MODULATION OF OXIDATIVE STRESS AND ANTIOXIDANTS BY LOSARTAN IN HEART FAILURE

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BY

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LIST OF ABBREVIATIONS

ACE	- Angiotensin converting enzyme
AT_1	- Angiotensin II type 1
AT_2	- Angiotensin II type 2
ADP	- Aortic diastolic pressure
ANG II	- Angiotensin II
ASP	- Aortic systolic pressure
BHA	- Butylated hydroxyanisole
CAT	- Catalase
cDNA	- Complementary deoxyribonucleic acid
CHF	- Congestive heart failure
CuZnSOD	- Copper zinc superoxide dismutase
DAG	- Diacylglycerol
DNA	- Deoxyribonucleic acid
ECM	- Extracellular matrix
ELITE	- Evaluation of losartan in the elderly
GSH	- Reduced glutathione
GSHPx	- Glutathione peroxidase
GSSG	- Oxidized glutathione
HPLC	- High performance liquid chromatography
IL-1α	- Interleukin-1α
IP_3	- Inositol 1, 4, 5-triphosphate
LVEDP	- Left ventricular end diastolic pressure
LVPSP	- Left ventricular peak systolic pressure
MDA	- Malondialdehyde
MHC	- Myosin heavy chain
MI	- Myocardial infarction
MnSOD	- Managenese superoxide dismutase
mRNA	- Messenger ribonucleic acid
NO	- Nitric oxide
PIP_2	- Phosphatidyinositol, 4, 5, biphosphate
PMI	- Post-myocardial infarction
PRFO	- Partially reduced forms of oxygen
RAS	- Renin Angiotensin system
SDS	- Sodium dodecyl sulfate
SOD	- Superoxide dismutase
TBARS	- Thiobarbituric acid reactive substances
TGF	- Transforming growth factor
TNF-α	- Tumor necrosis factor

ABSTRACT

In patients surviving a myocardial infarction (MI), the heart undergoes a remodeling process characterized by hypertrophy which can also lead to heart failure. Although hypertrophy is an early response that may temporarily preserve cardiac function, numerous studies have suggested that this long term process of remodeling is also associated with an increase in oxidative stress and cardiac decompensation. Although increased oxidative stress has been suggested to be involved, the precise mechanism/s underlying changes in the oxidative stress leading to the transition of the compensatory hypertrophy phase to the failure stage are poorly understood. Activation of the renin-angiotensin system (RAS) is another compensatory mechanism to sustain heart function in MI patients. However, prolonged activation of the RAS is also deleterious for sustained cardiac function. A selective and complete blockade of the RAS at the angiotensin II type I receptor (AT_1) site has been reported to improve cardiac function and survival in patients. However myocardial antioxidants and oxidative stress changes during the modulation of remodeling of the heart by blockade of the AT_1 receptors have not been examined.

The objectives of the present research on rat hearts subsequent to MI therefore, were to: i) characterize changes in the oxidative stress and enzymatic antioxidants, (superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase) in relation to their mRNA abundance and protein content; ii) study changes in nonenzymatic antioxidants at different stages of heart failure in relation to cardiac function in order to have a more comprehensive information about the antioxidant reserve, and iii) Study the effects of RAS inhibition at the AT_1 receptor site by losartan on the myocardial enzymatic and non-enzymatic antioxidants

(SOD, GSHPx, catalase, vitamins A and E) and oxidative stress (Lipid hydroperoxides, reduced and oxidized glutathione and the redox ratio) in relation to the changes in hemodynamic function during the sequelae of congestive heart failure.

MI in rats was produced by ligation of the left coronary artery and all the animals were hemodynamically assessed at different post-myocardial infarction (PMI) durations. Antioxidant enzyme activities as well as mRNA abundance were analyzed in controls and in the 1-week PMI; 4-week PMI and 16-week PMI hearts. In the 4-week PMI group, LVEDP was elevated and LVPSP was depressed with no signs of congestions. ADP and ASP in the 4-week sham control and PMI group were not different from each other. However, a significant decline in ASP accompanied by the greatest elevation in LVEDP and depression in LVPSP was seen in the 16-week PMI group. Pulmonary edema and liver congestion was noted in the 16-week PMI group and these animals also displayed overt clinical signs of heart failure consisting of dyspnea and lethargic behavior. Losartan treatment (2 mg/ml in drinking water, daily) was started at 4 weeks and continued for 12 weeks. Losartan treatment modulated the increase in LVEDP and normalized LVPSP in the 16-week PMI group. Furthermore, there was no lung and liver congestion in the losartan treated MI group.

The SOD enzyme activity remained unchanged at 1-week PMI, but was decreased by about 35% at 4 weeks and about 42% at 16 weeks. The mRNA levels for superoxide dismutase showed a biphasic response, where it was reduced by about 40% at 1-week PMI. At 4 weeks PMI, the levels returned back to the control level. At 16 weeks PMI, the levels in the PMI group were reduced by about 73% of the control value. The GSHPx enzyme

activity was slightly increased at 1 week PMI, but was reduced by about 26% and 38% at 4 and 16 weeks PMI duration, respectively. GSHPx mRNA levels remained unchanged at all time points. Catalase enzyme activity at 1 week was not changed, however, at 4 and 16 weeks PMI the activity was decreased by about 24% and 25% respectively. The mRNA levels for catalase remained unchanged at 1 and 4 weeks PMI and was significantly reduced by about 44% at 16 weeks PMI as compared to the controls.

SOD activity, which was decreased in the 16-week untreated PMI group, losartan treatment had no beneficial effect on this activity. GSHPx activity which was depressed in the untreated PMI group showed significant improvement in the PMI group treated with losartan such that the values at 16 weeks were no longer different than the control group. Catalase activity was significantly decreased in the 16-week untreated PMI group. It showed some improvement with losartan treatment but the increase was not significant. Interestingly, losartan treatment in the 16-week control animals resulted in a significant increase in the catalase activity. The protein levels for MnSOD, CuZnSOD, GSHPx and catalase at 1 and 16 weeks were also examined. The protein level for all these enzymes remained unchanged in the 1- and 16-week PMI groups compared to their respective controls. However, the protein levels for catalase was significantly increased in the control and PMI groups treated with losartan.

Myocardial vitamin A (retinol) and E (tocopherol) content were also analyzed at 4 and 16 weeks PMI duration. Vitamin A and E levels remained unchanged in the 4-week PMI group, but were significantly decreased in the 16-week PMI group. Treatment with losartan resulted in a significant increase in retinol levels in the 16-week treated PMI group. Losartan

treatment did not have any influence on tocopherol concentration in either of the groups. Collectively, data on the enzymatic and non-enzymatic antioxidants suggested a significant improvement in the myocardial antioxidant reserve of the MI animals treated with losartan.

Myocardial GSH and GSSG content in the 4-week untreated PMI group were no different as compared to the respective control. At 16 weeks PMI duration, the GSH content was decreased by about 40% and GSSG was increased by about 114% as compared to its respective sham control group. The GSH content in the losartan-treated control as well as PMI groups was significantly improved. GSSG content in the losartan-treated PMI group was decreased and the values no longer were different from its respective sham control group. Redox ratio was also assessed in the 4- and 16-week sham control and PMI group with and without losartan treatment. The redox ratio remained unchanged in the 4-week PMI group compared to its control but it was significantly depressed in the 16-week PMI group compared to its sham control group. This ratio was significantly improved in both the control and PMI groups treated with losartan. Lipid hydroperoxide content was significantly higher in the 4- and 16-week PMI group compared to its sham control group. This increase in the lipid hydroperoxide content was significantly attenuated in the 16-week PMI group treated with losartan. These data suggested a significant decrease in the oxidative stress due to losartan treatment of the MI animals.

It is concluded that inhibition of the RAS at the AT_1 receptor site with losartan, in addition to reducing cardiac remodeling and improving hemodynamic function, reduces oxidative stress and improves myocardial endogenous antioxidants subsequent to myocardial infarction. The study suggests a newer role for losartan in the treatment of heart failure.

Although changes in the SOD and catalase activities during heart failure correlated with changes in mRNA for these enzymes, the precise mechanism/s for decrease in oxidative stress and improvement in antioxidant reserve after losartan treatment is/are unclear at this time.

I. INTRODUCTION

Heart failure is a pathophysiologic state in which the failing heart is unable to deliver adequate amount of blood to the metabolizing tissues. Despite the advances made in the therapeutic strategies for heart failure, it still remains the number one cause of death. Some of the most common causes of heart failure include ischemic heart disease, valvular heart disease, hypertension, alcohol cardiomyopathy, myocarditis and drug-induced cardiomyopathy. When the heart fails, a complex sequence of compensatory mechanisms come into play to maintain the cardiac output.

Following a sudden occlusion of the coronary artery, the heart undergoes a remodeling process which is characterized by hypertrophy and heart failure. Although, hypertrophy is considered as an early response to preserve cardiac function, it is known that the long term process of remodeling has a deleterious effect. A number of responses are associated with the failing of the heart. Initially these changes are important for maintaining cardiac output however, over a period of time, it becomes maladaptive and contributes to the progression of heart failure. Although the mechanisms involved in the transition of the compensatory phase to the failure stage are poorly understood, chronic activation of the sympathetic and the renin-angiotensin systems (RAS), appears to play a role. Without the therapeutic intervention, some of these compensatory mechanisms continue to be activated, ultimately leading to heart failure.

Recent data from both animal and patient studies have provided strong evidence for the role of increased oxidative stress in the development of heart failure. Both a deficit in the antioxidant reserve and an increase in free radical mediated injury have been reported in

various pathophysiological conditions such as ischemia-reperfusion and adriamycin and diabetic cardiomyopathies. A strong correlation between vitamin intake and reduced risk of coronary artery disease have also been provided. Using the rat coronary artery ligation model, our laboratory has reported a deficit in the myocardial endogenous antioxidant reserve and an increase in oxidative stress which are associated with a poor cardiac function. However, there have been no studies examining the changes in antioxidant enzymes at the mRNA and protein level at different stages of post-myocardial infarction (PMI) duration. Therefore, we undertook this study, to characterize changes in the antioxidant enzymes (MnSOD, CuZnSOD, GSHPx and catalase at the mRNA and protein level at different PMI durations.

It is also known that non-enzymatic antioxidants are also an important component of the antioxidant reserve. However, nothing is known with respect to changes in the vitamins A and E in the heart at different PMI durations. Thus, in any study of antioxidants, analysis of non-enzymatic antioxidants is important to obtain a more comprehensive information. A direct analysis of oxidative stress changes is also required to compliment the information obtained from the study of myocardial antioxidants.

In the face of decreased cardiac output, activation of the RAS results in a series of enzymatic reaction that converts angiotensin I to angiotensin II via the angiotensin converting enzyme (ACE). Angiotensin II causes vasoconstriction as well as leads to the release of aldosterone resulting in increased preload and afterload. ACE-inhibitors have proven to be an effective treatment for heart failure both in animal and patient studies. This class of drugs have been shown to prevent cardiac remodeling and to prolong survival in both MI patients

and MI animals. We also recently reported that inhibition of the RAS by inhibiting ACE with captopril in MI rats not only resulted in improved hemodynamic function but also maintained the antioxidant reserve and decreased oxidative stress. Since captopril also possesses some free radical scavenging property and since inhibition of ACE is also known to increase bradykinin the exact property of captopril offering protection is not clear. In addition to inhibiting the production of angiotensin II, ACE-inhibitors also cause activation of prostaglandin synthesis and influence bradykinin metabolism. Therefore, angiotensin II receptor blockers such as losartan have been preferred in the management of heart failure. Losartan is a first of the new class of angiotensin II antagonist which selectively and completely blocks angiotensin II type I (AT_1) receptors. Losartan has been reported to reduce afterload, improve cardiac function and improve survival without altering the humoral factors such as bradykinin, vasopressin and prostaglandin. Recently there have also been evidence supporting the concept of angiotensin II as a source of free radicals. In various in vitro studies, production of superoxide anion by angiotensin II has been found to be inhibited by losartan. However, there have been no studies to date on the effects of losartan on myocardial endogenous enzymatic and non-enzymatic antioxidants and oxidative stress at different post-MI durations in rats. Thus, one of the objective of this research was also to study the effects of a specific angiotensin II blocker, losartan on antioxidants and oxidative stress in congestive heart failure in rats following MI.

We used a rat MI model of coronary artery ligation to explore the effects of RAS inhibition at the AT_1 receptor site by losartan on myocardial antioxidants and oxidative stress changes in congestive heart failure in relation to the hemodynamic function. Myocardial

antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) activities and non-enzymatic antioxidants (vitamins A and E) and changes in oxidative stress (lipid hydroperoxide contents, reduced and oxidized glutathione and their ratio) were recorded at different post-surgical durations. Using the northern and western blot technique, mRNA abundance and protein content for these enzymes were also determined. Effects of losartan on all the above mentioned parameters were also studied in relation to cardiac function. The treatment was started at 4-week PMI and continued for 12 weeks.

The findings from this study advance our knowledge of the role of oxidative stress in the pathogenesis of heart failure. The modulation of antioxidant changes as well as oxidative stress by a selective blocker of angiotensin II coupled with the improvement of the hemodynamic function suggest a newer role for losartan in the treatment of heart failure.

II. LITERATURE REVIEW

1. Background

Heart failure is a clinical condition whereby the heart is unable to pump blood adequately to tissues and organs throughout the body. It is currently the most frequent diagnosis for admissions in hospitals and is a major health problem. Congestive heart failure refers to the buildup of fluid in the dependent tissues. In spite of the remarkable gains made in the patient care and management, the incidence of heart failure remains high. Because of the ageing population and an improvement in surgical and medical treatments, the number of patients living with the condition of heart failure is on the rise (Sullivan, 1994). The most common symptoms of heart failure include fatigue, shortness of breath, chest pain, weakness and swelling of the legs (Cohn, 1997; Williams, 1990). Coronary artery disease, cardiomyopathy, congenital heart disease, hypertension, myocarditis, heart valve defects and drug abuse are all known to contribute to the incidence of heart failure. Thus, we do need to develop a better and thorough understanding of the pathogenesis of heart failure, if we are to reduce the cost of this disease to our health care system as well as the quality of life.

Extensive research in the field of heart failure has provided insight into the cellular and molecular bases of heart failure. As the pump function of the heart is compromised due to a variety of reasons cited above, compensatory mechanisms that are activated to maintain cardiac output and enhance contractile function of the heart come into play. Some of these mechanisms include activation of the sympathetic and the renin-angiotensin systems (RAS). Activation of the sympathetic system maintains blood pressure through activation of the adrenergic receptors (Lechat, 1998). This results in increased contractility leading to an

increase in cardiac output. The stimulation of the RAS in combination with the sympathetic system leads to vasoconstriction and enhances salt and water retention (McAlpine and Cobbe, 1988; Lechat, 1998). However, prolonged activation of these systems has been known to cause cardiac dysfunction and failure (Francis *et al.*, 1984; Packer, 1992).

In heart failure, defects in receptors and different pumps and proteins responsible for calcium movement have been reported, which may result in ionic imbalance and calcium overload. Sarcolemmal sodium calcium exchanger, $Na^+ K^+$ ATPase activity and calcium pumps have been shown to be depressed during heart failure following myocardial infarction (Dhalla *et al.*, 1991; Dixon *et al.*, 1990; Makino *et al.*, 1988; Dixon *et al.*, 1992a; Dixon *et al.*, 1992b). Decrease in expression of sarcoplasmic reticulum Ca^{2+}ATPase, and calcium release channel and phospholamban has also been reported (Arai et. al 1993; Brillantes *et al.*, 1992; Mercadier *et al.*, 1990). Collectively, these findings suggest that there are changes in the sarcoplasmic reticulum function which results in a disturbance in calcium homeostasis. Numerous studies have reported altered protein and gene expression of some of the contractile proteins such as the myosin heavy chain isoforms (Nakao *et al.*, 1997). Reactivation of fetal genes such as β-myosin heavy chain (MHC) and α-skeletal actin in adults also takes place (Schwartz *et al.*, 1993; Boheler *et al.*, 1991). Increased expression of β MHC is associated with the lower myosin ATPase activity and slower shortening velocity (Schwartz *et al.*, 1993). High energy phosphate stores are also reduced in heart failure conditions (Ingwall, 1993). Furthermore, reduction in creatine kinase activity have also been reported in many forms of heart failure (Braunwald and Bristow, 2000; Ye *et al.*, 2001).

Thus a variety of subcellular mechanisms have been documented to be defective in heart failure, but the cause and effect relationship is largely unknown.

1.1 Remodeling

Remodeling refers to changes in the ventricular chamber subsequent to changes at the cellular and molecular levels. In response to an increased workload, the heart undergoes hypertrophy, which is characterized by changes in ventricular chamber size and geometry (Grossman and Lorell, 1993; Cohn, 1997; Anversa *et al.*, 1993; Pfeffer *et al.*, 1991; Pfeffer *et al.*, 1993). Factors influencing such remodeling include the sympathetic and RAS systems, cytokines and oxidative stress. Increased sympathetic activation although at first may be beneficial, over a period of time may lead to myocardial cell loss and fibrosis (Anversa *et al.*, 1992; Olivetti *et al.*, 1997). Increased catecholamine levels also cause downregulation of β adrenergic receptors resulting in impaired cardiac function. In this regard, downregulation of β_1 receptors with either no change or downregulation of β_2 receptors have been reported (Bristow *et al.*, 1986; Bristow, 1993). The local RAS also interacts with the sympathetic system resulting in increased catecholamine release, which further leads to cardiac dysfunction.

Following myocardial infarction (MI), the viable myocardium is replaced with connective tissue and "infarct expansion" occurs which further results in cardiac remodeling (Pfeffer *et al.*, 1991; Hutchins and Bulkley, 1978). Furthermore, myocyte cell lengthening leads to myocyte slippage, which results in the thinning of the ventricular wall (Olivetti *et al.*, 1990; Weisman *et al.*, 1988; Pfeffer *et al.*, 1991). This derangement of myocytes lead to ventricular dilatation and cardiac dysfunction (McKay *et al.*, 1986; Anversa *et al.*, 1993;

Pfeffer *et al.*, 1991). Rearrangement of the myocytes resulting from side to side slippage has also been suggested to account for the occurrence of cell death (Anversa *et al.*, 1992).An increased expression of a number of extracellular matrix (ECM) proteins, including collagen has been described in the failing heart leading to increased fibrosis and stiffening of the ventricle (Pelouch *et al.*,1 993; Dixon *et al.*, 1996). In a recent study it has been suggested that cardiac fibrosis could be due either to an increased collagen synthesis or reduced collagen degradation (Bishop *et al.*, 1994; Ju *et al.*, 1997). Cardiac fibrosis in the failing hearts have been shown to be reduced by an inhibition of the RAS using losartan (Smits *et al.*, 1992; Ju *et al.*, 1997; Dixon *et al.*, 2000; Schieffer *et al.*, 1994).

Increasing evidence from both animal (Bozkurt *et al.*, 1998) and human studies (Levine *et al.*, 1990) have reported elevated levels of cytokines such as tumor necrosis factor α (TNF-α), interleukin-1α (IL-1α) and atrial naturietic factor in MI and failure conditions. Increased concentrations of these cytokines were shown to have a direct correlation with the severity of failure (Ferrari *et al.*, 1998; Levine *et al.*, 1990; Givertz and Colucci, 1998). Recent studies have highlighted the involvement of cytokines such as TNF-α, IL-1β and IL-6 during the remodeling process (Ono *et al.*, 1998). The deleterious effect of TNF-α is receptor-mediated and also directly activates nitric oxide, which is cytotoxic to myocardial cells by virtue of its ability to produce free radicals and cause apoptosis (Blum and Miller, 1998). The pathological potential of TNF-α is demonstrated in a study where overexpression of TNF-α in the transgenic mice developed cardiomyopathy associated with apoptosis (Bryant *et al.*, 1998). In a recent study, Nakamura and colleagues (1998) demonstrated that TNF-α and Ang II induce hypertrophy in cultured neonatal cardiac myocytes through their

free radical producing ability, and administration of antioxidants such as BHA, vitamin E and catalase inhibited this effect of TNF-α and Ang II by scavenging the radicals. In addition to producing free radicals, it is also suggested that TNF-α is also activated by hydrogen peroxide (Meldrum *et al.*, 1998).

