Türk Biyokimya Dergisi [Turkish Journal of Biochemistry - Turk J Biochem] 2006; 31 (2); 86–95.

Review Article [Derleme Makalesi]

Yayın tarihi 21 Haziran, 2006 © TurkJBiochem.com

[Published online 21 June, 2006]

# Oxidative / Nitrosative Stress in Chronic Heart Failure: A Critical Review

## Kronik Kalp Yetmezliğinde Oksidatif / Nitrozatif Stres: Eleştirel Bir Derleme

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Kayıt tarihi 13 Nisan 2006; kabul tarihi 11 Mayıs 2006
[Received 13 April 2006; accepted 11 May 2006]

**ABSTRACT**

Heart failure constitutes a major cardiovascular health hazard worldwide. It afflicts over 1% of the general population and nearly 1 in 10 of the elderly. Chronic heart failure (CHF) also accounts for an increasing proportion of available hospital resources. Moreover, despite intense medical care, the outlook for the patient with chronic heart failure continues to remain quite dismal; both quality of life and survival prospects are severely limited. Ventricular arrhythmias, thromboembolic phenomena and sudden death are common in patients with this syndrome. There is extensive experimental evidence from *in vitro* and animal experiments that chronic heart failure is a state of increased oxidative and nitrosative stress. Moreover, in animal models, the development of CHF is accompanied by changes in the antioxidant defense mechanisms of the myocardium as well as evidence of oxidative myocardial injury. On the other hand, in general, increased nitrosative stress is linked to increased oxidative stress. Alterations in nitric oxide synthase (NOS) activity and nitric oxide (•NO) may contribute to failing heart. These data have led to the hypothesis that increased oxidative and nitrosative stress at least may be a mechanism of disease progression in CHF. In this article, studies on oxidative / nitrosative stress in CHF are reviewed with all dimensions.

**Key Words:** Dextransucrase, dextran, polysaccharide, *Leuconostoc mesenteroides*

**ÖZET**

Kalp yetmezliği tüm dünyada kalp damar sağlığı açısından başlıca tehdittir. Genel olarak nüfusun %1'ini, yaşlı nüfusun ise onda birini etkiler. Kronik kalp yetmezliği (CHF) aynı zamanda hastane kaynaklarının gittikçe daha fazla oranda tüketilmesine yol açmaktadır. Üstelik, gösterilen yoğun tıbbi çabaya rağmen, kronik kalp yetmezliği hastasının durumu hala sıkıntılıdır; hem yaşam kalitesi, hem de yaşam süresi sınırlıdır. Bu sendromda, ventriküler aritmiler, tromboembolik olaylar ve birden ölüm ile sık karşılaşılır. Kronik kalp yetmezliğinde oksidatif ve nitrozatif stres artışını kanıtlayan pek çok in vitro çalışma ve hayvan deneyi vardır. Ayrıca, hayvan çalışmalarında CHF'nin gelişmesine kalbin antioksidan savunma mekanizmalarında değişiklikler ve oksidatif miyokart hasarının eşlik ettiği görülmüştür. Öte yandan, genel olarak nitrozatif stres artışı oksidatif stres artışı ile bağıntılıdır. Nitrik oksit sentaz (NOS) aktivitesi ve nitrik oksit (•NO) üretimindeki değişiklikler kalp yetmezliğini etkiler. Bu bilgiler, oksidatif / nitrozatif stres artışının, en azından CHF'nin ilerlemesine yol açan bir mekanizma olduğu düşüncesini doğurmuştur. Bu makalede, kronik kalp yetmezliğinde oksidatif ve nitrozatif stres artışı ile ilgili çalışmalar tüm boyutlarıyla derlendi.

**Anahtar Kelimeler:** Antioksidanlar, Kardiyomiyopati, Kronik kalp yetmezliği, Nitrik oksit, Nitrozatif stres, Oksidatif stres, Reaktif azot türleri, Reaktif oksijen türleri

## 1. INTRODUCTION

Heart failure is a complex clinical syndrome, most simply defined as a disorder in which the ventricles fail to pump adequate quantities of blood to meet the needs of peripheral organs. Chronic heart failure (CHF) is a common debilitating illness, associated with a high mortality and poor quality of life. It is the most frequent cause of hospitalization among persons older than 65 years of age (1). Perhaps there are additional many persons with asymptomatic left ventricular dysfunction who are at high risk of developing heart failure (2). Prognosis is poor, with median survival after onset only 1.7 years in men and 3.2 years in women (3). Ventricular arrhythmias, thromboembolic phenomena and sudden death are common in patients with this syndrome. Heart failure is a condition that affects principally the elderly, and with the progressive aging of the population, it is virtually certain that the prevalence of heart failure will continue to grow during the next decade both in developed and in developing nations. Nearly 5 million Americans have heart failure today (1). It affects 0.4%–2% of the total European population. Prevalence increases sharply with age, affecting 6%–10% of those older than 65 years (3,4). CHF is thus a disorder of the elderly, due to the increasing longevity of population, and increased survival rates from acute myocardial infarction. The average age at presentation is 76 years, with men being at a 75% higher risk of developing heart failure than women (5,6). Therefore CHF is an important and increasing public health problem because of the increasing incidence and prevalence of the disorder and enormous associated socioeconomic implications.

Reactive oxygen / nitrogen species (ROS/RNS) and free radicals are constantly formed in the human body and removed by an antioxidant defense system. A certain amount of ROS / RNS production is, in fact, necessary for proper health; for example ROS / RNS help the immune system to eliminate microorganisms. In healthy individuals, the generation of ROS / RNS appears to be approximately in balance with antioxidant defense. An imbalance between ROS / RNS and antioxidant defenses in favor of the former via excessive production of ROS / NOS, loss of antioxidant defenses, or both, has been described as oxidative / nitrosative stress (7,8). In some human diseases, increased oxidative / nitrosative stress may make an important contribution to disease pathology. However, increased oxidative / nitrosative stress may be a result of disease pathology. When ROS / RNS generation exceeds antioxidant defenses, the excess species react with all classes of biologic molecules, including lipids, proteins, and nucleic acid bases which can severely affect cell structure and viability. Especially cellular targets such as thiols, proteins and lipids, many of them have special roles for cellular signaling, are affected by increased ROS / RNS. Consequently, in conditions of increased ROS / RNS production, a variety of cellular responses through generation of secondary reactive products result in cell death by necrosis or apoptosis (Fig. 1).

