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FREE RADICALS IN CARDIOVASCULAR DISEASES

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Summary. *During the past several years, there has been a growing interest in the medical implications of free radicals. These chemical entities are common by-products of many oxidative biochemical reactions in the cell. Free radicals are essential to a number of normal biochemical and physiological processes. They are too reactive to be tolerated in living tissue, and aerobic organisms use sophisticated defense systems, enzymatic and nonenzymatic, for prevention of overload with free radicals and peroxides. In a number of pathophysiological conditions, the delicate equilibrium between free radical production and antioxidant capability can be altered in favor of the former, thus leading to oxidative stress and increased tissue injury. It is becoming well recognized that reactive oxygen species may be important mediators of cell injury in many cardiovascular diseases. This review concentrates on some of the considerable biochemical evidence concerning involvement of oxygen free radicals in several cardiovascular diseases: ischemia/reperfusion injury, atherosclerosis and hypertension.*
Since the current evidence shows that supplements and some of the cardiovascular drugs might possess useful antioxidant capacity, strategies for minimizing tissue injury caused by free radicals are briefly summarized.

Key words: *Free radicals, oxidative stress, cardiovascular diseases, antioxidants*

Introduction

The term "reactive oxygen species" (ROS) refers to an array of metabolites derived from molecular oxygen (O_2). During the past decade, there has been a growing interest in the medical implications of free oxygen radicals. Reactive oxygen radicals such as superoxide radical ($O^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and lipid peroxides (LOOH) are becoming increasingly implicated in human diseases. However, the question of whether such oxidants are a major cause of tissue injury in human diseases or are merely produced during such an injury has been difficult to answer because of inadequate experimental models.

In order to prove an association between a disease process and free radicals as its hypothesized cause, Halliwell, Cross and Gutteridge (1) state that three criteria must be met: 1) production of free radicals must be shown during the disease process, 2) there must be documented protection against injury by antioxidants that remove or nullify the free radicals, and 3) the disease process must be reproducible by the application of free radicals at the organ of interest (par example, the arterial endothelium). While some animal models of different diseases satisfied these criteria, extrapolation of animal studies to humans is difficult, and the need for more and larger clinical studies for most of oxidative stress-induced diseases is apparent.

Why should cardiologists be concerned? The cardiovascular disease is a heterogeneous group of disorders that affects the heart and blood vessels. The diseases are characterized by angina pectoris, hypertension, congestive heart failure, acute myocardial infarction (heart attacks), stroke, and arrhythmia. There is now considerable biochemical, physiological and pharmacological data to support a connection between free radical reactions and cardiovascular tissue injury. Evidence is accumulating that these disease conditions are directly or indirectly related to oxidative damage and that share common mechanisms of molecular and cellular damage. As these mechanisms are elucidated, it may be possible to improve the techniques for clinical and pharmacological intervention.

The present paper concentrates on the evidences concerning the involvement of free radicals in a few cardiovascular diseases and their relationship to specific pathophysiological events. Much of this information has been obtained from experimental investigations and some of them from clinical studies.

I. Oxidative stress in living cells

1. The birth of the concept

The first demonstration of the chemical existence of free radicals has been the isolation of the nitroxyl radical in the form of Fremy's salt $(KSO_3)_2NO$ in 1845. Surprisingly, it was only in 1987 that nitric oxide release was shown to be associated with the production of an endothelium-derived relaxing factor (EDRF) which is biosynthetized and released by the vascular system in mammals (2). Fundamental interest in free radicals began with the work of Moses Gomberg, who, in 1900, demonstrated the existence of the triphenylmethyl radical ($OH_3C^{\bullet}$) (3). In 1946, Michaelis proposed that free radicals were obligate intermediates in metabolic pathways of living cells (4), but this remarkable prediction was regarded with great skepticism by most biochemists of his generation.

The biochemistry of oxidative stress slowly emerged from the recognition that aerobic organisms were equipped with powerful and redundant antioxidant protections. Vitamin E was first discovered in 1922, and in 1957, an enzyme which used the reducing power of glutathione (GSH) to protect human red blood cells against hydrogen peroxide-mediated degradation of hemoglobin was identified. This enzyme, was called glutathione peroxidase, and has been shown to contain selenium at the active site (5).

However, the really exciting story of free radicals began in 1969 when Mc Cord and Fridovich (6) discovered that a copper-containing protein, erythrocuprein, isolated from red blood cells, catalyzed the dismutation of superoxide ($O_2^{\bullet-}$). This enzyme was renamed superoxide dismutase (SOD). "After the publication of the original paper identifying the enzymatic activity of erythrocuprein, there followed an intelectual and creative explosion which could be compared with the excitement generated by the discovery of double helical structure of DNA by Watson and Crick in 1953." (A. M. Michelson). The first discovered, the ubiquitous vitamin E, glutathione peroxidase and superoxide dismutases are now known to provide a primary protective barrier against the toxicity of free radicals and peroxides in mammalian cells.

2. The basic properties of free radicals

A free radical is any atom or molecule, capable of independent existence, that possesses one or more unpaired electrons. Electrons are more stable when paired together in orbitals: the two electrons in a pair have different directions of spin. Hence, radicals are generally less stable than nonradicals, although their reactivity vary (Table 1). The free radicals are capable of reacting indiscriminately with any molecules with which they come in contact. Once radicals are formed they can either react with another radical or with another nonradical molecule by various interactions. If two radicals meet they can combine their unpaired electron, thus forming a covalent bond. However, most molecules found *in vivo* are nonradicals. In this case a radical might donate its unpaired electron to the other molecule, or might take one electron from it, thus transforming its radical character. At the same time a new radical is formed. The feature that is becoming clear is that a radical generates another radical, leading to the chain reaction (7).

Table 1. Examples of Reactive Oxygen Species (ROS)

The Radicals		
Superoxide	$O_2^{\bullet-}$	Oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems, by autooxidation reactions and by nonenzymatic electron transfers that univalently reduce molecular oxygen. SOD accelerates the dismutation of $O_2^{\bullet-}$, converting it to hydrogen peroxide (H_2O_2) and oxygen (O_2).
Hydroxyl	$OH^{\bullet}$	A highly reactive oxygen-centered radical that attacks all molecules in the human body.
Peroxyl, alkoxyl	$RO_2^{\bullet}$, $RO^{\bullet}$	Typically, organic radicals often encountered as intermediates during the breakdown of peroxides of lipids in the free radical reaction of peroxidation.
Oxides of nitrogen	$NO^{\bullet}$, $NO_2^{\bullet}$	Nitric oxide is formed *in vivo* from the amino acid L-arginine. Nitrogen dioxide is formed when NO reacts with O_2 and is found in polluted air and smoke.
The Nonradicals		
Hydrogen peroxide	H_2O_2	Formed *in vivo* when $O_2^{\bullet-}$ dismutates and also by many oxidase enzymes. Higher levels of H_2O_2 can attack several cellular energy-producing systems. H_2O_2 also forms $OH^{\bullet}$ in the presence of transition metal ions (Fe^{2+}). $O_2^{\bullet-}$ can facilitate this reaction.
Hypochlorous acid	HOCl	A powerful oxidant formed in the human neutrophils at sites of inflammation by action of the enzyme myeloperoxidase. May also react with $O_2^{\bullet-}$ to generate $OH^{\bullet}$ in neutrophils.
Ozone	O_3	This noxious gas has been shown to deplete plasma antioxidants vitamin D, vitamin E, and uric acid.
Singlet oxygen	$^1O_2$	Here the spin of one of the electrons of the two outer orbitals is inverted, removing the quantum mechanical spin restrictions of molecular oxygen.