Autooxidation of catecholamines has also been suggested to cause cardiac dysfunction by virtue of producing free radicals (Singal *et al.*, 1982; Singal *et al.*, 1983). Thus, among different mechanisms, an increase in oxidative stress and a decrease inthe antioxidant reserve has also been suggested to play a role in the pathogenesis of heart failure (Singal and Kirshenbaum, 1990; Kaul *et al.*, 1993).

2. Free Radicals, Antioxidants and Oxidative Stress

2.1 General

The story of free radicals started more than two centuries ago. It was Lavoisier, in 1785, who made the first observation that oxygen has two main effects, i.e. it supports life but it also has toxic side effects (Lavoisier *et al.*, 1785). It was also demonstrated that increased oxygen tension results in lung congestion in mice, rats and pigs (Smith, 1899). Subsequent to that, Gomberg demonstrated the presence of triphenylmethyl molecule as a radical species in organic chemistry (Gomberg, 1900). In 1954, Gerschman and colleagues (1954) proposed that the damaging effects of oxygen were due to the formation of radical species (Gerschman *et al.*, 1954).

The discovery of superoxide dismutase by McCord and Fridovich, (1969), inspired biologists and clinicians around the world to study the role of free radicals in biology and medicine. Since then, a large body of evidence has accumulated showing that biological

systems are capable of producing a variety of reactive oxygen species which play an important role in various pathological conditions (Halliwell, 1987; Weiss, 1986). Our own laboratory has now provided evidence for the role of free radicals in the pathogenesis of cardiac dysfunction in a variety of conditions (Singal *et al.*, 1998; Singal *et al.*, 1996; Singal and Kirshenbaum, 1990; Kaul *et al.*, 1993) such as in catecholamine-induced (Singal *et al.*, 1982), adriamycin-induced (Singal *et al.*, 1987) and diabetic cardiomyopathy (Kaul *et al.*, 1995), pressure overload induced hypertrophy and heart failure (Dhalla and Singal, 1994). *In vitro* studies of the free radical effects of sarcoplasmic reticular function (Hess *et al.*, 1983) and stress-induced increase in lipid peroxidation (Meerson *et al.*, 1982) also provided more information on the role of oxygen radicals in myocardial dysfunction. The list of diseases and pathophysiological logical conditions where free radicals are involved continues to grow rapidly and has been the subject of several reviews (Kaul *et al.*, 1993; Singal *et al.*, 1998; Ball and Sole, 1998; Halliwelll, 1987; Halliwell and Gutteridge, 1984; Muskowitz and Kukin, 1999).

2.2 Oxygen free radicals

Free radicals are highly reactive atoms or molecules with an unpaired electron in their outermost orbits. The production of free radicals occurs either by the addition or by the removal of an electron in a reduction/oxidation reaction. Since oxygen has two electrons with a parallel spin in its outermost shell and is a diradical, it requires four electrons to be completely reduced to water (Kaul *et al.*, 1993; Singal *et al.*, 1988; Singh *et al.*, 1995). Oxygen is also the terminal acceptor of electrons for oxidative phosphorylation, and thus the simultaneous addition of four electrons is associated with the production of high energy

phosphates. However an addition of one electron at a time results in the formation of reactive oxygen species (Halliwell 1987; Weiss, 1986; Kaul *et al.*, 1993; Singal *et al.*, 1988; Singh *et al.*, 1995). In the univalent reduction pathway, the addition of one electron to molecular oxygen results in the production of superoxide anion radical ($O_2^{\cdot-}$). Mitochondria is the major source of $O_2^{\cdot-}$. Activated neutrophils, and cytoplasmic enzymes such as xanthine oxidase, NADPH oxidase are also the sources of $O_2^{\cdot-}$ (Muskowitz and Kukin, 1999). The addition of an electron to the $O2^{\cdot-}$ results in the formation of hydrogen peroxide (H_2O_2). The H_2O_2 is not a radical by itself, but it is capable of causing cell damage by interacting with transition metals such as iron. A single electron reduction of H_2O_2 results in the formation of the hydroxyl radical ($OH^{\cdot}$). The $OH^{\cdot}$ is a highly reactive molecule with an extremely short half-life, and therefore has a very limited diffusion capacity (Kaul *et al.*, 1993; Singal *et al.*, 1988; Singh *et al.*, 1995). Finally, the addition of a fourth electron results in the formation of water. The first excited state of oxygen i.e a singlet oxygen ($^1O_2$), can also initiate oxygen radical chain reactions (Singal *et al.*, 1988; Singh *et al.*, 1995; Kaul *et al.*, 1993).

These reactive oxygen intermediates such as $O2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$ are called activated oxygen species and are collectively known as partially reduced forms of oxygen (PRFO) (Saran *et al.*, 1989; Kaul *et al.*, 1993). Peroxynitrite, which is formed by the interaction of $O_2^{\cdot-}$ with nitric oxide (NO), is also harmful (Saran *et al.*, 1989; Yasmin *et al.*, 1994; Singal *et al.*, 1998). All these reactive species have the potential to interact with lipid and protein molecules and initiate free radical chain reactions resulting in cardiomyocyte damage (Freeman and Crapo, 1982; Singal *et al.*, 1988; Kaul *et al.*, 1993; Singal and Kirshenbaum 1990; Singal *et al.*, 1998; Moskowitz and Kukin, 1999).

2.3 Sources of free radicals

Although the precise mechanism for the production of free radicals is not known, studies suggest the involvement of increased prostaglandin biosynthesis (Dzau *et al.*, 1984), increased angiotensin II levels (Bech Laursen *et al.*, 1997) catecholamine autooxidation (Singal *et al.*, 1982; Singal *et al.*, 1983), neutrophilic NADPH oxidase and xanthine oxidase (McCord, 1988; Werns and Lucchesi, 1989) and cytokines (Ferrari *et al.*, 1998; Givertz and Colucci, 1998). Angiotensin II and catecholamines are elevated in congestive heart failure (Watkins *et al.*, 1976; Francis *et al.*, 1982). Angiotensin II stimulates the synthesis and release of prostaglandins during which free radicals are formed (Bech Laursen *et al.*, 1997; Oskarsson and Heistad, 1997). Autooxidation of catecholamines results in free radical production and cardiomyopathic changes (Singal *et al.*, 1982; Singal *et al.*, 1983). Increased levels of cytokines in heart failure conditions has been reported both in animal studies (Bozkurt *et al.*, 1998) as well as patient data (Levine *et al.*, 1990; Torre-Amione *et al.*, 1996). The deleterious effects of cytokines are shown to be mediated by free radicals (Blum and Miller, 1998). In a recent study by Nakamura and colleagues, it was reported that TNF-α and Ang II induced hypertrophy in cultured neonatal cardiomyocytes by virtue of producing free radicals. Antioxidants such as BHA, vitamin E and catalase inhibited this effect of Ang II and TNF-α (Nakamura *et al.*, 1998).

2.4 Free radical-mediated cell injury

Different pathophysiological conditions are known to influence the production of free radicals. Free radical-induced lipid peroxidation has been suggested to alter membrane structure and function (Kaul *et al.*, 1993; Singal *et al.*, 1998; Meerson *et al.*, 1982). The lipid

peroxidation process is initiated by the removal of a hydrogen atom from the unsaturated site in a fatty acid resulting in the production of a lipid radical. These radicals perpetuate a chain reaction leading to the formation of lipid peroxides (Kaul *et al.*, 1993; Singal *et al.*, 1988; Halliwell, 1987). The peroxidation of lipids is known to cause alterations in membrane fluidity (Eze *et al.*, 1992; Ceconi *et al.*, 1988). There is also evidence suggesting that free radicals can modify the protein structure and function (McCord, 1988). In this regard, proteins rich in sulphydryl groups are found to be more susceptible to free radical mediated attack (Singal *et al.*, 1988; Kaul *et al.*, 1993). Oxidation of the sulfhydryl groups in proteins results in the formation of toxic thiol compounds (Muskowitz and Kukin, 1999). Therefore, quantitation of glutathione, a sulphydryl compound, has been used to characterize radical-induced damage. In the myocardium, oxygen radicals have been shown to effect Na^+/Ca^{2+} exchanger, Na^+-K^+ ATPase and Ca^{2+} ATPase activities (Kaneko *et al.*, 1990; Reeves *et al.*, 1986; Kramer *et al.*, 1984; Dixon *et al.*, 1990). These activities are known to change during heart failure. Free radicals can also damage the DNA and chromosomes (Ozawa , 1995). Mitochondrial DNA is more susceptible than the nuclear DNA to free radical-induced damage (Ball and Sole, 1998). Such modifications have been shown to cause cellular abnormalities such as mutations and cell death (Halliwell, 1987; Singal *et al.*, 1988; Singal *et al.*, 1996; Kaul *et al.*, 1993). Thus the evidence that PRFO can cause subcellular abnormalities is overwhelming (Morris and Sulakhe, 1997; Kaul *et al.*, 1993; Ball and Sole, 1998; Singh *et al.*, 1995; Muskowitz and Kukin, 1999).

2.5 Methods of measuring free radical-mediated damage

Free radical-mediated damage includes oxidative modification of cellular proteins, lipids and nucleic acids. Lipid peroxidation is a free radical-mediated process, in which lipid peroxides are formed within cell membranes and organelles (Kaul *et al.*, 1993; Halliwell and Gutteridge, 1984; Kappus, 1985). Lipid peroxidation is measured by a variety of methods which include the estimation of malondialdehyde (MDA) either by ultraviolet light, HPLC or the thiobarbituric acid reactive substances (TBARS) method (Kaul *et al.*, 1993; Kappus, 1985; Ceconi *et al.*, 1991). The TBARS assay has been found to lack specificity and overestimates the MDA level by more than 10 fold, possibly due to the interaction with other aldehydes (Yeo *et al.*, 1994). Since the MDA assay has been shown to lack specificity, a new assay for detecting the dinitrophenylhydralazine derivative of MDA has recently been developed which is found to be more accurate than the TBARS assay (Cordis *et al.*, 1995). The direct detection of free radicals is done by electron spin resonance spectroscopy (Garlick *et al.*, 1987). Other methods of assessing free radical-mediated damage include determination of diene conjugates, lipid hydroperoxides, measurement of gases such as ethane, pentane and isoprotanes in exhaled air, measuring the reduced (GSH) and oxidized (GSSG) glutathione and the redox ratio and also measuring the activity of endogenous antioxidants. More recently, measurement of 8-iso-prostaglandin $F_{2\alpha}$ as a specific marker of oxidative stress has also been used using an enzyme immunoassay (Reilly *et al.*, 1997; Mallat *et al.*, 1998; Mankad *et al.*, 1998).

3. The Antioxidant Defense System

3.1 Enzymatic antioxidants

3.1.1 Superoxide dismutase (SOD). This enzyme is the first line of defense against free radical-induced damage. SOD is involved in the dismutation of superoxide radical where it specifically and efficiently catalyzes the conversion of $O_2^{\cdot-}$ to hydrogen peroxide. There are different types of SOD that differ based on the structure and localization. The CuZnSOD (molecular weight ~ 32, 000) is present in the cytoplasm and MnSOD (molecular weight ~ 80, 000) is found in the mitochondria. Hurt *et al.*, (1992) have reported that there are at least five transcripts for MnSOD. Superoxide dismutase activity has been reported to be significantly less in the heart as compared to the liver (Ferrari *et al.*, 1985; Ferrari *et al.*, 1998; Fridovich, 1978). It was the discovery of SOD which led to the realization that superoxide anion is formed *in vivo* in living organisms (McCord and Fridovich, 1969; Kaul *et al.*, 1993; Ferrari *et al.*, 1985; Ferrari *et al.*, 1998; Fridovich, 1978).

3.1.2 Glutathione peroxidase (GSHPx). This selenium-dependent enzyme (molecular weight ~84,000), is present both in the cytoplasm and mitochondria. It is involved in the detoxification of lipid hydroperoxides as well as catalyzes the reduction of hydrogen peroxides using glutathione (GSH) as a substrate. This enzyme is present in relatively high concentrations in the human heart (Kaul *et al.*, 1993; Ferrari *et al.*, 1985; Ferrari *et al.*, 1998; Fridovich, 1978; Kukreja *et al.*, 1997) and is therefore considered an important antioxidant enzyme in the heart. A selenium independent form of GSHPx, which dismutates organic peroxides is also present (Lawrence and Burk, 1978).

3.1.3 Catalase. This enzyme (molecular weight ~240,000) is also involved in the detoxification of hydrogen peroxide produced by superoxide dismutase. Present at relatively low concentrations in the heart, it converts H_2O_2 to water and oxygen. However, the difference between catalase and glutathione peroxidase is that GSHPx is more effective at low concentrations of H_2O_2, i.e. in μM range, whereas catalase is more effective at the mM concentrations of H_2O_2 (Kaul *et al.*, 1993; Singh *et al.*, 1995; Freeman and Crapo, 1982).

3.2 Non-enzymatic antioxidants

Non-enzymatic antioxidants include vitamins such as tocopherols, ascorbate and carotenes as well as other biological molecules including glutathione, uric acid and metal binding proteins.

3.2.1 Vitamin E. Tocopherol (vitamin E) is a group of eight structurally related compounds. These are further sub-divided into two groups: tocopherols and trienols. Of these, d-α-tocopherol is the most common type of vitamin E absorbed from the human diet (Packer, 1994; Packer, 1991; Burton and Traber 1990). It is a strong biological antioxidant. Because of its lipophillic nature, vitamin E offers maximum protection in cellular and subcellular membranes against free radical-mediated damage. It reacts with free radicals, yielding lipid hydroperoxides which can be removed by the GSHPx enzyme system. Vitamin E, thus, effectively terminates the lipid peroxide-mediated chain reaction and is therefore called a "chain breaking antioxidant" (Singal *et al.*, 1998; Packer , 1991; Packer *et al.*, 1979; Packer, 1994; Singal *et al.*, 1997a; Palace *et al.*, 1999a). Vitamin E also functions synergistically with ascorbic acid to terminate free radical chain reactions (Palace *et al.*, 1999a).

Increased vitamin E intake has been correlated with a reduced incidence of cardiovascular disease (Losonczy *et al,*. 1996; Kushi *et al.*, 1996; Gey *et al.*, 1993). Depleted concentrations of vitamin E have been found in the plasma as well as within the myocardium (Scragg *et al.*, 1989; Hill and Singal, 1997; Barsacchi *et al.*, 1992; Vaage *et al.*, 1997), in various cardiovascular diseases. Animals maintained on a vitamin E deficient diet were found to be more prone to catecholamine-induced (Singal *et al.*, 1983) and adriamycin-induced (Singal and Tong, 1988) cardiomyopathy. In a pressure overload model in guinea pigs, vitamin E therapy modulated the pathogenesis of heart failure including ultrastructural abnormalities (Dhalla *et al.*, 1996). Furthermore, vitamin E enriched membrane from rat hearts were found to be resistant to peroxidation (Janero and Burghart, 1989) suggesting that membrane α-tocopherol is important in the protection of myocardial phospholipids against oxidative damage. Vitamin E enrichment also reduced ischemia-reperfusion injury and improved contractile function of rat hearts (Massey and Burton, 1989; Ferrari *et al.*, 1989). Furthermore, vitamin E also maintained the redox ratio and antioxidant capacity and increased resistance to lipid peroxidation in guinea pigs (Rojas *et al.*, 1996). Vitamin E has also been reported to reduce infarct size in pigs and rabbits with MI (Klein *et al.*, 1989; Axford-Gatley and Wilson, 1991). Trolox, a water soluble analogue of α tocopherol, and ascorbic acid were also found to be effective in reducing myocardial necrosis after ischemia in a canine model (Mickle *et al.*, 1989).

However, some studies have reported no beneficial effects of tocopherol. Elevated myocardial tocopherol concentrations failed to reduce infarct size or improve contractile recovery after ischemic reperfusion in a pig model (Klein *et al.*, 1993). In fact, one study

even reported larger infarct sizes in vitamin E treated dogs subjected to coronary artery ligations, compared to controls (Sebbag *et al.*, 1994).

Recently, it has been discovered that vitamin E also possesses some other properties. Tocopherol reduces proliferation of smooth muscle cells, an important factor in atherogenesis, by activating TGF-β, a smooth muscle cell growth inhibitor (Ozer *et al.*, 1995; Chatelain *et al.*, 1993).

3.2.2 Vitamin C. Vitamin C is a water-soluble molecule. A direct correlation between the high intake of ascorbic acid and a lower incidence of cardiovascular mortality and risk of coronary artery disease (Enstrom *et al.*, 1992; Gey *et al.*, 1993b) has been established. Furthermore, lower dietary intake was associated with an increased risk of angina pectoris (Riemersma *et al.*, 1991) In rat hearts subjected to oxidative stress, treatment with vitamin C prevented myocardial damage (Bastounis *et al.*, 1994). Vitamin C also functions in synergism with vitamin E to promote regeneration of vitamin E (Frei *et al.*, 1990; Packer *et al.*, 1979). In addition to the antioxidant effect, vitamin C is also reported to increase nitric oxide generation (Heller *et al.*, 1999).

3.2.3 Carotene. Carotene, a precursor of vitamin A, is also known to quench free radicals. These reactions with carotene to operate maximally at low-oxygen tension as compared to vitamin E (Foote and Denny, 1968). Epidemiological studies have shown that increased levels of carotene and other carotenoids are associated with a decreased risk of cardiovascular diseases (Riemersma, 1994; Palace *et al.*, 1999b).

3.3 Glutathione

Glutathione is a tripeptide which protects cells against peroxides generated during aerobic metabolism. It undergoes redox cycling between the reduced (GSH) and oxidized (GSSG) forms (Kaul *et al.*, 1993; Clark *et al.*, 1990; Reed , 1990). In the heart, glutathione is predominantly in the GSH form. Glutathione also acts as a cosubstrate for GSHPx and plays an important role against free radical-mediated damage which results in the increased formation of GSSG. Depletion of glutathione by using L-buthionine-S, R-sulfoximine (BSO) renders the cell susceptible to free radical attack (Reed, 1984; Verma *et al.*, 1997). The redox ratio, which is the ratio of reduced to oxidized glutathione (GSH/GSSG) is used as a sensitive index of oxidative stress. An increase in the redox ratio indicates decreased oxidative stress and a decrease in the ratio means increased oxidative stress (Singal *et al.*, 1998; Kaul *et al.*, 1993; Verma *et al.*, 1997; Halliwell, 1995).

3.4 Other biological molecules

Various biological molecules such as uric acid and ubiquinone have been reported to possess antioxidant properties. Uric acid, has been shown to directly interact with OH^- and prevent oxidation (Ames *et al.*, 1981), suggesting that this molecule may possess an antioxidant property (Kaul *et al.*, 1993). Iron chelators, such as desferrioxamine1, inhibit lipid peroxidation and reduces the injury associated with ischemia-reperfusion (Bernier *et al.*, 1986; Chopra *et al.*, 1992). Ubiquinone, or coenzyme Q_{10}, also acts as a potent inhibitor of lipid peroxidation by directly quenching free radicals (Forsmark *et al.*, 1991). Coenzyme Q_{10} treatment has been found beneficial in patients undergoing coronary artery bypass graft surgery (Chello *et al.*, 1994). Various metal binding proteins, including ferritin, transferrin,

metallothioneins and ceruloplasmin, have been reported to inhibit lipid peroxidation (Halliwell and Gutteridge, 1984).

3.5 Drugs as antioxidants

3.5.1 Angiotensin converting enzyme (ACE) inhibitors. Captopril due to the presence of a sulfhydryl group has been shown to scavenge superoxide anions and protect the heart against oxidative stress-induced damage (Westlin and Mullane, 1988; Bartosz *et al.*, 1998; Chopra *et al.*, 1992). A protective effect of captopril against ischemia-reperfusion injury was shown. Both captopril and zofenoprilat exhibited dose-dependent inhibition of lipid peroxidation and prevented loss of cell viability (Weglicki *et al.*, 1990; Weglicki *et al.*, 1992; Mak *et al.*, 1990). In a recent study, we documented that treatment of MI rats with captopril resulted in an improvement of the myocardial endogenous antioxidants and a decrease in lipid peroxidation (Khaper and Singal, 1997a). In addition, captopril treatment resulted in an increase in redox state, indicating reduced oxidative stress (Khaper *et al.*, 1998). In patients with coronary artery disease, captopril, has been reported to decrease breath pentane levels (Sobotka *et al.*, 1993).

