Physiol. Res. 57: 327-338, 2008

MINIREVIEW

Neuroendocrine and Oxidoreductive Mechanisms of Stress-Induced Cardiovascular Diseases

S. B. PAJOVIĆ[1], M. B. RADOJČIĆ[1], D. T. KANAZIR[2]

[1]Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, [2]Serbian Academy of Sciences and Arts, Belgrade, Serbia

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

Received December 28, 2006
Accepted February 26, 2007
On-line April 25, 2007

Summary

The review concerns a number of basic molecular pathways that play a crucial role in perception, transmission, and modulation of the stress signals, and mediate the 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 the development of different pathophysiological states of the CVS, such as ischemia, hypertension, atherosclerosis and infarction. Stress-induced dysbalance 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

Corresponding author

Snežana B. Pajović, Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, P.O.Box 522, 11 001 Belgrade, Serbia. Fax: 381-11-8066-514. E-mail: pajovic@vin.bg.ac.yu

Introduction

Stress conditions represents a new dynamic state of the whole organism and the cardiovascular system (CVS) under the influence of either chemical (pharmaceutical products, pesticides, factors generating free radicals, etc.), physical (different types of radiation, heat), and/or biological 'stressors' (viruses, bacteria and their toxins) acting through endogenous hormonal and psychogenic pathways. These conditions represent a complex interaction between the circulating 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 the stress? As opposed to a short-lasting acute stress resulting 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 postischemic tissue damages and hypertension. At the molecular level, structural lesions are a common denominator for the CVS diseases including conformational changes of cellular components due to denaturation and oxidation. The review is aimed at detailed description of both structural and functional

© 2008 Institute of Physiology v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

alterations leading to deterioration of CVS function induced by stress. Our hope is that understanding of these mechanisms will help to develop better prophylactic and/or therapeutic strategies for prevention and/or treatment of stress-induced diseases of CVS in the future.

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, adrenaline keeps alertness, while steroids, primarily glucocorticoids, help to replenish energy supplies. 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 adrenaline 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 arise when the systems involved in these adaptive responses do not switch off when not needed or do not become active when needed. This may occur by prolonged exposure of the CVS to the stress, resulting in stress-associated dysfunctions related to deregulated concentrations of glucocorticoids (GCs) and mineralocorticoids (MCs) (Ng and Celermajer 2004).

Among the most important effects of GCs are regulation of the CVS fluid volume, electrolyte retention, and synthesis of angiotensin. In the physiological concentrations, GCs and MCs regulate the maintenance of normal blood pressure and normal heart action. However, when the circulating concentration of the major human GC cortisol is low during stress, these hormones are not capable to sustain the defense 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 are some data indicating that GCs potentiate the processes leading to atherosclerosis and thrombosis (Magiakou *et al.* 2006).

Generally, the effects of GCs on the CVS in acute stress should be considered as positive from the protective point of view. For example, synthetic GC dexamethasone is highly beneficial in a case of myocardial infarction, with its action leading to reduction of the myocardial infarction extent. Some MCs, such as aldosterone, have similar effect on release of the intracellular calcium (Ca^{++}) in vascular smooth muscle cells, maintaining the normal blood pressure and normal myocardial contractility (Seleznev *et al.* 1982, Guo *et al.* 2001). On the contrary, high GCs and MCs concentrations in chronic stress may have highly negative effects in the CVS due to sustained changes in the expression of 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 have not yet been sufficiently elucidated.

Another group of steroid hormones – estrogens (ES) is 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 the male compared to the female population of the same age before the menopause (30-45 years*) (*Barrett-Connor 2003). Clinical data indicate 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 the metabolism of coagulation factors. The studies carried out on the experimental animals described a sexual dimorphism in the presence of estrogen receptor (ER) in the different heart structures (Kanazir *et al.* 1982). Other studies showed that one of the possible mechanisms through which ES protect coronary arteries is by opening of the voltage-activated

K^+ channels and increasing of K^+ efflux. In addition, 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 (Baker *et al.* 1978, Pinthus *et al.* 2006).

