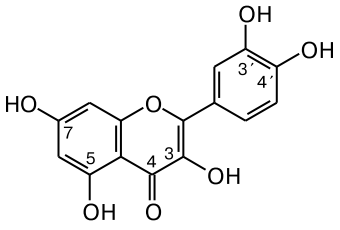
Vitamin E



Ubiquinone 10



Resveratrol



Quercetin

particular, querticin, flavones, and resveratrol, which is especially abundant in grapes and red wine, simple catechols, which are present in large amounts in green tea, and catechol oligomers, present in high concentrations in grapes, cocoa beans, *etc.*, carotenoids, tannins, anthocyanins, coumarins, hydroxycinnamic acid derivatives, *etc.*

With appropriate dosage, natural and synthetic antioxidants exert various favorable therapeutic effects and therefore they have been long used with success in medical practice. For example, BHT is used to treat burns, gunshot wounds, trophic ulcers, and bladder cancer. Mexidol (2-ethyl-3-hydroxy-6-methylpyridine succinate) is used to treat brain circulation disorders, and Emoxipine (hydrochloride of the same pyridine derivative) is used in ophthalmology.[9]

The same synthetic antioxidants proved to be effective geroprotectors, *i.e.*, compounds that extend the life span of laboratory animals when added to food or drinking water on a regular basis. For example, by adding BHT to food, it was possible in some cases to extend the lives of fruit flies and mice of some lines by, on average, 25—30%. The addition of 2-ethyl-3-hydroxy-6-methylpyridine to drinking water extended the average life spans of flies and mice by 24 and 38%, respectively (see, for example, reviews[19—23]). Currently the highest geroprotector effect has been found for resveratrol: the extension of the maximum life span by 59% in experiments on fishes and extension of the average life span by 30% in experiments on mice (on a fatty diet).[14]

The mitochondria-targeted antioxidants MitoVit E and Mito-Q had beneficial effects for treating sepsis.[10] Similar antioxidants based on plastoquinone (SkQ) were applied profitably against age pathologies in animals, in particular, for treating a number of cardiovascular and ocular diseases and even as geroprotectors in experiments on mice or other animal species.[11]

However, the classical natural antioxidant, α-tocopherol, was inefficient in analogous biomedical testing.[21] Quite a few examples of this type can be found in the literature.[20—23] The results of analysis of the rate constants and actual concentrations of antioxidants also raise doubts in the fact that antioxidants perform *in vivo* in as simple way as *in vitro*, *i.e.*, only as free-radical inhibitors.[24]

Oxygen radical anion (the superoxide radical, $O_2^{\cdot -}$), an important source of chemically reactive "toxic" oxygen species, is formed in the cells and tissues of aerobic organisms. Apparently it is also an important molecular component of the intracellular and intercellular regulation. The main bulk of $O_2^{\cdot -}$ is formed as by-product of electron transport in cell mitochondria, the organelles that use up to 99% of all oxygen consumed by the cells for ATP synthesis.[10—12,25] However, there are also specific enzymes, superoxide dismutases (SOD), which catalyze the dismutation of $O_2^{\cdot -}$ to hydrogen peroxide H_2O_2 and oxygen.[26,27] This enzyme, mitochondrial (Mn-SOD), cytosol (Cu,Zn-SOD), or periplasmatic bacterial enzyme (Fe-SOD), reacts with the $O_2^{\cdot -}$ radical anion with the rate constant $k \approx 2 \cdot 10^9$ L mol^{-1} s^{-1} (see Refs 24, 26—29). Meanwhile, the rate constants for the reactions of ascorbic acid and 5,7,8-trimethyltocol (water-soluble α-tocopherol derivative) with the $O_2^{\cdot -}$ radical do not exceed 10^5 L mol^{-1} s^{-1}, while those for hydroxypyridine antioxidants are no more than 10^2 L mol^{-1} s^{-1} (See Ref. 9). For Mito-Q (ubiquinone-based antioxidant), the rate constant for the reaction with $O_2^{\cdot -}$ in water can be as high as 10^8 L mol^{-1} s^{-1} according to pulse radiolysis data; however, in this case, too, it remains an order of magnitude lower than for SOD.[10] In principle, mitochondria-targeted antioxidants can be accumulated in mitochondria.[11] However, they can hardly be accumulated up to a concentration comparable with the amount of SOD (about 10^{-5} mol L^{-1})[26] without considerable disturbance of the operation of mitochondrial bionanoreactors.