In biological and related fields, the major free radical species of interest are oxygen free radicals (OFRs, Table 1). Step-wise single electron additions to (reduction of) molecular oxygen generates a unique spectrum of more reactive intermediates, the oxygen free radicals (Fig. 1 and Table 1). The term OFRs includes the superoxide anion free radical ($O_2^{\bullet-}$), the hydroxyl radical ($HO^{\bullet}$), and lipid (L) and other (X)

peroxy radicals ($LOO^{\bullet}$ and $XOO^{\bullet}$). More recently, through research into nitric oxide ($NO^{\bullet}$), the active moiety of endothelial derived relaxing factor, and into air-borne pollution, there has been growing interest in nitrogen-centered free radical species such as peroxynitrate ($^{\bullet}OONO_2$) and peroxynitrite ($^{\bullet}OONO$) (2, 8).

Oxygen free radicals (OFRs) are part of a greater group of molecules often called reactive oxygen species (ROS) that are all more strongly oxidizing than molecular oxygen itself. These include hydrogen peroxide (H_2O_2), lipid peroxide (LOOH), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and other *N*-chloramine compounds (Table 1).

OFRs are not the only free radicals species. For example, carbonyl, thiyl, and nitroxyl radicals can all exist. Many other free radical species can also be formed by various biological reactions (phenolic and aromatic species during xenobiotic metabolism as a part of natural drug detoxification mechanisms, par example).

3. Free radical-generating systems in normal processes *in vivo*

While a large number of articles focuses on the deleterious aspects of oxygen activation and oxygen-derived free radicals in mammalian cells, it is important to bear in mind that free radicals are molecular species which are both essential and harmful for aerobic cells.

OFRs and other free radicals are constantly formed in the human body by normal metabolic processes, as the reduction of oxygen to water by the mitochondrial electron transport chain. In this reaction oxygen itself is reduced in a such way that two electrons (and two pairs of protons) are accepted by each oxygen atom leading to the formation of a water molecule (Figs.1 and 2). A small percentage of electrons leaks away from the main stream of the mitochondrial respiratory chain, leading to univalent reduction of molecular oxygen, which generates superoxide anion ($O_2^{\bullet -}$). The quantitative importance of oxygen-derived free radicals can be realized by the fact that about 250 grams of oxygen are consumed every day by the human organism. Of this, about 2-5% would be converted to the superoxide (9).

In human cells superoxide is quickly transformed into hydrogen peroxide (H_2O_2). This reaction is greatly accelerated by superoxide dismutase (SOD), a widely distributed enzyme. H_2O_2 is a potent oxidant and, in sufficient concentrations, will kill any cell. The further reduction of H_2O_2 labilizes the interoxygen bond, resulting in a cleavage to produce OH^- and $OH^{\bullet}$. The latter one, hydroxyl radical, is a highly reactive radical species. A free transition metal ions (Fe, Cu) often act as electron donor necessary for generation of hydroxyl radical from H_2O_2 (Fig. 1). In the presence of excess iron, the toxicity of H_2O_2 may be magnified 10 to 1000 times. Because of this sequestration of transition metals can be considered as an important mechanism of antioxidant defense (10).

Free radicals are involved in several normal biological processes *in vivo*. For example, they are part of the cascade of events in the antimicrobial action of the phagocytic cells via NADPH-oxidase, in the arsenal of defense cells (neutrophils, monocytes, macrophages,

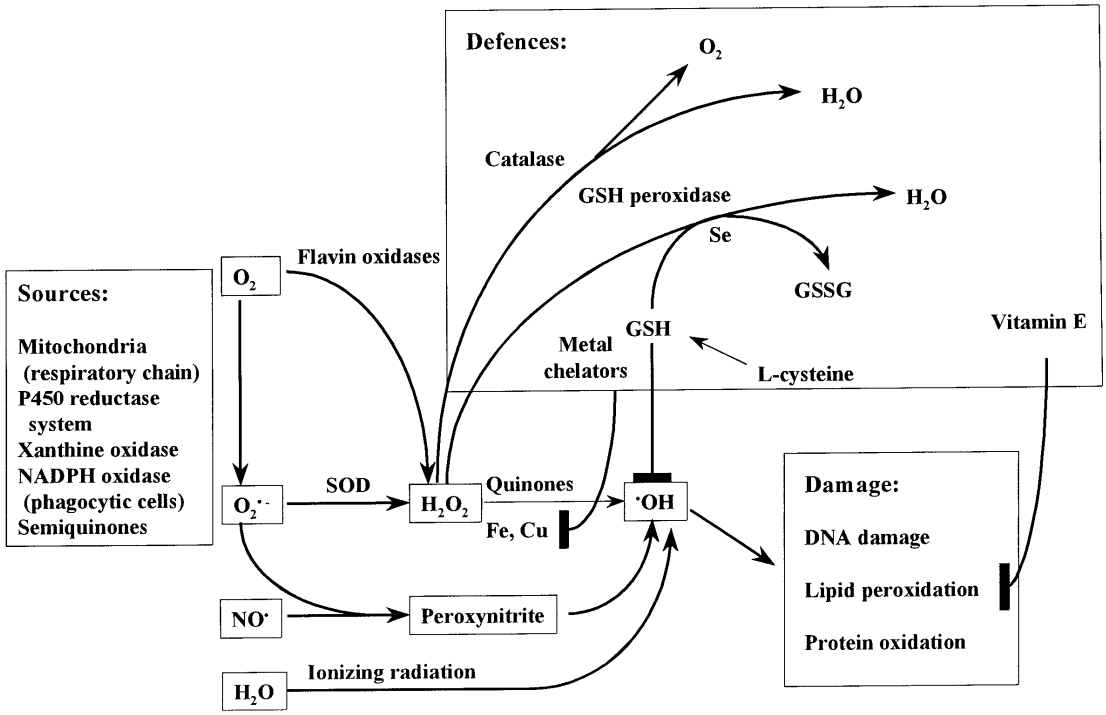

Fig. 1. Major reactive oxygen species pathways and antioxidant defences. These and other reactions are reviewed in more detail in Refs. 1 and 9. Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase. (Taken from Ref. 12)

eosinophils) with which human body is equipped. This process is central to the human antimicrobial defense system, and intended to damage the membranes, DNA and other cellular component of the invading organism (11). Free radicals can act as regulatory molecules in biochemical processes; for example, lymphocytes and fibroblasts constantly generate small amounts of superoxide radical as growth regulators (12). Other nonphagocytic cells, such as cell types including endothelial cells and arterial smooth muscle cells, can be stimulated to release superoxide. Nitric oxide from endothelial cells is involved in the regulation of vascular tone, inducing the relaxation of smooth muscle cells. Macrophage-derived nitric oxide has been implicated in the killing of tumor cells and bacteria (8). Free radicals are also involved in the mechanism of action of certain enzymes, for example, ribonucleoside diphosphate reductase, cytochrom P-450 and prostaglandin synthase (12).

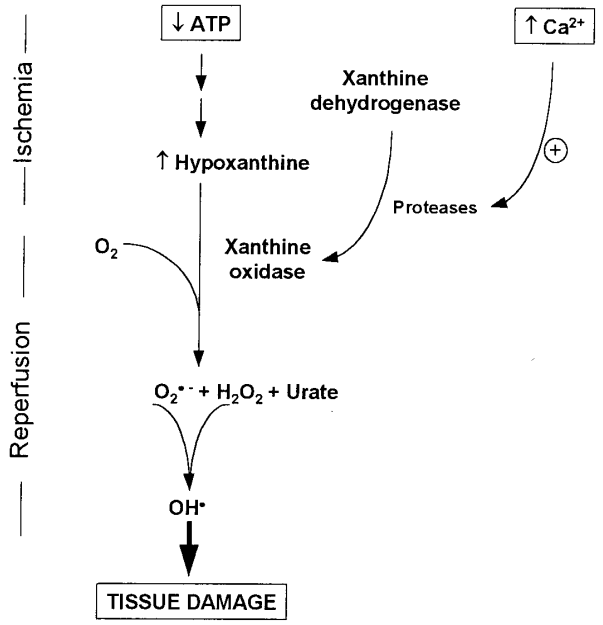

Fig. 2. Production of reactive oxygen species and tissue injury associated with ischemia/reperfusion events

The observations that a wide variety of normal and malignant cell types generate and release superoxide and hydrogen peroxide suggest that a role for such a species as cellular "messengers" is feasible (13).

