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SYMPOSIUM

An overview of free radical research+

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ABSTRACT The discovery of the pathogenetic role of free radicals in various human and animal diseases initiated a wide range of investigations, resulting in a number of important scientific dicoveries. These are reviewed in this paper, with an emphasis on preventing atherosclerotic complications and plaque development. The administration of antioxidants in the prevention and therapy of different diseases is listed, too. The questions of optimum antioxidant supply to prevent atherosclerosis, malignancies and other diseases are discussed.
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KEY WORDS

free radical
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The discovery of free radicals (FRs) and their pathogenetic role in the development of atherosclerosis (AS), tumours, teratogenesis and ageing initiated a wide-ranging research on this field (Horváth and Jávor 1985; Fehér et al. 1993; Frei 1994; Bergendy et al. 1999). This area includes the ecology and environmental medicine and has implications in the biological, biochemical or pharmacological investigations, as well as in the agriculture and in the veterinary and human medicine.

Medical and public health implications

In the "civilised" life the quantity of FRs has grown enormously. Oil the main source of energy in the world, the smoky atmosphere contains a lot of FRs generated by the burning process (Gracy et al. 1999; Hiura et al. 1999; Velichovszkij 2001). Xenobiotics are widely dispersed, as well. The organochlorides have become ubiquitous. These compounds are not only neurotoxic, but they also increase the superoxide production in the mitochondria and cause oxidative damages to the DNS (Stedeford et al. 2001).

These toxic substances might be concentrated in some foods. A sad consequence of this harmful accumulation is the diminished fertility of eskimoes brought about by the xenobiotics found in their principal nutrient, in the liver and oil of narwals.

It is an intriguing question as to how long time can nature compensate for this flood of FRs and which diseases or mutations could arise.

The mass of FRs affecting the mankind has been multiplied in the last 100 years, while, in contrast, the antioxidant (AO) intake of people has diminished. The consumption of fresh fruits and vegetables is very low, whereas smoking, alcohol, obesity, accompanied by a lot of faulty eating and culinary habits increase the FR load to the human organism (Fehér et al. 1993; Tulok and Matkovics A 1997).

The role of antioxidants in maintaining health

At the beginning of FR research a much expected possibility was to prevent the AS and tumour development by neutralizing FR pathogenicity (Horváth and Jávor 1985). A tremendous amount of scientific investigations was carried out. More and more AO substances have become known to provide the biomolecules an effective AO protection. Various kinds of fruits, vegetables and medicinal plants were examined and their abundance in AO came to light (Fehér et al. 1993).

The AOs differ in their affinity to the various FRs as well as in their binding site in the organism, *e.g.* vitamin E is highly effective with the superoxide radicals but it is very weak in scavenging hydroxyl ions. The "master of AOs" is vitamin E, but on the surface of biological membranes and of the blood lipids only, whereas in the fluid spaces vitamin C is the main AO, as glutathion acts intracellularly and melatonin in the nerve cells. Thus: the AOs can be classified according to their FR affinity and tissue concentrations (such as antibiotics can). It is beyond doubt that there is neither a generally effective AO compound, nor a universal AO treatment (Matkovics A 2001). There are only some substances scavenging certain FRs on defined cells or molecules.

When determining the AO content of a tissue or plant the result depends on the method employed (FRAP, TAS, TBARS, etc.). Testing different fruits in a peroxide system plums, bananas and red grapes were the most effective against FRs, whereas in a hypochlorite system the banana proved to be the weakest. Similarly, examining some food additives (butylhydroxytoluene, trinatriumpolyphosphate, phenol, propylgallate, etc.) and spices (rosemary, red pepper and oregano as the best ones) a different rank order was

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established in the two mentioned systems, except vitamin E, which proved to be the weakest one (Murcia et al. 2001). Garlic, carrots, apricots, onions, curry, citrus fruits and kale sorts had good AO properties as well.

The advantages of tea and fish consumption are undoubtedly proven, but the role of red wine in preventing AS is still debated. The publication of the "french paradox" prompted an intensive investigation. The results are controversial, but they suggest slightly, that a moderate drinking of red wine (1- 3 dl daily but not more!) may be protective against AS (Goldberg et al. 2001), and, possibly, against neoplastic diseases too. The differences of the studies on this subject may be explained by the individual polymorphism of alcohol metabolizing enzymes and by the different populations among which these investigations were performed.