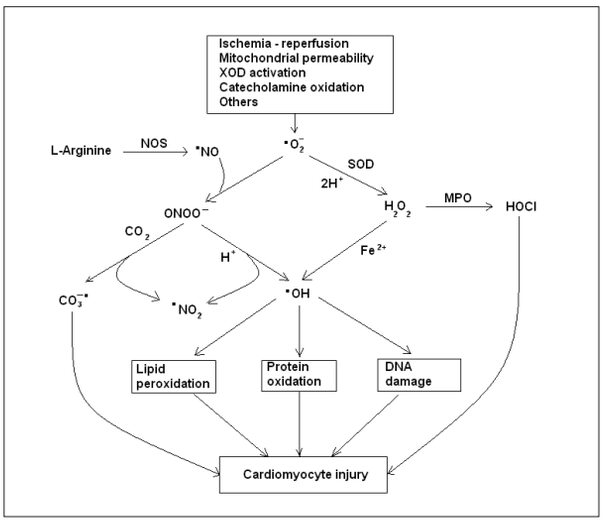


**Figure 1.** ROS / RNS formation and resultant cardiomyocyte injury in CHF.

Over the last 20 years, such a huge number of study relating to increased oxidative / nitrosative stress have been performed that few diseases remain that have not been linked in one way or another to oxidative stress. In this connection, investigators have probed for evidence of ROS / RNS – induced tissue injury and/or decreased concentrations of antioxidant defenses.

## 2. ROS / RNS AND THEIR SOURCES IN HUMAN BODY

Many ROS / RNS possess unpaired electrons and thus are free radicals. These include molecules such as superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), nitric oxide (nitrogen monoxide $^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$) and lipid radicals. Other ROS / RNS, such as hydrogen peroxide ($H_2O_2$), peroxynitrite ($ONOO^-$), peroxynitrous acid ($ONOOH$) and hypochlorous acid ($HOCl$), are not free radicals per se but have oxidizing effects that contribute to oxidant stress (Table 1).

The cellular production of one ROS may lead to the production of several others via radical chain reactions. For example, reactions between radicals and polyunsaturated fatty acids within cell membrane may result in a fatty acid peroxyl radical ($R\text{-}COO^{\bullet}$) that can attack adjacent fatty acid side chains and initiate production of other lipid radicals. Lipid radicals produced in this chain reaction accumulate in the cell membrane and may have a myriad of effects on cellular function, including leakage of the plasmalemma and dysfunction of membrane-bound receptors. Of note, end products of lipid peroxidation, including unsaturated aldehydes and other metabolites, have cytotoxic and mutagenic properties (9-11).

Oxidative stress in human biology comes from a variety of sources. In aerobic cells, incomplete reduction of oxygen in the mitochondrial electron transport chain releases $^{\bullet}O_2^-$ into the cytosol; 1-2% of molecular oxygen

Table 1. Major Reactive Oxygen and Nitrogen Species in Biological Systems.

| Reactive Oxygen Species | Formula |
|---|---|
| Superoxide anion | $^{\bullet}O_2^-$ |
| Hydroperoxyl radical | $HO^{\bullet}_2$ |
| Peroxyl radical | $RO^{\bullet}_2$ |
| Alkoxyl radical | $RO^{\bullet}$ |
| Hydroxyl radical | $^{\bullet}OH$ |
| Hydrogen peroxide | $H_2O_2$ |
| Hypochlorite | $HOCl^-$ |
| Hypochlorous acid | HOCl |
| Ozone | $O_3$ |
| Singlet oxygen | $^1\Delta gO_2$ |
| **Reactive Nitrogen Species** | **Formula** |
| Nitric oxide (nitrogen monoxide) | $^{\bullet}NO$ |
| Nitrogen dioxide | $^{\bullet}NO_2$ |
| Peroxynitrite | $ONOO^-$ |
| Peroxynitrous acid | ONOOH |
| Dinitrogen trioxide | $N_2O_3$ |
| Nitryl chloride | $NO_2Cl$ |
| Nitronium ion | $NO^+_2$ |
| Alkylperoxynitrite | ROONO |
| Nitrosothiols | RSNO |

[Adapted from Stocker R, Keany JF. (2003) Role of oxidative modifications in atherosclerosis. Physiol. Rev. 84, 1381-1478.]

consumed may be converted to $^{\bullet}O_2^-$ by this way. Free transition metal ions like iron and copper are strong catalysts for oxidation reactions in the presence of hydroperoxides. The superoxide anion interacts with these free transition metal ions to produce the highly reactive hydroxyl radical ($^{\bullet}OH$). (9,12,13). The enzyme xanthine oxidase (XO), an iron sulfur molybdenum flavoprotein with multiple functions, may also be a source of superoxide radicals during reperfusion of ischemic tissues (14,15). Macrophages and neutrophils produce also ROS such as $H_2O_2$ and HOCl as a means of bacterial killing (16,17). Myeloperoxidase (MPO) is a heme containing protein and the only human enzyme known to generate HOCl. Phagocytes, including neutrophils, monocytes and macrophages contain membrane-bound NAD(P)H oxidases generating $^{\bullet}O_2^-$ upon activation. Like phagocytes, endothelial cells, vascular smooth muscle cells and adventitial fibroblasts contain a membrane - bound NAD(P)H oxidase which generates $^{\bullet}O_2^-$ via reduction of molecular oxygen (19). ROS may be generated within the membranes, in association with the arachidonic acid cascade and with the auto-oxidation of catecholamines (20-22). Iron containing dioxygenases, lipoxygenases, are prooxidant enzymes which catalyze the insertion of molecular oxygen into polyunsaturated fatty acids to produce biologically active lipids such as prostaglandins, thromboxanes and leukotrienes (23). Drugs may exert toxic effects by promoting ROS formation during their metabolism, e.g. cardiomyopathy associated with daunomycin and doxorubicin (24). Smoking represents a major threat to health and many of its damaging effects can be attributes to its free radical content and a subsequent oxidative damage (25).

As newly biological aspects of $^{\bullet}NO$ have been discovered, speculation has emerged that this radical plays important roles in various pathophysiological mechanisms. $^{\bullet}NO$ is produced by NOS which are a family of enzymes that catalyze the oxidation of L-arginine to L-citrulline. There are three different isoforms of NOS. Two isoforms, neuronal and endothelial, are constitutively expressed while one is induced in response to cytokines and endotoxins among other stimuli. In the context of

cardiovascular system, endothelial NOS (eNOS; NOS3) and inducible NOS (iNOS; NOS2) are most relevant. In certain circumstances such as the absence of sufficient cofactors like tetrahydrobiopterin for enzyme catalysis, NOS becomes **"uncoupled".** When the enzyme becomes "uncoupled", generates $^{\bullet}O_2^-$ instead of $^{\bullet}NO$ by reducing molecular oxygen. $^{\bullet}NO$ forms RNS by reacting with $^{\bullet}O_2^-$, molecular oxygen, and metal complexes. $^{\bullet}NO$ rapidly reacts with $^{\bullet}O_2^-$ to give rise to $ONOO^-$. In biological systems, $ONOO^-$ results in a severely oxidizing environment. It can travel significant distances and readily crosses cell membranes, therefore it is considered a key trigger of DNA strand breakage. Its protonated form peroxynitrous acid (HNOOH) is also extremely reactive (26). Nitrogen dioxide ($^{\bullet}NO_2$) is a strongly oxidizing radical which is formed from $ONOO^-$ (27). Other important products derived from $^{\bullet}NO$ are dinitrogen trioxide ($N_2O_3$) which forms in the presence of molecular oxygen and S-nitrosothiols (RSNO) which form by nitrosation of thiols.