3.5.2 Beta (β)-blockers. β-blockers such as propranolol and carvedilol have been reported to offer protection against free radical-induced injury. Propranolol has been shown to inhibit membrane lipid peroxidation in a concentration dependent manner in a free radical generating system (Mak and Weglicki, 1988; Mak *et al.*, 1989a; Weglicki *et al.*, 1990). In rat hearts subjected to ischemia-reperfusion, we reported that pretreatment of rats with propranolol offered significant cardioprotection against I/R injury. Propranolol treatment not only decreased lipid peroxidation in the hearts, but also promoted endogenous antioxidants

such as catalase and glutathione peroxidase (Khaper *et al.*, 1997b). Carvedilol has also been shown to possess antioxidant properties primarily due to the presence of a carbazole moiety (Feuerstein and Ruffolo, 1996). Spin-trapping studies have demonstrated that carvedilol scavenges superoxide and hydroxyl radical (Yue *et al.*, 1995) and inhibits lipid peroxidation in swine ventricular membranes (Yue *et al.*, 1992). A decrease in lipid peroxidation in hearts of mice treated with carvedilol further confirmed the antioxidant property of carvedilol *in vivo* (Yue *et al.*, 1995). In patients with heart failure, carvedilol not only improved the function, but also modulated the antioxidants (Arumanayagam *et al.*, 2001).

3.5.3 Probucol. Probucol is also reported to possess strong antioxidant properties due to its unique chemical structure. In fact the antiperoxidative property of probucol was found to be comparable to that of α-tocopherol in inhibiting lipid peroxidation (Mak *et al.*, 1989). In adriamycin-induced congestive heart failure in rats, known to be mediated by oxidative stress, a decrease in antioxidant reserve and increase in oxidative stress has been reported to correlate with poor cardiac function (Siveski-Iliskovic *et al.*, 1994). Pretreatment of these rats with probucol provided complete protection against adriamycin-induced cardiomyopathy accompanied by a significant increase in myocardial GSHPx and SOD activities (Siveski- Iliskovic *et al.*, 1994; Singal *et al.*, 1998; Singal *et al.*, 1997b).

4. Oxidative Stress and Antioxidants in Heart Failure

The oxidative stress condition is defined as "a disturbance in the prooxidant and antioxidant balance, in favour of the former" (Sies, 1991). Atherosclerosis, diabetes, cancer, arrhythmia, rheumatoid arthritis, and neurodegenerative diseases are some of the pathological conditions where free radicals are known to play an important role. In fact, this list of

pathological conditions where oxidative stress is involved continues to grow rapidly. In recent years, substantial evidence has accumulated from acute and chronic studies to suggest the role of antioxidants and oxidative stress in the pathogenesis of heart dysfunction and failure both in animal studies and from clinical trials (Singal *et al.*, 1996; Kaul *et al.*, 1993; Ferrari *et al.*, 1998; Singal *et al.*, 1998; Belch *et al.*, 1991).

4.1 Free radicals in acute conditions

Free radicals have been shown to exert a direct inhibitory effect on myocardial dysfunction in *in vivo* and *in vitro* settings.

4.1.1 *In vitro* studies. Mitochondria, sarcoplasmic reticulum and sarcolemma are the major targets of free radical-induced damage. Free radicals are known to affect the activity of Na^{+}-K^{+} ATPase, the Na^{+}-Ca^{2+} exchanger (Kramer *et al.*, 1984; Reeves *et al.*, 1986; Kaneko *et al.*, 1990; Dixon *et al.*, 1990), which ultimately alter calcium homeostasis and thus cause contractile abnormalities. Free radicals have been shown to depress mitochondrial respiration, cytochrome oxidase and glucose-6-phosphatase, and increase malondialdehyde levels (Flamigni *et al.*, 1982; Kako, 1987; Paradies *et al.*, 1999). Free radicals have also been shown to reduce the ability of mitochondria to synthesize ATP while SOD and catalase have been reported to improve the ATP production (Ceconi *et al.*, 1988; Nohl and Hegner, 1978).

4.1.2 *Ex vivo* studies. Depressed contractile function, impairment in energy production, a rise in resting tension and an increase in lipid peroxidation by free radicals have been reported in various *ex vivo* preparations (Kaul *et al.*, 1993; Gupta and Singal, 1989; Kirshenbaum *et al.*, 1992; Kirshenbaum *et al.*, 1995). In intact cardiac myocytes from failing

heart, the hydroxyl radical has been reported to cause hypercontracture of the myocytes (Tsutsui *et al.*, 2001). Free radical-induced reduction of contractile function correlated with a decline in myocardial SOD, glutathione and α-tocopherol content, as well as with an increase in hydrogen peroxide content and lipid peroxidation (Gupta and Singal, 1989; Vaage *et al.*, 1997).

4.1.3 *In vivo* studies. Increased free radical production and a decrease in the antioxidant reserve has been reported to have serious functional consequences after ischemia-reperfusion. Many studies have reported the production of free radicals in reperfused hearts in animals (Zweier *et al.*, 1989; Ferrari *et al.*, 1998; Arroyo *et al.*, 1987; Przyklenk and Kloner, 1986; Garlick *et al.*, 1987; Bolli *et al.*, 1988) and humans (Currello *et al.*, 1995; Levy *et al.*, 1998; Ferrari *et al.*, 1990). Zweier and colleagues (1989) were among the first to directly measure free radical production in isolated perfused rabbit hearts subjected to ischemia-reperfusion. Studies on isolated perfused rat hearts showed increased production of H_2O_2 during ischemia as well as early reperfusion (Jeroudi *et al.*, 1994). In isolated perfused rabbit hearts, a decline in developed force during ischemia was accompanied by a progressive decline in tissue glutathione content and in the redox ratio indicating increased oxidative stress (Ferrari *et al.*, 1985). A decrease in tocopherol, retinol and ascorbic acid and redox ratio and an increase in lipid hydroperoxides has also recently been reported in rat hearts subjected to ischemia-reperfusion (Palace *et al.*, 1999c). Jolly and colleagues (1984) were the first to study the effects of antioxidant therapy in ischemia-reperfusion settings. In this study, a reduction in infarct size by combined administration of SOD and catalase was reported in dogs that underwent 90 min of ischemia and 24 hrs of reperfusion (Jolly *et al.*,

1984). Since then, a large number of studies have demonstrated the beneficial effects of antioxidants in ischmia/reperfusion injury (Ambrosio *et al.*, 1986; Werns et al ., 1998; Mehta *et al.*, 1989; Naslund *et al.*, 1986). In a transgenic mice model, which overexpressed MnSOD, it was demonstrated that MnSOD improved cardiac function and decreased release of lactate dehydrogenase in mice hearts subjected to ischemia-reperfusion (Chen *et al.*, 1998). Transgenic mice overexpressing GSHPx were found to be more resistant to ischemia-reperfusion injury (Yoshida *et al.*, 1996) whereas GSHPx knockout mice were more susceptible to ischemia-reperfusion induced damage (Yoshida *et al.*, 1997). Some studies have reported no protection with antioxidants. In patients undergoing thrombolytic therapy, administration of recombinant human SOD failed to provide any significant improvement of the ventricular function (Murohara *et al.*, 1991).

It has been demonstrated that oxidative stress also plays a major role in myocardial stunning (Bolli, 1988; Bolli, 1998; Hess and Kukreja, 1995). Antioxidant therapy suppressed the production of free radicals and attenuated myocardial stunning, suggesting a cause and effect relationship (Bolli, 1988; Bolli, 1998). An elegant study by Li and colleagues, using adenovirus- mediated gene transfer technique to introduce SOD in the rabbits, demonstrated protection against myocardial stunning (Li *et al.*, 1998).

5. Animal models of heart failure

In the recent years, a number of animal models of heart failure have been developed that closely mimics the heart failure condition in the clinical settings. One such model is the coronary artery ligation model of MI was initially produced in the dog with not much success due to the presence of collateral vessels. Following that chronic MI was produced in rats

which resembled the condition observed in humans (Selye *et al.*, 1960; Johns and Olson, 1954). The rat MI model has been shown to result in left ventricular remodeling, progressive ventricular dilatation and eventual failure with clinical signs of heart failure including dyspnea and pulmonary edema. Thus the rat MI model is highly reproducible, relatively less expensive and resembles closely to the clinical condition of heart failure. This model however has some limitations such as it is more invasive and there is a large variation in the infarct size (Dixon *et al.*, 1990; Hill and Singal, 1996; Fishbein *et al.*, 1978).

Other commonly used model of heart failure include the low cardiac output model by ventricular pacing. This model is characterized by bi-ventricular dilatation and impairment of left ventricular myocardial contractility. This model is considered to be valuable to study neurohumoral and peripheral circulatory changes in heart failure (Zile *et al.*, 1995). The pressure overload model produced by ascending aorta constriction is a good model to study the transition from hypertrophy to heart failure (Feldman *et al.*, 1993; Dhalla and Singal, 1994). Volume overload produced by either arteriovenous shunt or mitral regurgitation has been shown to result in progressive left ventricular dysfunction and heart failure (Liu *et al.*, 1991).

6. Oxidative Stress in Chronic Heart Failure

There are many clinical and basic studies where production of free radicals and increased oxidative stress has been documented.

6.1 Catecholamine-induced cardiomyopathy

Increased plasma levels of catecholamines have been reported in acute MI and heart failure conditions (Francis *et al.*, 1982). Excess catecholamines have been shown to cause

arrhythmias as well as cardiomyopathy (Singal *et al.*, 1982; Singal *et al.*, 1985). Production of free radicals upon autooxidation of catecholamines play a critical role in cardiomyopathy (Singal *et al.*, 1982; Singal *et al.*, 1983). A recent study reported increased lipid peroxidation in isoproterenol administered rat hearts (Rathore *et al.*, 1998). Pretreatment of rats with vitamin E reduced catecholamine-induced arrhythmia and other functional changes (Kirshenbaum *et al.*, 1990). In another recent study, it was reported that catecholamine-induced heart dysfunction was associated with calcium overload, increased malondialdehyde content, increased conjugated dienes and lower redox ratio (Tappia *et al.*, 2001). Pretreatment of these animals with vitamin E modulated all these changes (Tappia *et al.*, 2001).

6.2 Adriamycin cardiomyopathy

Adriamycin cardiomyopathy is associated with increased free radicals and decreased antioxidant status in the heart (Doroshow, 1983; Singal *et al.*, 1997b; Singal and Iliskovic, 1998; Li and Singal, 2000). Direct evidence for free radical involvement was provided by studies which showed that vitamin E-treated mice were more resistant to adriamycin cardiotoxicity (Myers *et al.*, 1977). Moreover, rats maintained on a vitamin E deficient diet were more susceptible to adriamycin cardiotoxicity (Singal *et al.*, 1987; Singal and Tong, 1988). Treatment with probucol, an antioxidant as well as a lipid-lowering drug, modulated the pathogenesis of heart failure due to adriamycin (Siveski-Iliskovic *et al.*, 1994; Siveski-Iliskovic *et al.*, 1995; Singal *et al.*, 1997b; Li and Singal, 2000).

6.3 Diabetic cardiomyopathy

Evidence is available to suggest the role of increased oxidative stress and depressed antioxidant enzyme activities in the pathogenesis of diabetic cardiomyopathy (Guigliano *et al.*, 1995). A decrease in myocardial SOD and catalase activity and an increase in oxidative stress has been demonstrated in streptozotocin-induced diabetic rats (Kaul *et al.*, 1995). Furthermore, treatment with probucol in these rats resulted in improved cardiac function (Kaul *et al.*, 1995). Supplementation with vitamin E reduced oxidative stress in animals and patients with diabetes (Paolisso *et al.*, 1993; Wohaieb *et al.*, 1987).

6.4 Hypertrophy and heart failure

An increase in endogenous antioxidant enzyme activities and a decrease in lipid peroxidation have been reported in the hypertrophy stage induced by chronic pressure overload in rats (Gupta and Singal, 1989; Kirshenbaum and Singal, 1993) and guinea pigs (Dhalla and Singal, 1994; Dhalla *et al.*, 1996). Myocytes isolated from hypertrophied rat hearts also showed increased antioxidants and reduced lipid peroxidation (Kirshenbaum *et al.*, 1995). Antioxidant treatment of the guinea pigs with vitamin E decreased oxidative stress and delayed the occurrence of heart failure (Dhalla *et al.*, 1996).

A decrease in the activity of SOD, catalase and GSHPx of both the right and left ventricles and also an increase in the levels of lipid peroxidation has been reported in volume overload-induced heart failure in dogs (Prasad *et al.*, 1996). Treatment of these animals with vitamin E was effective in increasing the GSHPx activity, improving cardiac contractility and reducing lipid-peroxidation (Prasad *et al.*, 1996).

6.5 Myocardial infarction and congestive heart failure

Changes in myocardial antioxidants as well as oxidative stress have been described in the surviving myocardium of rats subjected to MI (Hill and Singal, 1996). In this study, maintenance of hemodynamic function in early stages (non-failure stage) was accompanied by a significant decrease in oxidative stress and lipid peroxidation while the antioxidant reserve was maintained. In late stages, where hemodynamic function was depressed, the myocardial antioxidants GSHPx, catalase, SOD and vitamin E were also significantly decreased while oxidative stress was increased (Hill and Singal, 1996). In addition to the myocardium, the levels of vitamin E and A were also depleted in the storage organs liver and kidney in rats with severe heart failure (Palace *et al.*, 1999a). Pretreatment of these rats with vitamin E before subjecting them to coronary artery ligation surgery modulated these changes (Palace *et al.*, 1999a).

In another study of regional changes in the two ventricles during the development of heart failure, the antioxidant deficit and an increase in oxidative stress was found to occur first in the left ventricle (Hill and Singal, 1997). In moderate to severe heart failure stages, these changes also occurred in the right ventricle (Hill and Singal, 1997). In another study, using the same animal model, we reported that improved cardiac function after treatment with the afterload reducing drugs captopril or prazosin was accompanied by the maintenance of myocardial endogenous antioxidant status and decrease in oxidative stress (Khaper and Singal, 1997; Khaper *et al.*, 1998). In a recent study, gene therapy with extracellular form of SOD was shown to protect rabbits against MI (Li *et al.*, 2001). Evidence of an increased oxidative stress in heart failure was also provided by another study in MI rats. In this study, increased xanthine oxidoreductase (a free radical generating system) activity was reported and the

enzyme was found to be localized in the inflammatory cells (deJong *et al.*, 2000). Antioxidant vitamins such as vitamin C, carotenoids and vitamin E have been shown to decrease lipid peroxidation and reduce atherogenesis and the risk of coronary heart disease. Pretreatment with vitamin E and C limited myocardial necrosis (Axford-Gatley and Wilson, 1991; Mickle *et al.*, 1989; Massey *et al.*, 1989).

6.6 Right heart failure

Myocardial oxidative stress changes during compensated right heart failure in rats have also been studied in our laboratory (Pichardo *et al.*, 1999). In this study, right heart failure was induced by the administration of monocrotaline to rats. There was no difference in myocardial SOD, GSHPx and catalase activity in the hypertrophied hearts as compared to control hearts. Although the antioxidant enzyme levels did not change, there was a significant increase in lipid hydroperoxide content, suggesting that the animals were in a compensated heart failure stage — a step before the development of overt heart failure (Pichardo *et al.*, 1999). Free radical generating system, xanthine oxidoreductase was also reported to be significantly increased in monocrotaline-induced right heart failure (de Jong *et al.*, 2001).

6.7 Ventricular pacing

A direct evidence for the production of free radicals has also been provided in a dog model of heart failure-induced by ventricular pacing. Using ESR techniques, it was demonstrated that OH^- originated from $O_2^{\cdot -}$ via H_2O_2. Further, it was also demonstrated that oxidative stress was associated with a parallel decrease in left ventricular function (Ide *et al.*, 2001).

7. Clinical Studies

Changes in antioxidants and oxidative stress has also recently also been documented in heart failure patients (Belch *et al.*, 1991; McMurray *et al.*, 1990; Diaz-Velez *et al.*, 1996). Breath pentane (a byproduct of lipid peroxidation) levels was reported to be elevated in exhaled air of CHF patients and treatment with captopril attenuated this rise and improved the patient's clinical condition (Sobotka *et al.*, 1993; Weitz *et al.*, 1991). Furthermore, it has also been demonstrated that the increase in lipid peroxidation correlates with the severity of heart failure (Diaz-Velez *et al.*, 1996; Charney *et al.*, 1997; Keith *et al.*, 1998). Using, a more specific marker of oxidative stress, one study reported a strong correlation between pericardial fluid levels of 8-iso-prostaglandin $F_{2\alpha}$ and the severity of heart failure in patients with ischemic and valvular heart disease (Mallat *et al.*, 1998). In addition to an increase in free radical activity, a decrease in enzymatic and non-enzymatic antioxidants has also been reported in patients with congestive heart failure (Belch *et al.*, 1991; McMurray *et al.*, 1990; Diaz-Velez *et al.*, 1996) and this increase in oxidative stress has been suggested to be related to exercise intolerance in patients with heart failure (Nishiyama *et al.*, 1998). A correlation between plasma lipid peroxide and malondialdehyde levels and the clinical class of heart failure has also been established (Keith *et al.*, 1998). A decrease in catalase enzyme activity has also been reported in heart failure patients due to idiopathic dilated cardiomyopathy (Baumer *et al.*, 2000). In another recent study, Yucel and colleagues reported a significant decrease in blood glutathione and erythrocyte SOD activity and an increase in lipid peroxidation in patients with dilated cardiomyopathy (Yucel *et al.*, 1998). In contrast in another recent study on patients with end stage heart failure, an upregulation of catalase enzyme activity was associated with an increase in the mRNA abundance and protein contents

for these enzyme (Dieterich *et al.*, 2000). All of these clinical studies provide strong support for the involvement of oxidative stress in the progression of heart failure.

Various clinical trials have examined the beneficial effects of antioxidant vitamins in MI and heart failure conditions. The Health Professional Follow-up Study (Rimm *et al.*, 1993) and the Nurses Health Study (Stampfer *et al.*, 1993) found a decrease in the incidence of coronary artery disease in men and women who were taking vitamin E. In another study, a cocktail containing antioxidant vitamins A, C, E and β-carotene resulted in a decrease in oxidative stress as well as the infarct size in MI patients (Singh *et al.*, 1996). The Cambridge Heart Antioxidant Study (CHAOS) reported a substantial decrease in the incidence of MI in patients receiving vitamin E (Stephens *et al.*, 1996). Vitamin E also preserves the activity of enzymatic antioxidants, reducing lipid peroxidation in serum (Chen *et al.*, 1997). Two recent studies (Yusuf *et al.*, 2000; Keith *et al.*, 2001) failed to show a correlation between vitamin E intake and its effect on cardiovascular events. On the other hand, some studies found no correlation between alpha-tocopherol and improvement in functional aspect or quality of life of patients (Kok *et al.*, 1987; Hense *et al.*, 1993; Keith *et al.*, 2001). In another study, in patients with CHF, the increase in free radicals and TBARS was significantly reduced by vitamin C therapy which was suggested to be due to reduced neutrophil mediated superoxide anion generating capacity(Ellis *et al.*, 2000).

Collectively, these studies suggest that increased free radicals and decreased antioxidants are involved in heart failure.

8. Oxidative Stress, Apoptosis and Heart Failure

More recently, apoptosis or programmed cell death, has been reported in the infarct regions of myocardium from MI patients (Sarasate *et al.*, 1997) as well as patients with end stage heart failure (Narula *et al.*, 1996; Olivetti *et al.*, 1997). Findings from several in vitro studies and animal models also suggest that apoptosis occurs in response to ischemia-reperfusion, myocardial infarction, and chronic pressure overload (Gottleib *et al.*, 1994; Kajstura *et al.*, 1996; Teiger *et al.*, 1996), all of which are conditions known to generate oxidative stress (Kaul *et al.*, 1993; Singal *et al.*, 2000). Recent studies have also documented the role of cytokines (Krown *et al.*, 1996) and angiotensin II in triggering apoptosis which is mediated by oxidative stress. Although the role of oxidative stress in apoptosis has been documented, the exact contribution of apoptosis in the development of heart failure has yet to be established. Direct involvement of oxidative stress in apoptosis has been demonstrated in a variety of cell types (Hockenberry *et al.*, 1993). Adriamycin, UV radiation and TNF-α have all been reported to produce free radicals and to cause apoptosis (Butke and Sandstorm, 1994; Kumar *et al.*, 1999). Furthermore, apoptosis is inhibited by antioxidants such as catalase, SOD, vitamin E and trolox (Butke and Sandstorm *et al.*, 1994; Kumar *et al.*, 1999; Forrest *et al.*, 1994 Haendeler *et al.*, 1996). In a swine model of ischemia-reperfusion, it was demonstrated that cardiomyocyte undergo apoptosis during reperfusion. The study also suggested that oxidative stress may be one of the causative factors for the development of apoptosis. Furthermore, addition of ebselen, a synthetic mimic of GSHPx reduced apoptosis (Maulik and Yoshida, 2000).