Thus, free radicals are involved, in a controlled fashion, in many normal biochemical mechanism *in vivo,* but they became highly reactive if not tightly controlled.

4. Protective pathways in mammalian cells and tissues

Free radicals and reactive oxygen species are too reactive to be tolerated in living tissues and their removal and control may have had a dominating evolutionary pressure with the first appearance of O_2 in the atmosphere. A hierarchy of mechanisms has been evolved to deal with these reactive intermediates (9, 11, 14).

To prevent an overload in free radicals and peroxides, aerobic organisms use a sophisticated defense system which operates both in intra- and extracellular aqueous phases and in membranes. Antioxidant defense strategies are committed to counteract the oxidative attack in its early moments i.e., formation of priming radicals, as well as during the initiation and chain propagation processes.

Antioxidant protection can be viewed as consisting of four sequential levels of defensive activity: preventive; chain-breaking; repairing and adaptive (Fig. 1). The first level of defense, which is largely enzymatic, involves enzymes whose activity depends principally on trace amounts of minerals Mn, Cu, Zn and Se (superoxide dismutases, glutathione peroxidases and catalase); it is concerned with the control of formation and proliferation of primary radical species derived from molecular oxygen (Figs. 1 and 2). The second, which involves the two vitamins C and E, and probably carotenoids, is concerned with the prevention of the proliferation of secondary radicals in chain reactions such as lipid peroxidation, initiated and driven by primary radicals. The third level is the enzymatic prevention of formation of secondary radicals from chain-terminated derivatives and enabling the removal of such molecules from an environment in which metal-catalyzed reactions might causes further oxidative damage. Finally, adaptation can also be included in antioxidant mechanisms. Namely, free radicals also work as a signal capable of inducing the synthesis and the transport of the appropriate antioxidant to its site of action (9, 14).

5. Free radical-mediated tissue damage

In human diseases an increased free radical activity can occur as the consequence of either primary (e.g. excess radiation exposure) or secondary (e.g. tissue damage by trauma) events through the setting of different biochemical processes: extracellular release of ROS by polymorphonuclears, xanthine oxidase activation, iron release from sequestrated sites, phospholipases activation, alteration of electron transport in mitochondrial chain, etc. Xenobiotics and environmental pollutants may increase the intracellular formation of ROS, for example, through the Fenton reaction involving trace metals such as iron and copper (9, 10, 15). Consequently, in these reactions the antioxidant defense will be rapidly overwhelmed and free radicals will become highly destructive to cells and tissues. Thus, an "oxidative stress" occurres when, as defined by Sies (15), there is a profound disturbance in the prooxidant-antioxidant balance in favor of former, leading to lipid peroxidation, denaturation of proteins or enzymes or mutagenic damage to nucleic acid. Such a view could be illustrated with activated neutrophiles which can evolve in both physiological (killing of

microorganisms with intracellular and controlled production of ROS) and pathological (inflammation with uncontrolled extracellular production of ROS) events.

Oxidative stress may be due to endogenous stresses or exogenous sources of free radicals (9). Increased radical input arises exogenously from:

i) ionizing radiation,
ii) excess availability of transition metals,
iii) side effects of drugs and toxic chemicals,
iv) oxygen excess and increased oxygen concentration.

Oxidative stress accompanying disease state may arise from endogenous factors:

i) phagocyte recruitment and activation at the site of injury,
ii) leakage from the disrupted mitochondrial electron chain,
iii) delocalization of transition metal ion and leakage of haem protein.
iv) decreased protective capacity including reduced antioxidant enzymes,
v) reduced levels of antioxidant,
vi) leakage or destruction of antioxidants.

These factors may be mutually interactive and lead to generation of radical species. Due to their highly reactive nature they can readily combine with other molecules, such as enzymes, receptors, and ion pumps, causing oxidation directly, and inactivating or inhibiting their normal function (12, 16, 17). Some of the products of oxygen free radicals attack on other molecules can interfere with nucleic acid function, generating alterations in the base sequence with the potential for mutations, leading to cancer or germ-line mutations (13). Changes in normal proteins and other structures by free radical species can also generate novel immunogenic structures (17). One of the most destructive effects of oxygen free radicals is the initiation of lipid peroxidation which can result in run-away reactions (18). If not terminated, this chain reaction will lead to destruction of the cell membranes, breakdown compartmentalization and release of lysosomal enzymes and subsequent autolysis. Termination of this chain of events can only be achieved by the reaction going to completion or by chain-breaking antioxidants that destroy the free radicals produced.

II. Free radicals in cardiovasular diseases

1. Free radicals in ischemia-reperfusion myocardial injury

Exposure of myocardial tissue to a brief, transient ischemia, followed by reperfusion, has attracted remarkable attention in recent years. Myocardial ischemia occurs when myocardial oxygen demand exceeds oxygen supply. Unless reversed, this situation results in cell injury, and clinical event is myocardial infarction. Logically, reperfusion of ischemic myocardium is recognized as a potentially beneficial because mortality is directly proportional to infarct size, and this latter to the severity and duration of ischemia. Reperfusion of the ischemic myocardium can restore oxygen and substrates to the ischemic myocardial cells, but this process may create another form of myocardial damage, termed "reperfusion injury" (19-21). Thus, restoration of a normal blood flow in the heart by methods such as angioplasty, thrombolytic agents or cardiopulmonary bypass can lead to specific lesions (arrhythmias, deficit in contractility, necrosis), the importance of which also depends on the duration of ischemia.

Evidence suggests that damage to the myocardial cell induced by the cycle of ischemia and reperfusion may be due, in part, to the generation of toxic, reactive oxygen species such as superoxide radical, hydrogen peroxide, and the hydroxyl radical (21- 25). The active involvement of free radicals in the ischemia-reperfusion damage is demonstrated by direct and indirect experimental evidences. Direct evidences arise from the possibility of measuring radicals in myocardial tissue by electron spin resonance (ESR) and spin trapping methodology (26, 27); indirect evidences by the measurement of the products of free radical attack on biological substrates (usually malondialdehyde as a measure of lipid peroxidation extent), and intracellular and extracellular antioxidant capacity (28-31).

Using electron spin resonance methodology, an increased free radical production in blood after reperfusion of infarcting tissue has been reported (26). ESR signals are also recorded in blood taken from coronary sinus of patients undergoing percutaneous transluminal coronary angioplasty, an ideal model of myocardial ischemia -reperfusion (30). In patients undergoing cardiopulmonary bypass, an increased free radical activity and reduction of blood antioxidant capacity in plasma has been shown to occur following aortic declamping (32, 33).

Furthermore, it is currently believed, based on the experimental findings, that in the ischemic tissue there is an impairment of antioxidant mechanisms (33). Evidence to support this statement comes also from the cardioprotective effects of agents capable of inducing antioxidant enzymes in the heart and from the beneficial effects of several enzymatic free radical scavengers, antioxidants and iron chelators in reperfused myocardium (33-37).

The proposed concept of the role of free radicals in reperfusion injury has enormous implications in the setting of cardiac surgery and heart transplantation. Conditions of ischemia and reperfusion are created routinely by the surgeon during open heart procedures, such as cardiopulmonary bypass and aortic cross-clamping (21). Similar conditions prevail during heart transplantation when ischemic donor heart is rapidly

deoxygenated by the recipient's blood.