Since SOD protection has limited reliability, there exists a finite probability that the $O_2^{\cdot -}$ radical would penetrate the "SOD-barrier" and then react with H_2O_2 to give the hydroxyl radical ($OH^{\cdot}$).[11,12,17,24,28,29] However, it is known that specialized enzymes, catalase and/or glutathione peroxidase, which catalyze hydrogen peroxide decomposition to water and oxygen, always occur near SOD.[27] The rate constant for the reaction of the antioxidant α-tocopherol with the $OH^{\cdot}$ radical can be as high as $8 \cdot 10^{10}$ L mol^{-1} s^{-1} (see Ref. 9). The $OH^{\cdot}$ radical is known to react with any organic molecules as a strong oxidant with rate constants close to the diffusion limit (>10^{10} L mol^{-1} s^{-1}).[7] Therefore, none of antioxidants can compete for hydroxyl radicals *in vivo* with other organic molecules, which are obviously always present around this radical in considerably greater numbers than the molecules of any antioxidant.

The reactions of $OH^{\cdot}$ radicals with lipids, for example, with mitochondrial cardiolipin can produce peroxyl radicals ($RO_2^{\cdot}$). In addition, $OH^{\cdot}$ radicals can initiate oxidation of proteins, oxidative degradation of DNA with formation of 8-oxo-7,8-dihydroguanine and 5,6-dihydroxy-5,6-dihydrothymine, and so on.[12,17] In model reactions, the rate constants for reactions of synthetic and natural antioxidants with peroxyl radicals of lipids ($RO_2^{\cdot}$) and protein radicals range up to about 10^6 L mol^{-1} s^{-1} (see Refs 9, 10). However, *in vivo* these radicals arise mainly as secondary products of free radical reactions that accompany cell death on apoptosis and autophagocytosis during utilization of the cellular waste by lyzosomes and peroxysomes (see, for example, a review[30]). Antioxidants are unlikely to be highly necessary for scavenging active radicals in the catabolism. The reports on *in vivo* yields of the DNA oxidation products for both mitochondrial and nuclear DNA are also overestimated due to various artifacts.[12]

Thus, manifold effects of antioxidants *in vivo* can hardly be interpreted only on the basis of simple chemical analogy with the action of the same antioxidants as radical scavengers *in vitro*.

Bioantioxidants as system regulators of protection from free radicals

The most efficient way to increase the reliability of complex systems is well-timed prevention of malfunction (failure) of functional elements.[28,29] Antioxidants can provide exactly this preventive protection from free radicals *in vivo*. The particular protection mechanisms may be different for antioxidants of different types.

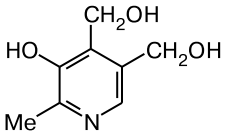
For example, BHT prevents the generation of $O_2^{\cdot -}$ radicals as by-products of electron transport in mitochondria. In a study of low-temperature ESR signals of rat tissues, we found that BHT increases the degree of myocardium oxygenation.[31] In addition, BHT injections gave rise to an ESR signal from the nitrosyl complex of haemoglobin (NO—Hb) in animal blood.[32] Nitric oxide serves as the signal molecule, which causes, in particular, relaxation of arterial smooth muscles, thus enhancing oxygen supply of the myocardium.[33] It is also known[34] that hypoxia results in structural damage in mitochondrial membranes, and this leads to considerable decrease in the reliability of electron transport, and mitochondria become generators of intense $O_2^{\cdot -}$ fluxes. Thus, by increasing the degree of myocardium oxygenation, BHT prevents the development of hypoxia and thus prevents the transformation of mitochondria into $O_2^{\cdot -}$ generators.

This antioxidant preventive action is mediated through the hormonal regulation system, as after BHT injections not only nitric oxide appeared in the rat blood but also dramatic increase in the corticotropin and corticosteroid concentrations took place simultaneously with dramatic decrease in the thyrotropin and L-3,3′,5-triiodothyronine concentrations. Hence, BHT induces substantial shifts in the functions of adenohypophysis, the source of corticotropin and thyrotropin hormones, and peripheral endocrine glands: the adrenal cortex (corticosteroids) and the thyroid gland (triiodothyronine).[35] The corticotropin release into blood followed by increase in the synthesis of corticosteroids and decrease in the synthesis of thyroid hormones is a significant phase of system adaptation to stress. This suggests that BHT being introduced into the diet of animals on a regular basis also acts as a mild stress training factor, as it stimulates the endocrine system and thus increases the reliability (adaptative capability) of the organism.