It has been documented that the tumor suppression protein p53 triggers and Bcl2 inhibits the apoptosis process in cardiomyocytes (Kirshenbaum and de Mossaic, 1997; Olivetti

et al., 1997). The mechanism of action of Bcl2 for the prevention of apoptosis has also been suggested to be mediated by an antioxidant pathway (Hockenberry *et al.*, 1993). Overexpression of Bcl2 in neural cells inhibits apoptosis mediated by reactive oxygen species (Kane *et al.*, 1993). Bcl2 also blocks apoptosis-induced by α-irradiation which produces hydroxyl radicals, causing oxidative damage to proteins and DNA (Sentman *et al.*, 1991).

The importance of oxidative stress in apoptosis is also confirmed by the observation that knock-out mice lacking MnSOD die early due to cardiomyopathy as compared to normal mice (Lebovitz *et al.*, 1996). The underlying molecular mechanisms that regulate this remodelling event are still not fully understood.

9. Renin Angiotensin System

The different components of the RAS include angiotensinogen, renin, angiotensin I and angiotensin II. Angiotensin II formation is initiated by renin, a proteolytic enzyme that is stored and secreted by the juxtaglomerular apparatus in the kidney. Upon secretion, renin acts on angiotensinigen to form the decapeptide Angiotensin I. Angiotensin I is cleaved by the action of angiotensin converting enzyme (ACE) which leads to the generation of the effector peptide Angiotensin II (Ang II) (Dostal and Baker, 1995; Dzau, 1989; Dzau, 1988). Although the circulatory RAS has been discovered for a long time, the presence of cardiac RAS has only become recently evident. Various biochemical and molecular studies have provided evidence for the presence of renin, angiotensinogen and ACE genes and angiotensin receptors in cardiac tissues (Danser, 1996; Campbell, 1987; Baker *et al.*, 1984). The pathogenic role of RAS is linked to the biological effects of Ang II, which includes vasoconstriction, sodium and water retention, aldosterone secretion, growth and proliferation

(Dzau, 1995; Brunner La Rocca *et al.*, 1999; Ju *et al.*, 1997). Ang II is a very potent vasopressor which exerts a direct effect on smooth muscle cells and may induce positive inotropism and chronotropism in the heart (Goodfriend *et al.*, 1996). Ang II also increases the preload and the afterload on the heart via enhanced sympathetic drive and by stimulating aldosterone synthesis (Goodfriend *et al.*, 1996). Furthermore, Ang II has been shown to act as a growth factor involved in a signaling cascade leading to ventricular hypertrophy (Sadoshima *et al.*, 1995).

More recently, the role of angiotensin II in producing free radicals have also been documented. Bech Laursen and colleagues (1997) have reported that angiotensin II induced hypertension in rats is associated with a large increase in vascular production of superoxide radical, which is accompanied by NO dependent vasodilation (Bech Laursen *et al.*, 1997). The authors also observed that chronic infusion of SOD, resulted in the normalization of superoxide release in the rat aorta accompanied by a reduction in mean arterial pressure. This mechanism could also contribute to the pathogenesis of heart failure, where RAS is known to be activated (Fig 1).

9.1 Angiotensin converting enzyme inhibitors

The ACE-inhibitors have been reported to reduce mortality and morbidity and significantly reduce the incidence of recurrent MI (Yusuf *et al.*, 1992; Pfeffer *et al.*, 1992). The CONSENSUS (Cooperative North Scandinavian Enalapril Survival Study) trial (Swedberg *et al.*, 1999) demonstrated a significant reduction in one year mortality with enalapril in class IV heart failure patients. The influence of captopril in MI patients showed

a 17% reduction in mortality in the SAVE (Survival and Ventricular Enlargement) trial (Pfeffer *et al.*, 1992).

9.2 Angiotensin II receptors

Numerous studies on binding have demonstrated cardiac Ang II binding sites in sarcolemmal vesicles from rat, rabbit, guinea pigs and human myocardium. The biologic effects of Ang II are mediated by two types of specific receptors, i.e AT_1 and AT_2 (Dzau, 1995; Timmermans *et al.*, 1992; Timmermans and Smith, 1994). Other angiotensin receptors such as AT_3 and AT_4 have also been proposed in other tissues (Brunner La Rocca, *et al.*, 1999). Recent studies have reported the presence of AT_1 and AT_2 receptors subtypes in rabbit and rat ventricular myocardium (Dostal and Baker, 1995). Almost all the known actions of angiotensin II in adult tissues are known to be mediated by the AT_1 receptor (Moser, 1997; Dostal and Baker, 1995). The AT_1 receptor is a member of the seven transmembrane, G protein-coupled receptor superfamily (Chiu *et al.*, 1989; Dzau, 1995). Pharmacological and ligand binding studies have indicated that there are sub-types of AT_{1A} and AT_{1B} in some species (Chiu *et al.*, 1989; Dzau, 1995; Timmermans *et al.*, 1992; Timmermans *et al.*, 1993). Studies have reported no difference in the ligand binding characteristics and signal transduction mechanism between AT_{1A} and AT_{1B}. The AT_{1A} receptor is localized mainly in the vascular smooth muscle cells, lung, kidney, brain and liver (Timmermans *et al.*, 1992). The AT_{1B} is localized in the adrenal medulla, uterine, pituitary and renal tissues (Timmermans *et al.*, 1992; Dzau, 1995; Dostal and Baker, 1995). The AT_2

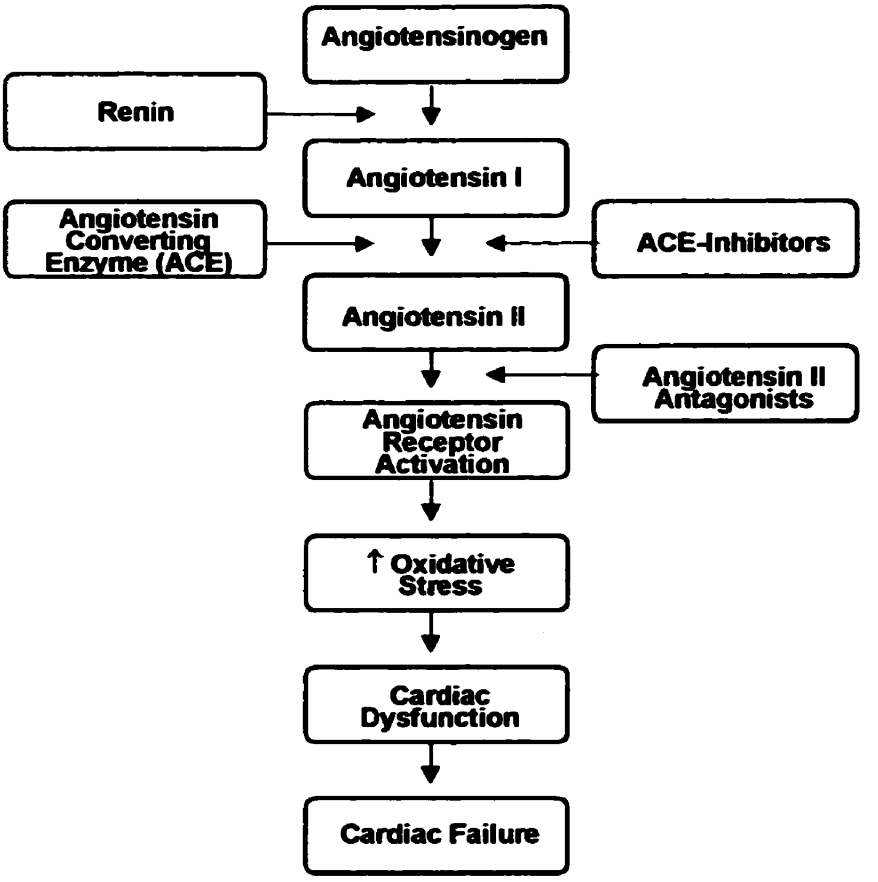


Fig. 1: Role of RAS and oxidative stress in heart failure.

receptor is also a seven-transmembrane domain receptor protein (Inagami, 1995). The AT_2 receptor is hardly expressed in most adult tissue. Its expression is controlled based on the stage of development of the tissue. It has been shown that, the AT_2 receptor is present in high concentration in the fetus, but very low concentration in adult. The AT_2 receptors have also

been further divided into AT_{2A} and AT_{2B} (Timmermans *et al.*, 1992). The AT_{2A} receptors are found mainly in the brain whereas the AT_{2B} receptors are found mainly in the adrenal medulla and uterine (Dzau, 1995). The AT_2 receptor has an anti-growth effect. The AT_1 and AT_2 receptors have recently been shown to be distributed differently in the normal and failing human hearts (Wharton et., al 1998). In this regard, AT_2 receptors were found to be localized in the area of fibroblast and collagen deposition suggesting RAS to be involved in cardiac remodeling (Brunner La Rocca, 1999).

9.3 Signal transduction

Angiotensin II mediated activation of the AT_1 receptors is G protein coupled. Experimental studies have reported that activation of the receptor leads to the stimulation of Gqα which in turn stimulates phospholipase C. Activation of phospholipase C facilitates the breakdown of phosphoionositol 4,5 biphosphate (PIP_2) to inositol 1,4,5 triphosphate (IP_3) and 1,2 diacylglycerol (DAG). IP_3 in turn leads to an increase in intracellular calcium, which exerts a positive inotropic effect on the heart (Baker *et al.*, 1989; Sadoshima *et al.*, 1993). DAG further activates protein kinase C which has a downstream effect on both Na H and Na Ca exchanger leading to the contraction of smooth muscle. However, in the liver, activation of the AT_1 receptors leads to the activation of Gi which in turn inhibits adenylate cyclase and subsequent reduction of cAMP (Dzau, 1995). Although the signal transduction pathway of AT_2 has not studied in details. Studies report that activation of AT_2 receptor activates MAP kinase phosphatase (Hayashida *et al.*, 1996).

9.4 Clinical relevance of the RAS

The existence of RAS has been confirmed in both the circulatory system and at the tissue level (Danser, 1996; Dzau, 1988; Campbell and Habener, 1986). The pathophysiological role of RAS has been extensively studied in congestive heart failure and myocardial ischemia. Elevated plasma levels of renin and Ang II were observed in patients with acute MI (Michorowski and Ceremuzynski, 1983). Enhanced activity of RAS has also been observed in animal models of heart failure (Baker *et al.*, 1990). Angiotensin converting enzyme inhibitors have proven to be very effective in improving the cardiac function and symptoms of CHF (Dzau *et al.*, 1980; Pfeffer *et al.*, 1992).

10. Losartan

Losartan is a new class of Ang II receptor type 1 antagonists, which is now clinically used for the treatment of hypertension (Johnston, 1995). It was first introduced in Canada in October 1995, for the management of hypertension. AT_1 antagonists such as losartan selectively and completely block the effects of Ang II regardless of the pathway by which Ang II is formed (Timmermans and Smith, 1994). Unlike ACE-inhibitors, which has an effect on angiotensin I and bradykinin and causes cough (Skidgel *et al.*, 1987). it does not cause cough or hypotension.

10.1 Structure and pharmacokinetics

Losartan is a phenyl tetrazole substituted imidazole. It is a low molecular weight (MW 461), orally active agent which does not accumulate in plasma (Timmermans, 1999; Timmermans *et al.*, 1993; Lacourciere, 1995). The oral availability of losartan is about 33% and about 14% of losartan is converted to an active metabolite (E-3174), which is a more potent inhibitor of AT_1 receptor than losartan.. Mean peak concentrations for losartan are

reached at 1 hour following oral administration. The elimination half life for losartan is 1.5-2 hrs. It is eliminated by both urinary and biliary excretion (Johnston, 1995).

10.2 Losartan in hypertension

Losartan is a vasodilator and reduces both afterload and preload (Rush and Rajfer, 1993). There have been a number of studies reporting the beneficial effects of losartan in patients (Oparil *et al.*, 2001; Hedner *et al.*, 1999) and animals (Kahonen *et al.*, 1999; Varo *et al.*, 1999; Varo *et al.*, 2000) with hypertension. In patients with mild to moderate hypertension, losartan was reported to have a long acting hypotensive effect with a hypouricemic action (Tsunoda *et al.*, 1993).

10.3 Losartan in heart failure

Due to its selectivity and specificity AII receptor antagonists such as losartan has advantage over ACE inhibitors in congestive heart failure. The ELITE (Evaluation of losartan in the Elderly) trial, marks the first major clinical study of losartan. This trial was a prospective, randomized, double blind trial which compared the effects of captopril to that of losartan (Pitt *et al.*, 1997). The results from this trial suggested that losartan was better tolerated than captopril, with fewer adverse effects (Pitt *et al.*, 1997). Furthermore, patients taking losartan had no cough as compared to the captopril group and there was a decrease in overall mortality in the losartan group. In other studies, losartan has been reported to have beneficial effects on hemodynamics in patient studies and animal studies with heart failure, in terms of reducing systemic vascular resistance, and raising the cardiac index and had no severe adverse effect (Gottlieb *et al.*, 1993; Crozier *et al.*, 1995; Lang *et al.*, 1997; Dickstein *et al.*, 1995; Mankad *et al.*, 2001).

10.4 Other angiotensin II type 1 receptor antagonists

There have been only a few studies on the effects of other AII type 1 receptor antagonists in heart failure. In RESOLVD (Design of the Randomized Evaluation of Strategies for Left Ventricular Dysfunction), trial in patients with mild heart failure, individual and combined effects of enalapril and candesartan were studied on ventricular remodeling and exercise capacity. It was found that the combination therapy had more favourable effect on ventricular remodeling although a trend towards increase in mortality was seen with candesartan treatment (Tsuyuki *et al.*, 1997). More recently, one study looked at the individual and combined effects of endothelin I receptor antagonist bosentan and AII type 1 antagonist valsartan on cardiac remodeling in post MI rats (Tzanidis *et al.*, 2001). The study reported that combined treatment had beneficial effects on myocardial fibrinogenesis over individual treatment (Tzanidis *et al.*, 2001). Inhibition of apoptosis by irbesartan has also been reported in rats with CHF (Dalla Libera *et al.*, 2001).

10.5 Effects of losartan on oxidative stress

Although the role of angiotensin II in producing free radicals has been established (Bech Laursen *et al.*, 1997; Rajagopalan et al. 1996; Kitamoto *et al.*, 2000), there have been no studies to date reporting the effects of losartan on oxidative stress in heart failure condition. A recent study from atherosclerosis apo-E deficient mice documented that treatment with losartan reduced LDL peroxidation in the atherosclerosis lesion area (Keidar, 1998). In another study performed on rabbits, losartan treatment was associated with normalized vascular $O2^{-}$ production in a dose dependent manner and prevented tolerance to nitroglycerine (Kurz *et al.*, 1999). A similar study showed that exogenous infusion with Ang

II in hypertensive rats was associated with increased vascular smooth muscle cell $O2^{-}$ production via NADH/NADPH oxidase activation. Losartan treatment to these rats normalized $O2^{-}$ production (Rajagopalan *et al.*, 1996). Peroxynitrite, a potent oxidant was decreased by losartan in cultured rat aortic endothelial cells (Pueyo *et al.*, 1998). In this study, the endothelial cells were exposed to angiotensin II, which is known to elicit the production of nitric oxide. Nitric oxide has been shown to interact with superoxide anion to form peroxynitrite (Yasmin *et al.*, 1997). In a human study on vascular endothelial cells, the production of superoxide anion by angiotensin II was significantly decreased by losartan (Zhang *et al.*, 1999). Inhibition of apoptosis by losartan in a rat model of ischemia reperfusion suggests another indirect mechanism by which losartan may modulate oxidative stress changes (Moudgil *et al.*, 2001), since apoptosis is known to involve oxidative stress (Hockenberry *et al.*, 1993). All these studies suggests the involvement of angiotensin II in producing free radicals and by analogy losartan modulates the free radical mediated damage. In the present study, we have observed that treatment of MI animals with losartan results in improved cardiac function and modulates oxidative stress.

III. MATERIALS AND METHODS

1. Animal Model

Male Sprague-Dawley rats weighing 150 ± 10 g were maintained on standard rat chow and water *ad libitum* unless otherwise mentioned. Myocardial infarction (MI) was produced by occlusion of the left anterior descending coronary artery according to an established procedure first described by Johns and Olson (1954) and later modified by different investigators (Selye, 1960; Dixon *et al.*, 1990; Hill and Singal, 1997). The animals were anesthetized with 2% isoflurane and the skin was then incised along the left sternal border. The third and fourth ribs were cut above the sternum with the subsequent insertion of retractors. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated with a 6-0 silk thread. After the ligation, the heart was gently placed back in the chest. Excess air was drawn using a syringe after which the chest was closed with a purse string suture. The rats were maintained on a positive pressure ventilation, delivering 2% isofurane. The entire surgical procedure was carried out in sterile conditions. Sham control animals were similarly handled, except that the suture around the coronary artery was not tied, and the silk thread was passed only through the muscle as described before (Khaper and Singal, 1997). Following the surgery the animals were allowed to recover on the table. The animals were monitored on a regular basis for their food and water intake, body weight, general behavior, mortality etc.

2. Study Groups

Sham and Post-MI (PMI) animals were divided into eight groups as follows: 1-week sham control without coronary ligation and without drug), 1-week PMI (with coronary ligation but no drug treatment), 4-week sham control (without coronary ligation and without drug; 4-week PMI (with coronary ligation but no drug treatment); 16-week sham control (without coronary ligation and without drug); 16-week PMI (with coronary ligation but no drug treatment); 16-week sham + losartan (no coronary ligation, and the drug was given; and 16-week PMI + losartan (coronary-ligated group with the drug treatment). The treatment with losartan (2 mg/ml, in drinking water, daily) was started at 4 weeks after the surgery and was continued for 12 weeks. Similar dosage for losartan has been used in other studies (Milavetz *et al.*, 1996). Daily average water consumption in drug treated control and experimental animals was about 30 ml.

3. Hemodynamic Measurements

Rats were anesthetized with an injection (i. p) of ketamine (60 mg/kg) and xylazine (10 mg/kg). A miniature pressure transducer catheter (Millar Micro-Tip, model PR 249) was inserted into the right carotid artery and then advanced into the left ventricle. The catheter was secured with a silk ligature around the artery (Khaper and Singal, 1997). Left ventricular end-diastolic (LVEDP), left ventricular peak systolic (LVPSP), aortic diastolic (ADP) and aortic systolic (ASP) pressures were recorded for an on-line analysis. After these assessments, the rats were killed, and the heart and other organs were removed for further studies.

4. Tissue Weight Determination

For ventricular weight in sham control group, both right and left ventricles were included as described before (Hill and Singal, 1996). In the PMI group, right and viable left ventricle were used without the infarcted tissue. In order to obtain the wet/dry weight ratio of the lungs and liver, these organs were removed and freed from adhering tissue. In each case, the sample tissue was weighed, chopped into smaller pieces, and placed in the oven (65° C) until a constant weight was obtained, which was usually after 24 h.

5. Biochemical Assays for Antioxidants

For all biochemical assessments, atria, scar and the adhering tissue from the heart were removed and only the viable ventricular tissue was used.

A: Enzymatic

5.1 Glutathione peroxidase (GSHPx) assay

GSHPx activity was expressed as nanomoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized to nicotinamide adenine dinucleotide phosphate (NADH) per minute per milligram protein, with a molar extinction coefficient for NADPH of 6.22×10^6 (Paglia and Valentine, 1967). Cytosolic GSHPx was assayed in a 3 ml cuvette containing 2.4 ml of 75 mM phosphate buffer (pH 7.0). The following solutions were then added: 50 µl of 60 mM reduced glutathione, 100 µl glutathione reductase (30 U/ml), 50 µl of 120 mM NaN_3, 100 µl of 15 mM Na_2EDTA, 100 µl of 3.0 mM NADPH, and 100 µl of cytosolic fraction obtained after centrifugation of the heart homogenate at 20,000 x g for 25 min. The reaction was initiated by the addition of 100 µl of 7.5 mM H_2O_2, and the conversion

of NADPH to NADP was assayed by measuring the absorbance at 340 nm at 1 min intervals for 5 min.

