

Neuroendocrine and oxidoreductive mechanisms of stress induced cardiovascular diseases

Snežana B. Pajović¹, Marija B. Radojčić¹, Dušan T. Kanazir²

¹ Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences

² Serbian Academy of Sciences and Arts

This article dedicated to Prof. Dr. Dušan T. Kanazir, on the occasion of his 85th birthday

Correspondence to:

Snežana B. Pajović, Ph.D.

Laboratory of Molecular Biology and Endocrinology

Vinča Institute of Nuclear Sciences,

P.O.Box 522,

11 001 Belgrade, Serbia

Phone/fax: 381- 11- 245-55-61

381-11- 80 – 66- 514

e-mail : pajovic@ vin.bg.ac.yu

Running title: Molecular mechanism of stress induced cardiovascular disease

Summary

The presented review addresses a number of basic molecular pathways that play crucial role in perception, transmission and modulation of the stress signals, and conduct adaptation of the vital processes in the cardiovascular system (CVS). These, highly complex systems for intracellular transfer of information include stress hormones and their receptors, stress-activated phosphoprotein kinases, stress-activated heat shock proteins, and antioxidant enzymes maintaining oxidoreductive homeostasis of the CVS. Failure to compensate for the deleterious effects of stress may result in development of different pathophysiologies in the CVS, such as, ischemia, hypertension, atherosclerosis and infarction. Stress-induced disbalance in each of the CVS molecular signaling systems and their contribution to the CVS malfunctioning is reviewed. The general picture of the molecular mechanisms of the stress-induced pathophysiology in the CVS pointed out the importance of stress duration and intensity as etiological factors, and suggested that future studies should be complemented by the careful insights into the individual factors of susceptibility to stress, prophylactic effects of 'healthy' life styles and beneficial action of antioxidant rich nutrition.

Key words: neuroendocrine response, oxidative stress, cardiovascular diseases

Introduction

Stress conditions represents new, dynamic state of the whole organism and the cardiovascular system (CVS) under the influence of either chemical (pesticides, pharmaceutical products, factors generating free radicals, etc.), physical (different types of radiation, heat), and/or biological 'stressors' (bacteria, viruses, microorganism and their toxins) acting through endogenous hormonal and psychogenic pathways. These conditions represent a complex interaction between the circulatory hormones, cellular receptors, stress-activated phosphoprotein kinases, stress-induced proteins, and enzymes maintaining oxidoreductive balance in the CVS, which finally translate 'stressor' message into biological responses (McEwen, 2002). How the CVS responds to stress? As opposed to short lasting, acute, stress which mostly results in time-limited homeostatic changes of the CVS, prolonged, chronic, stress leads to the permanent changes in the gene expression resulting in persistent homeostatic disorders of the CVS. Prolonged changes in the expression of these adaptation genes may cause ill-health conditions of the CVS, starting from myocardial ischemia to atherosclerotic post ischemic tissue damages and hypertension. At the molecular level a common denominator for the CVS diseases are structural lesions including conformational changes of cellular components due to denaturation and oxidation. The review is aimed at detailed description of both structural and functional alterations laying in the basis of the CVS malfunctioning induced by stress. Our hope is that understanding of those mechanisms will help in future to develop better prophylactic and/or therapeutic strategies for prevention and/or treatment of stress induced diseases of the CVS.

Stress hormones and receptors in the cardiovascular system

When exposed to stress the internal balance of the CVS is disrupted, but it can be regained through the brain controlled outputs of two classes of hormones, steroids and catecholamines (McEwen, 2002). In acute stress conditions adrenalin keeps alertness, while steroids, primarily glucocorticoids (GCs) help replenish energy supplies. The adrenaline plays a major role in adaptation, as well as in adverse stress effects. The molecular mechanism of its signal transmission includes binding to membrane G-protein coupled receptors, increase or fall of cyclic AMP production, and final stimulatory or inhibitory response. Cyclic AMP, as a secondary messenger, activates a series of phosphoprotein kinases, which activate cAMP-regulatory protein, CREB, and transcription of genes adapting the CVS to the stressogenic factor. For example, the excessive emotional stress, acting through adrenalin signaling pathway leads to altered expression of the early gene products within the CVS (Ueyama 2004). If these mechanisms are insufficient for stress adaptation of the CVS, an antagonistic pathway through cyclic GMP is also included (Feil *et al.* 2003). The real problems in adaptation of the CVS arises when the systems involved in these adaptive responses don't switch off when not needed or don't become active when needed. This may occur by prolonged exposure of the CVS to stress, resulting in stress associated dysfunctions related to deregulated concentration of GCs and mineralocorticoids (MCs) (Ng and Celermajer 2004).