1.1 Cellular sources of free radicals and mechanisms of damage in reperfusion injury

Reactive oxygen radicals in reperfusion injury can originate from intracellular sources, such as mitochondria and xanthine oxidase, or from extracellular sources, such as neutrophiles and macrophages.

Central to any discussion of the pathophysiology of ischemic lesions is energy depletion. Energy failure alone cannot explain the functional damage occuring during the reperfusion phase. At present, the following features of the ischemic and postischemic heart are the focus of interest: development of acidosis, edema formation, calcium overload, free radical formation and nitric oxide overproduction. These events, together with other less known ones, contribute to the occurrence of irreversible damage. The rapid decrease of oxygen in ischemic tissue causes a switch from oxidative to anaerobic metabolism. Within minutes of the onset of ischemia, energy demands exceed the heart's capacity to synthetize ATP anaerobically. Energy depletion has fundamental importance in the genesis of subsequent injurious events. Lactate and unbuffered hydrogen ions accumulate in tissue leading to the rapid change in tissue acid-base status. The failure of all energy dependent mechanisms leads to the deterioration of membrane ion gradients, opening of selective and unselective ion channels and equilibration of most intracellular and extracellular ions. As a consequence of this "anoxic depolarization", potassium ions leave the cell, sodium chloride and calcium ions enter. Cellular accumulation of ions causes formation of cytotoxic edema. Intracellular Ca^{2+} overload can also set off a cascade of events which may lead to the formation of ROS. The elevated Ca concentration activates proteases that can convert xanthine dehydrogenase to xanthine oxidase. During reoxygenation, xanthine oxidase can use O_2 as an electron acceptor, leading to formation of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H2O2), which can react to produce hydroxyl radicals ($OH^{\bullet}$, Fig. 2). These reactive species are responsible for the tissue damage (Fig. 2). Xanthine oxidase production of oxygen free radicals plays a major part in generating tissue damage seen in ischemia/reperfusion injury, as seen by the ability of inhibitors of xanthine oxidase (allopurinol) and antioxidants, to protect against such damage in experimental models of myocardial injury (23, 34, 35).

However, xanthine oxidase may not be the only source of OFRs during reperfusion. Activation of complement by proteases and other mechanisms leads to activation of neutrophils and their subsequent recruitment into reperfused tissue. The fact that depletion of neutrophils or prevention of their adhesion to endothelium, the first stage in their recruitment, is also protective in ischemia/reperfusion injury suggests that these may be equally, if not more important sources of ROS (23). Injury to myocardial tissue may also release iron ions that can stimulate free radical reactions (38).

One more source of oxygen free radicals is the intramitochondrial electron transfer chain (Fig. 1). Free radicals produced in mitochondria could also have the ability to cause point mutations, DNA cross link and DNA strand breaks in mitochondrial genes. Damage to the mitochondrial genome results in impaired respiration, further increasing the possibility of oxygen radical production. Impaired mitochondrial function and increased production of superoxide are very common reperfusion-associated events. The activities of components of mitochondrial respiratory chain have been documented to be markedly reduced during postischemic reperfusion or posthypoxic reoxygenation (39-41). Experimental studies have suggested that mitochondrial dysfunction results in increased production of $O_2^{\bullet-}$ by this organelle after exposure of cardiac muscle to ischemia/reperfusion (36).

The increased formation of ROS following hypoxia/reoxygenation is, unfortunatelly, associated with low antioxidant capacity of myocardial tissue. Namely, in myocytes, as well as in endothelial cells, catalase concentration is very low and most of it is compartmentalized within peroxisomes. This subcellular localization prevents catalase from being an efficient acceptor of H_2O_2 resulting from SOD activity in the cytosol (36). Insufficient antioxidant capacity of tissue to scavenge the increased content of ROS following hypoxia/reoxygenation appear to be an important contributing factor to tissue dysfunction, restenosis of bypass grafts, and postbaloon angioplasty myocyte proliferation (23, 36). These complications worsen efficiency of interventions used in the treatment of coronary artery disease (36).

Hydrogen peroxide has been reported to increase vascular permeability, prostacyclin release, and translocation of P-selectine to the endothelial cell surface (42-44). ROS oxidatively modify lipid and protein in membranes resulting in cellular dysfunction and tissue damage associated with reperfusion injury. Increase in oxidative stress in reperfusion injury is associated with functional impairment of vascular reactivity and release into bloodstream of creatine kinase, lactate dehydrogenase, myoglobin and troponin C from the cytosolic compartment (45).

The cascade of events associated with ischemia/reperfusion injury, besides free radical generation, includes the release of cytokines and growth factors, leukocyte adhesion, platelets aggregation, smooth muscle proliferation, and mechanical injury (13, 43, 44).

1.2 Nitric oxide in myocardial ischemia

Nitric oxide (NO) has recently emerged as an important mediator of cellular and molecular events which impacts the pathophysiology of myocardial ischemia. An increase in intracellular Ca^{2+} (resulting from the activation of voltage-gated Ca^{2+} channels or ligand-gated Ca^{2+} channels or from the mobilization of

intracellular Ca^{2+} stores) could activate the enzyme NO synthase (8) which catalyzes the synthesis of NO from L-arginine and molecular oxygen. Nitric oxide may causes cytotoxicity through formation of iron-NO complexes with several enzymes including mitochondrial electron transport chain, oxidation of protein sulphydryls and DNA nitration (8, 46). NO may also mediate cell death through formation of the potent oxidant peroxynitrite ($ONOO^-$), the reaction product of NO with $O_2^{\bullet-}$ (12). Peroxynitrite decomposes to the hydroxyl free radical ($OH^\bullet$) and to radical nitrogen dioxide ($NO_2^\bullet$) which is potent activator of lipid peroxidation. Recently, have been reported that nitric oxide and peroxynitrite, could contribute to cardiac dysfunction in situation such as hypoxia/reoxygenation (46). On the other hand, vascular NO as a potent vasodilatator and an inhibitor of platelet aggregation, may be beneficial to the early stages of focal myocardial ischemia. It may also facilitate collateral blood flow to the ischemic territory (8).

2. Free radical hypothesis of atherosclerosis

The development of atherosclerosis is a multifactorial process in which both elevated plasma cholesterol levels and proliferation of smooth muscle cells play a central role. Atherogenesis is an alteration of the artery wall that includes two major phases: 1. Adhesion of monocytes to the endothelium and their migration into the subendothelial space and differentiation into macrophages. These cells ingest (oxidized) low density lipoproteins and through this process they are transformed into "foam cell". 2. Vascular smooth muscle cells migration from the media into the intima and their proliferation with the formation of atherosclerotic plaques.

The importance of oxidative stress in the development of atherosclerosis seems to be widely accepted. The statement that free radicals are involved throughout the atherogenic process, beginning from endothelial dysfunction in an otherwise intact vessel wall, up to the rupture of a lipid-rich atherosclerotic plaque, leading to acute myocardial infarction or sudden death, has been reported recently (47).

2.1 Oxidation of low density lipoprotein

Considerable *in vivo* evidence, animal and human, supports the important role of oxygen-free reactions in atherogenesis and atherosclerotic coronary heart disease (47-50). While the exact mechanisms for atherogenesis are not completely understood, recent studies suggest that oxidative modification of low-density lipoproteins (LDL) is a critical factor (49,50). Thus, LDL is the "bad actor" in the free-radical hypothesis of atherosclerosis.