At the intracellular level, the steroids, GCs, MCs, ES, and AN exert 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 factors occurring in a heterocomplex with heat shock proteins. Upon steroid binding, they dissociate from the complex and regulate gene expression in various tissues including the CVS. Some functions of SRs are controlled through phosphorylation by the stress-activated kinases MAPKs, cyclin-dependent kinases (CDKs) and sumoylation (Krstić *et al*. 1997*)*. Such modifications lead to sequential recruiting of 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. MRs regulate expression of the membrane transporters for Ca^{2+}, K^+ and Na^+ ions regulating blood pressure, while ERs are involved in the regulation of oxidoreductive equilibrium of the CVS.

Stress-induced phosphoprotein kinases of the cardiovascular system

Protein phosphoprotein kinase 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 adrenoreceptors, 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 amplified several times. 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 (Ravingerová *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. 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 and Emslie 1992). The last component of the activated cascade, c-Jun, functions primarily 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-synthase, 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 the 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 postischemic cardiac injuries (Libby *et al.* 2002). The role of p38 kinase in response to ischemia is less clear, although it is not less important than the role of SAPK. These kinases also play an important role in the regulation of synthesis of the inflammatory cytokines in the postischemic tissue, which is considered to accelerate postischemic 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 cell death, apoptosis (Feuerstein 2001). At the cellular level, the key determinants of this outcome is the duration of stress and its intensity. Thus, CVS cells exposed to ischemic shock are dying in the case of high intensity or prolonged ischemia. However, during short-lasting ischemic shock 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 physiological conditions, they participate in the maintenance of the correct tertiary structure of numerous structural and regulatory proteins of the CVS assuring its homeostasis. As the 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 and McMillan 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 cells *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 under 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 normally 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. 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 vascular endothelial cells. On the other hand, reperfusion, i.e. recovery of the normal blood oxygen concentration, may disturb intracellular mechanisms of oxidoreductive balance regulation due to the increased Ca^{++} influx and therefore 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, these 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, the induction of HSPs may be overcome in 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 their 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, xanthine 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 up 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 biomolecules 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 non-enzymatic 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. Selenium-containing GSH-Px reduces hydrogen peroxide to water and organic hydroperoxides in the presence of glutathione (GSH) as a second substrate. Selenium-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 under 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 indicate that the so-called mini-oxidative explosion plays an important role in signal transduction. It was found 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 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, the immune system cells produce not only NO but also considerable quantities of superoxide anion radicals ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2). In contrast, other cell types, such as vascular smooth muscle cells, chondrocytes and fibroblasts produce significantly lower quantities of these reactive species ($O_2^{\cdot}$, H_2O_2).

Although there are some data indicating that ROS exert their secondary messenger function depending on the

type of the cell of its origin, other results undoubtedly suggest that oxidative stress may also activate the same signaling pathways leading to the same transcription and translation changes in different cells. This is the case with the regulation of expression of the nuclear transcription factor kappa-B (NF-κB) in numerous cell types. Synthesis of this 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 κβ) subunit. It was observed that NF-κB activation might be blocked by antioxidative treatment and that ROS also play an important role in activation of the transcription factor (Schreck *et al.* 1991). On the other hand, direct oxidative stress, such as hypoxic reoxygenation, activates NF-κB. However, the activation is mediated through another mechanism in absence of proteolytic degradation of I κβ subunit (Imbert *et al.* 1996). Similarly, the addition of H_2O_2 may activate enzymes from the protein kinase C (PKC) family (Konishi *et al.* 1997). However, PKC activation induced by oxidants is independent from lipid cofactors and thus it is distinguishable from the classic ligand-stimulated pathway.