Human body controls and survives the continuous ROS production due to a delicate balance between cellular systems generating the various oxidants and those maintaining the antioxidant defense mechanisms. There are three antioxidant defence mechanisms existing inside (intracellular) and outside (extracellular) the cells and in membranes. Intracellular defense mechanisms include the enzymes superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione peroxidase (GPO). These enzymes eliminate toxic reduction intermediates of oxygen inside the cell, prevent radical formation and so restrict oxygen toxicity. SOD removes $^{\bullet}O_2^-$ catalytically by promoting the dismutation of $^{\bullet}O_2^-$ to $H_2O_2$ and $O_2$; catalase removes $H_2O_2$ when present in high concentrations; GPO removes $H_2O_2$ when present in low concentrations and can remove organic hydroperoxides by conversion of reduced glutathione (GSH) to oxidized form (GSSG); and GR substitutes the GSSG for the reaction of GPO. Extracellular defence mechanisms include plasma antioxidants such as albumin, bilirubin, urate, ceruloplasmin and ascorbic acid, and extracellular forms of SOD and GPO. Albumin binds copper and heme and scavenges HOCl; bilirubin scavenges peroxyl radicals; urate is a radical scavenger and metal binder; ceruloplasmin scavenges $^{\bullet}O_2^-$ and binds copper; ascorbic acid scavenges $^{\bullet}OH$ radical and plays a role in recycling of vitamin E, a membrane antioxidant. Defence mechanisms in memranes include vitamin E, beta-carotene and coenzyme Q. Vitamin E ($\alpha$-tocopherol) is a very effective, lipid-soluble, chain-breaking antioxidant. Beta-carotene is also a lipid-soluble antioxidant and plays a role as a radical scavenger and singlet oxygen quencher. These lipid-soluble antioxidants have also antioxidant effects in lipoproteins. Coenzyme Q is another membrane antioxidant in addition to its major role in cellular energy metabolism (7, 9, 12).

## 3. EVIDENCE OF INCREASED OXIDATIVE / NITROSATIVE STRESS IN CHF

Heart failure is not only an abnormality of the heart, but a disorder of the circulation. Some patients have structural cardiac damage that adversely affects systolic or diastolic function, but they do not have heart failure because of the compensatory mechanisms that maintain cardiac output and peripheral perfusion. Therefore, hemodynamic effects and neurohormonal compensatory mechanisms take together place in patients with heart failure. When the peripheral perfusion is decreased in CHF, a number of neurohormonal mechanisms are activated to preserve circulatory homeostasis. However, neurohormonal activation fails to correct the hemodynamic abnormalities (2,128,29). On the contrary, sympathetic activation in CHF may even exacerbate these abnormalities. Repeated ischemia reperfusion cycles occur in the heart and peripheral tissues. The release of catecholamines by sympathetic activation initiates a series of self-reinforcing events that lead to progressive left ventricular dysfunction and death (30-32). In addition, increased activity of renin-angiotensin-aldosterone system, particularly high angiotensin II concentration, contributes to progressive ventricular dysfunction (33). Although some investigators have suggested that noradrenaline and angiotensin II cause a state of calcium overload in the failing heart, recent evidence suggests that the main mechanism of neurohormonally mediated tissue injury may involve ROS / RNS (34-37).

The present evidence suggests that oxidative stress is increased in patients with CHF. There is extensive experimental evidence from *in vitro* and *in vivo* animal experiments that CHF is a state of oxidative stress. Several studies have been published demonstrating elevated plasma concentrations of malondialdehyde (MDA), an end-product of lipid peroxidation, or MDA-like material (thiobarbituric acid reactive substances: TBARS) in patients with CHF (38-44). Previously we showed elevated TBARS and increased susceptibility to peroxidation in erythrocyte membranes of patients with CHF (45). However, most of these investigations have been criticized for the small study populations (46). Also an elevated 4-hydroxy-nonenal level, an unsaturated toxic aldehyde end-product of lipid peroxidation, has been reported in patients with CHF (47). The detection of unsaturated aldehydes is much more important than that of MDA because of their higher toxicity (48). One of the sources of ROS in the body is polymorphonuclear leucocytes. Prasad *et al* (49) showed that polymorphonuclear leukocyte production of oxygen-derived free radicals is increased 4-fold in patients with heart failure compared with controls. Recently $F_2$-isoprostanes have become prominent as a sensitive and specific indicator of oxidative stress. Mallat *et al* (50) reported a strong correlation between

the concentration of pericardial 8-iso-prostaglandin $F_2\alpha$ ($iPF_{2\alpha}$) and left ventricle dimensions measured echocardiographically. Additionally, these authors reported elevated levels of immunoreactive $iPF_{2\alpha}$ - III in pericardial fluid of patients with heart failure. Ide *et al* (51) recently demonstrated that $\bullet O_2^-$ production is increased in the submitochondrial particle fractions isolated from canine failing hearts. The same authors extended their earlier observations (52) by showing that the production of ROS is increased in the failing (vs nonfailing) myocardium. In a new study, urinary 15-$F_{2t}$-isoprostane, plasma brain natriuretic peptide (BNP), and serum IL-6 concentrations in patients with severe CHF were found significantly higher than those in control subjects or in patients with mild CHF (53).

Another means of detecting oxidative stress have been to quantify antioxidant defenses. Dhalla and Singal (54) and Hill and Singal (55) have found that antioxidant capacity is decreased in models of heart failure. Consistent with these observations, Keith *et al* (56) showed reduced antioxidant activity and vitamin C concentrations in plasma from patients with chronic heart failure. We found decreased thiol groups in plasma and erythrocyte membranes and decreased reduced glutathione (GSH) in whole blood of patients with CHF (44, 45). Thiol groups and GSH act as free radical scavenger and help in regenerating other antioxidants; they are depleted during such reactions (57). We also found depressed erythrocyte superoxide dismutase activity in patients with ischemic or idiopathic dilated cardiomyopathy compared to controls (45). Other studies mostly showed decreased plasma and erythrocyte thiols or GSH and decreased SOD activity (38,39,58). Landmesser *et al* (59) observed a marked reduction of endothelium-bound extracellular SOD activity, a major antioxidant defense system of the arterial wall, in patients with CHF. In the heart, overexpression of another antioxidant enzyme, GPO, after myocardial infarction prevents failure and left ventricular remodeling in mice (60). On the other hand, in the Keshan area of China, cardiomyopathy is endemic (Keshan disease) among children under 15 years of age and is associated with selenium deficiency and controlled by selenium supplementation. Selenium is necessary for the activity of GPO as a cofactor of the enzyme. Patients with cardiomyopathy have been reported to have decreased serum selenium (61). However requirement of selenium in endemic cardiomyopathy needs further large-scale investigations (62). Baumer *et al* (63) found a significant decrease in catalase activity in human hearts with idiopathic dilated cardiomyopathy, although direct ROS mediated damage was not detected. At the cellular level naturally occurring enzymatic antioxidants, such as SOD, GPO, and catalase play an important role in the conversion of ROS to oxygen and water. Accominotti *et al* (64) showed lower vitamin C and beta-carotene levels in CHF. Keith *et al* (56) showed decreased vitamin C levels with increased lipid peroxidation in patients with CHF. Serdar *et al* (65) found a negative correlation between the New York Heart Association functional class and vitamin E and beta-carotene levels, and Polidori *et al* (66) found directly correlated plasma vitamin E and A levels with ejection fraction.