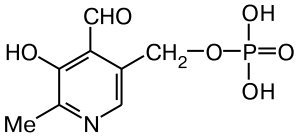
Thus, the antioxidant BHT is actually able to decrease the level of active oxygen species in cells but its effect is manifested not through direct radical elimination (scavenge), but in a preventive manner, *i.e.*, upon decrease in the probability of their generation. It was shown by spin probe technique that hydrophobic BHT molecules react with serum albumin, a blood protein, performing a number of important functions, in particular, transport of the hormone aldosterone.[31] Thus, hormone transport proteins can serve as the molecular targets of the antioxidant.

The so-called mitochondria-targeted antioxidants can also act in the preventive manner. Phenolic compounds like MitoVit-E and SkQ1 have weakly acidic properties and can serve as protonophore uncouplers, as, for example, 2,4-dinitrophenol.[27] In addition, hydrophobic cations can transfer counter-ions (anions) through a mitochondrial lipid membrane, which also decreases the transmembrane potential, and, according to the Mitchell theory, has a uncoupling effect.[27] The molecules combining a hydrophobic part and a cationic group, in particular, a triphenylphosphonium group in their structure serve as efficient uncouplers of the oxidative phosphorylation in mitochondria (they were synthesized for this purpose 40 years ago as uncouplers[36]). It is also known that the electron transport in mitochondria experiences a "back pressure" of the transmembrane potential. Therefore, oxidative phosphorylation uncouplers, in particular, transmembrane transfer agents of protons and anions, decrease the transmembrane potential and thus decrease the generation of $O_2^{\cdot -}$ radicals and other reactive oxygen species in mitochondria.[37] This provides grounds for believing that mitochondria-targeted antioxidants not only and not so much scavenge directly the $O_2^{\cdot -}$ radical (or its protonated form $HO_2^{\cdot}$) as prevent the formation of these radicals in mitochondria. It is noteworthy that SkQ1 synthesized from plant plastoquinone[11] proved to be a substantially more efficient therapeutic agent in biomedical investigations than similar mitochondria-targeted antioxidants MitoVit E and Mito-Q synthesized from vitamin E and animal ubiquinone, respectively.[10]

Like BHT, the antioxidant 2-ethyl-3-hydroxy-6-methylpyridine increases the reliability of the electron transporting bionanoreactors but the mechanisms of "antioxidant prophylaxis" can in this case differ from those for BHT. The antioxidants based on hydroxypyridines are analogs of pyridoxine (**1**) and pyridoxal phosphate (**2**), which are group B_6 vitamins.



1



2

Pyridoxal phosphate (**2**) is a cofactor of glutamate aspartate aminotransferase, RNA polymerase, and some other enzymes of the biosynthesis of nitrogen-containing compounds.[27] This implies that hydroxypyridine antioxidants are vitamin B_6 antimetabolites and thus inhibit the activity of key enzymes of the synthesis of amino acids and nucleotides. In particular, this allows an understanding of the efficiency of 2-ethyl-3-hydroxy-6-methylpyridine as a radioprotector in yeast cell experiments.[38] The inhibition of biosynthesis retards the cell division and thus provides the cells with additional time for restoring the genetic structures damaged by ionizing radiation.

Recently, it has been shown that the magnetic isotope ^{25}Mg (nuclear spin $I = 5/2$) is a much more efficient cofactor of oxidative phosphorylation in mitochondria than non-magnetic ^{24}Mg and ^{26}Mg isotopes ($I = 0$).[39] Meanwhile, the slower the ATP synthesis in the active site of ATP-synthase, the higher the probability of electron leakage on to oxygen to give $O_2^{\cdot -}$ during the operation of electron-transport chains.[28,29,37] One can expect the development of new geroprotectors based on the magnetic isotope ^{25}Mg that would ensure a decrease in the oxygen radical generation as a result of the preventive antioxidant effect of the stable magnetic magnesium isotope *in vivo*.[40]

Most of flavonoids, which are traditionally regarded as natural antioxidants, refer to the extensive class of physiologically active compounds long known as phytoalexins. They are synthesized in plant tissues for fighting against bacterial and fungal infections like antibiotics and for acting as inhibitors of transcription and translation of particular proteins in the cells of the infecting organism.[41] The direct antioxidant activity exhibited by flavonoid molecules in model peroxide oxidation reactions has a minor physiological role, like, for example, the ability of the hormone melatonin or ethanol molecules to scavenge the $OH^{\cdot}$ radical. However, flavonoids can provide the preventive protection from oxygen radicals by induction of specific antioxidant enzymes. For example, a considerable increase in the activity of SOD enzymes and catalase was detected in blood erythrocytes of humans who have received the food additive Protandim (extracts of five medical plants).[13] The natural antioxidant resveratrol induces the expression and activity of mitochondrial Mn-SOD in mice.[16]