Extracellular signaling molecules, such as growth factors and cytokines, induce cellular 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 and Hunter 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), H_2O_2 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) and collagenase (Brenneisen *et al.* 1997), influence post-transcriptional ferritin control, induce activation of the transcriptional factors such as Myb (Myrset *et al.* 1993) and Erg-1 (Huang and Adamson 1993), and stimulate binding of Fos-Jun (AP-1) protein complex to early gene promoter regions (Toledano and Leonard 1991). 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 H_2O_2 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 cell proliferation. 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 an increased mitotic activity, the same is true during tissue regeneration process or 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 phases 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 confirm that oxidative stress, which is characterized by increased ROS production, generates simultaneously a wide spectrum of signaling and regulatory factors, some of them being 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 molecular mechanisms has enabled us not only to better understand 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 wide range of 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, both incidence and intensity of the CVS diseases are more prominent in menopausal than in premenopausal women. The former coincides with the drastic changes of both hormonal and antioxidative status (White *et al.* 1997). Dose- and time-dependent 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 of 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 and Paulson 1997). Krstevska *et al.* (2001) studied the 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). It has been known that ROS are generated in the vascular wall by 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 mechanical stress, different environmental factors, angiotensin II, cytokines, natural lipoproteins (LDL), in the presence of metal ions as catalysts. ROS are modified by LDL, while oxidative LDL form interacts thereafter with the capillary endothelium, which results in increased production of nitric oxide as well as peroxynitrate in the later phase. The reaction between NO 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 the 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. Ferro and Webb (1997) emphasized that capillary endothelial cells have a 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 results in increase of the peripheral vascular tone and blood pressure (Paravicini and Touyz 2006). This further leads to the development of vascular hypertrophy, enhanced endothelial adhesion of the monocytes, atherosclerosis and myocardial infraction. 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, these authors emphasized that modulation of endothelial function represents a modern therapeutic option in treatment of hypertension (Ferro and Webb 1997). They 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 also be 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 the 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 D) and inducible oxygenase isoform HO^{-1} (molecular mass 32 000 D) of heme oxygenase were 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 oxygenase and carbon monoxide (Torti FM and Torti SV 2000).

The role of oxidative stress in the development of CVS diseases is studied from different aspects, both at the molecular and cellular levels. Some of the results are contradictory, but it is evident 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). It may be concluded that antioxidative defense system plays a key role in defense the organism against the 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 cardio-vascular diseases

Both enzymatic and non-enzymatic 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 includes the intake of natural antioxidants. This type of nutrition represents a 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 should contain necessary amounts of important and active components in order to supply the organism with necessary quantities of the organic substance, vitamins, and microelements. Antioxidative properties are highly important food quality parameters. This involves a 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 of Fang *et al*. (2002) reached in collaboration with a group of researchers from the Texas University. They have presented a detailed role of proteins, lipids, vitamins, minerals and phytochemical antioxidants in the 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 an 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 the 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 is one of the essential prerequisites for appropriate cell differentiation and proliferation. Formation and maturation of all systems, particularly the immune and neuroendocrine systems, is to a certain extent related to the existing antioxidative status of the organism. The fact that 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 exerts significant physiological activity in the natural lipoprotein milieu (Kiyosawa *et al.* 1993, Kasapovič *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 (Filipovič *et al.* 2005). It was documented 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 good as the human milk, is recommended as a source for manufacturing of baby foods.

Increasing number of the recent studies suggests a relationship between the frequency of certain diseases and

dietary habits. The antioxidant food components may represent certain kind of "protectors" against different pathological conditions, starting from inflammatory up 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 the 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 enzymes (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 existing AO status of the organisms as a consequence of every day exposure to different types of stress increases the risk of disease onset. 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.