LDL may be oxidatively modified by all major cell types of the arterial wall, including endothelial cells, smooth muscle cells, and macrophages (47,49,50) via their extracellular release of reactive oxygen species (ROS). Hydroxyl radical (thus formed) may initiate the peroxidation of long-chain polyunsaturated fatty acids within LDL molecule, giving rise to conjugated dienes and lipid hydroperoxy radicals ($LOO^\bullet$) This process is self-propagating, such that $LOO^\bullet$ can attack adjacent fatty acids until complete fatty acid chains fragmentation occurs. A number of highly reactive products then accumulate in the LDL particle, including malondialdehyde and lysophosphatides. These products interact with the amino side chain of the apoprotein B 100 and modify it to form new epitopes that are not recognized by the LDL receptor. OxLDL is avidly taken up by subendothelial macrophages via the "scavenger" receptor pathway which does not recognize native, unmodified LDL. Through the scavenger receptor, unlimited amounts of modified LDL are ingested by the monocyte/macrophage, which is now a "foam cell" in the arterial intima. The "foam cell" recruits more monocyte/macrophages to converts in foam cells. Accumulation of LDL-laden foam cells beneath arterial endothelium lays the foundation for the "fatty streak", the earliest histopathological evidence of the development of atherosclerotic plaque (47). Oxidized LDL also stimulates the release of monocyte-derived TNFα and IL-1β, leading to smooth muscle cell proliferation. Elaboration of collagen and elastin by smooth muscle cells lay the foundation for plaque formation and ultimately fibrosis (47, 50). Lipid peroxides also inhibit synthesis of prostacyclin, an antiplatelet-aggregation substance, which can results in platelet adherence and aggregation. Platelets release growth factors, subsequently leading to smooth muscle cell proliferation and migration to intima. Besides, aggregation of platelets lays the foundation for formation of thrombus (47).

Oxidatively modified LDL (oxLDL) has additional atherogenic and many pro-inflammatory properties. It stimulates the expression of macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF) and monocyte chemotactic protein-1 (MCP-1) by endothelial cells and is also cytotoxic to these cells. OxLDL is chemotactic for monocytes, and inhibits the motility of macrophages (50).

Finally, oxLDL is highly immunogenic, forming immune complexes in the arterial wall that can also be taken by macrophages. Antibodies against oxidized LDL have been detected in rabbit atherosclerotic lesions, and the plasma of rabbits and humans contains autoantibodies that react with several forms of oxidized LDL (50-52). Atherosclerotic lesions from human aorta contain lipid peroxides, and the peroxide content correlates with the extent of atheroma (53). Detectable levels of oxLDL are also found in human plasma, and elevated plasma peroxide levels have been found in diabetics, smokers and patients with coronary disease (54, 55).

2.2 The role of nitric oxide in atherosclerosis

Since the discovery of the role of nitric oxide (NO) as a vasodilatator, there has been intense experimental

interest in this substance. It has turned out to have a variety of physiological roles, involving virtually every tissue in the body. Nitric oxide is synthetized in the heart by both inducible and constitutive nitric oxide synthases (iNOS and cNOS, respectively). The constitutive NOS (cNOS) is present in coronary endothelium, endocardium, and, unlike vascular smooth muscle, it is also located in the myocardium (56). The inducible NOS (iNOS) is present in endocardium and the myocardium, as well as in vascular smooth muscle cells.

Physiological production of NO from constitutive NOS in endothelium is important to maintain cardiovascular homeostasis. Despite the clear biochemical and molecular biological evidence for the presence of iNOS protein in human vascular smooth muscle cells, determining its contribution to pathophysiological changes has been less straightforward. Within cardiovascular system, a dual opposite effect of expression of iNOS, beneficial and deleterious has been demonstrated. Nitric oxide causes myocyte relaxation, and plays an important role in the regulation of coronary circulation and myocardial contractility (57). It has also been recognized that nitric oxide (NO), by inhibition of platelet aggregation and leukocyte adhesion, may defend endothelial cell against damage (8). Overproduction of NO in the blood vessel wall is associated with vasodilatation, and resistance to constrictor stimuli. However, it has been shown that endothelial cell damage and increased vascular permeability may result from increased synthesis of NO (58). Besides, overexpression of iNOS is implicated as a mechanism of tissue dysfunction and damage in many acute and chronic diseases of cardiovascular system, which are characterized by an inflamatory response. There is biochemical and functional evidence to support a role for iNOS in the vasodilatation and hypotension of septic shock in patients.

Atherogenesis is also considered a chronic inflammatory disease of the vessel wall (47). Thus, in this condition, after exposure to endotoxins and certain cytokines (TNF α, IL-1), expression of iNOS in vascular endothelial cells, smooth muscle cells, endocardium, and macrophages located within the vessel wall, leads to prolonged synthesis of large amounts of NO, and also to the endothelial cell damage or dysfunction. The initial hypothesis for deleterious effects of NO has been based on its free radical nature and its high reactivity. NO diffuses out and can reach adjacent cells where it reacts with the iron-sulfur centers of several important enzymes from the mitochondrial electron transport chain and/or ribonucleotide reductase, the enzyme necessary for DNA synthesis (8). Recently it has been suggested that iNOS is expressed in aneurysmal atherosclerotic human aorta (59) and in megakaryocytes of patients with atherosclerosis (58). Besides, studies with NO-donor drugs suggest that overproduction of NO in the human heart might decrease contractility and impair diastolic relaxation.

According to these findings it may be proposed that the net effect of nitric oxide modulation in cardiovascular system probably results from a balance between beneficial hemodynamic effects and cytotoxicity. It remains to be determined why normal physiological production of NO is protective in cardiovascular system and may prevent atheroma formation, whereas overproduction of NO after induction of iNOS is potentially harmful.

3. Free radicals in hypertension

Reactive oxygen species (superoxide radical, hydrogen peroxide, hydroxyl radical) and oxidized low density lipoproteins (oxLDL) may play a critical role in the pathobiology of hypertension, as well in other conditions mentioned above, such as atherosclerosis, reperfusion injury and myocardial infarction. However, the relationship between oxygen free radicals and essential hypertension has received relatively limited attention.

Several reports have documented that essential hypertension (EH) is associated with increased superoxide anion and hydrogen peroxide production as well as decreased antioxidant capacity (60-63). The involvement of reactive oxygen intermediates in EH is also suggested by the observation of increased level of lipid peroxides and decreased concentrations of antioxidant vitamin E in plasma of EH patients. Recently, we have shown that patients with EH have plasma concentrations of free radical scavengers lower than healthy normotensive subjects (64). The elevated consumption of plasma antioxidants was accompanied by increased activity of extracellular antioxidant enzymes (glutathione peroxidase and superoxide dismutase). Our results suggest that free radical production in EH overwhelmes antioxidant defense capacity. Oxidative stress in EH patients is accompanied with the decreased red blood cells (64) and neutrophils (63) superoxide dismutase and glutathione peroxidase activity.

However, whether the oxidative process is a mere consequence of the increased blood pressure or a concomitant feature of a still unknown pathological processes is not clear. The question of whether elevated blood pressure alone constitutes a risk for cardiovascular complications in hypertensive subjects is unresolved. Besides, the underlying mechanism that leads to the oxidative stress remains largely unexplored. Reactive oxygen radicals may play a dual role in essential hypertension. On one hand, they may inactivate nitric oxide converting them in peroxynitrite in reaction with superoxide anion, thereby causing arteriolar vasoconstriction and elevation of peripheral hemodynamic resistance (65). On the other hand, enhanced production of free radicals may serve as trigger mechanism for oxidative damage of numerous macromolecules (par example low-density lipoprotein). The enhanced LDL oxidation has been observed in patients with essential hypertension (47,53). This

conclusion was based on findings obtained in isolated LDL (which appeared more prone to oxidation triggered by exogenous stimuli) and on demonstration of autoantibodies directed against epitopes generated during oxidative modification of apoprotein B 100 (47). Little is known, however, about the molecular processes underlying LDL oxidation in essential hypertension.