Among the most important effects of GCs are regulation of the CVS volume, electrolyte retention and synthesis of angiotensin. In the physiological concentrations GCs and MCs regulate maintenance of normal blood pressure and normal heart actions. But, if for example, the circulatory concentrations of the major human GCs, cortisol, is low during stress, these hormones are not capable to sustain the defence mechanisms that would protect the CVS (Crown and Lightman 2005). Thus, reduced level of GCs decreases blood pressure, alters myocardial arteriolar tone, increases capillary permeability, alters vasomotor response to neural stimuli, and even changes heart size. On the other hand, increased GCs concentration, in case of the impaired negative feedback mechanism at the level of hypothalamo-pituitary-adrenal axis, is frequently noxious to the CVS (Whitworth *et al.* 2000). Hypercorticism, that may be a consequence of prolonged stress, may lead to the chronic arterial hypertension (Das and O'Keefe 2006). Moreover, there is some data indicating that GCs potentiate the processes leading to atherosclerosis and thrombosis (Magiakou *et al.* 2006).

Generally, the effects of GCs in acute stress of the CVS should be considered as positive from the protective point of view. For example, synthetic GC, dexamethasone, is highly beneficial in case of myocardial infarction, with its action leading to reduction of the myocardial infarction extent. Also, some MCs, such as aldosterone, have similar effect on release of the intracellular calcium (Ca^{++}) in the smooth muscle cells of the CVS blood vessels, maintaining the normal blood pressure and normal myocardial contractility (Seleznev YM *et al.* 1982, Guo *et al.* 2001). As opposed to that high GCs and MCs concentrations in chronic stress may have highly negative effects in the CVS due to sustained changes in expression of the genes regulating reception, transmission, processing and response to other cellular signals. Hypercorticism and hyperaldosteronism are risk factors for high blood pressure, although the precise mechanisms through which they influence the blood pressure has not yet been sufficiently elucidated.

Another group of steroid hormones-estrogens (ES), are also considered to be exceptionally important for regulation of the CVS homeostasis. The action of these hormones is sexually determined. Namely, it has been evidenced that the frequency of the CVS diseases is considerably higher in male in comparison of the female population of the same age before the menopause (30-45 years, Barrett-Connor 2003). Clinical data indicated that ES ameliorate processes leading to development of hypertension, as well as the onset of myocardial ischemia and tachycardia (Barrett-

Connor 2003). At the molecular level the ES effects are explained by the expression of genes regulating metabolism of the coagulation factors. The studies carried out on the experimental animals evidenced sexual dimorphism in the presence of estrogen receptor (ER) in the different heart structures (Kanazir *et al.* 1982). Other studies evidenced that one of the possible mechanisms through which ES protect coronary arteries is by opening of the voltage-activated K^+ canals and increasing of K^+ efflux. In addition to that, ES were shown to regulate expression of vascular adhesion molecule-1 gene of endothelial cells of the CVS in the sex-dependent manner (Marui *et al.* 1993). Interestingly, some natural androgens (AN) were also shown to be capable of preventing the onset of atherosclerosis (Pinthius *et al.* 2006).

At the intracellular level the steroids, GCs, MCs, ES, and AN, achieve their action through binding to the respective steroid receptor (SRs): glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and/or estradiol/androgen receptors (ER/AR). The SRs are ligand regulated transcription factor occurring in heterocomplex with heat shock proteins. Upon steroid binding they dissociate from the complex and regulate gene expression in various tissues including the CVS. Some of SRs functions are controlled through phosphorylation by the stress activated kinases MAPKs, cyclin dependent kinases (CDKs) and sumoylation (Krstić M *et al.* 1997). Such modifications lead to sequential recruiting of the different components of the transcription machinery to the promoter regions of the genes under steroid regulation. Thus, it is known that GR primarily regulates genes of the intermediate metabolism of glucose thus maintaining the energy homeostasis of the CVS. The MR regulates expression of the membrane transporters for Ca^{++} , K^+ and Na^+ ions regulating blood pressure, while the ER are involved in the regulation of oxidoreductive equilibrium of the CVS.