Conclusions

Stress acts on the cardiovascular system (CVS) through the numerous signaling pathways involving hormonal (cateholamine, steroid), peptide (inflammatory cytokines IL-1β, TNF, etc) and cell redox-state regulating factors (low molecular antioxidants, antioxidant enzymes). These pathways regulate blood pressure, heart volume, electrolyte balance, adrenaline 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 etiological factors.

While under the acute stress the CVS adapting mechanisms successfully restore the homeostasis, under the chronic stress their capacity may become 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 the 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.

Thus, 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.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work supported by the Ministry of Sciences, Technology and Environmental Protection of the Republic of Serbia, Grants No. 143035 B and 143042 B.

References

ALLEN RG: Oxygen-reactive species and antioxidant responses during development: the metabolic paradox of cellular differentiation. *Proc Soc Exp Biol Med* **196**: 117-129, 1991.

ALLEN RG: Oxidative stress and superoxide dismutase in development, aging and gene regulation. *Age* **21**: 47-76, 1998.

ALLEN RG, TRESINI M: Oxidative stress and gene regulation. *Free Rad Biol Med* **28**: 463-499, 2000.

BAKER PJ, RAMEY ER, RAMWELL PW: Androgen-mediated sex differences of cardiovascular responses in rats. *Am J Physiol* **235**: H242-H246, 1978.

BARRETT-CONNOR E: Cardiovascular endocrinology 3: an epidemiologist looks at hormones and heart disease in women. *J Clin Endocrinol Metab* **88**: 4031-4042, 2003.

BENJAMIN IJ, MCMILLAN DR: Stress (heat shock) proteins-molecular chaperons in cardiovascular biology and disease. *Circ Res* **83**: 117-132, 1998.

BESSE S, BULTEAU AL, BOUCHER F, RIOU B, SWYNGHEDAUW B, DE LEIRIS J: Antioxidant treatment prevents cardiac protein oxidation after ischemia-reperfusion and improves myocardial function and coronary perfusion in senescent hearts. *J Physiol Pharmacol* **57**: 541-552, 2006.

BRENNEISEN P, BRIVIBA K, WLASCHEK M., WENK J, SCHARFFETTER-KOCHANEK K: Hydrogen peroxide (H_2O_2) increases the steady-state mRNA level of collagenase/MMP-1 in human dermal fibroblasts. *Free Radic Biol Med* **22**: 515-524, 1997.

BRUNDEL BJ, SHIROSHITA-TAKESHITA A, QI X, YEH YH, CHARTIER D, VAN GELDER IC, HENNING RH, KAMPINGA HH, NATTEL S: Induction of heat shock response protects the heart against atrial fibrillation. *Circ Res* **99**: 1394-1402, 2006.

CADENAS E: Biochemistry of oxygen toxicity. *Annu Rev Biochem* **58**: 79-110, 1989.

CHOI AMK, PIGNOLO RJ, RHYS CMJ, CRISTOFALO VJ, HOLBROOK NJ: Alterations in the molecular response to DNA damage during cellular aging of cultured fibroblasts: reduced AP-1 activation and collagenase gene expression. *J Cell Physiol* **164**: 65-73, 1995.

CHOJKIER M, HOUGLUM K, SOLIS-HERRUZO J, BRENNER DA: Stimulation of collagen gene expression by ascorbic acid in cultured human fibroblasts. *J Biol Chem* **264**: 16957-16962, 1989.

COTGREAVE IA., MOLDEUS P, ORRENIUS S: Host biochemical defense mechanisms against prooxidants. *Pharmacol Toxicol* **28**: 189-212, 1988.

CROWN A, LIGHTMAN S: Why is the management of glucocorticoid deficiency still controversial: a review of the literature. *Clin Endocrinol* **63**: 483-492, 2005.

DAS S, O'KEEFE JH: Behavioral cardiology: recognizing and addressing the profound impact of psychosocial stress on cardiovascular health. *Curr Atheroscler Rep* **8**: 111-118, 2006.