deBelder *et al* (67) discovered that iNOS is expressed in the myocardium of patients with heart failure. After this discovery, *in vitro* studies showed that iNOS expression can induce cardiac contraction dysfunction (68,69). These early studies have led to considerable interest on the role of $^{\bullet}NO$ in heart failure. Now, there is increasing evidence on alterations in $^{\bullet}NO$ generation are of pathological importance in heart failure. We measured $^{\bullet}NO$ metabolites, nitrite and nitrate in a group of patients with ischemic, dilated, and hypertrophic cardiomyopathy and age- and sex- matched healthy controls, and we found higher nitrite and nitrate concentrations in the whole cardiomyopathy group than control group with the highest levels in patients with dilated cardiomyopathy (70). In another study, an extremely high nitrite level was reported in patients with ischemic and idiopathic dilated cardiomyopathy (71). Sugamori *et al* (72) reported increased concentrations of $^{\bullet}NO$ metabolites and tumor necrosis factor α (TNFα) in proportion with the severity of heart failure in patients with dilated cardiomyopathy. In these patients enhanced production of basal $^{\bullet}NO$ could be caused by iNOS which can generate large amounts of $^{\bullet}NO$ and is induced by cytokines. However, plasma concentrations of $^{\bullet}NO$ metabolites are affected by exogenous factors like drugs (nitrates, ACE inhibitors), diet, and renal excretion. Therefore, increased concentrations of $^{\bullet}NO$ metabolites may not reflect actual tissue $^{\bullet}NO$ synthesis. In this connection, iNOS expression and activity could more actually reflect the $^{\bullet}NO$ status. Drexler *et al* (73) found increased iNOS gene expression in failing human myocardium, but eNOS mRNA was decreased. Additionally, neuronal NOS (nNOS) gene expression is also increased in failing myocardium (74). However, increased iNOS and nNOS expression does not reflect the actual amount of bioavailable $^{\bullet}NO$ produced. Because of the phenomenon of "NOS uncoupling", the enzyme produce $^{\bullet}O_2^-$ rather than $^{\bullet}NO$. In this setting, $^{\bullet}NO$ is inactivated by $^{\bullet}O_2^-$ in a reaction that can generate $ONOO^-$ so that bioactive $^{\bullet}NO$ concentrations are decreased. And, $ONOO^-$ is a toxic species itself that can damage various proteins by nitration and oxidation reactions.

In summary, CHF, regardless of etiology, is associated with an increase in oxidative / nitrosative stress, and an increase in ROS / RNS, a relative deficiency in the endogenous antioxidant reserve, or both may be one of the contributing factors in the pathogenesis of heart failure.

## 4. MECHANISMS AND CONSEQUENCES OF INCREASED OXIDATIVE / NITROSATIVE STRESS IN CHF

A number of mechanisms have been suggested for this situation. Most patients with CHF exhibit excessive activation of the sympathetic nervous system at rest or during exercise; consequently there is an increase in the circulating catecholamines. Auto-oxidation of catecholamines, which are abundantly present in plasma in CHF, can provide oxygen free radicals through adrenochrome formation (30,75). It has been suggested that, when mechanisms for metabolism of circulating catecholamines are saturated or impaired, increased free radical and adrenochrome production may become available in the body (76). Adrenochrome has been reported to affect cardiac function and viability (77). Free radicals may reveal cardiotoxic effect by causing peroxidation of membrane phospholipids, which can result in permeability changes in the membrane, as well as intracellular calcium overload and by oxidation of sulfhydryl groups of membrane proteins (78). Furthermore, free radicals are capable of reducing beta - receptor density in cardiac tissue (79). In fact, the ability of the failing heart to respond to endogenous or exogenous catecholamines becomes markedly attenuated (80).

During CHF there is an increase in the production of prostaglandin $E_2$ and $I_2$ through activation of phospholipases, and the production of these prostaglandins is associated with the production of free radicals through the arachidonic acid metabolic pathway (49,81**).** In CHF, recurring ischemia - reperfusion cycles may contribute to ROS generation. In the capillary endothelial cell, the enzyme xanthine dehydrogenase (XD) is converted to xanthine oxidase (XO) during ischemia. This enzyme, by using oxygen, catalyzes the conversion of xanthine and hypoxanthine to uric acid. On reperfusion, the delivered oxygen is reduced by XO system, producing ROS. At the same time, serum levels of uric acid, the product of xanthine-dehydrogenase / oxidase, are elevated in patients with CHF (82). Mitochondrial electron - transport chain is another source for ROS in the failing heart. The density of mitochondria in cardiac myocytes and the high rate of oxidative phosphorylation can result in a substantial flux of $^{\bullet}O_2^-$. During ischemia, the components of electron – transport chain are reduced, increasing electron leakage from the respiratory chain, which reacts with residual molecular oxygen and forms superoxide radical (16). Reperfusion re-energizes the mitochondria, but electron egress through cytochrome oxidase is reduced because of the lack of adenosine diphosphate (ADP), forming oxygen radicals (83).

In CHF, there is also an increase in angiotensin II. Angiotensin II stimulates the production of ROS and activates molecules associated with redox regulation in the vasculature (34) and heart (84). In rats made hypertensive by angiotensin II infusion, superoxide production and NAD(P)H oxidase expression are increased (34). It has been demonstrated that a major source of oxygen radical production in vascular cells is a membrane-bound, flavin-containing NADH/NADPH-dependent oxidase, which is regulated *in vitro* and *in vivo* by angiotensin II (85). Therefore, in CHF, in which the renin angiotensin system is activated, one would expect superoxide production to be increased.