Adverse effects of antioxidant administration

A great number of data shows that any AO can be transformed to a prooxidant, especially if it is given in a high dose and without other AOs. When being not able to step in to the chain of electron transport the AO will be saturated and become a FR source. Probably this is the explanation of the failure of the „Natural" Cancer Prevention Trial (Peterson 1996). Later it was experimentally proved that high doses of ß-carotene produce superoxide radicals.

It can, thus, be stated: "A single antioxidant is not an antioxidant!" (Tulok and Matkovics 1997).

A new term called "antioxidative stress" has also been introduced. This includes all adverse effects of AOs and the indirect disadvantages of them. For example, the beneficial action of exercise by the induction of aortic catalase activity and endothelial NO synthase expression was counteracted by the administration of vitamin E (Meilhac et al. 2001). It means that an adaptative reaction may be hindered by the administration of an AO. Certainly the physiological FR reactions must not be blocked. The AOs have a Janus face!

Prevention of diseases by antioxidants

The best results of AO administration were attained in halting the progression of AS.

The first interventional trials with the administration of vitamin E did not yield convincing results. Although vitamin E in chronic renal insufficiency reduced the number of cardiovascular events by 40%, it is known, that in this condition the atherosclerotic process quickens by up to 6 – 26% faster, than in the age-matched control people (Boaz et al. 2000). Other studies gave minimal or negative results. These latter ones, however,were performed on people who probably had had AS plaques already at the beginning of the study.

Hence it can be suggested that vitamin E really hinders only the initial phase of AS, but has not any important influence on the later processes of plaque development and rupture, leading to myocardial infarction or arterial occlusion, to the so- called "hard endpoints."

Although the early captopril studies (SAVE, SOLVD) showed a significant reduction in stroke and cardiovasular events, the HOPE study (The Heart Outcomes Prevention Evaluation Study Investigators, 2000) provides the convincing evidence for the effectivity of ACE inhibitors in the impeding the AS process in the vascular wall.

Contemporary laboratory investigations have pointed out the pathogenetic role of angiotensin-II (which is also a ROS producer) in the process of AS: oxidizing lipoproteins, activating macrophages and smooth muscle cells, triggering the excretion of cytokines, adhesion – and apoptotic factors, leading to plaque formation – thus verifying theoretically the beneficial effects observed by the ACEi administration.

The administration of calcium channel antagonists and the angiotensin AT_1 blockers in hypertensive patients and HMG-CoA reductases in hyperlipidemic patients provided similar positive results to the aforementioned ones.

Corti and his co-workers measured the size of AS plaques with a high resolution MRI method and found out that after a year of simvastatin therapy both the diameter of AS plaques and the thickening of the arterial wall were signif-icantly diminished (Corti et al. 2001).

Such regression of AS plaques (in 15-18% of treated patients) has been already observed in some studies on antihyperlipidemic treatment both in the ELSA study with lacipidin and in other statin and fibrate studies.

The atherogenetic role of hyperhomoscystinemia was discovered in the latest years, with the hope to prevent AS in these cases with the administration of folic acid and vitamin B_6.

The main causes of AS are summarized in Table 1.

In the prevention of neoplastic diseases no similar achievements could have been reached. A number of studies on successful prevention were published (Shanghai, New Zealand, Uruguay study, etc.) but it is not clear, what kinds of materials and in which combination and doses are necessary.

The key question of cancer prevention and avoidance of premature ageing seems to be the AO protection of the DNS and the mitochondria (Van Remmer and Richardson 2001; László and Falus 2002). An immense research is going on for newer and better mitochondrial and DNS-protecting AO substances.

Antioxidants in the therapy

Every illness or tissue damage leads to an increased FR generation and these radicals cause further injuries (Matkovics 1993). Therefore each disease leads to a common, unspecific complication: the oxidative stress. So: the administration of

Table 1. Factors of atherogenesis.