DHALLA NS, TEMSAH RM, NETTICADAN T: Role of oxidative stress in cardiovascular diseases. *J Hypertens* **18**: 655-673, 2000.

FANG YZ, YANG S, WU G: Free radicals, antioxidants and nutrition. *Nutrition* **18**: 872-879, 2002.

FEIL R, LOHMANN SM, DEJONGE H, WALTER U, HOFMANN F: Insights from genetically modified mice cyclic GMP-dependent protein kinases and the cardiovascular system. *Circ Res* **93**: 907-916, 2003.

FERRO CJ, WEBB DJ: Endothelial dysfunction and hypertension. *Drugs* **53**: 30-41, 1997.

FEUERSTEIN GZ: Apoptosis – new opportunities for novel therapeutics for heart diseases. *Cardiovasc Drugs Ther* **15**: 547-551, 2001.

FILIPOVIĆ D, KASAPOVIĆ J, PEJIĆ S, NICIFOROVIĆ A, PAJOVIĆ SB, RADOJČIĆ MB: Superoxide dismutase activity in various fractions of full bovine milk. *Acta Aliment* **3**: 219-226, 2005.

GUISASOLA MC, DESCO MDEL M, GONZALEZ FS, ASENSIO F, DULIN E, SUAREZ A, GARCIA BARRENO P: Heat shock proteins, end effectors of myocardium ischemic preconditioning? *Cell Stress Chaperones* **11**: 250-258, 2006.

GUO DF, SUN YL, HAMET P, INAGAMI T: The angiotensin II type 1 receptor and receptor-associated proteins. *Cell Res* **11**: 165-180, 2001.

HALLIWELL B: Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *NutrRev* **57**: 104-113, 1999.

HASLER CM, KUNDRAT S, WOOL D: Functional foods and cardiovascular disease. *Curr Atheroscler Rep* **2**: 467-475, 2000.

HOFFMAN RM, GAREWAL HS: Antioxidants and the prevention of coronary disease. *Arch Intern Med* **155**: 241-246, 1995.

HOLVOET P: Endothelial dysfunction, oxidation of low-density lipoprotein, and cardiovascular disease. *Ther Apher* **3**: 287-293, 1999.

HUANG RP, ADAMSON ED: Characterization of the DNA- binding properties of the early growth response-1 (ERG-1) transcription factor: evidence for modulation by a redox mechanism. *DNA Cell Biol* **12**: 265-273, 1993.

HUTTER MM, SIEVERS RE, DARBOSA V, WOLFE CL: Heat-shock protein induction in rat hearts: a direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection. *Circulation* **89**: 355-360, 1994.

IMBERT V, RUPEC RA, LIVOLSI A, PAHL HL., TRAENCKNER BM., MUELLER-DIECKMANN C, FARAHIFA D, ROSSI B, AUBERGER P, BAEUERLE PA, PEYRON JF: Tyrosine phosphorylation of IκB activities NF-κB without proteolytic degradation of IκB-α. *Cell* **86**: 787-798, 1996.

ISHII T, YANAGAWA T, YUKI K, KAWANE T, YOSHIDA H, BANNAI S: Low micromolar levels of hydrogen peroxide and proteasome inhibitors induce the 60-kDa A 170 stress protein in murine peritoneal macrophages. *Biochem Biophys Res Commun* **232**: 33-37, 1997.

KANAZIR DT, RIBARAC-STEPIĆ N, DJORDJEVIĆ-MARKOVIĆ R, STEFANOVIĆ D, KATAN M, POPIĆ S, RADOJČIĆ M, KOVAČIĆ-MILIVOJEVIĆ B: Extragenomic effects of glucocorticoids. In: *Cell Function and Differentiation*, C. Sekeris (ed.), Alan R. Liss, New York, 1982, pp 193-205.

KARIN M, HUNTER T: Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. *Curr Biol* **5**: 747-757, 1995.