5.2 Superoxide dismutase assay

Supernatant dismutase was assayed for SOD activity by following the inhibition of pyrogallol autooxidation (Marklund, 1985). The ventricles were homogenized (1:10) in 50 mmol/litre Tris-HCL, pH 8.20, containing 1mmol/litre diethyltriamine pentaacetic acid. The homogenate was centrifuged at 20, 000 g for 20 minutes. The supernatant was aspirated and assayed for total SODactivity. Pyrogallol (24 m mol/litre) was prepared in 10 m mol/litre HCl and stored at 4°C before use. Catalase 30 µmol/litre stock solution was made in an alkaline buffer (pH 9.0). Aliquots of supernatant (150 µg protein) were added to Tris-HCl buffer containing 25 µl pyrogallol and 10 µl catalase stock solutions. Using the same Tris-HCl buffer the total reaction mixture was made to 3 ml. Auto-oxidation of pyrogallol was monitored by measuring absorbance at 420 nm at 1 min intervals for 5 min. SOD activity was expressed as units per milligram protein determined from a standard curve of percentage inhibition of pyrogallol autooxidation with a known SOD activity.

5.3 Catalase assay

The ventricles were homogenized in 50 mM potassium phosphate buffer (pH 7.4) using a weight to volume ratio of 1:10. The homogenate was centrifuged at 40,000 x g for 30 min. Supernatant of 50 µl was added to a cuvette containing 2.95 ml of 19 mM H_2O_2 solution prepared in potassium phosphate buffer (Clairborne, 1985). H_2O_2 was monitored at 240 nm wave length at 1 min intervals for 5 min. Catalase was expressed as micromoles of H_2O_2 consumed per minute per milligram protein.

B. Non-enzymatic (Tocopherol and Retinol:

Both tocopherol and retinol were measured using a modification of the extraction procedures and reverse phase HPLC detection method described by Palace and Brown, (1994). Briefly, a known amount of the ventricular tissue was homogenized in 20 volumes of ice-cold double distilled water. Proteins in the homogenate were then precipitated by adding an equal volume of ice-cold methanol containing 60 μg/ml tocopherol acetate. Tocopherol acetate is a synthetic form of tocopherol that was used as an internal standard to correct for extraction efficiency in each sample. Tocopherol, retinol and the tocopherol acetate internal standard were then extracted from the homogenate by mixing with 2 ml of ice-cold ethyl acetate:hexane (3:2; v:v). After kept on the ice for 15 min, capped and shielded from the light, the two phases were separated by centrifugation at 3000 g for 5 min and a 1 ml aliquot of the top ethyl acetate:hexane layer was recovered. The aliquot was dried under vacuum in a rotary evaporator and the remaining oily residue was reconstituted in 60μl of HPLC mobile phase before injecting directly into the HPLC system. Recovery of all samples ranged between 79 and 93% based on the internal standard and regular spikes of the authentic compounds (Palace et., al 1999a).

The HPLC mobile phase contained 70:20:10 (v:v:v)acetonitrile:dichloromethane:methanol and was delivered at 1 ml/min through an Adsorbosphere HS C18 column (250x4.6 mm, 5 μm pore size) preceded by a 5 mm guard column with the same packing material. UV detection of peaks was accomplished by monitoring at 325 nm until 7 min for retinol and then switching to 292 nm for detection of

tocopherol. Total run time was 30 min, with typical retention times for retinol and tocopherol (internal standard) of 4.5, 10.7 and 12.5 min. respectively(Palace *et al.*, 1999a).

6. Biochemical assays for oxidative Stress

In a rigorous approach, oxidative stress was assessed by measuring myocardial reduced (GSH), oxidized (GSSG) glutathione and the ratio (GSH/GSSG) as well as by quantitating lipid hydroperoxides.

6.1 Glutathione and redox ratio

Concentration of total glutathione (GSSG + GSH) was measured in the myocardium by the glutathione reductase/ 5,5' –dithiobis-(2-nitrobenzoic acid) (DTNB) recycling assay described earlier (Anderson, 1985). The rate of DTNB formation was followed at 412 nm and is proportional to the sum of GSH and GSSG present. Myocardial tissue was homogenized in 5% sulfosalicylic acid. The tissue homogenate was centrifuged for 10 min at 10,000 g. Supernatant was stored in the fridge until assayed. GSSG alone was measured by treating the sulfosalicylic acid supernatant with 2-vinylpyridine and triethanolamine. The solution was vigorously mixed and final pH of the solution was checked to be between 6 and 7. After 60 min, the derivatized sample were assayed as described above in the DTNB-GSSG reductase recycling assay. GSH values were calculated as the difference between total (GSSG + GSH) and GSSG concentrations.

6.2 Lipid hydroperoxides

This assay was done with a commercial kit (LPO-CC assay, Kamiya Biomed Co., Seattle, WA, USA) that specifically detects lipid hydroperoxides as described earlier (Palace et., al 1999c). This procedure uses a derivative of methylene blue (10-N-methylcarbonyl-3,7-

dimethylamino-10 H phenothiazine) which is specifically cleaved by lipid hydroperoxides to yield methylene blue dye, that can be quantified spectrophotometrically at 675 nm and compared to standard curve based on the same reaction with cumene hydroperoxide (Ohishi *et al.*, 1985; Palace *et al.*, 1999c). For this assay, 300 mg of myocardial tissue were homogenized in 2 ml of double distilled water and 2 ml of the homogenate was suspended in 3 ml of 2:1 (v:v) chloroform: ethanol which was then clarified with 0.6 ml of 0.9% saline. After centrifugation, at 30, 000 g for 5 minutes, 1 ml of the bottom chloroform layer was completely dried under vacuum in a rotary evaporator and reconstituted in 100µl of isopropanol. A 45 µl aliquot of this solution was used in this assay. This is compared to a standard curve based on the same reaction with cumene hydroperoxide (50-12.5 nmol/litre) (Palace et., al 1999c; Pichardo et al., 1999).

7. **Northern Blot Analysis**

Total RNA from the 1-, 4- and 16-week sham control and MI rats was isolated by using the acid guanidium isothiocyanate-cesium-chloride extraction method (Chirgwin *et al.*, 1979). The OD of the RNA was assessed spectrophotometrically at a wavelength of 260 nm. 50 µg of the RNA was separated electrophoretically on 1% agarose gel, 2.2 M formaldehyde gels and transferred to a nitrocellulose filter by capillary blot. The membranes were baked at 80°C for 2 hours and prehybridized at 42°C for 2-4 h in a solution containing 50% deionized formamide, 20 m mol/litre NaH_2PO4 (pH 7.0), 4x SSC, 2 m mol/ litre EDTA , 5x Denhardts solution (1x = 0.02 % BSA ficol and polyvinylpyrrolidane), 0.01% sodium dodecyl sulfate (SDS) and 100 µg/ µl sonicated salmon sperm DNA . Hybridization was carried out in the same solution at 42°C for 16-18 hour under the standard conditions with

^{32}P-labelled cDNA probes (specific activity > 10^9 cpm/ g DNA). Gel purified cDNA inserts of human GSHPx (Cowan *et al.*, 1992), MnSOD (Xiang *et al.*, 1987) and Catalase (Quan *et al.*, 1986) were purchased from the ATCC (Bethesda, MD, USA). These cDNA inserts were nick translated to a specific activity of 10^8 d/ min./µg DNA and used as probes. 28s ribosomal RNA was used to confirm equal loading (Tiemeier *et al.*, 1977). The membranes were washed for 15 min at room temperature with a solution of 2x SSC/0.1% SDS, and followed by a wash at 42°C in 0.1x SSC/0.1% SDS. The autoradiograph was established by exposing the filter for 24-48 h to X-ray film (X-OMAT™) at -80°C with intensifying screens (Khaper et al., 1997). The bands were quantitatively evaluated by densitometric analysis (Bio-Rad imaging densitometer GS 670). The mRNA message was presented as the ratio of expression of enzyme vs. 28S rRNA. The MnSOD scanning values represented the total densities of 3.8, 2.7, 2.2, 1.3, 1.1 kb corresponding to polyadenylated isoforms (Hurt *et al.*, 1992). All the quantitative data were presented as percentage of values in control group.

8. <u>Western Blot Analysis</u>

For protein isolation, the tissue samples were thawed in ice-cold Tris/EDTA buffer (100 mM Tris-HCl, 5 mM EDTA, pH 7.4) and homogenized with a Polytron homogenizer. Aprotinin (10 µg/ml), Leupepsin (10 µg/ml), Pepstinin A (10 µg/ml), and phenylmethysulfonyl fluoride (20 µM) were added to the homogenizing buffer to avoid protein degradation. Protein concentrations were determined according to the procedure described by Lowry and Colleagues (1951) and used to normalize the protein loading. 20µg of protein were subjected to one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) by using 15% separating gel and 5% stacking gel (Laemmli,

1970). The separated proteins were electrophoretically transferred to nitrocellulose membranes using a modified Towbin buffer (20 mM Tris, 150 mM glycine, 20% methanol, 0.02% SDS (pH8.3). After tat the nonspecific protein-binding sites were blocked with 5% nonfat milk in Tris-buffered saline/0.1% Tween-20 for about an hour and then the membranes were processed by using rabbit anti-human GSHPx antibody (kindly provided by Dr. I. Singh, Charleston, SC), rabbit anti-MnSOD and CuZnSOD antibodies (kindly provided by Dr. L. W. Oberley, Iowa City, IA) and sheep anti-CAT polyclonal antibody (The Binding Site, Birmingham, UK) as primary antibody. The bound primary antibodies were detected using anti-rabbit/sheep horse-radish peroxidase-conjugated secondary antibody and using an ECL Western blotting detection system (Amersham Inc. Arlington Height, IL). The molecular weights of the protein bands were determined by comparing to the standard molecular weight markers obtained from Bio-Rad, CA, USA. The analysis for the GSHPx, MnSOD, CuZnSOD and CAT protein levels were done with a Bio-Rad GS-670 image densitometer.

9. <u>Protein Determination and Statistical Analysis</u>

Proteins were determined by the methods of Lowry and associates (Lowry *et al.*, 1951). Data were expressed as the means ● SEM. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) and Bonferroni' s test was used to identify differences between groups. Statistical significance was acceptable to a level of $P<0.05$.

IV. RESULTS

1. General Characteristics

Sham control and post-myocardial infarction (PMI) rats from the untreated and losartan-treated groups were monitored periodically for their general behavior and food and water intake throughout the study for a 16-week period. In terms of general appearance and behavior, nothing unusual was noted in any of the animals in the sham control group. However, the rats in the untreated, 16-week coronary-ligated (PMI) group appeared lethargic, with clear signs of heart failure indicated by dyspnea and cyanosis of the peripheral extremities. In contrast, coronary ligated animals treated with losartan did not display any of the clinical signs of heart failure which were so apparent in the 16-week untreated PMI group. Mortality in the coronary artery-ligated animals during or immediately after the surgery was about 20%. Another ~15% of the animals died within 24 hrs following the surgery. Losartan treatment of the sham controls also did not have any apparent effect on the general behavior and food and water intake.

2. Body And Tissue Weights

The body weight gain in both the losartan-treated and untreated PMI groups was slightly lower than their respective control groups, but the differences were not significant ($p>0.05$) (Fig 2). The left ventricular weight remained unchanged in the 4-week PMI group compared with its respective control. In the 16-week untreated PMI group the left ventricular weight was slightly increased compared with its respective control (Table 1) although the increase was not statistically significant. Although the left ventricular weight in the losartan-treated PMI group remained unchanged as compared to the control group, it was

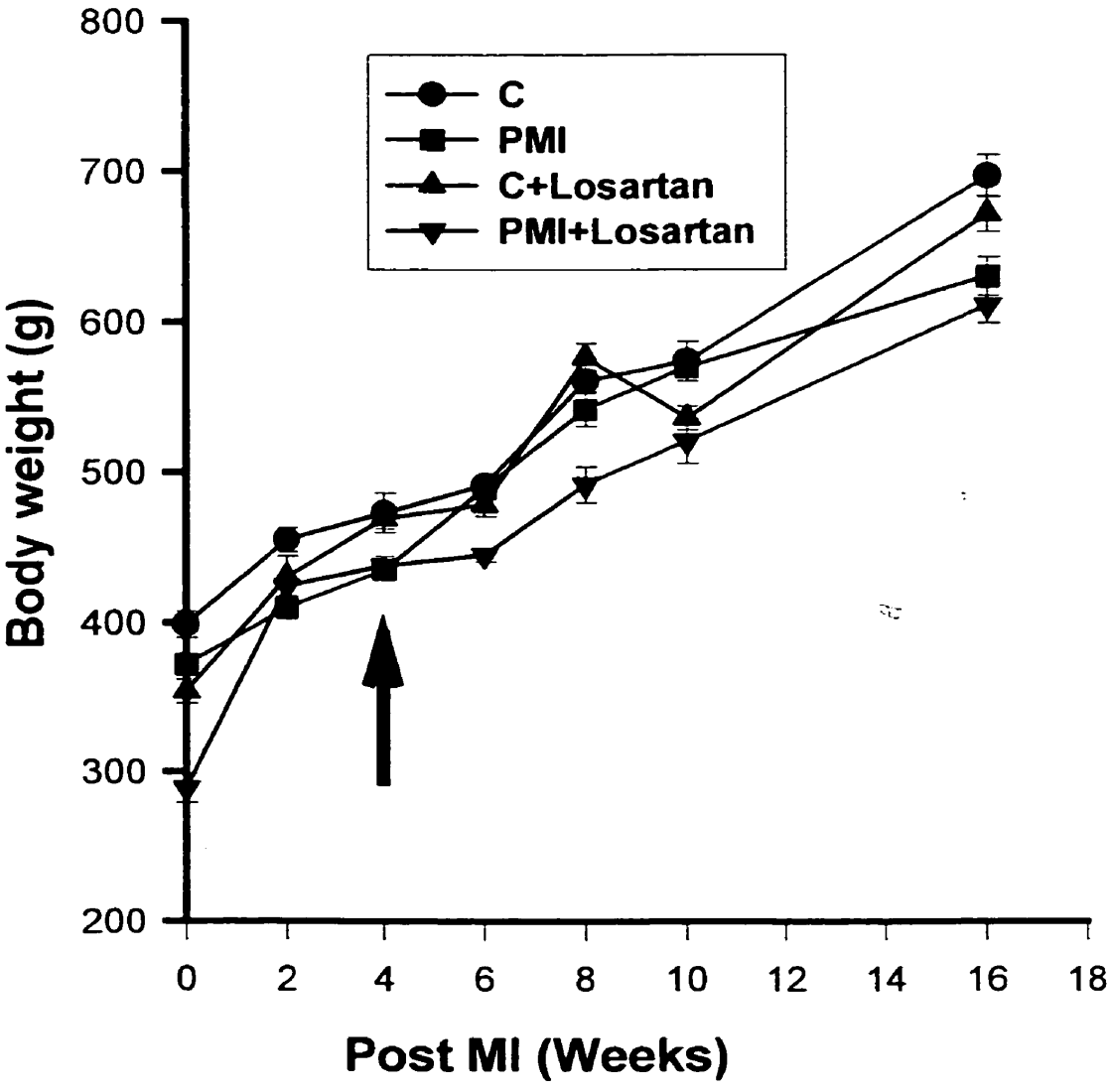


Fig 2: Effect of losartan on body weight gain subsequent to myocardial infarction (MI). Values are mean ± SEM of 7-9 animals C) Sham control; PMI) Infarcted group. Arrow indicates the time at which the drug treatment was started.

Table 1: Body weight, ventricular weight and ventricular to body weight ratio in sham control and PMI rats at 4 and 16 weeks PMI duration without losartan and at 16 weeks with losartan treatment.

	Body Weight(g)		Ventricular Weight(g)		VW/BW(10^3)	
Post-surgery Duration	C	PMI	C	PMI	C	PMI
4 weeks	465± 8.2	457± 9.6	0.92± 0.2	1.2± 0.3	2.0± 0.8	2.4 ± 0.5
16 weeks	681.8± 11.4	667.0± 13	1.6±0.4	1.89± 0.6	2.3± 1.2	2.8 ±0.2
16 weeks+ losartan	671.9± 12	611± 11.6	1.25±0.1	1.2± 0.02@	1.90±0.2	1.96± 0.3

Values are mean ± SE of 6-8 animals. @) Significantly different from the respective untreated PMI group. C) control; PMI) Post-myocardial infarction.

significantly less as compared to the respective untreated group (Table 1). The ventricular to body weight ratio remained unchanged in the untreated and losartan-treated PMI group as compared to their respective control.

At the time of sacrifice, wet/dry weight ratio for the lungs and liver was determined. At 4 weeks PMI, the lung and liver wet/dry weight ratios of infarcted animals were no different from their respective controls (Fig 3A and 3B). This ratio for both lungs and liver was significantly higher in the 16-week untreated PMI group (Fig 3A and 3B). Treatment with losartan prevented the increase in these ratios such that these values were not statistically different from their respective controls (Fig 3A and 3B).

3. Hemodynamics

Cardiac function as well as blood pressure readings were taken by inserting a catheter with a micro-tip pressure transducer through the right carotid artery, in the aorta and then into the left ventricle. The data on aortic systolic (ASP), aortic diastolic (ADP), left ventricular peak systolic (LVPSP) and left ventricular end diastolic (LVEDP), pressures are given in Tables 2 and 3. ASP and ADP in the 4-week sham control and PMI group were not different from each other. However a significant decline in ASP was noted in the 16-week untreated PMI group compared with its respective sham control group (Table 2). ASP and ADP in the PMI group treated with losartan was not different from the losartan treated control (Table 2).

A significant increase in the LVEDP as well as a significant decline in the LVPSP was noted in the PMI rats compared to their controls at 4 weeks PMI duration. These changes were more pronounced and progressive at 16 weeks PMI (Table 3). Losartan

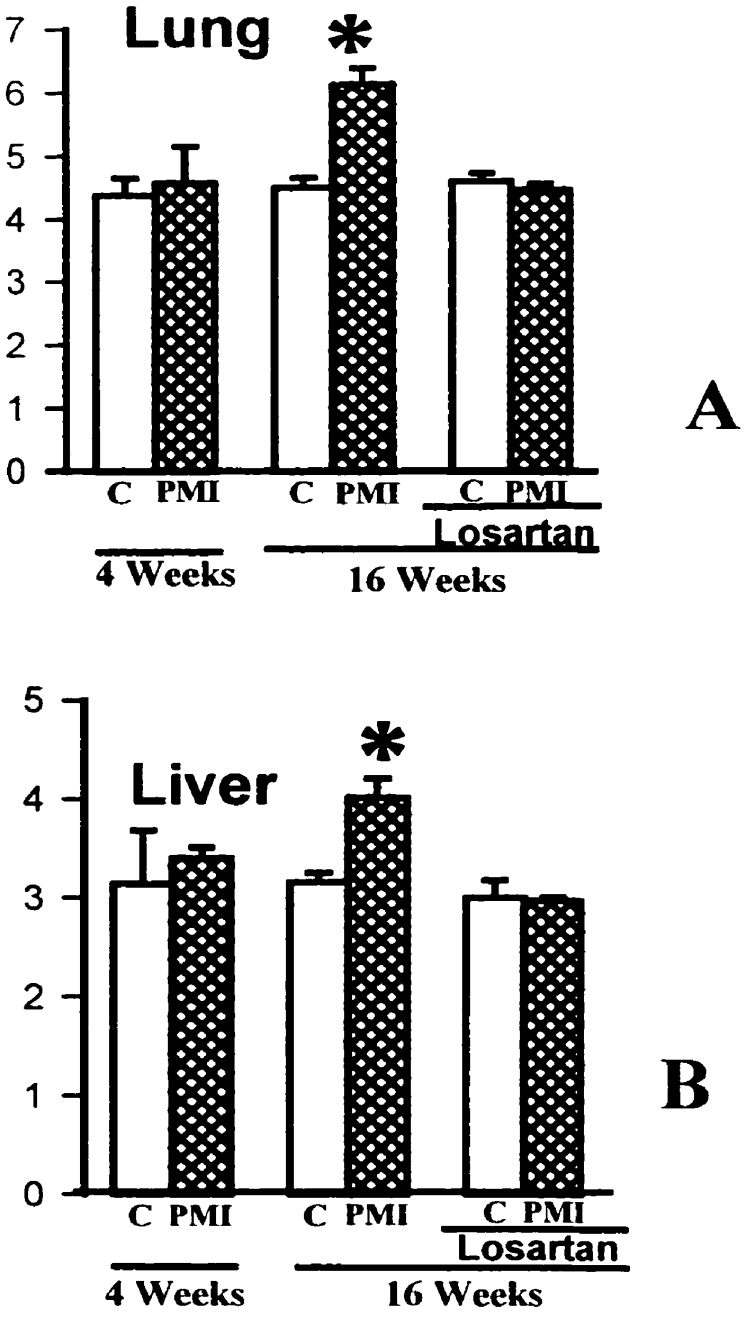


Fig 3 (A and B): Lung and Liver wet/dry weight ratios in the sham control (C) and post-myocardial infarction (PMI) rats at 4 and 16 weeks PMI duration without losartan and at 16 weeks with losartan. Data are mean ± SEM from 8-10 animals. *) Significantly different ($p<0.05$) from the respective control group.