Stress-induced phosphoprotein kinases of the cardiovascular system

Protein phosphoprotein kinases cascade is a part of molecular mechanism which enables adaptive CVS response to the stress signals. It is triggered in response to activation of receptor systems from the cell membrane, such as adreno-receptors, growth factor receptors and others, acting *via* cAMP, cGMP or inositol-triphosphate mechanism. The signals are transmitted to the first kinase in the chain, which thereafter phosphorylates the following kinase in the chain. Since the quantity of each phosphokinase activity product in the chain is greater than the level of the starting enzyme, the signal from the membrane is manifoldly amplified. Kinases of MAPK family known as stress-activated kinases (SAPK), p38 kinase and ERK-1/-2 kinase are found at the end of the cascade chain. The MAPK cascade is activated in response to a large number of stress signals, including environmental temperature changes, ischemia and presence of metabolic inhibitors (Ravingerova *et al.* 2003)

The SAPK phosphorylates transcription factors of AP1 family, c-Jun and ATF-2 which thereafter activate expression of early genes jun and fos. Kinase p38, also activates transcription factor ATF-2. The kinases belonging to SAPK cascade are activated by phosphorylation, which takes place either on threonine or tyrosine residue. Subsequent events on the molecular level are determined by the balance between kinase and phosphatases (Keyse, Emslie 1992). The last component of the activated cascade, c-Jun, primary functions as a heterodimer, with c-Fos or ATF 2 protein. As a heterodimer Jun-Fos binds to gene promoter encoding the enzyme collagenase. The other regulatory factor ATF-2 activates genes coding inducible NO-synthetase, interleukin-8 and proliferating nuclear cellular antigen. Inflammatory cytokines are shown to induce synthesis of matrix metalloprotease (MMP) collagenase, stromelysin and gelatinase involved in regeneration of extracellular matrix and decomposition of collagen in the fibrous cap of the atherosclerotic plaque. Gene promoter encoding MMP collagenase was among the first identified to be under control of AP-1 (Jun-Fos dimer). However, the process may also lead to weakening of the fibrous cap and possible rupture of the atherosclerotic

plaque. Thus, SAPK are not only included in regulation of the inflammatory processes, but also in progression of the atherosclerotic plaques and post ischemic cardiac injuries (Libby 2002). The role of p38 in response to ischemia is less clear, although it is not less important than the role of SAPK. These kinases also play important role in the regulation of synthesis of the inflammatory cytokines in the post ischemic tissue, which is considered to accelerate post ischemic injury.

It may be concluded that the activity of all MAPK members and the balance between them determines whether the CVS cells exposed to stress signals will adapt and survive, or undergo patophysiological changes leading to programmed CVS cell death, apoptosis (Feuerstein 2001). The key determinants of this outcome at the cellular level are duration of stress and its intensity. Thus, CVS cells exposed to ischemic shock are dying in the case of high intensity or prolonged ischemia. But if the ischemic shock is only short-lasting, the CVS cells may be repaired and undergo further division, thus successfully adapting to the stress stimulus.

Induction of stress proteins in cardiovascular system diseases

Stressful conditions result in the induction and synthesis of another important class of regulatory proteins in the CVS, known as 'heat-shock proteins', 'molecular chaperons' or 'stress proteins', HSPs (Nadeau and Landry 2007). HSPs belong to the multigene family of proteins with different molecular masses found in all cell compartments, cytoplasm, nucleus, and cell organelles. Under homeostatic conditions they participate in the maintenance of the correct tertiary structure of numerous structural and regulatory proteins of the CVS assuring its homeostasis. As most of the stress conditions result in the increased concentration of denatured, structurally altered and incorrectly folded proteins, they impose high demands for the new HSPs synthesis (Wang *et al.* 2006). HSPs thus participate in maintenance and physiological adaptation of the CVS during stress induced heart ischemia and injuries of blood vessels, inflammatory conditions and oxidative stress (Guisasola *et al.* 2006).

Genetic studies offer the information suggesting that excessive expression of HSPs represents a highly potent mechanism of cell protection, even in the myocardial infarction (Benjamin *et al.* 1998). At the molecular level, physiological stressors, such as increased temperature and ischemia, induce heat shock factor HSF-1 monomer oligomerization *i.e.* formation of HSF-1 homotrimer, which binds to specific sequences in the gene promoters for HSPs. The induction of one of the most potent HSPs, Hsp 70 improves myocardial tissue regeneration and reduces size of ischemic infarction (Mestril *et al.* 1996). Tissue-specific Hsp 70 expression prevents apoptosis in some myocardial calls *via* the mechanism which includes control of the cell cycle through previously described SAPK pathway (Kim *et al.* 1997).

In addition to Hsp 70, other important HSPs, such as Hsp 27 and crystalline provide primary protection of the CVS cells from the injuries induced by ischemia. The *de novo* synthesis of these HSPs is believed to alleviate detrimental effects of stress on the CVS proteins, resulting in their renaturation and leading to reduction of arrhythmia and degree of ischemia in such conditions (Brundel *et al.* 2006). A number of recent studies indicate that Hsp 27 plays major role in the CVS cell survival *via* the mechanism that includes reparation of the oxidative lesions and re-establishment of the characteristics of the normal differentiated CVS cells. Increased expression of Hsp 27, similarly to anti-apoptotic protein Bcl-2, increased the level of low molecular mass antioxidants, such as glutathione, and stimulated reparation processes thus protecting the CVS from apoptosis.