Quite a lot of data have now been accumulated demonstrating that even vitamin E, a "key antioxidant", can hardly serve as a free radical inhibitor *in vivo* and, hence, this issue in handbooks should be revised.[43] Four isomers of vitamin E are known: α-, β-, γ-, and δ-tocopherols. All four isomers react *in vitro* with the peroxyl radical $RO_2^{\cdot}$ with approximately the same rate constants of about 10^6 L mol^{-1} s^{-1}. In the living nature, mainly α-tocopherol is encountered. As shown in recent experiments on cell cultures and isolated enzymes, this form of vitamin E inhibits a key regulator enzyme of biosynthesis, protein kinase C, inhibits 5-lipoxygenase and phospholipase A_2, and also activates protein phosphatase 2A and diacylglycerol kinase. It was proved that α-tocopherol modulates the expression of genes encoding the synthesis of a number of protective proteins including α-TTP, α-tropomyosin, and collagenase. Probably, α-tocopherol serves as the ligand for yet unidentified specific proteins: transcription factors, membrane receptors, and so on. Moreover, α-tocophenyl phosphate rather than the antioxidant phenolic form of vitamin E serves as the bioregulator.[42]

The activity of SOD and other antioxidant enzymes in human and animal cells and tissues is under hormone control. Experiments with *Macaca mulatta* monkeys showed a tight positive correlation between the cortisol and dehydroepiandrosterone sulfate (DHEAS) circadian rhythms in blood plasma and the circadian rhythm of SOD activity in erythrocytes.[43] Like the above-mentioned experiments with dibunol, this implies that corticosteroid hormones play an important role in the "antioxidant prophylaxis" regulation.

It is well known that stress is accompanied by intensification of breathing; this, in turn, leads to enhanced generation of the $O_2^{\cdot -}$ radicals as by-products of breathing. In experiments on monkeys when the animals were subjected to a psychoemotional stress (two-hour immobilization), the plasma levels of cortisol and DHEAS sharply increased but the activities of SOD and glutathione peroxidase, two key antioxidant enzymes, in erythrocytes remained invariable during the stress and even during several hours after the stress release.[44] Thus, an acute stress is accompanied by kinetic deficiency of antioxidant enzymes. This means that a timely (before the stress) introduction of bioantioxidants, for example, flavonoids inducing the SOD synthesis may considerably increase the reliability of cell and tissue protection from oxygen free radicals.

Resveratrol was found to activate the expression of so-called sirtuin proteins.[14] The first of this protein family, Sir2 (Silent Information Regulator Two), was discovered in yeast cells. In human and animal organisms, seven sirtuins (Sirt1—7) orthological to yeast cell Sir2 have been found by now. These are localized in the cell nucleus (Sirt1, Sirt2, Sirt6, Sirt7), cytoplasm (Sirt1, Sirt2), and mitochondria (Sirt3, Sirt4, Sirt5). These proteins, NAD^+-dependent deacetylases/ADP-monoribosyltransferase, serve as key regulators of many important cell processes including glucose metabolism, insulin secretion, and adaptation to the oxidative stress and hypoxia.[14,45] For example, Sirt1 whose gene is sensitive to the redox state of intracellular medium produces structural changes in chromatin, which activate the synthesis of protective proteins, including antioxidant enzymes, and increases the number of mitochondria in the cells.[46] Some data indicate that Sirt1 and, possibly, other sirtuins regulate the amplitude and duration of the expression of circadian genes (metabolic clock).[47]

Thus, more and more experimental results indicate that the molecular mechanisms of the "antioxidant prophylaxis" should be studied on the ways of molecular systems biology.

Conclusions

Antioxidants increase the reliability of cell and tissue protection from chemically reactive oxygen species. However, their efficiency as scavengers of oxygen free radicals in living systems is low compared to natural antioxidant enzymes. *In vivo* antioxidants decrease the concentrations of free radicals but they function not only as inhibitors of free radical processes, but rather in the preventive manner as physiologically active compounds that prevent the for-

mation of oxygen radicals and free radical oxidation products. For example, the synthetic antioxidant BHT (dibunol) prevents the formation of the $O_2^{\cdot -}$ radical as a by-product of electron transport in mitochondria; the natural antioxidant resveratrol also has a preventive antioxidant action but through induction of synthesis of the antioxidant enzyme SOD. In all cases, a significant role is played by the endocrine system and the regulatory transcription and translation factors. Therefore, elucidation of molecular mechanisms of "antioxidant prophylaxis" is a task of molecular systems biology.