To understand the mechanism for oxygen free radical formation in hypertension, the cellular source must be identified. The endothelial cell, which is recognized as a source of NO, has also been identified as a potential site of oxygen free radical production (8, 66). Superoxide radicals **in** and **around** vascular endothelial cells were found to play a critical role in the pathogenesis of hypertension. Recent studies by Prabha et al (66) and Kumar and Das (67) indicate that essential hypertension is associated with increased superoxide anion and hydrogen peroxide production by circulating leukocytes. Besides, there is evidence that spontaneously hypertensive rats (Dahl-S) have an elevated number of circulating leukocytes that produce superoxide compared with its normotensive control (Dahl-R rats) (68).

3.1 The role of nitric oxide in hypertension

Nitric oxide (NO), the recently discovered mediator of cell communication, may be involved in pathogenesis of hypertension as well as its complications. NO is produced from the arginino guanidino group, by an enzyme called nitric oxide synthase (NOS) in cells lining parts of the vascular system. The NO diffuses into the smooth muscle of blood vessels, causing cGMP production which, in turn, causes muscle relaxation and vessel dilatation. Production of NO can also be stimulated by acetylcholine so that it can mediate nervous control. It is also produced in response to a shearing force exerted by blood flow on the endothelial cells lining the vessels, thus resulting in vasodilatation and reduction of shearing force (8). Trinitroglycerine, drug used since 1867 in the treatment of angina, slowly produced nitric oxide thereby relaxing blood vessels, thus reducing the work load of the heart muscle. NO lasts only about 100 msec in blood, and only a few seconds in tissues because it combines with O_2 to form nitrite. Nitrite is converted to nitrate and excreted in the urine.

The cardiovascular system is exposed to a continuous NO-dependent vasodilatatory tone (56,69) and withdrawal of it mimics many features of human hypertension including target organ damage. Of particular interest in this context is the fact that NO not only act as a vasodilatator, but also inhibits platelet adhesion and aggregation (8,69) as well as migration and proliferation of vascular smooth muscle cells (70).

The role of endothelium and NO in systemic hypertension, however is still controversial. In spontaneous hypertensive rats the production of NO seems to be increased (71). These data suggest that blood pressure *per se* is a stimulus for NO release. It is likely, however, that NO, although its production is augmented in spontaneous hypertension, is inefficacious due to its increased inactivation, probably because of an imbalance between level of superoxide and the activity of superoxide dismutase in the vessel wall (66-68). NO has very high reactivity towards superoxide anion and in the presence of high level of superoxide nitric oxide is converted in toxic peroxynitrite ($ONOO^{\bullet}$) Thus, interaction between superoxide and NO reduces the physiological vasodilatatory and protective effects of NO.

However, an unbalanced antioxidative status with overproduction of ROS, shown in essential hypertension, may also enhance oxidative damage in endothelial cell, contributing to the impaired endothelium-dependent relaxations of blood vessels (68,69). Several other reports suggest the role of superoxide radical in the depressed nitric oxide production by the endothelium in genetically hypertensive rats (68, 72). Although an impaired release of NO may partly underlie the pathogenesis of hypertension, it now appears that endothelium-dependent relaxation is heterogeneously affected in this condition. In some vascular beds of hypertensive rats, such as aorta, mesenteric, carotid and cerebral vessels, endothelium-dependent relaxation is impaired (56). In contrast, in coronary and renal arteries of spontaneously hypertensive rats, endothelial function does not seem to be affected by high blood pressure (56, 73).

4. Strategies for inhibition of free-radical damage

Antioxidants have attracted scientific and clinical attention in medicine, and intensive investigations are underway. Experimental *in vivo* and *in vitro* studies, as well epidemiological studies, suggest an inverse correlation between severity of oxidative stress-induced diseases and levels of antioxidants, indicating the likelihood that antioxidant status may be relevant to human health and disease.

A wide range of natural and synthetic antioxidant drugs and/or supplements for prophylactic and therapeutic purposes have been suggested.

Of growing interest are the small antioxidant molecules (some of them vitamins), such as α-tocopherol, ascorbic acid, carotenoids, coenzyme Q, uric acid, vitamin A, melatonin, and aminoindoles. Of physiological and/or pharmacological interest also are antioxidants, such as flavonoids and polyphenols, those contained in herbal antioxidants, and α-lipoic acid. Comprehensive and authoritative treatises on the chemical, biological, and clinical aspects of natural antioxidants, were published recently (74-76). Likewise, synthetic antioxidants are currently being developed as therapeutic agent against oxidative stress. Such compounds include, among other, derivatives of natural antioxidants (e.g. α-tocopherol analogs), phenolic antioxidants (such as Probucol and Nitecapone), 21-aminosteroids or lazaroids, sulphydryl-containing compounds (thiazolidine, Ebselen, dithiolethiones), and

low-molecular weight mimics od superoxide dismutase. An example of tissue-directed α-tocopherol analogs are the water-soluble, quaternary ammonium tocopherol derivatives, which accumulate in heart tissue and have a potential use as cardioprotectors.

A number of drugs such as β-blockers, ACE (angiotensin-converting enzyme) inhibitors, calcium antagonists, and H_2-receptor antagonists, either in clinical use or at certain stages of development, have been reported to scavenge ROS, inhibit lipid peroxidation, and modulate oxidation of low density lipoprotein (74, 76).

The β-blockers which can attenuate lipid peroxidation and oxidation of low-density lipoprotein are relevant to the management of cardiovascular disorders. The drug carvedilol, third-generation β-blocking agent with cardiovascular activity, reacts rapidly with model peroxyl radical, prevents oxidation of LDL and is able to scavenge hypochlorous acid (77). H_2 antagonists have also been investigated for their ability to scavenge ROS. The results published have shown that famatidine, cimetidine and ranitidine react rapidly with hydroxyl radical (78). The ACE inhibitors (captopril, enalapril, lisinopril), used for the treatment of arterial hypertension and cardiac failure on patients with myocardial infarction, have beneficial effects on the reoxygenated myocardium. It has been suggested that this effect is due to the ability to scavenge ROS. It has also been proposed that sulphydryl groups in catopril and related compounds are responsible for the observed scavenging properties (79).

Finally, one of the most successful approaches to prevention of free-radical damage has been metal chelation. Desferrioxamine (Desferal), an iron chelator, has been used in experimental infarction models, where it exerted some level of protection. A beneficial effect of desferrioxamine may be explain by its capability of reducing, i.e. inactivating, ferryl myoglobin (74).

Antioxidant therapy is gaining the significance in coronary heart disease, atherosclerosis and inflammation. However, final proof of beneficial effect of antioxidant therapy in patients with coronary heart disease is still lacking. It can be important to take a look at the one of the latest Hotline Editorials in European Heart Journal entitled "Should we prescribe antioxidants to patients with coronary heart disease?" (80). Nonetheless B. Sies (1997) concluded that the implementation of antioxidant therapies requires a better understanding of the involvement of free radicals and molecular mechanisms by which they exert cytotoxicity in diseases states.