KASAPOVIĆ J, PEJIĆ S, MLADENOVIĆ M, RADLOVIĆ N, PAJOVIĆ SB: Superoxide dismutase activity in colostrums, transitional and mature human milk. *Turkish J Pediatr* **47**: 343-347, 2005.

KEYSE SM, EMSLIE EA: Oxidative stress and heat shock induce a human gene encoding a protein-tyrosin phosphatase. *Nature* **359**: 644-647, 1992.

KIM J, NUEDA A, MENG XH, DYNAN WS, MIVERCHI NF: Analysis of the phosphorylation of human heat shock transcription factor-1 by MAP kinase family members. *J Cell Biochem* **67**: 43-54, 1997.

KIYOSAWA I, MATUYAMA J, NYUI S, YOSHIDA K: Cu,Zn- and Mn-superoxide dismutase in human colostrum and mature milk. *Biosci Biotech Biochem* **57**: 676-677, 1993.

KONISHI H, TANAKA M, TAKEMURA Y, MATSUZAKI H, ONO Y, KIKKAWA U, NISHIZUKA Y: Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. *Proc Natl Acad Sci USA* **94**: 11233-11237, 1997.

KRSTEVSKA M, DZHEKOVA-STOJKOVA S, BOSILKPVA G: Menopause, coronary artery disease and antioxidants. *Clin Chem Lab Med* **39**: 641-644, 2001.

KRSTIC MD, ROGATSKY I, YAMAMOTO KR, GARABEDIAN MJ: Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol Cell Biol* **17**: 3947-3954, 1997.

LANDER HM: An essential role for free radicals and derived species in signal transduction. *FASEB J* **11**: 118-124, 1997.

LIBBY P, RIDKER PM, MASERI A: Inflammation and atherosclerosis. *Circulation* **105**: 1135-1143, 2002.

LIN SJ, SHYUE SK, HUNG YY, CHEN YH, KU HH, CHEN JW, TAM KB, CHEN YL: Superoxide dismutase inhibits the expression of vascular cell adhesion molecule-1 and intracellular cell adhesion molecule-1 induced by tumor necrosis factor-alpha in human endothelial cells through the JNK/p38 pathways. *Arterioscler Thromb Vasc Biol* **25**: 334-340, 2005.

MAGIAKOU MA, SMYRNAKI P, CHROUSOS GP.: Hypertension in Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab* **20**: 467-482, 2006.

MARUI N, OFFERMANN MK, SWERLICK R, KUNSCH C, ROSEN CA, AHMAD M, ALEXANDER RW, MEDFORD RM: Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* **92**: 1866-1874, 1993.

MCEWEN B: *The End of Stress as We Know It*. Joseph Henry Press, New York, 2002, 262 p.

MESTRIL R, CHI SH, SAYEN MR, O'REILLY K, DILLMANN WH, WOLFE CL: Overexpression of heat shock protein 72 in transgenic mice decreases infarct size in vivo. *Circulation* **94**: 1408-1411, 1996.

MICHELSON AM: Medical aspects of superoxide dismutase. In: *Life Chemistry Reports*. Harwood Academic Pub., United Kingdom, 1987, pp 1-142.

MYRSET AH., BOSTARD A, JAMIN N, LIRSAC PN, TOMA F, GABRIELSEN OS: DNA and redox state induced conformational changes in the DNA-binding domain of the Myb oncoprotein. *EMBO J* **12**: 4625-4633, 1993.

NADEAU SI, LANDRY J: Mechanisms of activation and regulation of the heat shock-sensitive signaling pathways. *Adv Exp Med Biol* **594**: 100-113, 2007.

NG MKC, CELERMAJER DS: Glucocorticoid treatment and cardiovascular disease. *Heart* **90**: 829-830, 2004.

NUTTALL SL, KENDALL MJ, MARTIN U: Antioxidant therapy for the prevention of cardiovascular disease. *QJM* **92**: 239-244, 1999.