This work was supported by the Russian Foundation for Basic Research (Project No. 07-03-00897).

References

1. B. N. Tarusov, *Osnovy Biologicheskogo Deistviya Radioaktivnykh Izluchenii* (*Foundations of the Biological Action of Radioactive Radiation*), Medgiz, Moscow, 1954, 140 pp. (in Rusaian).
2. *Oxygen and Living Processes: An Interdisciplinary Approach*, Ed. D. L. Gilbert, Springer-Verlag, New York, 1981, 401 pp.
3. B. Commoner, J. Townsend, G. E. Pake, *Nature*, 1954, **174**, 689.
4. D. Harman, *J.Gerontol.*, 1956, **11**, 298.
5. D. Harman, in *Free Radicals and Aging*, Ed. I. Emerit, B. Chance, Birkhauser, Basel, 1992, 1.
6. N. M. Emanuel´, L. P. Lipchina, *Dokl. Akad. Nauk SSSR*, 1958, **121**, 141 [*Rep. USSR Acad. Sci.* (*Engl. Transl.*), 1958].
7. N. M. Emanuel´, A.A. Buchachenko, *Khimicheskaya Fizika Stareniya i Stabilizatsii Polymerov* (*Chemical Physics of Aging and Stabilization of Polymers*), Nauka, Moscow, 1982, 360 pp. (in Russian).
8. E. B. Burlakova, *Ros. Khim. zhurn. (Zh. Ros. Khim. o-va im. D. I. Mendeleeva)*, 2007, **51**, 3 [*Mendeleev. Chem. J.* (*Engl. Transl.*), 2007, **51**].
9. H. Babich, *Environ. Res.*, 1982, **29**, 1.
10. (a) M. P. Murphy, *Biochim. Biophys. Acta*, 2008, **1777**, 1028; (b) A. Maroz, R. F. Anderson, R. A. J. Smith, M. P. Murphy, *Free Radic. Biol. Med.*, 2009, **46**, 105.
11. V. P. Skulachev, V. N. Anisimov, Y. N. Antonenko, L. E. Bakeeva, B. V. Chernyak, V. P. Erichev, O. F. Filenko, N. I. Kalinina, V. I. Kapelko, N. G. Kolosova, B. P. Kopnin, G. A. Korshunova, M. R. Lichinitser, L. A. Obukhova, E. G. Pasyukova, O. I. Pisarenko, V. A. Roginsky, E. K. Ruuge, I. I. Senin, I. I. Severina, M. V. Skulachev, I. M. Spivak, V. N. Tashlitsky, V. A. Tkachuk, M. Yu. Vyssokikh, L. S. Yaguzhinsky, D. B. Zorov, *Biochim. Biophys. Acta*, 2009, **1787**, 437.
12. A. F. Cadet, T. Douki, J. L. Ravanat, *Accounts Chem. Res.*, 2008, **41**, 1075.
13. S. K. Nelson, S. K. Bose, G. K. Grunwald, P. Myhill, J. M. McCord, *Free Radic. Biol. Med.*, 2006, **40**, 341.
14. J. A. Baur, D. A. Sinclair, *Nature Rev. Drug Discovery*, 2006, **5**, 493.
15. T. Schewe, Y. Steffen, H. Sies, *Arch. Biochem. Biophys.*, 2008, **476**, 102.
16. E. L. Robb, L. Winkelmolen, N. Visanji, J. Brotchie, J. A. Stuart, *Biochem. Biophys. Res. Commun.*, 2008, **372**, 254.
17. C. C. Winterbourn, *Nature Chem. Biol.*, 2008, **4**, 278.
18. L. S. Kozina, A. V. Arutjunyan, V. Kh. Khavinson, *Arch. Gerontol. Geriatrics*, 2007, **44**, 213.
19. D. Harman, *AGE (J. Amer. Aging Ass.)*, 2000, **23**, 25.
20. L. K. Obukhova, *Usp. Gerontologii* (*Advances in Gerontology*) 1999, **3**, 27 (in Russian).
21. C. Driver, A. Georgeou, *Biogerontology*, 2003, **4**, 91.
22. V. K. Koltover, *Problemy stareniya i dolgoletiya* [*Problems of Aging and Longevity*], 2000, **9**, No. 1, 78 (in Russian).