References

1. Halliwell B, Cross CE, Gutteridge MC. Free radicals, antioxidants and human diseases. Where are we now? J Lab Clin Med 1992; 119: 598-620.
2. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-526.
3. Gomberg M. An incidence of trivalent carbon trimethylphenyl. J Am Chem Soc 1900; 22: 757-771.
4. Michaelis L. in: Currents in biochemical research (Green DE, ed) Willey Interscience, New York, 1946: 207-227.
5. Halliwell B. Superoxide dismutase, catalase and glutathione peroxidase: solutions to the problems of living with O_2. New Pathologist 1974; 73: 1075-1086.
6. Mc Cord J, Fridovich I. Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-6055.
7. Nonhebel DC, Tedder JM, Walton JC. Radicals. Cambridge University Press 1979; 200-231.
8. Bredt DS, Snyder SH. Nitric oxide: a physiological messenger molecule. Annu Rev Biochem 1994; 63: 175-195.
9. Rice-Evans CA. Formation of free radicals and mechanisms of action in normal biochemical processes and pathological states. In: CA Rice-Evans and RH Burdone (eds), Free Radical Damage and its Control. Elsevier Science 1994; 131-153.
10. Halliwel B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219: 1-14.
11. Del Maestro RF. An approach to free radicals in medicine and biology. Acta Physiol Scand 1980; 492: 153-168.
12. Jacobson MD. Reactive oxygen species and programmed cell death. Trends Biochem Sci 1996; 243(21): 81-119.
13. Burdon RH. Free radicals and cell proliferation. In: CA Rice-Evans and RH Burdone (eds), Free Radical Damage and its Control. Elsevier Science 1994: 155-189.
14. Diplock AT. Antioxidant and disease prevention. Mol Asp Med 1994; 15: 293-376.
15. Sies H. Oxidative stress: from basic research to clinical application. Am J Med 1991; 91(Suppl. 3C): 31-38.
16. Stadtman ER. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu Rev Biochem 1993; 62: 797-821.
17. Davies KJA. Protein modification by oxidants and the role of proteolytic enzymes. Biochem Soc Trans 1993; 21: 346-353.
18. Schoneich C, Dillinger V, von Bruchhausen KD. Oxidation of polyunsaturated fatty acids and lipids. Reaction kinetics, and influence of oxygen and structure of thyil radicals. Arch Biochem Biophys 1992; 292: 456-467.
19. Bolli R. Postischemic myocardial injury. Pathogenesis, pathophysiology, and clinical relevance. In: Yellon DM, Jennings RB (eds). Myocardial Protection: the Pathophysiology of Reperfusion and Reperfusion Injury., Raven Press, New York 1992: 105-149.
20. Ferrari R, Ceconi C, Curello S. et al. Myocardial damage during ischemia and reperfusion. Eur Heart J 1993; 14(Suppl G): 25-30.
21. Bolli R. Oxygen-derived free radicals and myocardial reperfusion injury: An overview. Cardiovasc Drugs Ther 1991; 5: 249-268.
22. Starkopf J, Zilmer K, Vihalemm T. et al. Time-course of oxidative stress during open-heart surgery. Scand J Thorac Cardiovasc Surg 1995; 29(4): 181-186.
23. Fox KAA. Reperfusion injury: a clinical perspective. In: Yellon DM, Jennings RB (eds). Myocardial Protection: the Pathophysiology of Reperfusion and Reperfusion Injury., Raven Press, New York, 1992: 151-165.
24. De Lorgeril M, Richard MJ, Arnaud J. et al. Lipid peroxides and antioxidant defenses in the accelerated transplantation-associated coronary arteriosclerosis. Am Heart J 1993, 125: 974-980.
25. Valen G, Vaage J. Toxic oxygen metabolites in reperfusion injury. Scand J Thor Cardiovasc Surg 1993; 41(Suppl): 19-29.
26. Garlick PB, Davies MJ, Hearse DJ, Slater TF. Direct detection of free radicals in the reperfused rat heart using electron spin