PAJOVIĆ S., NIKEZIĆ G, MARTINOVIĆ JV: Effects of ovarian hormones on superoxide dismutase activity in rat brain synaptosomes. *Neuroendocrinol Lett* **16**: 291-296, 1994.

PAJOVIĆ S, SAIČIĆ ZS, SPASIĆ MB, PETROVIĆ VM, MARTINOVI JV: Effects of progesterone and estradiol benzoate on superoxide dismutase activity in the brain of male rats. *Experientia* **52**: 221-224, 1996.

PALMER HJ, PAULSON KE: Reactive oxygen species and antioxidants in signal transduction and gene expression. *Nutr Rev* **55**: 353-361, 1997.

PARAVICINI TM, TOUYZ RM: Redox signaling in hypertension. *Cardiovasc Res* **71**: 247-258, 2006.

PARTHASARATHY S, KHANMERCHANT N, PENUMETCHA M, SANTANAM: Oxidative stress in cardiovascular disease. *J Nucl Cardiol* **8**: 379-389, 2001.

PINTHUS JH, TRACHTENBERG J, KLOTZ L: Cardiovascular effects of androgen depletion and replacement therapy. *Urology* **67**: 1126-1132, 2006.

RAVINGEROVÁ T, BARANČÍK M, STRNISKOVÁ M: Mitogen-activated protein kinases: a new therapeutic target in cardiac pathology. *Mol Cell Biochem* **247**: 127-138, 2003.

SCHRECK R, RIEBER R, BAEUERELE PA: Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *EMBO J* **10**: 2247-2258, 1991.

SELEZNEV YM, MARTINOV AV: Permissive effect of glucocorticoids in catecholamine action in the heart: possible mechanism. *J Mol Cell Cardiol* **14**: 49-58, 1982.

SHACKELFORD RE, KAUFMANN WK, PAULES RS: Oxidative stress and cell cycle checkpoint function. *Free Radic Biol Med* **28**: 1387-1404, 2000.

SIOW RCM, SATO H, MANN GE: Heme oxygenase carbon monoxide signaling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide. *Cardiovasc Res* **41**: 385-394, 1999.

TOLEDANO MB, LEONARD WJ: Modulation of transcription factor NF-κB binding activity by oxidation-reduction in vitro. *Proc Natl Acad Sci USA* **88**: 4328-4332, 1991.

TORTI FM, TORTI SV: Regulation of ferritin genes and protein. *Blood* **99**: 3505-3516, 2002.

TOUYZ RM, SCHIFFRIN EL: Reactive oxygen species in vascular biology: implications in hypertension. *Histochem Cell Biol* **122**: 339-352, 2004.

UEYAMA T: Emotional stress-induced Tako-tsubo cardiomyopathy: animal model and molecular mechanism. *Ann N Y Acad Sci* **1018**: 437-444, 1999.

USHIO-FUKAI M: Localizing NADPH oxidase-derived ROS. *Sci STKE* **349**: re8, 2006.

USHIO-FUKAI M, ALEXANDER RW: Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. *Mol Cell Biochem* **264**: 85-97, 2004.

WANG X, XU C, WANG X, WANG D, WANG Q, ZHANG B: Heat shock response and mammal adaptation to high elevation (hypoxia). *Sci China C Life Sci* **49**: 500-512, 2006.

WHITE CR, DARLEYUSMAR V, OPARIL S: Gender and cardiovascular disease-recent insights. *Trends Cardiovasc Med* **7**: 94-100, 1997.

WHITWORTH JA., MANGOS GJ, KELLY JJ: Cushing, cortisol, and cardiovascular disease *Hypertension* **36**: 912-916, 2000.

YUNG LM, LEUNG FP, YAO X, CHEN ZY, HUANG Y: Reactive oxygen species in vascular wall. *Cardiovasc Hematol Disord Drug Targets* **6**: 1-19, 2006.