23. V. K. Koltover, *Usp. Gerontologii* (*Advances in Gerontology*) 2000, No. 4, 33 (in Russian).
24. V. K. Koltover, in *Vitality, Mortality and Aging*, Ed. A. Viidik and G. Hofecker, Facultas, Vienna, 1996, 173.
25. B. D´Autreaux, M. B. Toledano, *Nature Reviews Mol. Cell Biol.*, 2007, **8**, 813.
26. I. Fridovich, *J. Biol. Chem.*, 1997, **272**, 18515.
27. D. L. Nelson, M. M. Cox, *Lehninger Principles of Biochemistry*, W.H. Freeman, New York, 2008.
28. V. K. Koltover, in *Free Radicals and Aging*, Ed. I. Emerit and B. Chance, Birkhauser, Basel, 1992, 11.
29. V. K. Koltover, *Problemy Upravleniya* (*Control Sciences*), 2004, No. 4, 40 (in Russian).
30. T. Kurz, A. Terman, B. Gustafsson, U. T. Brunk, *Biochim. Biophys. Acta*, 2008, **1780**, 1291.
31. V. K. Koltover, E. N. Gorban´, P. S. Maior, *Dokl. Akad. Nauk SSSR*, 1984, 277, 497 [*Dokl. Biophys.* (*Engl. Transl.*), 1984, 277, 130].
32. V. K. Koltover, *AGE (J. Amer. Aging Ass.)*, 1995, **18**, 85.
33. F. Murad, *Biosci. Rep.*, 2004, **24**, 452.
34. H. Nohl, V. Koltover, K. Stolze, *Free Radical Res. Commun.*, 1993, **18**, 127.
35. V. V. Frol´kis, E. N. Gorban´, V. K. Kol´tover, *Dokl. Akad. Nauk SSSR*, 1985, **284**, 499 [*Dokl. Biophys.* (*Engl. Transl.*), 1985, **284**, 210].
36. E. A. Liberman, V. P. Topaly, L. M. Tsofina, A. A. Jasaitis, V. P. Skulachev, *Nature*, 1969, **222**, 1076.
37. K. S. Echtay, *Free Radic. Biol. Med.*, 2007, **43**, 1351.
38. V. K. Kol´tover, Yu. A. Kutlakhmedov, E. L. Afanas´eva, *Dokl. Akad. Nauk SSSR*, 1980, **254**, 760 [*Dokl. Biophys.* (*Engl. Transl.*), 1980, 254, 159].
39. A. L. Buchachenko, D. A. Kouznetsov, N. N. Breslavskaya, M. A. Orlova, *J. Phys. Chem.*, 2008, **112**, 2548.
40. V. K. Koltover, *Free. Radic. Biol. Med.*, 2007, **43**, S67.
41. *Phytoalexins*, Eds J. A. Bailey, J. W. Mansfield, Blackie, Glasgow—London, 1982.
42. A. Azzi, *Free Radic. Biol. Med.*, 2007, **43**, 16.
43. N. D. Goncharova, A. V. Shmaliy, T. N. Bogatyrenko, V. K. Koltover, *Exp. Gerontol.*, 2006, **41**, 778.
44. V. K. Koltover, T. N. Bogatyrenko, A. V. Shmaliy, N. D. Goncharova, *Free. Radic. Biol. Med.*, 2006, **41**, S164.
45. K. J. Pearson, A. J. Baur, K. N. Lewis, L. Peshkin, N. L. Price, N. Labinskyy, W. R. Swindell, D. Kamara, P. K. Minor, E. Perez, H. A. Jamieson, Y. Zhang, S. R. Dunn, K. Sharma, N. Pleshko, L. A. Woollett, A. Csiszar, Y. Ikeno, D. Le Couteur, P. J. Elliott, K. G. Becker, P. Navas, D. K. Ingram, N. S. Wolf, Z. Ungvari, D. A. Sinclair, R. de Cabo, *Cell Metab.*, 2008, **8**, 157.
46. E. M. Dioum, R. Chen, M. S. Alexander, Q. Zhang, R. T. Hogg, R. D. Gerard, J. A. Garcia, *Science*, 2009, **324**, 1289.
47. S. Imai, *Cell Biochem. Biophys.*, 2009, **53**, 65.

Received June 25, 2009;
in revised form November 13, 2009