Although effective under various acute stress conditions, a question arises if HSPs may successfully adapt and repair the CVS under the chronic stress conditions? Namely, some studies suggest that the increased level of HSPs

expression may be associated with chronic elevation of arterial blood pressure *i.e.* hypertension (Hutter *et al.* 1994). In contrast to this finding, the reduced HSPs gene expression and reduced levels of HSP proteins were observed in animal myocardium during the process of aging. Chronic ischemia, which is ubiquitously associated with the process of ageing, is shown to cause oxidative lesions, such as lipid and protein peroxidation of the CVS cells (Besse *et al.* 2006). Increased concentration of oxidized proteins and lipids results in significant alterations in cell transport and signal transduction. Extracellular sources of reactive oxygen species (ROS) produced in the course of ischemia, may significantly damage external surface of the endothelial blood vessel cells of the CVS. On the other hand, reperfusion, *i.e.*, recovery of the normal blood oxygen concentration may, due to the increased Ca^{++} influx, disturb intracellular mechanisms of oxidoreductive balance regulation and thus result in further myocardial lesions. Cytoskeleton protein oxidation developing under such conditions is considered to be an early event leading to chronic ventricular heart dysfunction. Moreover, this oxidized cell and tissue components may serve as a signal for activation of the inflammatory process. They lead to accumulation of polymorphonuclear lymphocytes in the ischemic region, which subsequently release derivatives of the ROS in the ischemic region, thus building a vicious feed-forward cycle of the CVS damage. In addition to that, lymphocytes excrete extracellular lysosomal enzymes with markedly cytotoxic effects. Thus, although powerful against the acute stress of the CVS, induction of HSPs may be overcome in the case of persistently elevated concentrations of oxidatively modified cell components found under the chronic stress conditions. In that case, additional line of defense, including various antioxidants, is of utmost importance for restoration of homeostasis in the CVS.

Reactive oxygen species, antioxidative enzymes and cardiovascular diseases

A wide range of interesting discoveries has been published over the last several decades in the field of formation and role of free radicals in the biological systems. Free radicals are the molecules, ions or atoms having one or more unpaired electrons in their structure. In the physiological conditions, even small quantity of free radicals is dangerous for the organism due to the high reactivity and possible damaging of DNA, RNA, enzyme or lipid components of the membranes (Michelson 1987). Free radicals are produced in the processes of the oxidative phosphorylation and catalytic activity of some enzymes (e.g. aldehyde oxidase, xanthin oxidase). Free radicals are also produced in numerous pathological conditions. They are considered to play significant role in etiopathogenesis of more than 200 human and animal diseases, starting from the cardiovascular diseases to Parkinson disease. Additionally, some cytostatics, certain airborne waste materials, hyperoxia, pesticides, some herbicides (paraquat), cigarette smoke, alcohol, anesthetics as well as majority of carbohydrates induce cell damages due to free radicals.

The part of the cell (proteins, nucleic acids, membrane lipids, cytosol molecules) or extracellular component (hyaluronic acid, collagen) that will react with free radicals depends on the nature of radicals, site and source of their production (e.g. cytosol components, mitochondria, endoplasmic reticulum, peroxisomes, cell membrane).

Antioxidative defense (AO) system reflects dynamic balance of free radicals in the cell, which protects biomolecule from the serious damages that may be caused by free radicals. AO system includes primary and secondary antioxidative protection. According to Cotgreave *et al.* (1988) and Cadenas (1989), the secondary AO system comprises: protein-specific oxidoreductases, protein-ADP-ribosyl-transferases and non-ATP and non- Ca^{2+} - dependent proteases. Primary AO system comprises enzymatic and nonenzymatic components (Cadenas 1989). Non-enzymatic components include vitamin E, vitamin A, bilirubin, vitamin C, uric acid, glutathione, cysteine, glucose, cysteamine, albumin, ceruloplasmin, transferrin, lactoferrin, flavonides, etc. An exceptionally important part of the AO system includes

enzymatic components that eliminate toxic products of the molecular oxygen, such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen. AO system is composed of the following enzymes: superoxide dismutases (FeSOD, MnSOD, CuZnSOD and EcSOD) which catalyze conversion of the superoxide anion radicals into the molecular oxygen and hydrogen peroxide, catalase, which catalyses reduction of hydrogen peroxide in water and molecular oxygen and enzymes of the glutathione redox cycle. Glutathione redox cycle is a major mechanism of reduction of the endogenous hydroperoxides. Glutathione peroxidase (GSH-Px) is the crucial enzyme. The enzyme that contains selenium GSH-Px reduces hydrogen peroxide to water and organic hydroperoxides, in presence of glutathione (GSH) as a second substrate. Selenium – the independent form of GSH-Px, which belongs to the family of glutathione -S-transferases, catalyses GSH conjugation reactions with different organic compounds. Glutathione reductase (GR) reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of reduced NADPH.