On the other hand, myocyte cell loss through apoptosis, programmed cell death has been reported in end-stage heart failure and adriamycin cardiomyopathy (86). More recently, increased oxidative stress has been shown to promote apoptosis, and antioxidants have been shown to inhibit this process (87). Increased tumor necrosis factor (TNFα), natriuretic peptides, and angiotensin II, may contribute to apoptosis in human CHF (88). $^{\bullet}$NO regulates apoptosis in two opposite way. Physiological $^{\bullet}$NO concentrations seem to suppress the apoptotic pathway, while excessive $^{\bullet}$NO concentrations may overwhelm cellular protective mechanisms and exert cytotoxic and pro-apoptotic effects in patients with CHF (89). Additionally, increased $^{\bullet}$NO in CHF reacts with thiols and produces S-nitrosothiols. S-nitrosothiols are potent inducers of apoptosis in the heart (90). Thiol groups are reactive constituents of protein molecules and essential in the protection against the deleterious effects of ROS. Many enzymes and ion pumps e.g., $Ca^{2+}$-ATPase of sarcoplasmic reticulum, $Na^+K^+$-ATPase of plasma membrane, and creatine kinase, include essential SH groups in or near their active sites. Therefore, thiol oxidation or nitrosilation may exert deleterious effects on the activities of these enzymes and ion pumps of cardiomyocytes and disrupts excitation-contraction coupling (44, 45). In the myocytes, ion pumps regulate especially calcium cycle responsible for normal systolic and diastolic function. Moreover, in the conditions of low oxygen saturation of CHF, ROS and s-nitrosohemoglobin are generated from hemoglobin and these alterations accompanying with oxidase activity of hemoglobin contribute to tissue ischemia (91).

Additionally, oxidative stress may contribute to endothelial dysfunction in heart failure, pathology with growing interest in the setting of heart failure. $^{\bullet}$NO is the main molecule derived from endothelium that (in addition to the regulation of vascular tone) inhibits platelet activity, vascular smooth muscle cell growth, and adhesion of inflammatory cells to the endothelial surface (92-94). Increased oxidative stress in the coronary vascular bed in CHF not only deplete bioactive $^{\bullet}$NO and causes endothelial dysfunction, but also impairs coronary flow reserve (95-96). Elevated levels of ROS, especially superoxide anion, deplete bioavailable $^{\bullet}$NO and exacerbate local oxidative / nitrosative stress by reacting directly with $^{\bullet}$NO to form peroxynitrite ($ONOO^-$), which, in turn, imparts further oxidative /

nitrosative injury to the endothelium by uncoupling of NOS, thereby switching a beneficial enzyme for tissue to an enzyme that may initiate or even accelerate the oxidative / nitrosative stress (97). Moreover, decreased $^{\bullet}NO$ bioavailability disrupts vascular endothelium by rising in adhesion on the surface of endothelium for neutrophils (98). $^{\bullet}NO$ induces a concentration-dependent biphasic contractile response with low concentrations result in a positive inotropic effect, but high concentrations cause a negative inotropic effect (73). Therefore, negative inotropic effect of high $^{\bullet}NO$ concentrations due to iNOS activation may be responsible for contractile dysfunction in CHF patients.

Reduced coronary flow reserve in patients with CHF has been reported to be associated with myocardial ischemia (99), suggesting that a reduced coronary flow reserve in CHF may initiate myocardial hypoperfusion and thus lead to the progression of CHF. There is a close relationship between activation of neutrophils and inflammatory response. Activated neutrophils are sources of ROS / RNS and cytokines. Overexpressed cytokines such as TNFα produce ROS / RNS in CHF (72,100). Elevated angiotensin II concentration resulted from the activation of renin – angiotensin axis, causes suppression of $^{\bullet}NO$ synthesis (101), in addition to stimulation of the pro-inflammatory cytokines and ROS / RNS generation (84, 85). Increased ROS - generating capacity in neutrophils from patients with heart failure (49, 102) may be based on these alterations. In addition, the activation of neutrophils and migration to areas of inflammation may play a significant role in the immunologic response of chronic heart failure (103).

Extracellular matrix undergoes extensive and continuous turnover in the transition to heart failure and during the progression of left ventricular dysfunction. Matrix metalloproteinases (MMP) are extracellular proteinases stored in tissue and play a role in extracellular matrix turnover. The activity of MMPs is tightly controlled in normal circumstances. Increased oxidative / nitrosative stress have also been implicated in the activation of matrix metalloproteinases (MMP). Activation of MMPs by $ONOO^-$ result in progression of heart failure through ventricular remodeling, dilation and increased fibrosis which contribute to diminished systolic performance (104-105). Another enzyme activated by $ONOO^-$ (also $^{\bullet}OH$ and $H_2O_2$) is Poly (ADP-ribose) polymerase (PARP). PARP is an energy-consuming, abundant nuclear enzyme with complex regulatory functions. PARP is a DNA sensor and signaling enzyme and binds both single- and double- strand DNA breaks; when becomes activated it transfers ADP ribose units to nuclear proteins so that intracellular oxidized nicotinamide adenine dinucleotide ($NAD^+$) and ATP are depleted. These alterations result in cell dysfunction and cell death via slowing the rate of glycolysis and mitochondrial respiration. It has been reported that oxidative / nitrosative stress triggers the activation of the PARP via DNA strand breakage (105,106).

## 5. CONCLUSIONS

In summary, although ROS / RNS are not totally responsible for CHF, the evidence suggests that their involvement often appears to play an important role by increasing each other in the disease process. Increased oxidative / nitrosative stress may play an important role in myocardial remodeling, cardiomyocyte apoptosis, endothelial dysfunction, myocardial excitation-contraction uncoupling, fatal ventricular arrhythmias, and sudden death in patient with CHF. This situation opens up potentially new, simple, and attractive prevention and treatment approaches. In fact, there is growing potential benefit in therapies that can decrease oxidative / nitrosative stress in CHF. At least, many cardiovascular agents with characteristics against oxidative / nitrosative stress currently used, e.g., ACE inhibitors, statins, calcium channel blockers, beta blockers, probucol and hydralazine, have already given hopeful results. However, the results should be interpreted carefully because of the commercial motivation of medicine companies.

In general, antioxidant supplements (vitamin C and E), enzyme inhibition (inhibitors of XO, iNOS, MMP, PARP), thiol agents (N-acetylcysteine, dimethylthiourea, thioredoxin), some minerals (selenium, magnesium) have potential benefit in therapy of CHF. However, still there are very few large, prospective, longitudinal, cohort randomized-controlled multicenter studies performed with these agents.

## 6. References


[1] Jessup M, Brozena S. (2003) Heart failure. N. Eng. J. Med. 348, 2007-2018.

[2] Braunwald E, Bristow MR. (2000) Congestive heart failure: Fifty years of progress. Circulation 102, IV-14 – IV-23.

[3] Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A, Deckers JW, Witteman JC, Stricker BH. (2004) Quantifying the the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure. The Rotterdam Study. Eur. Heart J. 25, 1614-1619.