Table 2: Aortic systolic and diastolic pressures in sham control (C) and post-Myocardial infarction (PMI) rats at 4 and 16 weeks post-surgery duration without losartan and at 16 weeks with losartan treatment.

Post-MI Duration	ASP		ADP	
	C	PMI	C	PMI
4 wks	92.14 ± 4.5	97.17 ± 4.2	68.8 ± 3.6	71.8 ± 1.5
16 wks	99.6 ± 5.7	88.5 ± 4.8 †	63.7 ± 4.1	74.3 ± 7.1
16wks+ Losartan	86.49 ● 3.7	91.5±6.3	63.54 ± 4.2	73.16 ± 6.1

Values are mean ● SEM of 4-6 animals. ADP) Aortic diastolic pressure, ASP) aortic systolic pressure. †) Significantly different from the respective sham control by using ANOVA.

Table 3: Left Ventricular peak systolic and end diastolic pressures in sham control and post-myocardial infarction rats at 4 and 16 weeks PMI duration without losartan and at 16 weeks with losartan treatment.

PMI Duration	LVPSP		LVEDP	
	C	PMI	C	PMI
4 weeks	121.6 ± 7.4	102.9 ± 2.4*	2.46 ± 0.8	6.8 ± 0.7†
16 weeks	124.3 ± 1.9	89.58 ± 2.9 *	3.38 ± 0.4	26.11 ± 1.5*
16weeks+ losartan	117 ± 12.66	95.27 ± 3.9	2.83 ± 0.4	8.62 ± 1*@

Values are mean ± SE of 6-8 animals. LVEDP) left ventricular end diastolic and LVPSP) left ventricular peak systolic pressures, †) Significantly different ($p < 0.05$) from the respective sham control group by using ANOVA and *) ANOVA followed by Bonferroni test. @) Significantly different from the respective untreated PMI group. C) Sham control; PMI) Post-Myocardial infarction.

treatment attenuated the rise in LVEDP (Table 3). LVPSP in the losartan treated group was not significantly different from the sham control (Table 3).

4. Myocardial Endogenous Antioxidants

In order to establish baseline differences in the myocardial enzyme activities, mRNA abundance and proteins content for different antioxidant enzymes were examined at different PMI durations. Non-enzymatic antioxidants such as tocopherol and retinol were also examined at different PMI durations.

4.1 Antioxidant enzyme activities

Myocardial superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase activities were examined in the viable myocardium at 1, 4 and 16 weeks PMI duration without any treatment and these data are shown in Table 4. The SOD enzyme activity remained unchanged at 1 week PMI as compared to its respective control. However, it was significantly decreased at 4 and 16 weeks by 35% and 42% respectively (Table 4). Glutathione peroxidase was slightly increased at 1 week PMI duration, but the increase was not significant. At 4 and 16 weeks PMI, the activity was depressed by about 26% and 38% respectively (Table 4). The catalase enzyme activity remained unchanged at 1 week, but was significantly depressed by about 24% and 25% respectively (Table 4).

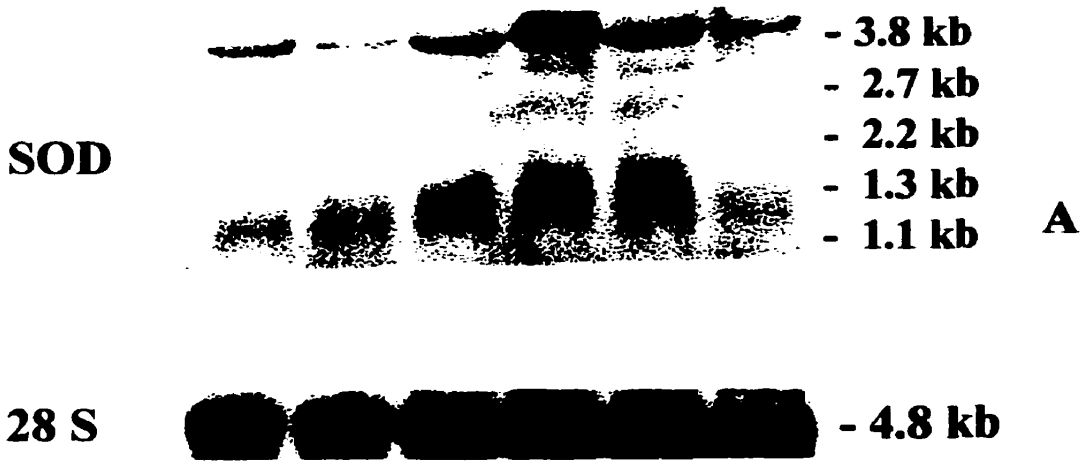
4.2 mRNA abundance

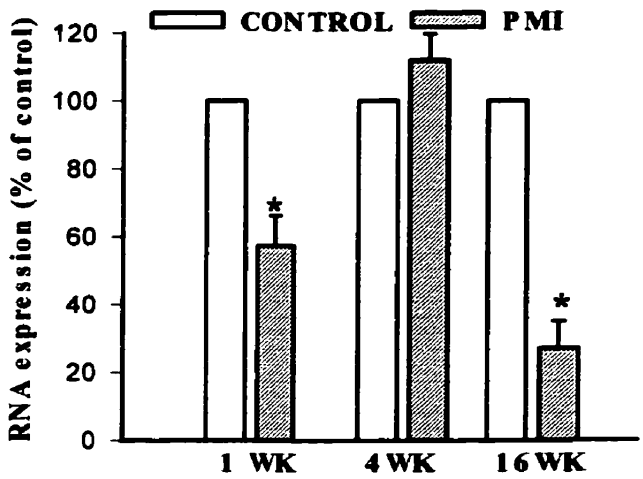
In order to examine the molecular changes underlying the differences seen in the enzyme activities of SOD, GSHPx and catalase, the mRNA abundance of these enzymes were examined at 1, 4 and 16 weeks in the sham control and PMI group and the data are presented in Figs 4, 5 and 6, respectively. The mRNA levels for SOD showed a biphasic

Table 4: Antioxidant enzyme activities at 1, 4 and 16 weeks post surgery duration in control and PMI rats.

PMI Duration	Superoxide Dismutase (U/mg protein)		Glutathione Peroxidase (nmol/mg protein)		Catalase (μmole of H_2O_2/min/mg protein)	
	Sham	PMI	Sham	PMI	Sham	PMI
1 weeks	40.2 ± 4.2	38.6 ± 4.7	78.4 ± 1.4	86.4 ± 4.1	24.0±2.7	23.4±3.8
4 weeks	36.0 ± 5.1	23.4 ±4.2†	86.4 ± 1.6	63.3±1.9†	31.6±3.12	24±1.7†
16weeks	31.78±3.21	17.86±1.0*	78.68± 2.5	49.6±2.27*	33.03±1.1	25.31±.92†

Values are mean ± S.E. of 4-6 animals. †) Significantly ($p<0.05$) different from the respective sham control group by Anova and *) Anova followed by Bonferroni test.

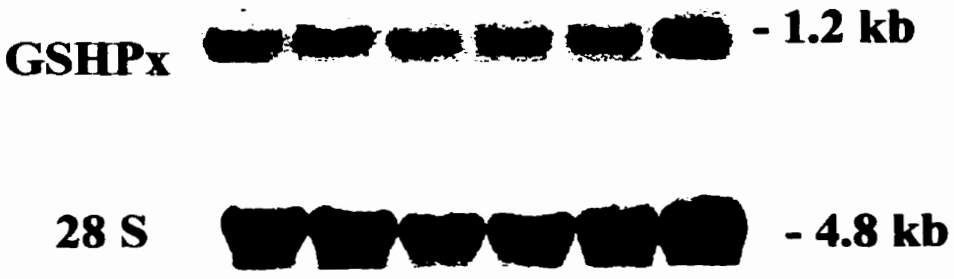




B

Fig 4: A) mRNA abundance for superoxide dismutase at 1, 4 and 16 weeks post-myocardial infarction (PMI) duration in the control (C) and PMI rats. B) Data expressed as % of control. Data are mean ± SEM from 3 animals

***) significantly different ($p<0.05$) as compared to the control group.**



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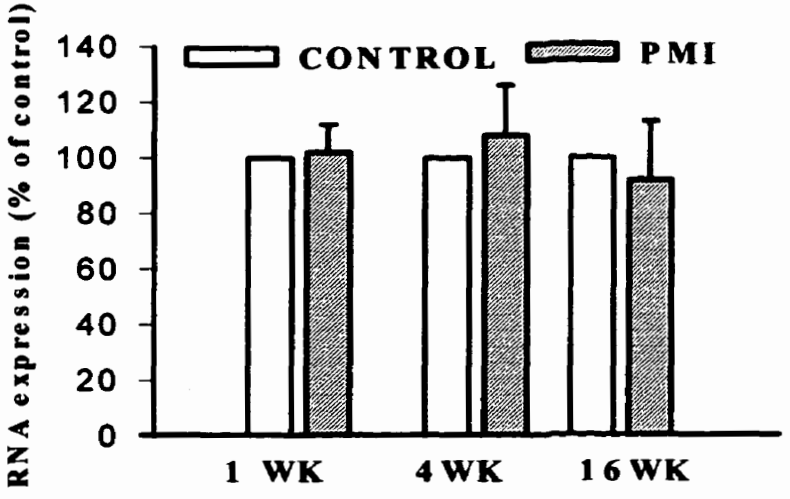
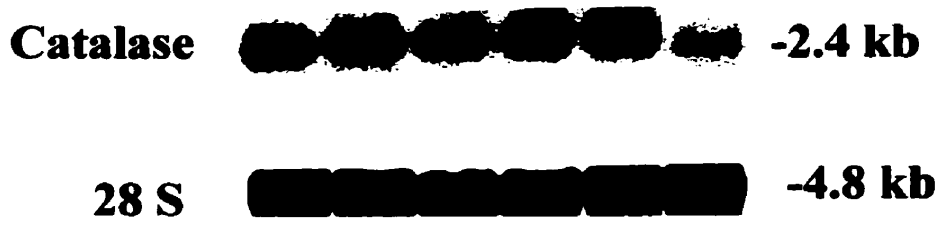


B

Fig 5: A) mRNA abundance for glutathione peroxidase at 1, 4 and 16 weeks post-myocardial infarction (PMI) duration in the control (C) and PMI rats. B) Data expressed as % of control. Data are mean ± SEM from 3 animals



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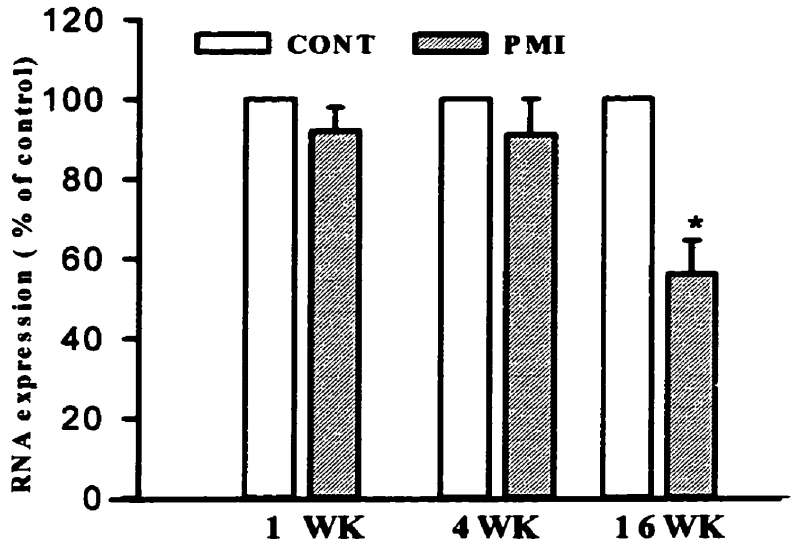


B

Fig 6: A) mRNA abundance for catalase at 1, 4 and 16 weeks post-myocardial infarction (PMI) duration in the control (C) and PMI rats. B) Data expressed as % of control. Data are mean ± SEM from 3 animals *) significantly different ($p<0.05$) as compared to the control group.

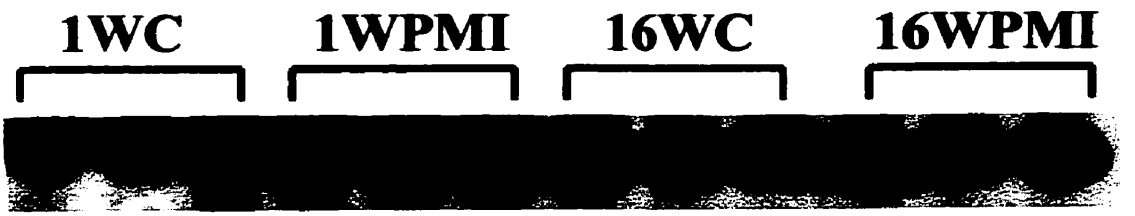
response, where it was reduced by about 40% at 1 week PMI and at 4 weeks PMI, the levels returned back to the control level and at 16 weeks PMI, the levels in the PMI group were reduced by about 73% of the control (Fig 4). GSHPx mRNA levels remained unchanged at all time points (Fig 5). The mRNA levels for catalase remained unchanged at 1 and 4 weeks PMI and was significantly reduced by about 44% at 16 weeks PMI as compared to the control group (Fig 6).

4.3 Proteins

The protein levels for MnSOD, CuZnSOD, GSHPx and catalase at 1 and 16 weeks PMI duration were also examined and the results are presented in Fig 7, 8, 9 and 10 respectively. The protein levels for MnSOD, CuZnSOD, GSHPx and catalase remained unchanged in the 1- and 16-week PMI group at all time points compared to their respective sham controls.

4.4 Non-enzymatic antioxidants

Myocardial vitamin A (retinol) and E (α-tocopherol) content were analyzed by quantitating retinol and α-tocopherol by the HPLC method and these data are shown in Table 5. Vitamin A levels remained unchanged in the 4-week PMI group, however, it was significantly decreased in the 16-week PMI group as compared to its respective control (Table 5). Vitamin E content in the 4-week PMI group remained unchanged as compared to the respective sham control. In the 16-week untreated PMI group, the concentration of vitamin E was significantly decreased as compared to its respective sham control (Table 5).



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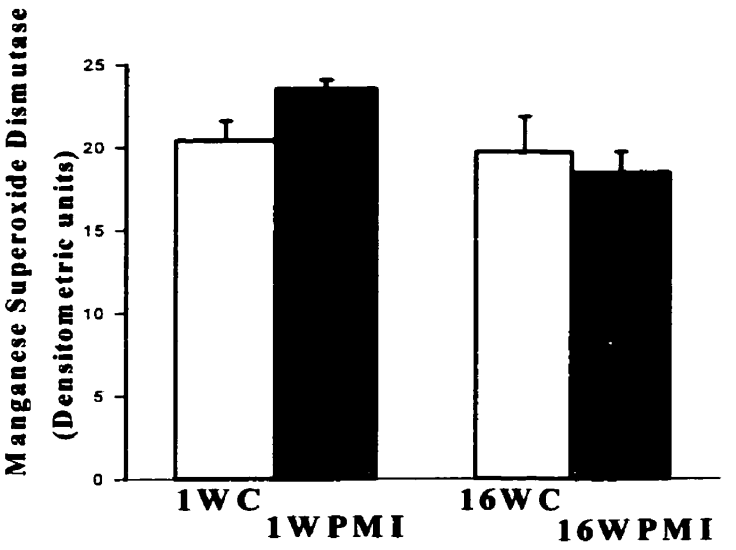
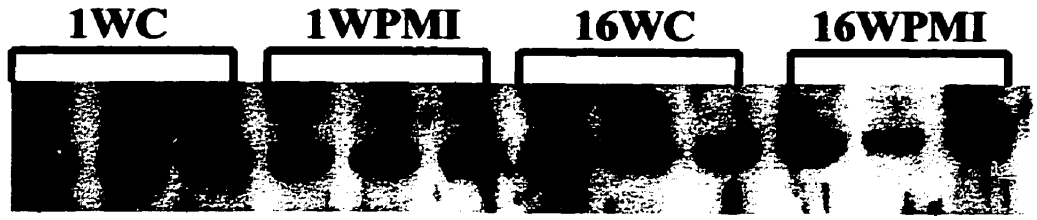


C

Fig. 7: A) Western blot for MnSOD in the sham control (C) and infarcted (PMI) rats at 1 (1W) and 16 (16W) weeks PMI duration. B) Protein loading control by Ponceau staining. C) Quantified data of MnSOD protein concentration in C and PMI animals. Data are expressed as mean ± SEM of 3 animals.



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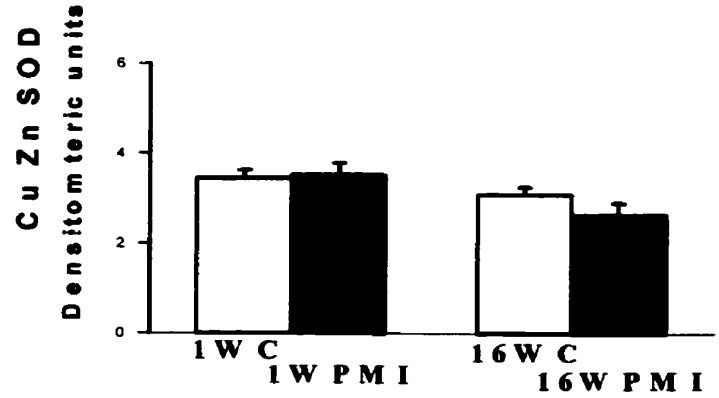
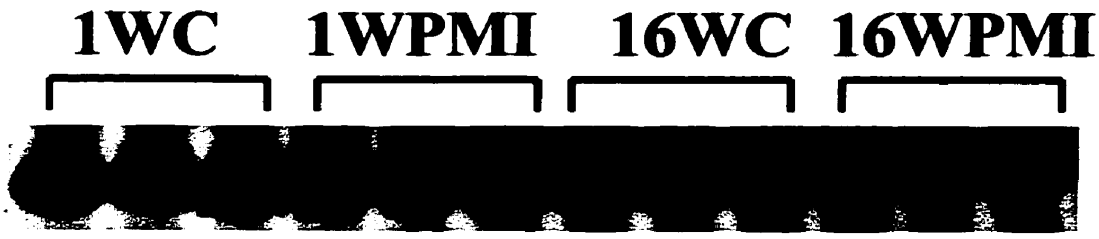


C

Fig 8: A) Western blot for CuZnSOD in the sham control (C) and infarcted (PMI) rats at 1 (1W) and 16 (16W) weeks PMI duration. B) Protein loading control by Ponceau staining. C) Quantified data of CuZnSOD protein concentration in C and PMI animals. Data are expressed as mean ±SEM of 3 animals



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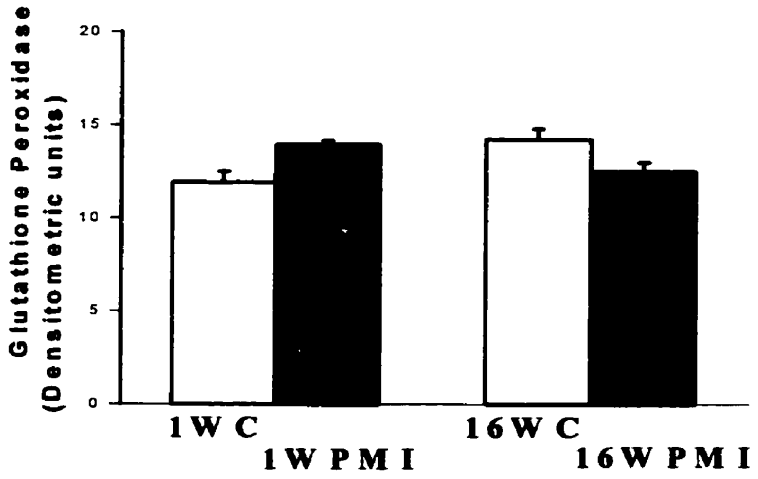
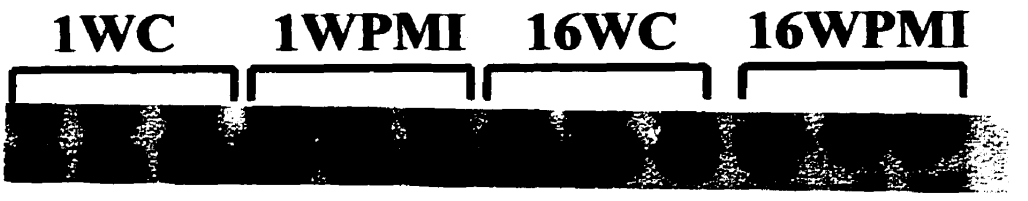


C

Fig 9: A) Western blot for GSHPx in the sham control (C) and infarcted (PMI) rats at 1 (1W) and 16 (16W) weeks PMI duration. B) Protein loading control by Ponceau staining. C) Quantified data of GSHPx protein concentration in C and MI animals. Data are expressed as mean ±SEM of 3 animals.