Cause	Pathomechanism
Hypertension	Endothel dysfunction and lesion Biomechanical (shear) stress Overproduction of endothelin and angiotensin
Hyperlipidemias	Oxidised LDL Lipoprotein-A ; remnant- lipoproteins
Hypercholesterinemia Dyslipidemia Obesity Metabolic X syndrome	Triglyceride and free fatty acids Leptin Deficiency in HDL
Hyperfibrinogenemia Hypercoagulation syndromes	Enhanced activity of thrombocyte mitogenes and LDL binding capacity of the vascular wall ncreased smooth cell proliferation and migration.
Smoking	Increased lipid peroxidation Oxidative stress in the vascular wall Vasoconstriction, ischaemia , generation of ROS
Hyperhomocysteinemia	
a) congenital, (b) deficiency in vitamine B_6 and c) folic acid d.) Diabetes mellitus type 2. (NIDDM)	Autooxidation products of ROS (peroxide and superoxide radicals) Inhibition of glutathion peroxidase
Generalized oxidative stress Critically severe conditions:burns, polytraumatization, respiratory distress syndrome, serious infection, etc.)	Increased lipid peroxidation Endothelial damage caused by ROS
Nutritional AO deficiencies, alcoholism, Abundant lipid intake (including PUFA)	Weakness in defense to ROS Oxidative stress Increased lipid peroxidation
Ageing	Increased production of mitochondrial ROS. Oxidation of membrane lipids Lipid peroxidation enhanced.
Diabetes mellitus (mainly Type 2)	Glycation products, glycotoxicity, lipotoxicity
Chronic renal insufficiency	A general increase in ROS production Enhanced lipid peroxidation
Infections, inflammations, autoimmune diseases (SLE) (Chlamydia, CMV, herpes viruses, oral pathogenes, focal infections, etc.)	Increased inflammatory reactions in the vascular wall

AOs is reasonable in all diseases, in the form of the poly-AO therapy. The results of this approach have already been published in a lot of articles. In cases of polytraumatized patients (Porter et al. 1999) burns and in other critically serious conditions a higher percentage of recovery was observed during the AO treatment (N-acetylcysteine, selenium, Vitamin C+E, allopurinol and lazaroid combination): with a smaller rate of infections, cardiovascular or other complications (Bulger and Maier 2001). In subarachnoidal hemorrhages a smaller percent of brain damages was found in the AO group than in the control ones (Asano and Matsui 1999). Similarly, Goode (1993) observed hopeful outcomes in sepsis cases by the combined AO administration.

Professor Boda had been investigating the uric acid metabolism since the 60's and based on his own results he saved the life of many children with serious conditions (such as shock, or respiratory distress syndrome) by the allopurinol treatment (Boda and Németh 1983). It came to light only later, that in such clinical states the xanthine oxidase activity has increased, resulting in a high overproduction of superoxide radicals.

In the course of the influenza the ROS derived from the macrophages causes more damages than the influenza virus itself (Jacoby 1994). Hemila and Douglas (1999) pointed out, that vitamin C is really effective in acute respiratory infections, but it must be given in high doses, such as 2000 mg daily.

The combinations of AO compounds (mostly based on silymarin with other AOs) has proved to be effective in the therapy of different (toxic and infective) hepatic diseases (Berkson 1990; Fehér and Vereckei 1990; Fehér 2002). Good effect was observed with a vitamin C, coenzyme $Q_{10,}$ ß-carotene combination in primary biliary cirrhosis (Watson et

al. 1999). The chronic pancreatitis was influenced favourably with selenium compounds (Bowrey et al. 1992).

A vast body of literature testifies that a row of renal (Annuk et al. 2001; Clermont et al. 2001), hematological (Németh et al. 2000), autoimmune, gynaecologic, neurologic and metabolic disorders have been influenced favourably by the AO treatment, as summarized by Matkovics (2001).

There is a large quantity of materials which are effective against experimental carcinogenesis or they cause apoptosis of tumour cells – but not in curing human malignancies. In the malignant cell lines the pycnogenol, allicin and resveratrol showed the greatest antitumour activity. The clinical results have been scarce until now. Lockwood et al. (1994) published surprisingly good effects with a megadose coenzyme Q_{10} and multivitamin therapy, but these are still not confirmed by other investigators.

In clinical oncology the AOs have at present a rather adjuvant role, *e.g.* glutathion alleviates the toxic side effects of cysplatin therapy.

Some open questions

According to our present knowledge, the most important questions are still to be answered, *e.g.*: What does the optimal AO supply consist of? How many AOs must be taken and in what dosage? How can we provide a safe AO protection for the most important biomolecules without any harmful effect?

Nowadays the wisest advice might be: taking as many kinds of natural AO-s as it is possible, and in proportions resembling natural foods.

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