[4] McKenzie DB, Cowley AJ. (2003) Drug therapy in chronic heart failure. Postgrad. Med. J. 79, 634-642.

[5] Cowie MR, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, Suresh V, Sutton GC. (1999) Incidence and aetiology of heart failure; a population-based study. Eur. Heart. J. 20, 421-428.

[6] Cowie MR, Fox KF, Wood DA, Metcalfe C, Thompson SG, Coats AJ, Poole-Wilson PA, Sutton GC. (2002) Hospitalization of patients with heart failure: a population-based study. Eur. Heart. J. 23, 877-885.

[7] Halliwell B. (1993) The role of oxygen radicals in human disease, with particular reference to the vascular system. Haemostasis 23(Suppl 1), 118-126.

[8] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. (2006) Biomarkers of oxidative damage in human disease. Clin. Chem. 52, 601-623.

[9] Freeman BA, Crapo JD. (1982) Biology of disease. Free radicals and tissue injury. Lab. Invest. 47, 412-426.

[10] Herbst U, Toborek M, Kaiser S, Mattson MP, Hennig B. (1999) 4-Hydroxynonenal induces dysfunction and apoptosis of cultured endothelial cells. J. Cell. Physiol. 181, 295–303.

[11] Cai H, Harrison DG. (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation 87, 840-844.

[12] Halliwell B. (1991) Reactive oxygen species in livins systems: source, biochemistry, and role of in human disease. Am. J. Med. 91(Suppl), 14S-22S.

[13] Rowley DA, Halliwell B. (1982) Superoxide-dependent formation of hydroxyl radicals in the presence of thiol compounds. FEBS Lett . 138, 33-36.

[14] Braunwald E, Kloner RA. (1985) Myocardial reperfusion: a double edged sword. J. Clin. Invest. 76, 1713-1719.

[15] McCord JM. (1985) Oxygen-derived free radicals in post - ischaemic tissue injury. N. Eng. J. Med. 312, 159-163.

[16] McCord JM. (1988) Free radicals and myocardial ischaemia: overview and outlook. Free Rad. Biol. Med. 4, 9-14.

[17] Rossi F. (1986) •$O2^-$ forming NADPH oxidase of the phagocytes: nature, mechanisims of activation and function. Biochim. Biophys. Acta 853, 65-89.

[18] Chatcart MK. (2004) Regulation of superoxide anion production by NADPH oxidase in monocytes/macrophages: contributions to atherosclerosis. Arterioscler Thromb Vasc Biol 24, 23-28.

[19] Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, Shah AM. (2003) Increased myocardial NADPH oxidase activity in human heart failure. J. Am. Coll. Cardiol. 41, 2164-2171.

[20] Hsueh W, Needleman P. (1978) Sites of lipase activation and prostaglandin synthesis in isolated perfused rabbit hearts and hydronephrotic kidneys. Prostaglandins 16, 661-681.

[21] Misra HP, Fridovich I. (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170-3175.

[22] Singal PK, Beamish RE, Dhalla NS. (1983) Potential oxidative pathways of catecholamines in the formation of lipid peroxidation. Adv. Exp. Med. Biol. 161, 391-401.

[23] Kukreja RC, Kontos HA, Hess ML, Ellis EF. (1986) PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. Circ. Res. 59, 612–619.

[24] Goodman J, Hochstein PL. (1977) Generation of free radicals and lipid proxidation by redox cycling of adriamycin and daunamycin. Biochem. Biophys. Res. Commun. 77, 797-803.

[25] Pryor WA, Tamura M, Church DF. (1984) An ESR spin trapping study of the radicals produced in NO/olefin reactions: a mechanism for production of the apparently long-lived radicals in gas-phase cigarette smoke. J. Am. Chem. Soc. 106, 5073-5079.

[26] Kissner R, Nauser T, Bugnon P, Lye PG, Koppenol WH. (1997) Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. Chem. Res. Toxicol. 10, 1285-1292.

[27] Kirsch M, Korth HG, Sustmann R, de Groot H. (2002) The pathobiochemistry of nitrogen dioxide. Biol. Chem. 383, 389–399.

[28] Packer M. (1992) Pathophysiology of chronic heart failure. Lancet 340, 88-92.

[29] Packer M. (1992) The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J. Am. Coll. Cardiol. 20, 248-254.

[30] Ganguly PK. (1989) Catecholamines and cardiovascular disorders: pathophysiologic considerations. Am. Heart J. 118, 868-872.

[31] Leier CV. (1992) Regional blood flow in human congestive heart failure. Am. Heart J. 124, 726-738.

[32] Poole-Wilson PA. (1993) Relation of pathophysiologic mechanisms to outcome in heart failure. J. Am. Coll. Cardiol. 22(Suppl A), 22A-29A.

[33] Tan LB, Jalil JE, Pick R, Janicki JS, Weber KT. (1991) Cardiac myocyte necrosis induced by angiotensin II. Circ. Res. 69, 1185-1195.

[34] Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. (1994) Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ. Res. 74, 1141-1148.

[35] Griendling KK, FitzGerald GA. (2003) Oxidative stress and cardiovascular injury. Part II. Animal and human studies. Circulation 108, 2034-2040.

[36] Cotton JM, Kearny MT, Shah AM. (2002) Nitric oxide and myocardial function in heart failure: friend or foe? Heart 88, 564-566.

[37] Damy T, Ratajczak P, Shah AM, Camors E, Marty I, Hasenfuss G, Marotte F, Samuel JL, Heymes C. (2004) Increased neuronal nitric oxide synthase-derived NO production in the failing human heart. Lancet 363, 1365-1367.

[38] McMurray J, McLay J, Chopra M, Bridges A, Belch JJF. (1990) Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease. Am. J. Cardiol. 65, 1261-1262.

[39] Belch JJF, Bridges AB, Scott N, Chopra M. (1991) Oxygen free radicals and congestive heart failure. Br. Heart J. 65, 245-248.

[40] McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ. (1993) Evidence of oxidative stress in chronic heart failure in humans. Eur. Heart J. 14, 1493-1498.

[41] Diaz-Velez CR, Garcia-Castineiras S, Mendoza-Ramos E, Hernandez-Lopez E. (1996) Increased malondialdehyde in peripheral blood of patients with congestive heart failure. Am. Heart J. 131, 146-152.

[42] Nishiyama Y, Ikeda H, Haramaki N, Yoshida N, Imaizumi T. (1998) Oxidative stress is related to exercise intolerance in patients with heart failure. Am. Heart J. 135, 115-120.

[43] Moriyama Y, Yasue H, Yoshimura M, Mizuno Y, Nishiyama K, Tsunoda R, Kawano H, Kugiyama K, Ogawa H, Saito Y, Nakao K. (2000). The plasma level of dehydroepiandrosterone sulfate are decreased in patients with chronic heart failure in proportion to the severity. J. Clin. Endocrin. Metab. 85, 1834, 1840.