Oxidative stress and gene regulation

Free radicals and other reactive oxygen species (ROS) produced in the cells in aerobic conditions, modulate cell homeostasis and induce gene expression changes at all levels, which are proportional to the intensity of ROS production as well as to the types of generated ROS. Numerous data indicates that the so-called mini-oxidative explosion plays an important role in signal transduction. Thus it was confirmed, for example, that in the mammalian cell, in addition to nitric oxide (NO), whose physiological role is best explained, superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) in certain concentrations, also have significant roles as intracellular secondary messengers (Allen and Tresini 2000). The analysis of NO role indicates two possible functions. Increased NO production by macrophages and other effector cells of the immune system primarily emphasizes its defensive role, that is, predominant role in regulation of the immune response. On the other hand, NO synthesis in the endothelial cells and neurons is suggestive of NO role in signal transduction. Fine biological balance between the two opposed physiological NO functions (destruction of "foreign" cells and signal transduction within the cell) depends on production of other reactive oxygen species (ROS).

Thus, for example, in addition to NO, the immune system cells also produce considerable quantities of superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2). As opposed to this, other cell types, such as vascular smooth muscle cells, chondrocytes and fibroblasts produce significantly lower quantities of these reactive species (O_2^- , H_2O_2).

Although there is some data indicating that ROS exert their secondary messenger function depending on the type of the cell of its origin, the results that undoubtedly suggest that oxidative stress may also activate the same signaling pathways leading to the same transcription and translation changes in different cells are also important. This is the case with regulation of expression of the nuclear transcription factor kappa-B (NF- κ B) in numerous cell types. Synthesis of the factor is stimulated by the majority of ligands which induce serine phosphorylation on the targeted substrate (e.g. kinases) and proteosomal degradation of the inhibitory kappa-beta (I κ B) subunit. It was evidenced in certain cases that NF- κ B activation may be blocked by antioxidative treatment, as well as that ROS play an important role in activation of the transcription factor (Schreck *et al.* 1991). On the other hand, direct oxidative stress, such as hypoxic re-oxygenation, activates NF- κ B, however, the activation is effectuated through another mechanism, in absence of proteolytic degradation of I κ B subunit (Imbert *et al.* 1996). Similarly, addition of H_2O_2 may activate the enzymes from the protein kinase C (PKC) family (Konishi *et al.* 1997). However, PKC activation induced by oxidants is independent from lipid co-factors and thus it is distinguishable from the classic ligand-stimulated pathway.

Extracellular signaling molecules, such as growth factors and cytokines, induce cell behavior changes *via* the complex mechanisms, which include signal transmission from the plasma membrane to the nucleus, and they also lead to gene expression changes (Karin *et al.* 1995). The first step of the signaling cascade usually includes activation of receptors, which either have their own protein kinase activity or activate protein kinases in the cytoplasm. The signal is transmitted to the nucleus, where the transcription factors regulating gene expression are being activated. As it was already mentioned, several MAP kinase families were identified: ERK, JNK/SAPK, p38 kinase and large MAP kinase (BMK/ERK5). All the signaling pathways passing through MAP kinases contain the components sensitive to oxidoreductive balance in the cell. Among the oxygen reactive species (ROS), H₂O₂ is particularly important as a regulatory factor influencing oxidoreductive cell balance. (Ishii *et al.* 1997). According to Lander (1997), cell response to altered ROS concentration may be classified in five categories: 1) modulation of activity and secretion of cytokines, growth factors and hormones, 2) ion transport changes 3) transcription changes, 4) neuromodulation, and 5) induction of the programmed cell death, apoptosis. In this way, free radicals participate in biochemical reactions that directly cause cell changes in the course of differentiation, aging and transformation. Over the last decade, the corpus of information indicating that expression of numerous genes is influenced by redox changes in the cell caused by ROS concentration changes has grown abruptly. It has also been evidenced that redox changes in the cell may also modulate transcriptional activation of collagen (Chojkier *et al.* 1989) i collagenase (Brenneisen *et al.* 1997), influence post-transcriptional ferritin control (Frank *et al.* 2002), induce activation of the transcriptional factors such as Myb (Myrset *et al.* 1993) and Erg-1 (Huang and Adamson 1993), as well as activational binding of Fos-Jun (AP-1) protein complex to early gene promoter regions (Toledano and Leonard 1991). It has been known that transcription of several oncogenes may be activated by increase of unrepaired oxidation products within the cell.. Thus, for example, exposure of normal or transformed cells to UV radiation and increased H₂O₂ concentration stimulates increased expression of jun-B, jun-D, c-fos and fos-B genes (Choi *et al.* 1995).