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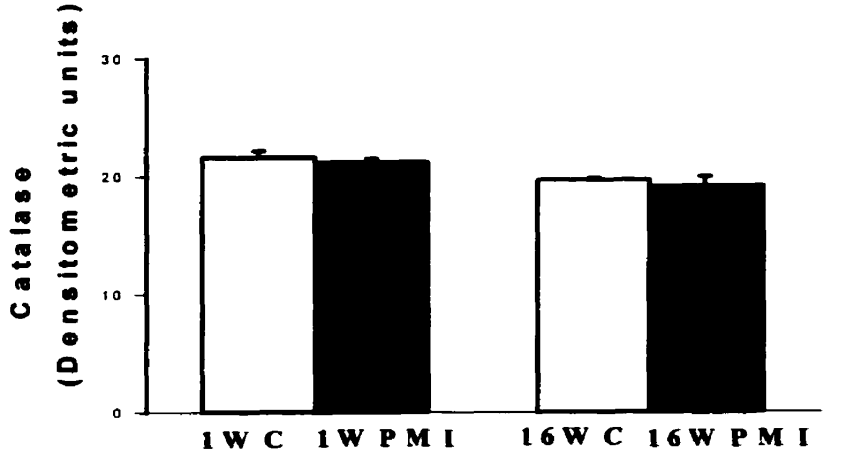


C

Fig 10: A) Western blot for catalase in the sham control (C) and infarcted (PMI) rats at 1(1W) and 16 weeks (16W) PMI duration. B) Protein loading control by Ponceau staining. C) Quantified data of catalase protein concentration. Data are expressed as mean ± SEM of 3 animals.

Table 5: Myocardial retinol and tocopherol levels in sham control and PMI rats at 4 and 16 weeks post surgery duration

Post-surgery Duration	Retinol (µg/g)		Tocopherol (µg/g)	
	C	PMI	C	PMI
4 weeks	1.54 ±0.3	1.59 ± 0.41	47.01±3.8	52.56±6.2
16 weeks	1.39 ±0.2	0.94 ±0.3*	70 ±15.3	48.76±7.5*

Data expressed as mean ± S.E. of 3-5 animals. *) Significantly ($p<0.05$) different from the respective sham control group. C) sham control; PMI) post-myocardial infarction.

5. Effects of Losartan on Enzymatic and Nonenzymatic Antioxidants and Protein Level

5.1 Antioxidant enzymes and protein

Myocardial SOD, GSHPx and catalase activities were examined in the viable myocardium at 4 and 16 weeks PMI duration without losartan treatment and at 16 weeks with losartan treatment (Figs. 11, 12 and 13). SOD activity was decreased by about 35% in the 4 weeks and about 42% in the 16-week untreated PMI group compared with its respective sham control (Fig. 11). In the 16-week PMI group treated with losartan, SOD activity remained unchanged compared with the 16-week PMI untreated group (Fig 11). GSHPx activity was depressed by about 26% in the 4-week PMI group compared with its respective sham control and was decreased by about 38% in the 16-week untreated PMI group relative to sham control values (Fig 12). Losartan treatment resulted in significant improvement in the GSHPx activity in the PMI group as compared to the 16-week untreated PMI group and the values were no more different among these groups (Fig 12). Catalase activity was significantly decreased at 4 by about 24% and by about 25% at 16-week untreated PMI group compared with its respective sham control group (Fig 13). The catalase activity in the 16-week PMI group treated with losartan showed some improvement, however, this change was not statistically significant. Losartan treatment in the 16-week control animals resulted in a significant increase in the catalase activity (Fig. 13). The protein levels for catalase were significantly increased in the losartan-treated control and MI groups (Fig 14).

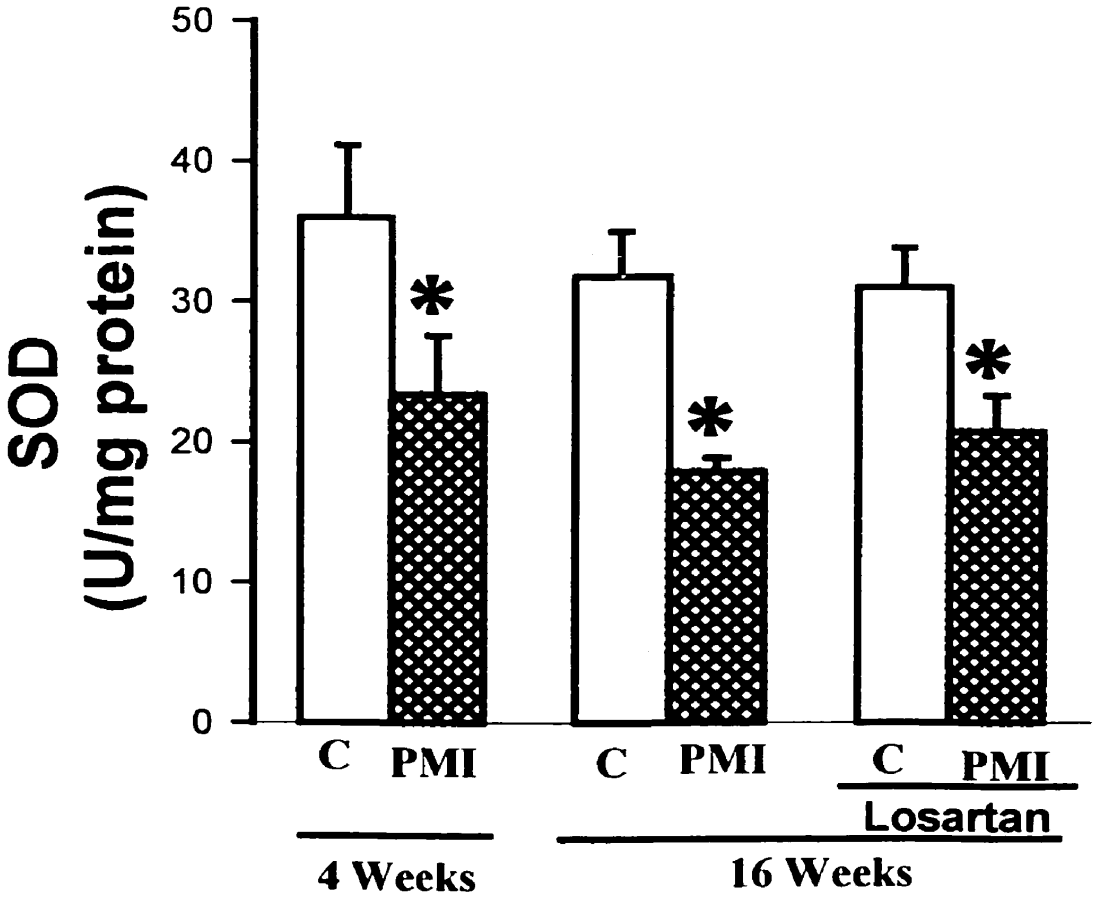


Fig 11:Myocardial superoxide dismutase activity in the 4 and 16 weeks shamcontrol (C) and post-Myocardial infarction (PMI) rats without losartan and at 16 weeks with losartan. Data are presented as mean±SEM from 4-6 animals. *)Significantly different ($p<0.05$) from the respective control group.

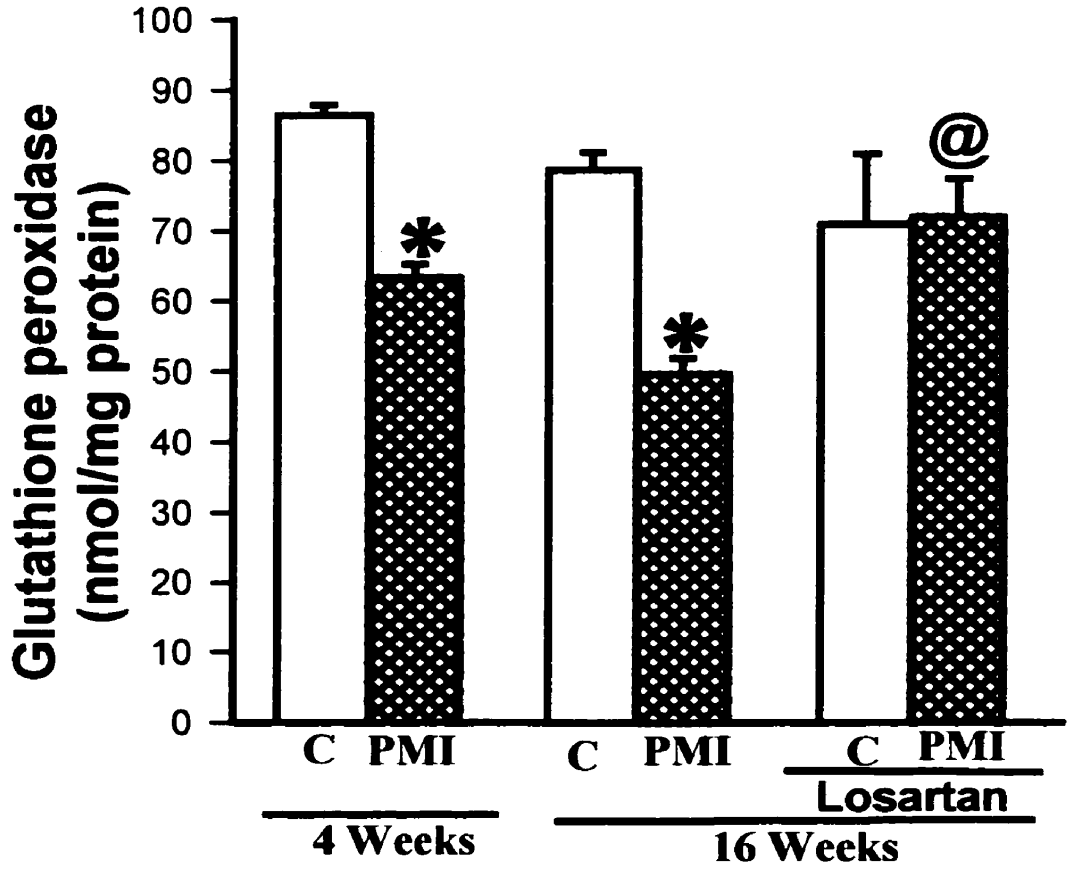


Fig. 12: Myocardial glutathione peroxidase activity in the 4 and 16 weeks sham control (C) and post-Myocardial infarction (PMI) rats without losartan and at 16 weeks with losartan treatment. Data are presented as mean ± SEM from 5-7 animals. Significantly different ($p<0.05$) from the respective control group;@) significantly different ($p<0.05$) from the respective untreated PMI group.

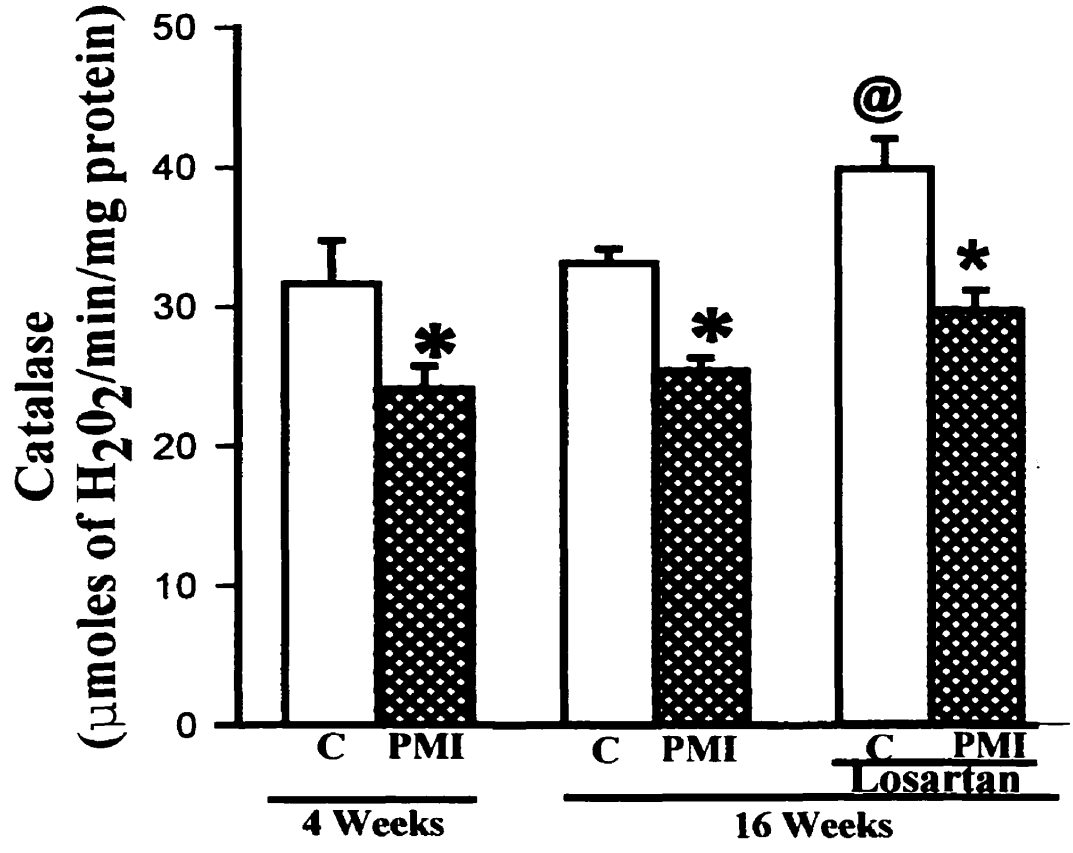
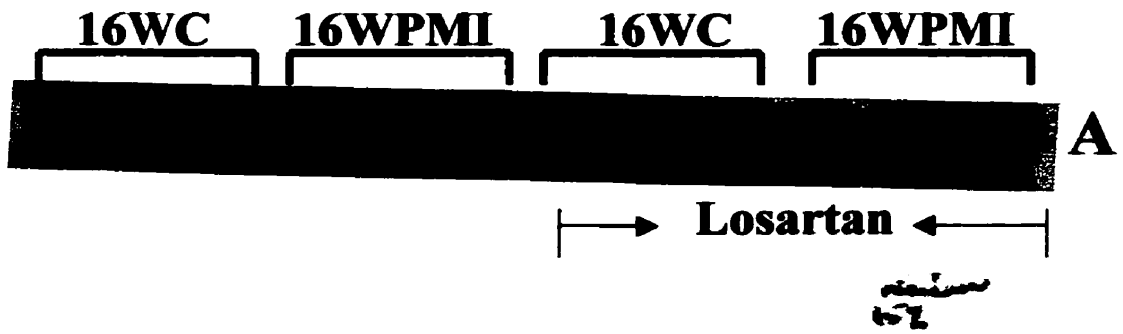


Fig 13: Myocardial CAT activity in the 4 and 16 weeks control (C) and post-Myocardial infarction (PMI) rats without losartan and at 16 weeks with losartan treatment. Data are presented as mean SEM from 5-7 animals. *) Significantly different ($p<0.05$) from the respective control group;@) significantly different ($p<0.05$) from the respective untreated 16 weeks control and PMI group.



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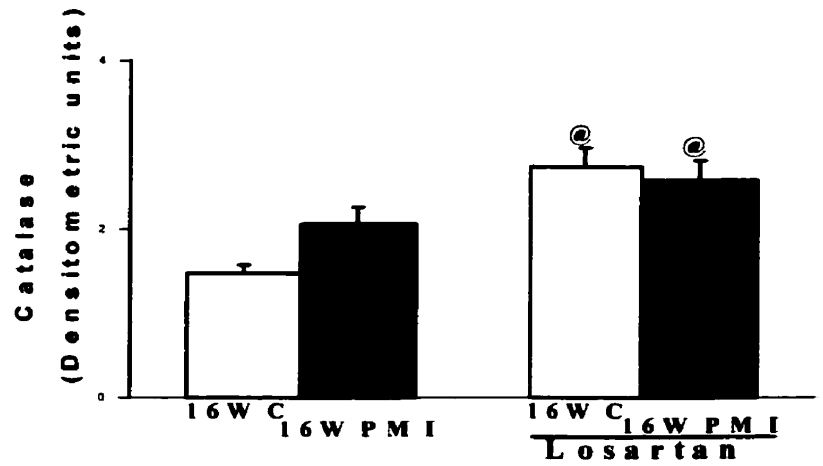

C

Fig 14: A) Western blot for catalase in the sham control (C) and infarcted (PMI) rats at 16 weeks (16 W)PMI duration with and without losartan treatment. Representative blot showing specific blot for catalase B)Protein control loading by Ponceau staining. C) Quantified data of catalase protein concentration. Data are expressed as mean ±SEM of 3 animals. @) Significantly different from the untreated C and PMI group.

5.2 Vitamins A and E

In the 16-week PMI group, retinol levels were significantly below ($p<0.05$) the control level. Treatment with losartan resulted in a significant increase in retinol levels in the 16-week treated PMI group (Fig 15). Vitamin E levels in the untreated PMI animals were significantly ($p<0.05$) less than the sham control. Losartan treatment did not have any influence on vitamin E concentration in either of the groups (Fig 16).

6. Oxidative Stress

6.1 Glutathione (reduced and oxidized)

Myocardial oxidative stress changes due to coronary ligation and their modulation by losartan were examined using several approaches including GSH levels, redox ratio and lipid peroxidation. Myocardial reduced (GSH) and oxidized (GSSG) glutathione contents were examined in the 4- and 16-week sham and PMI groups without any treatment and in the 16-week sham and MI group with losartan treatment and these data are presented in Table 6. There was a slight but insignificant decrease in the myocardial GSH content in the 4-week untreated PMI group as compared to the respective sham control group. In the 16- week PMI group the GSH content was decreased by about 40% compared to its respective sham control group. GSSG content remained unchanged in the 4-week PMI group as compared to its respective control. At 16 weeks PMI duration, the GSSG content was increased by about 114% (Table 6). The GSH content in the losartan-treated control as well as MI groups was significantly improved. GSSG content in the losartan-treated PMI group was decreased and the values no longer were different from its respective sham control group (Table 6).