[44] Yucel D, Aydoğdu S, Şeneş M, Topkaya BÇ, Nebioğlu S. (2002) Evidence of increased oxidative stress in patients with dilated cardiomyopathy. Scand. J. Clin. Lab. Invest. 62, 463-468.

[45] Yucel D, Aydogdu S, Çehreli S, Saydam G, Canatan H, Şeneş M, Topkaya BÇ, Nebioğlu S. (1998) Increased oxidative stress in dilated cardiomyopathic heart failure. Clin. Chem. 44, 148-154.

[46] Mak S, Newton GE. (2001) The oxidative stress hypothesis in congestive heart failure. Chest 120, 2035-2046.

[47] Mak S, Lehotay DC, Yazdanpanah M, Azevedo ER, Liu PP, Newton GE. (2000) Unsaturated aldehydes including 4-OH-nonenal are elevated in patients with congestive heart failure. J. Card. Fail. 6, 108-114.

[48] Esterbauer H, Schaur RJ, Zollner H. (1991) Chemistry and biochemistry of 4-hydoxynonenal, malondialdehyde and related aldehydes. Free Radic. Biol. Med. 11, 81-128.

[49] Prasad K, Gupta JB, Karla J, Bharadwaj B. (1992) Oxygen free radicals in volume overload heart failure. Mol. Cell. Biochem. 111, 55-59.

[50] Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. (1998) Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure. Circulation 28, 1536-1539.

[51] Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, Utsumi H, Machida Y, Egashira K, Takeshita A. (2000) Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. Circ. Res. 86, 152–157.

[52] Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, Uchida K, Arimura K, Egashira K, Takeshita A. (1999) Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ. Res. 85, 357-363.

[53] Nonaka-Sarukawa M, Yamamoto K, Aoki H, Takano H, Katsuki T, Ikeda U, Shimada K. (2003) Increased urinary 15-F2t-isoprostane concentrations in patients with non-ischaemic congestive heart failure: a marker of oxidative stress. Heart 89, 871-874.

[54] Dhalla AK, Singal PK. (1994) Antioxidant changes in hypertrophied and failing guinea pig hearts. Am. J. Physiol. 266, H1280-H1285.

[55] Hill MF, Singal PK. (1996) Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. Am. J. Physiol. 148, 291-300.

[56] Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Orman AS, Jeejeebhoy KN (1998) Increased oxidative stress in patients with congestive heart failure. J. Am. Coll. Cardiol. 31, 1352-1356.

[57] Meister A, Anderson ME. (1983) Glutathione. Annu. Rev. Biochem. 52, 711-760.

[58] McMurray J, Chopra M, Reglinski J, Smith WE, Dargie HJ. (1990) Disclosure of increased free radical activity in heart failure by biochemical markers and proton nuclear magnetic resonance spectroscopy. Br. Heart J. 64, 60.

[59] Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, Harrison DG, Hornig B, Drexler H. (2002) Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure. Role of xanthine – oxidase and extracellular superoxide dismutase. Circulation 106, 3073-3078.

[60] Shiomi T, Tsutsui H, Matsusaka H, Murakami K, Hayashidani S, Ikeuchi M, Wen J, Kubota T, Utsumi H, Takeshita A. (2004) Overexpression of glutathione peroxidase (GPO) prevents left ventricular remodeling and failure after myocardial infarction in mice. Circulation 109, 544-549.

[61] Oster O, Prellwitz W, Kasper W, Meinertz T. (1983) Congestive cardiomyopathy and the selenium content of serum. Clin. Chim. Acta 128, 125-132.

[62] Thomson CD. (2004) Assessment of requirements for selenium and adequacy of selenium status: a review. Eur. J. Clin. Nutr. 58, 391-402.

[63] Baumer AT, Flesch M, Wang X, Shen Q, Feuerstein GZ, Bohm M. (2000) Antioxidative enzymes in human hearts with idiopathic dilated cardiomyopathy. J. Mol. Cell. Cardiol. 32, 121-130.

[64] e Lorgeril M, Salen P, Accominotti M, Cadau M, Steghens JP, Boucher F, de Leiris J. (2001) Dietary and blood antioxidants in patients with chronic heart failure. Insights into the potential importance of selenium in heart failure. Eur. J. Heart Fail. 3, 661-669.

[65] Serdar A, Yesilbursa D, Serdar Z, Dirican M, Turel B, Cordan J. (2001) Relation of functional capacity with the oxidative stress and antioxidants in chronic heart failure. Congest. Heart Fail. 7, 309-311.

[66] Polidori MC, Savino K, Alunni G, Stahl W, Freddio M, Senin U, Sies H, Stahl W, Mecocci P. (2002) Plasma lipophilic antioxidants and malondialdehyde in congestive heart failure patients: relationship to disease severity. Free Radic. Biol. Med. 32, 148-152.

[67] de Belder AJ, Radomski MW, Why HJ, Richardson PJ, Bucknall CA, Salas E, Martin JF, Moncada S. (1993) Nitric oxide synthase activities in human myocardium. Lancet 341, 84–85.

[68] Balligand JL, Ungureanu-Longrois D, Simmons WW, Pimental D, Malinski TA, Kapturczak M, Taha Z, Lowenstein CJ, Davidoff AJ, Kelly RA. (1994) Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes. Characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes in vitro. J. Biol. Chem.269, 27580–27588.

[69] Shah AM, MacCarthy PA. (2000) Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol. Ther. 86, 49–86.

[70] Uncugil F, Öğüş E, Erbay AR, Düzoylum A, Yücel D. (2004) Nitric oxide metabolites and and cardiac troponin I levels in cardiomyopathies. Turk. J. Biochem. 29, 199-203.

[71] Rakhit RD, Tousoulis D, Lefroy DC, Davies GJ. (1999) Differential nitric oxide synthase activity in ischemic and idiopathic dilated cardiomyopathy. Am. J. Cardiol. 84, 737-738.

[72] Sugamori T, Ishibashi Y, Shimada T, Takahashi N, Sakane T, Ohata S, Kunizawa Y, Inoue S, Nakaamura K, Ohta Y, Shimizu H, Katoh H, Oyake N, Murqakami Y, Hashimoto M. (2002) Increased nitric oxide in proportion to the severity of heart failure in patients with dilated cardiomyopathy. Circ. Res. 66, 627-632.

[73] Drexler H, Kastner S, Strobel S, Studer R, Brodde OE, Hasenfuss G. (1998) Expression, activity, and functional significance of inducible nitric oxide synthase in the failing human heart. J. Am. Coll. Cardiol. 32, 955-963.

[74] Damy T, Ratajczak P, Shah AM, Camors E, Marty I, Hasenfuss G, Marotte F, Samuel JL, Heymes C. (2004) Increased neuronal nitric oxide synthase – derived NO production in the failing human heart. Lancet 363, 1365-1367.