Changes of oxidoreductive cell balance stimulate corresponding changes related to differentiation and proliferative cell capacity. Cell development and differentiation is closely related to oxidation parameter changes. Thus, increased sensitivity to lipid peroxidation has been observed in majority of the organisms during development (Allen *et al.* 1998). It has also been evidenced that lipid peroxidation is reduced in the period of the increased mitotic activity, as it is the case during the tissue regeneration process and cell transformation (Shackelford *et al.* 2000). Antioxidative enzyme activity changes, particularly those affecting superoxide dismutase (SOD) are also observed to be closely correlated with certain phase of differentiation (Allen 1998). These studies indicate that changes in cell redox status in the critical moment of development may be a signal for initiation of the next phase of the developmental program. (Allen 1991).

The above data confirms that oxidative stress, which is characterized by increased ROS production, generates simultaneously a wide spectrum of signaling and regulatory factors, out of which some are highly cell- or tissue-specific. Elucidation of the specific sites and mechanisms of oxidoreductive effects will lead to better understanding of the physiological role of ROS in life processes.

The role of oxidative stress in development of cardiovascular diseases

Epidemiological studies on the molecular level indicate increasingly clear correlation between the increased level of the reactive oxygen species (ROS) and onset of cardiopathy (Dhalla *et al.* 2000; Parthasarathy *et al.* 2001). The insight into the molecular mechanisms has enabled us not only to understand better the above-mentioned process, but also to carry out appropriate, timely and effective preventive measures aimed at prevention of onset of different forms of cardiomyopathy

(Nuttall *et al.* 1999). Degree and type of oxidative lesions on the DNA molecules as well as the level of lipid peroxidation represent reliable biomarkers indicative of the efficacy of antioxidative defense of the organism. (Halliwell 1999). Antioxidative status is changed over the life since it is modulated by a range of both external and internal factors. Accordingly, the incidence of CVS diseases may also be correlated with certain life phases, which are, among others, clearly defined by hormonal status changes (Krstevska *et al.* 2001). For example, it has been well known that both incidence and intensity of the CVS diseases are more prominent in menopausal in comparison to premenopausal women. The former coincides with the drastic changes of both hormonal and antioxidative status (White *et al.* 1997). Dose and time modulation of the antioxidant enzyme status influenced by sex steroid hormones (SHs) has been a subject of our research for years (Pajović *et al.* 1994, 1996). The obtained results indicate that SHs, PR and ES, modulate activity of the antioxidative enzymes and thus influence oxidoreductive balance in the organism. The fact that reduced capacity of antioxidant defense results in increased level of free radicals, which disturb regular functioning of the genome, DNA and proteins synthesis owing to their reactive effects, further suggests that hormonal changes may be the initial event for onset of both cardiopathic and other pathological processes in the organism (Palmer *et al.* 1997). Krstevska *et al.*, (2001) studied influence of menopause on the activity of primary intracellular antioxidative enzymes, SOD and GSH-Px, as well as on the level of the total antioxidative status (TAS) in healthy women as well as in women with coronary arterial diseases (CAD). Based on the obtained results, they concluded that antioxidant level is significantly reduced during menopause, particularly in women with CAD. The former is supported by the theory based on the role of reactive oxygen species (ROS) in the development of atherosclerosis (Yung *et al.* 2006). Namely, it has been known that ROS are generated between the vascular walls through different mechanisms, including the activity of vascular NAD(P)H oxidase (Ushio-Fukai and Alexander 2004, Ushio-Fukai 2006). Production of ROS may be stimulated by mechanic stress, different environmental factors, peptide angiotensin II, cytokines, natural lipoproteins (LDL), in the presence of metal ions as catalysts. ROS are modified by LDL, while oxidative LDL form interact thereafter with the capillary endothelium, which results in increased production of nitric oxide as well as peroxy nitrate in the later phase. Namely, the reaction between nitric oxide and $O_2^{\cdot -}$ is faster than the catalytic SOD reaction, which eliminates surplus of $O_2^{\cdot -}$, thus leading to defects of the structural cell elements. These reactive molecular species enhance expression of different genes important for intensification of leukocyte activity in the arterial wall, which represents basic mechanism of the oxidant injury theory of atherosclerosis. The authors Ferro and Webb, (1997) emphasize that capillary endothelial cells have key role in the CVS regulation. Capillary endothelium produces different vasoactive components, such as nitric oxide (NO), which is well known vasodilator and peptide endothelin (ET-1), which is a vasoconstrictor. Dysfunction of the vascular endothelium is included in pathophysiology of numerous CVS diseases accompanied by increased blood pressure. If the level of $O_2^{\cdot -}$ is increased, NO inactivation is also increased, which consequentially results in increase of the peripheral vascular tone and blood pressure (Paravicini and Touyz 2006). The former further leads to development of vascular hypertrophy, enhanced endothelial adhesion of the monocytes, atherosclerosis and myocardial infarction. On the other hand, increased ET-1 synthesis or increased smooth muscle sensitivity to ET-1, may also cause increased peripheral vascular tone and vascular hypertrophy. Anyway, the authors emphasize that modulation of endothelial function represents a modern therapeutic option in treatment of hypertension (Ferro and Webb 1997). The above-mentioned authors have also pointed out that calcium antagonists enhance NO effects and inhibit ET-1 effects at the level of vascular smooth muscle cells. Owing to the fact that calcium antagonists have both antiatherogenic and antioxidative properties, they may be also used in prevention of complications induced by hypertension (Touyz and Schiffrin, 2004).