resonance spectroscopy. Circul Res 1987; 61: 757-760.
27. Pietri S, Culcasi M, Cozzone PJ. Real-time continous-flow spin trapping of hydroxyl radical in the ischemic and post-ischemic myocardium. Eur J Biochem 1989; 186: 163-173.
28. Ytrehus K, Hegstad AC. Lipid peroxidation and membrane damage of heart. Acta Physiol Scand 1991; S599: 81-91.
29. Dousset JC, Trouilh M, Foglietti MJ. Plasma malondialdehyde levels during myocardial infarction. Clin Chim Acta 1983; 129: 319-322.
30. Coghlan JG, Flitter W, Holley AE et al. Detection of free radicals and cholesterol hydroperoxides in blood taken from the coronary sinus of man during coronary of man during percutaneous transluminal coronary angioplasty. Free Radic Biol Med 1993; 14: 339-342.
31. Tortolani A, Powell S, Misik V. et al. Detection of alkoxyl and carbon-centered free radicals in coronary sinus blood from patients undergoing elective cardioplegia, Free Radic Biol Med 1993; 14: 421-426.
32. Murphy ME, Kolvenbach R, Aleksis M, Sies H. Antioxidant depletion in aortic crossclamping ischemia: increase of the plasma alpha-tocopheryl quinone/alpha-tocopherol ratio. Free radic Biol Med 1992; 13: 95-100.
33. Starkopf J, Tamme K, Zilmer M et al. The evidence of oxidative stress in cardiac and septic patients: a comparative study. Clin Chim Acta 1997; 262: 77-88.
34. Jeroudi MO, Hartley CJ, Bolli R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. Am J Cardiol 1994; 73: 2B-7B.
35. Otani R, Engelman RM, Roussou JA. Et al. Cardiac performance during reperfusion improved by pretreatment with oxygen-free radicals scavengers. J Thorac Cardiovasc Surg 1986; 91: 290-295.
36. Kalyanaraman B, Konorev EA, Joseph J, Baker JE. Radical generation and detection in myocardial injury. In: CA Rice-Evans and RH Burdon (eds), Free Radical Damage and its Control. Elsevier Science 1994; 333-355.
37. Axford-Gately RA, Wilson GJ. Myocardial infarct size reduction by single high-dose of repeated low-dose vitamin E supplementation in rabbits. Can J Cardiol 1993; 9: 94-98.
38. Galaris D, Eddy L, Arduini A et al. Mechanism of reoxygenation injury in myocardial infarction: Implications of myoglobin redox cycle. Biochem Biophys Res Commun 1989; 160: 1162-1168.
39. Veitch K, Hombroeckx A, Caucheteux D et al. Global ischemia induces a biphasic response of mitochondrial respiratory chain. Biochem J 1992; 281: 709-715.
40. Hardy L, Clark JB, Darley-Usmar VM et al. Reoxygenation-dependent decrease in mitochondrial NADH:CoQ reductase complex activity in the hypoxic/reoxygenated rat heart. Biochem J 1991; 274: 133-137.
41. Xie Y-W, Shen W, Zhao G. et al. Role of endothelium-derived nitric oxide in modulation of canine myocardial mitochondrial respiration in vitro: implications for the development of heart failure. Circ Res 1996; 79: 381-387.
42. Shasby MD, Lind SE, Shasby SS et al. Reversible oxidant-induced increases in albumin transfer across cultured endothelium: Alterations in cell shape and calcium homeostasis. Blood 1985; 65: 605-612.
43. Shatos MA, Doherty JM, Hoak JC. Alterations in human vascular endothelial cell function by oxygen free radicals: Platelets adherence and prostacyclin release. Arteriosclerosis Thromb 1991; 11: 594-601.
44. Patel KD, Zimmerman GA, Prescott SM, McEver RP. Oxygen radicals induce human endothelial cells to express GMP-149 and bind neutrophils. J Cell Biol 1992; 112; 749-759.
45. Mair J. Cardiac troponin I and troponin T: Are enzymes still relevant as cardiac markers? Clin Chim Acta 1997; 257: 99-115.
46. Xie Y. W, Michael MW. Role of nitric oxide and its interaction with superoxide in the suppression of cardiac muscle mitochondrial respiration. Involvement in response to hypoxia/reoxygenation. Circulation 1996; 94: 2580-2586.
47. Fruchart JC, Duriez P. Free radicals and atherosclerosis. In: CA Rice-Evans and RH Burdon (eds), Free Radical Damage and its Control. Elsevier Science 1994; 257-281.
48. White CR, Brock TA, Chang LY. Superoxide and peroxynitrite in atherosclerosis. Proc Natl Acad Sci 1994; 91: 1044-1048.
49. Parthasaratry S, Rankin SM. Role of oxidized low density lipoproteins in atherogenesis. 1992; Progr Lipid Res 31: 127-143.
50. Morel DW, DiCorleto PE, Chisolm GM. Endothelial and smooth muscle cells alter low density lipoprotein by free radical oxidation. Arteriosclerosis 1984; 4: 357-364.
51. Esterbauer H, Rosenfeld ME, Waeg G. et al. Biochemical, structural and functional properties of oxidized low-density lipoproteins. Chem Res Toxicol 1990; 3: 77-92.
52. Juergens G, Ashy A, Esterbauer H. Detection of new epitopes formed upon oxidation of low density lipoprotein, lipoprotein (a) and very-low-density lipoproteins. Use of an antiserum against 4-hydroxynonenal-modified low-density lipoprotein. Biochem J 1990; 265: 605-608.
53. Glavind J, Hartmann S, Clemmesen J. et al. Studies on the role of lipoperoxides in human pathology. II: The presence of peroxidized lipids in the atherosclerotic aorta. Acta Pathol Microbiol Scand 1952; 30: 1-6.
54. Blackman B, White P, Tsou W, Finkel D. Peroxidation of plasma and platelet lipids in chronic cigarette smokers and insulin-dependent diabetics. Ann N Y Acad Sci 1984; 435: 385-387.
55. Sato Y, Hotta N, Sakamato N. et al. Lipid peroxide level in plasma of diabetic patients. Biochem Med 1979; 21: 104-107.
56. Belder AJ, Radomski MW, Why HJF, et al. Nitric oxide synthase activities in human myocardium. Lancet 1993; 341: 84-85.
57. Amezcua JL, Palmer RMJ, Souza BM, Moncada S. Nitric oxide synthetized from L-arginine regulates vascular tone in the coronary circulation. Br J Pharmacol 1989; 97: 1119-1124.
58. Bhagat K, Vallance P. Inducible nitric oxide sunthase in the cardiovascular system. Heart 1996; 75: 218-220.
59. Morris SM, Billiar TR. New insights into the regulation of inducible nitric oxide sunthesis. Am J Physiol 1994; 266: E829-839.
60. Kuman KV, Das UN. Are free radicals involved in the pathobiology of human essential hypertension? Free Rad Res Commun 1993; 19: 59-66.
61. Jun T, Ke-Yan F, Catalano M. Increased superoxide anion production in humans: a possible mechanism for the pathogenesis of hypertension. J Human Hypertens 1996; 10: 305-309.
62. Nakazono K. Does superoxide anion underline the pathogenesis of hypertension. Proc Natl Acad Sci USA 1991; 88: 10045-10048.
63. Tse WY, Maxwell Sr, Thomason H. et al. Antioxidant status in controlled and uncontrolled hypertension and its relationship to endothelial damage. J Hum Hypertens 1994; 8: 843-849.
64. Simi} D, Simi} T, Mimi}-Oka J. et al. Antioxidant status in patients with essential hypertension. XIII World Congress of Cardiology, Monduzzi Editore Sp. A. Bologna, 1998, pp. 421-425.
65. Rafi JA, Boegehold MA. Microvascular responses to oxygen and muscle contraction in hypertensive Dahl rats. Int J Microcirc Clin Exp 1993; 13: 83-97.
66. Prabha PS, das UN, Koratkar R. et al. Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. Prostaglandins Leukot Essent Fatty Acids 1990; 41: 27-33.
67. Kumar KV, Das UN. Are free radicals involved in the pathobiology of human essential hypertension? Free Radic Res Commun 1993; 19: 59-66.
68. Swei A, Lacy F, DeLano FA. et al. Oxidative stress in the Dahl hypertensive rat. Hypertension 1997; 30: 1628-1633.
69. Nava E, Noll G, Luscher TF. Nitric oxide in cardiovascular diseases. Ann Med 1995; 27: 343-351.
70. Dubey RK, Luscher TF. Nitric oxide inhibits angiotensin II-induced migration of vascular smooth cells. Hypertension 1993; 22: 412-415.
71. Kelm M, Feelisch M, Krebber T. et al. The role of nitric oxide in the regulation of coronary vascular resistance in arterial

hypertension: comparison of normotensive and spontaneously hypertensive rats. J Cardiovasc Pharmacol 1992; 20: 183-186.
72. Grunfeld S, Hamilton CA, Mesaros S. et al. Role of superoxide in the depressed nitric oxide production by the endothelium of genetically hypertensive rats. Hypertension 1995; 9: 854-857.
73. Tschudi MR, Criscione L, Luscher TF. Effect of aging and hypertension on endothelial function of rat coronary arteries. J Hypertension 1991; 9: 164-165.
74. Aruoma OI. Characterization of drugs as antioxidant prophylactics. Free Radic Biol Med 1996; 20: 675-705.
75. Halliwell B. Drug antioxidant effect. A basis for drug selection Drugs 1991; 42: 569-605.
76. Rice-Evans CA, Diplock AT. Current status of antioxidant therapy. Free Radic Biol Med 1993; 15: 77-96.
77. Yue TL, McKenna PJ, Lysko PG. Et al. Carvedilol, a new antihypertensive, prevents oxidation of human low-density lipoproteins by macrophages and copper. Atherosclerosis. 1992; 97: 209-216.
78. Hinrichsen H, Halabi A, Kirch W. Clinical aspects of cardiovascular effects of H_2 -receptor antagonists. Eur J Clin Invest 1995; 25(Suppl 1): 47-56.
79. McMurray J, Chopra M. Influence of ACE inhibitors on free radicals and reperfusion injury, pharmacological curiosity or therapeutic hope? Br J Clin Pharmacol 1991; 31: 373-379.
80. Rapola JM. Should we prescribe antioxidants to patients with coronary heart disease? Europ Heart J 1998; 19: 530-532.

SLOBODNI RADIKALI U KARDIOVASKULARNIM OBOLJENJIMA

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Kratak sadržaj: *Rezultati brojnih istraživanja poslednjih godina nedvosmisleno su pokazali da slobodni radikali imaju značajnu ulogu u humanoj patologiji. Slobodni radikali se stvaraju u mnogim biohemijskim reakcijama u ćeliji, i neophodni su za odvijanje brojnih normalnih biohemijskih i fizioloških procesa. Slobodni radikali su veoma reaktivni, pa su aerobni organizmi razvili sna`an enzimski i neenzimski antioksidantni zaštitni sistem za sprečavanje stvaranja i hvatanje slobodnih radikala. Medjutim, u brojnim patološkim uslovima slobodni radikali se mogu intenzivnije stvarati, pa delikatna ravnoteža izmedju stvaranja slobodnih radikala i antioksidantnog kapaciteta ćelija i tkiva može biti naručena. Posledica je nastanak oksidativnog stresa i oštećenja tkiva izazvana veoma reaktivnim slobodnim radikalima. Brojni podaci ukazuju da su kiseonični slobodni radikali značajni medijatori oštećenja ćelija i u kardiovaskularnim oboljenjima. U ovom radu rezimirani su najznačajniji rezultati istraživanja uloge slobodnih radikala u ishemijsko/reperfuzionom oštećenju miokarda, aterosklerozi i hipertenziji.*
Dat je i kratak prikaz lekova koji se koriste u terapiji kardiovaskularnih oboljenja, a imaju i korisne osobine antioksidanasa.

Ključne reči: *Slobodni radikali, oksidativni stres, kardiovaskularna oboljenja, antioksidansi.*

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