[75] Ganguly PK, Beamish RE, Dhalla NS. (1989) Catecholamine cardiotoxicity in pheochromacytoma. Am. Heart J. 117, 1399-1400.

[76] Ganguly PK. (1991) Antioxidant therapy in congestive heart failure: is there any advantage? J. Intern. Med. 229, 205-208.

[77] Singal PK, Dhillon KS, Beamish RE, Kapur N, Dhalla NS. (1982) Myocardial cell damage and cardiovascular changes due to IV infusion of adrenochrome in rats. Br. J. Exp. Pathol. 63, 167-176.

[78] Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O. (1991) Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. Am. J. Med. 91(Suppl 3C), 3C-95S – 3C-105S.

[79] Kaneko M, Chapman DC, Ganguly PK, Beamish RE, Dhalla NS. (1991) Modification of cardiac adrenergic receptors by oxygen free radicals. Am. J. Physiol. 260, H821-H826.

[80] Covell JW, Chidsey CA, Braunwald E. (1966) Reduction in the cardiac response to postganglionic sympathetic nevre stimulation in experimental heart failure. Circ. Res. 19, 51-8.

[81] Dzau VJ, Packer M, Lilly LS, Swartz SL, Hollenberg NK, Williams GH. (1989) Prostaglandins in severe congestive heart failure. Relation to activation of the renin-angiotensin system and hyponatremia. N. Eng. J. Med. 310, 347-352.

[82] Sawyer DB, Colucci WS. (2000) Mitochondrial oxidative stress in heart failure. "Oxygen wastage" revisited. Circ. Res. 86, 119-120.

[83] Boveris A, Cadenas E, Stoppani AO. (1976) Role of ubiquinone in the mitochondrial generation of hydrogen peroxide. Biochem. J. 156, 435–444.

[84] Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, Namba M. (1998) Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. Circulation 98, 794–799.

[85] Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. (1996) Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J. Clin. Invest. 97, 1916-1923.

[86] Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. (1996) Apoptosis in myocytes in end-stage heart failure. N. Eng. J. Med. 335, 1182-1189.

[87] Kumar D, Lou H, Singal PK. (2002) Oxidative stress and apoptosis in heart dysfunction. Herz 27, 662-668.

[88] Stefanec T. (2000) Endothelial apoptosis. Could it have a role in the pathogenesis and treatment of disease? Chest 117, 841-854.

[89] Kim YM, Bombeck CA, Billiar TR. (1999) Nitric oxide as a bifunctional regulator of apoptosis. Circ. Res. 84, 253-256.

[90] Jekabsone A, Dapkunas Z, Brown GC, Borutaite V. (2003) S-nitrosothiol-induced rapid cytochrome c release, caspase activation and mitochondrial permeability transition in perfused heart. Biochem. Pharmacol. 66, 1513-1519.

[91] Hare JM, Stamler JS. (2005) NO/redox disequilibrium in the failing heart and cardiovascular system. J. Clin. Invest. 115, 509-517.

[92] Moncada S, Higgs A. (1993) L-arginine-nitric oxide pathway. N. Eng. J. Med. 329, 2002-2012.

[93] Kubes P, Suzuki M, Granger DN. (1991) Nitric oxide: an endothelial modulator of leukocyte adhesion. Proc. Natl. Acad. Sci. USA 88, 587-590.

[94] Lopez-Farre Caramelo C, Esteban A, Alberola ML, Milas I, Monton M, Casado S. (1995) Effects of aspirin on platelet-neutrophil interactions: role of nitric oxide and endothelin-1. Circulation 91, 2080-2088.

[95] Arimura K, Egashira K, Nakamura R, Ide T, Tsutsui H, Shimokawa H, Takeshita A. (2001) Increased inactivation of nitric oxide is involved in coronary endothelial dysfunction in heart failure. Am. J. Physiol. Heart Circ. Physiol. 280, H68-H75.

[96] Nakamura R, Egashira K, Arimura K, Machida Y, Ide T, Tsutsui H, Shimokawa H, Takeshita A. (2001) Increased inactivation is involved in impaired coronary flow reserve in heart failure. Am. J. Physiol. Circ. Physiol. 281, H2619-H2625.

[97] Munzel T, Daiber A, Ulrich V, Mulsch A. (2005) Vascular consequences of endothelial nitric oxide synthase uncoupling fort he activity and expression of the soluble guanylyl cyclase and the cGMP – dependent protein kinase. Arterioscler. Thromb. Vasc. Biol. 25, 1551-1557.

[98] Niu XF, Smith W, Kubes P. (1994) Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion to neutrophils. Circ. Res. 74, 1133-1140.

[99] van den Heuvel AF, van Veldhuisen DJ, van der Wall EE, Blanksma PK, Siebelink HM, Vaalburg WM, van Gilst WH, Crijns HJ. (2000) Regional myocardial blood flow reserve impairment and metabolic changes suggesting myocardial ischemia in patients with idiopathic dilated cardiomyopathy. J. Am. Coll. Cardiol. 35, 19-28.

[100] Machida Y, Kubota T, Kawamura N, Funakoshi H, Ide T, Utsumi H, Li YY, Feldman AM, Tsutsui H, Shimokawa H, Takeshita A. (2003) Overexpression of tumor necrosis factor-α increases production of hydroxyl radical in murine myocardium. Am. J. Physiol. Heart Circ. Physiol. 284, H449-H455.

[101] Das UN. (2005) Is angiotensin-II an endogenous pro-inflammatory molecule? Med. Sci. Monit. 11, RA155-RA162.

[102] Ellis GR, Anderson RA, Lang D, Blackman DJ, Mornis RHK, Morris-Thurgood J, McDowell IF, Jackson SK, Lewis MJ. (2000) Neutrophil superoxide anion-generating capacity, endothelial function and oxidative stress in chronic heart failure: effects of short- and long-term vitamin C therapy. J. Am. Coll. Cardiol. 36, 1474-1482.

[103] Mann DL. (1999) Inflammatory mediators in heart failure. homogeneity through heterogeneity. Lancet 353, 1812-1813.

[104] Moshal KS, Tyagi N, Moss V, Henderson B, Steed M, Ovechkin A, Aru GM, Tyagi SC. (2005) Early induction of matrix metalloproteinase-9 transduces signaling in human heart end stage failure. J. Cell. Mol. Med. 9, 704-713.

[105] Pacher P, Schulz R, Liaudet L, Szabo C. (2005) Nitrosative stress and pharmacological modulation of heart failure. Trends. Pharmacol. Sci. 26, 302-310.

[106] Xiao C-Y, Chen M, Zsengeller Z, Li H, Kiss L, Kollai M, Szabo C. (2005) Poly(ADP-ribose) polymerase promotes cardiac remodeling, contractile failure, and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure. J. Pharmacol. Exp. Ther. 312, 891-898.