Siow *et al.* (1999) indicated that, in addition to NO, carbon monoxide (CO) also represents an important cell messenger included in regulation of vascular smooth muscle tone. Microsomal heme oxygenase degrade heme to biliverdin

and CO, while cytosol enzyme, biliverdin reductase catalyses reduction of biliverdin to bilirubin, a compound with prominent antioxidative properties. Two major isoenzymatic forms, constitutive oxygenase isoform HO⁻² (molecular mass 34.000 g/mol) and inducible oxygenase isoform HO⁻¹ (molecular mass 32.000 g/mol) of heme oxygenase are identified in the capillary endothelium and capillary smooth muscle cells. They are induced in the presence of heavy metals, increased oxidative stress, in presence of inflammatory mediators as well as in presence of increased concentration of oxidized lipoproteins (LDL). The studies described herein clearly indicate antiatherogenic role of the signaling pathway in which participate heme oxydase and carbon monoxide.

The role of oxidative stress in development of CVS diseases is studied from different aspects, both at the molecular and at the cellular levels. Some of the results are contradictory, however it is sure that hypertension and increased serum cholesterol level develop as consequences of oxidative stress, which disturbs cell homeostasis and activates a range of parameters that accelerate pathogenesis of the CVS diseases (Holvoet 1999). Having in mind above-mentioned, it may be concluded that antioxidative defense system play key roles in defense of organism against stress in general, likewise in case of the CVS system (Lin *et al.* 2005). Recent studies indicate that regular physical activity, intensive rest and sleep as well as healthy and balanced diet may significantly reduce risk of onset of CVS diseases caused by stress (Hoffman and Garewal 1995).

Dietary regimen, minimization of adverse stress effects and prevention of cardiovascular diseases

Both enzyme and non-enzyme components of antioxidant protection are present in each organism, tissue and cell and they represent our genetically determined potential for defense from oxidative stress. Our defense system may be supplemented and enhanced by appropriate, healthy diet which included intake of the nutritive components that contain natural antioxidants. This type of nutrition is generally accepted trend in the world, since previous practice has suggested the possibility that this kind of nutrition may reduce the risk of carcinogenesis, chronic heart diseases, nerve diseases, etc.. Namely, quality food must contain necessary quantity of important and active components in order to supply the organism with necessary and increased quantities of the organic substance, vitamins and microelements. Antioxidative properties are highly important food quality parameters. This involves control of the level of high molecular (enzymatic) and low molecular (vitamins A,C,E etc.) components of the antioxidant defense system in the food products during the manufacturing process, preparation and distribution to consumers, as well as possibility of additional enrichment with the above-mentioned components. The argument that healthy food represents an important component of the preventive medicine is supported by the increasing number of experimental and clinical studies indicating that spontaneous miscarriages, as well as birth of children with inborn defects, are frequently the consequence of lack of certain antioxidant dietary components (Hasler *et al.* 2000, Fang *et al.* 2002). We are obviously coming closer to the Hippocrates words said in the 4th century BC.: ***Let food be thy medicine and medicine be thy food.***

The above assertion is supported by conclusions presented by Yun - Zhong Fanga *et al.* (2002) and reached in collaboration with a group of researchers from the Texas University. Namely, they have presented in detail roles of proteins, lipids, vitamins, minerals and phytochemical antioxidants in maintenance of oxidoreductive balance, which is a key factor in preservation of the homeostasis of the organism in general.. They have finally concluded that recent knowledge in the field of free radical biology represents a base for practical application of antioxidants for improvement of human health and prevention of the CVS diseases. Having in mind the fact that quality food among other properties must have antioxidant properties necessary for maintenance of the general homeostasis of the organism, it becomes clear that healthy food has an important place in modern medicine, primarily as a means of prevention. Maintenance of the good antioxidative status

during infant growth and development is crucial, since it enables one of the essential prerequisites for appropriate cell differentiation and proliferation to be fulfilled. Formation and maturation of all systems, particularly the immune and neuroendocrine systems is to the certain extent correlated with the existing antioxidative status of the organism. Having in mind the above fact, the nature took care to supply human milk, in addition to low molecular antioxidative components, with high molecular enzyme components, cytosolic and mitochondrial SOD, which in the natural lipoprotein milieu express significant physiological activity (Kiyosawa *et al.* 1993, Kasapovic *et al.* 2005). Complex conformational structure of the above-mentioned enzyme molecules and their natural lipoprotein coating enable preservation of the structure and function of the enzyme molecule. In addition to the human milk, SOD is also present in cow's milk (Filipovic *et al.* 2005). Statistical analysis evidenced that even in case of absence of significant difference between the cow's milk samples related to protein concentration, the difference in specific SOD activity still exists. The sample containing the highest SOD activity, which makes it as quality as the human milk, is recommended as a starting material for baby formulas.

Increasing number of the recent studies suggests the correlation between the frequency of onset of certain diseases and dietary habit. The antioxidant food components may be reliably said to represent certain kind of "protectors" against different pathological conditions, starting from inflammatory, to cardiovascular and carcinogenic ones. In the chain of biochemical events, antioxidants eliminate surplus of free radicals and thus prevent inflammatory reactions that initiate conditions such as psoriasis, arthritis and lupus, as well as blood vessel lesions preceding development of atherosclerotic plaques.

As for the CVS diseases, food rich with functional antioxidant components reduces significantly the risk of their development through several possible mechanisms: reduction of blood cholesterol level, reduction of lipid peroxidation, reduction of atherosclerotic plaque formation, elimination of free radicals and inhibition of protein (Hasler *et al.* 2000). Determination of the total antioxidant status (TAS) is used in clinical practice as a biomarker in different prognostic and diagnostic approaches. Based on the existing AO status, the predisposition to certain diseases may be determined. Disturbance of the exiting AO status of the organisms as a consequence of every day exposure to different types of stress increases the risk of onset of the disease. Thus, it is clear how important is to preserve the existing AO defense of the organism. Consumption of food rich in antioxidants may prevent formation of surplus of free radicals, or, if they have already been formed, it may prevent disturbance of the cell homeostasis through prevention of their interaction with other biomolecules. This type of nutrition enables us to control stress and ameliorate its consequences.

Conclusion

Stress acts on the cardiovascular system (CVS) through the numerous signaling pathways involving hormonal (catecholamine, steroid), peptide (inflammatory cytokines IL-1 β , TNF, etc) and cell redox-state regulating factors (low molecular antioxidants, antioxidant enzymes). These pathways regulate blood pressure, heart volume, electrolyte balance, adrenalin metabolism, angiotensin level and other CVS functions both under the normal and stressful conditions. The general picture of the molecular mechanisms of stress-induced CVS pathophysiology covered by the present review points out the major influence of its duration and intensity as ethological factors.

While under the acute stress the CVS adapting mechanisms successfully restore the homeostasis, under the chronic stress their capacity may be insufficient leading to different CVS pathologies. For example, chronic stress induces overactivity in the TNF pathway implicated in myocardial ischemia and programmed myocyte death. Stress-hypertensive individuals have increased blood levels of steroids and catecholamines. The overactivity of MAPK/SAPK cascade under chronic stress may lead to formation of atherosclerotic plaques and postischemic cardiac injuries. In addition to that, excessive levels of unrepaired structurally damaged proteins generated under chronic stress conditions

may lead to myocardial infarction. Cardiovascular diseases have also been associated with oxidative stress due to increased concentration of free radicals and decreased antioxidant status. These findings are supported by the numerous experimental data demonstrating prophylactic effects of various antioxidant-rich diets.

In conclusion, the data presented in this review strongly suggest the need for evaluation of the individual risk factors and individual sensitivity to different stressors as imperative of the future investigations in the area of stress-induced CVS diseases. The risk evaluation for the onset of CVS diseases may, at least in part, be performed by the careful monitoring of the antioxidant status in the individual's blood, which should lead to significant prevention and reduction of stress related CVS disorders.

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