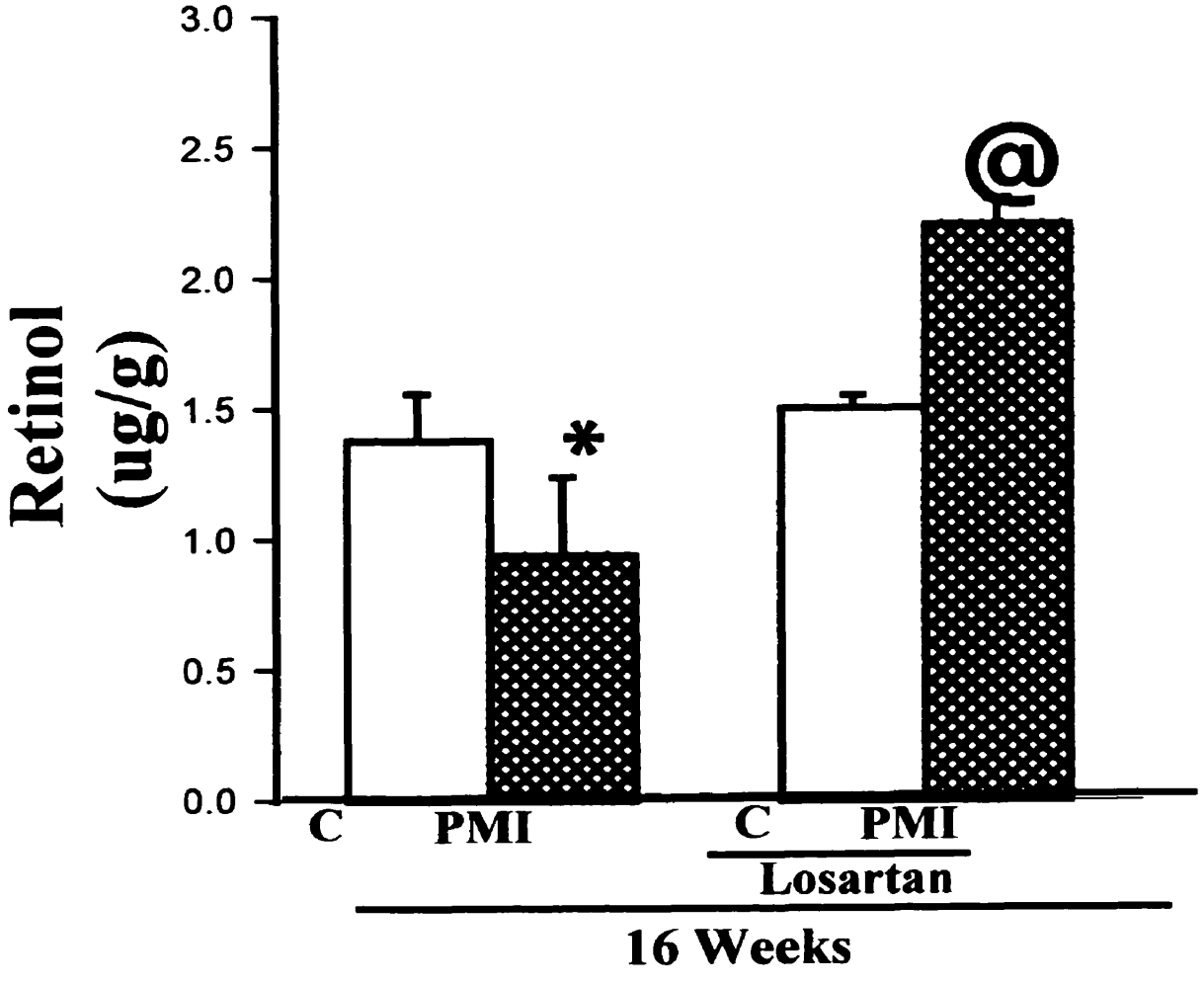

Fig 15: Myocardial retinol content in the 16 weeks sham control (C) and post-myocardial infarction (PMI) rats with and without losartan. Data are expressed as mean " SEM from 3-5 rats. *) Significantly different ($p<0.05$) from the respective control group. @) significantly different ($p<0.05$) from the respective untreated group.

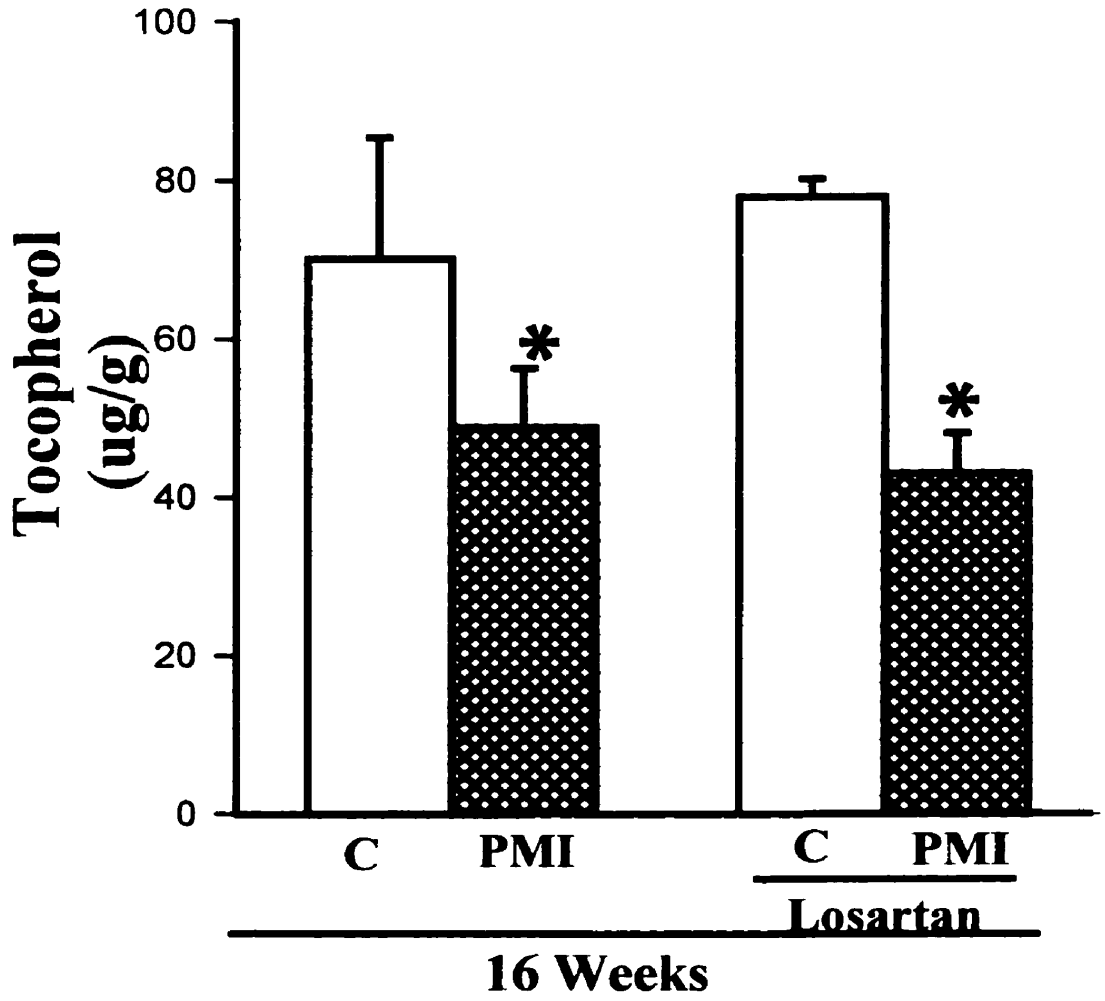

Fig 16: Myocardial tocopherol content in the 16 weeks sham control (C) and post Myocardial infarction (PMI) rats with and without losartan. Data are expressed as mean ±SEM from 3-5 rats.*)Significantly different ($p<0.05$) from the respective control group.

Table 6: Myocardial reduced and oxidized glutathione levels at 4 and 16 weeks in sham control and PMI rats without losartan and at 16 weeks PMI with losartan treatment.

Post-MI Duration	GSH (µmol /g tissue wt)		GSSG (µmol /g tissue wt)	
	C	PMI	C	PMI
4 wks	67.8 ± 2.1	58.5 ± 1.4	8.4 ± 1.4	8.3 ± 0.7
16 wks	71.53 ± 1.32	42.68 ± 1.3*	8.63 ± 0.51	18.51 ±1.2*
16wks+ Losartan	88.66 ± 4.1@	77.73 ±7.1@	7.25 ± 0.4	8.12 ± 1.32†

Values are mean ± SEM of 5-7 hearts. *) Significantly different ($p<0.05$) from respective sham controls. @) Significantly different from the 4 week and 16 week untreated group. †) Significantly different from the 16 week untreated group. C) control; PMI) post-myocardial infarction.

6.2 Redox ratio

Redox ratio was also assessed in the 4- and 16-week sham control and MI group without losartan treatment and at 16 weeks PMI duration with losartan treatment and the results are presented in Fig 17. The redox ratio remained unchanged in the 4-week PMI group compared to its respective sham control group, however, it was significantly depressed in the 16-week PMI group compared to its respective sham control group (Fig 17). This ratio was significantly improved in both the control and MI groups treated with losartan (Fig 17).

6.3 Lipid hydroperoxides

In order to assess the degree of lipid peroxidation during heart failure, lipid hydroperoxide formation was determined (Fig 18). Lipid hydroperoxide content was significantly higher ($p < 0.05$) in the 4- and 16-week MI group compared to its respective sham control group. This increase in the lipid hydroperoxide content was significantly attenuated by losartan (Fig 18).

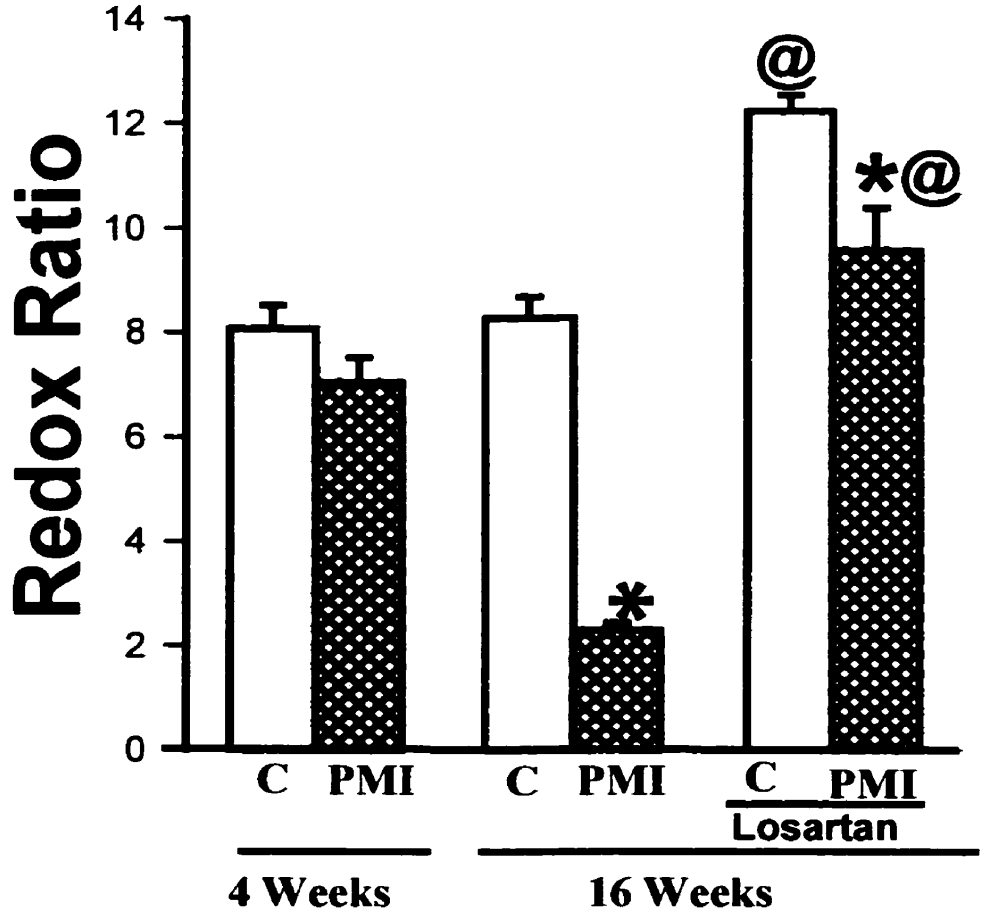

Fig 17: Redox (GSH/GSSG) ratio in the 4 and 16 weeks control (C) and post-Myocardial infarction (PMI) rats without losartan and at 16 weeks with losartan. Data are presented as mean ± SEM from 8-10 animals. *)Significantly different ($p<0.05$) from the respective control group. @) Significantly different ($p<0.05$) from the respective untreated control and PMI groups.

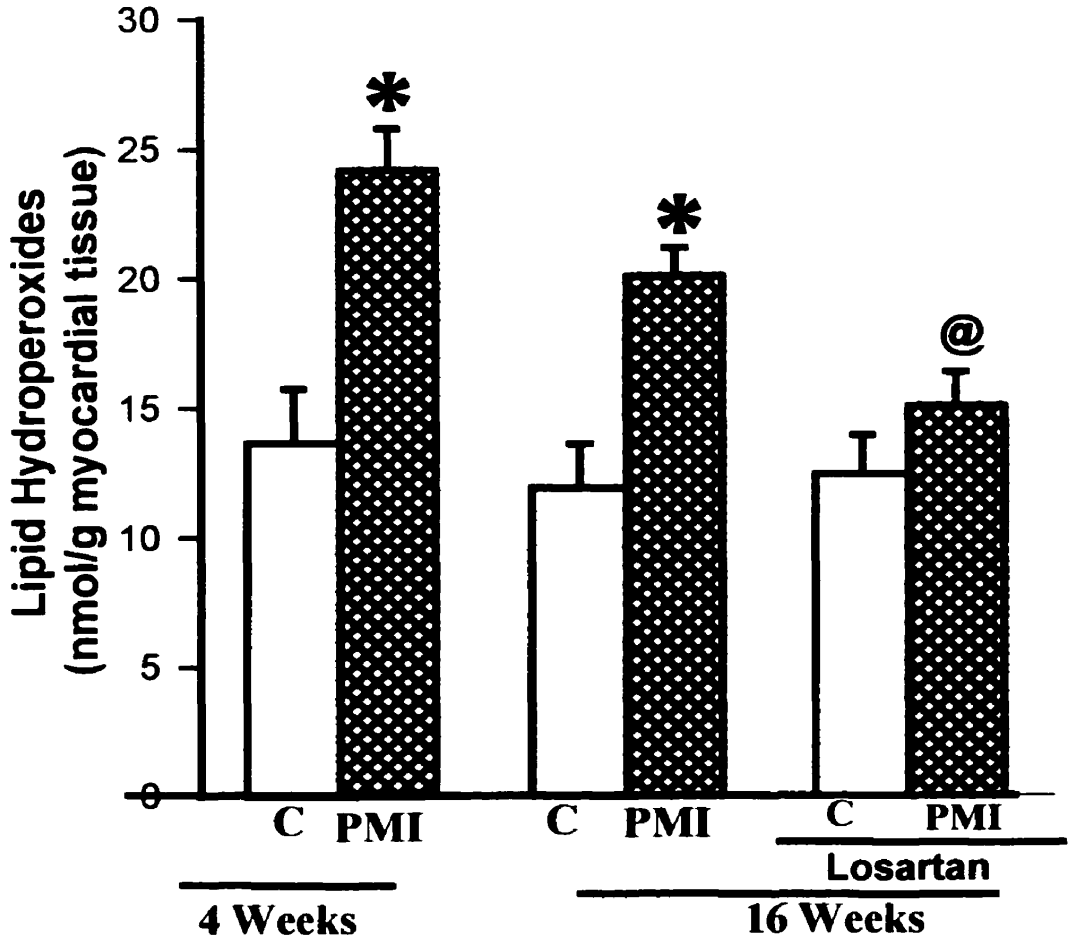

Fig 18: Myocardial lipid hydroperoxide content in the 4 and16 weeks control (C) and post Myocardial infarction (PMI) rats without losartan and at 16 weeks with losartan treatment. Data are presented as mean ± SEM from 4-6 rats. *) Significantly different ($p<0.05$) from the respective control group. @) Significantly different from the respective untreated group.

V. DISCUSSION

In the present study, we have confirmed that heart failure following myocardial infarction (MI) is associated with depressed hemodynamic function which correlated with a decrease in the antioxidants (enzymatic and non-enzymatic) and an increase in oxidative stress. The study shows for the first time a correlation between the decrease in the mRNA abundance for the antioxidant enzymes superoxide dismutase (SOD) and catalase, and their activities. Glutathione peroxidase (GSHPx) activity was also depressed in the myocardial infarction group, however, the mRNA abundance for this enzyme was not changed. Both vitamins A and E were also decreased in the myocardial infarction group. Losartan treatment not only improved the cardiac function, but also maintained the glutathione peroxidase activity and improved vitamin A levels. Oxidative stress parameters were significantly decreased in the MI group treated with losartan. The study documents for the first time that the beneficial effects of inhibition of angiotensin II type 1 receptors are associated with an improvement in myocardial antioxidants and decreased oxidative stress.

1. Heart Failure and Renin-Angiotesin System

Heart failure is a major cause of mortality and morbidity in the industrialized nations. Research in the field of heart failure has shown that the transition from the compensatory stage to the decompensated, or failing, stage include abnormalities in the energy metabolism (Lindenmayer *et al.*, 1970), defects in intracellular calcium handling (Afzal and Dhalla, 1992; Dixon *et al.*, 1992a) alterations in the contractile protein and gene expression (Schwartz *et al.*, 1993; Boheler *et al.*, 1991), activation of the sympathetic system (Francis *et al.*, 1984), abnormal collagen deposition and altered extracellular matrix structure (Dixon *et al.*, 1996),

and excessive activation of the renin-angiotensin system (RAS) (Baker *et al.*, 1990). Despite significant advances, both the mortality and morbidity still remain high in heart failure patients. Clearly, the precise mechanism involved in the pathogenesis of heart failure remains undetermined. During the past decade, various experimental and clinical research efforts have resulted in the development of different therapeutic strategies for the management of heart failure.

Although, activation of the RAS is a compensatory response, over a period of time this compensation becomes detrimental to heart function. This is the reason that angiotensin converting enzyme (ACE)-inhibitors have been successfully used in the management of heart failure patients. Numerous studies have documented the beneficial effects of ACE-inhibitors in reducing mortality (Pfeffer *et al.*, 1992; Dzau *et al.*, 1980; Levine *et al.*, 1980). However, due to the limited efficacy of ACE-inhibitors in blocking the RAS as well as their effects on the bradykinin and prostaglandin metabolism (Gohlke *et al.*, 1994; Martorana *et al.*, 1991; Linz and Scholkens, 1992; Schror 1992), angiotensin II (AII)-receptor blockers are considered as an attractive alternative to ACE-inhibitors. Losartan is an orally active, non-peptide angiotensin receptor blocker that has beneficial effects similar to ACE-inhibitors without the side effect of cough. Other advantages of losartan over other ACE-inhibitors include its long duration of action and better oral absorption. Losartan selectively and specifically inhibits all the angiotensin II type 1 (AT_1)-mediated actions of AII irrespective of the pathway by which the AII is formed (Wong *et al.*, 1990). It is considered to be about 30,000 times more selective for AT_1 (Chiu *et al.*, 1990; Chiu *et al.*, 1989). Furthermore, losartan is known to have no inhibitory effects on vasopressin, bradykinin or prostaglandin

metabolism (Eberhardt *et al.*, 1993). Numerous studies have demonstrated reversal of left ventricular hypertrophy, fibrosis, improvement in coronary flow and cardiac function following losartan treatment (Sladek *et al.*, 1996; Milavetz *et al.*, 1996; Frimm *et al.*, 1997; Thai *et al.*, 1999; Dixon *et al.*, 1996; Awan and Mason *et al.*, 1996). The Evaluation of Losartan In The Elderly (ELITE) study indicated that losartan had a better overall tolerability than captopril in elderly patients with New York Heart Association classes II to IV failure (Pitt *et al.*, 1997). An improvement in survival and clinical outcomes with losartan was also noted (Pitt *et al.*, 1997; Rump, 1998).

2. Heart Failure Model and Hemodynamics

We chose the coronary artery ligation model in rats based on the fact that this procedure produces more consistent infarction as compared to the dog model (Fishbein *et al.*, 1978). In this model, the clinical signs of heart failure such as dyspnea, lethargic behavior, and congestion of the lungs and liver were clearly evident. The heart failure is reproducible, and thus the animal model is reliable and this model has been extensively used in experimental studies (Dixon *et al.*, 1992a; Hill and Singal, 1996; Hill and Singal, 1997; Khaper and Singal, 1997; Khaper and Singal, 2001).

In the present study, the LVEDP, LVPSP, ASP and ADP were maintained at 1 week MI duration with no signs of congestive heart failure. Furthermore, there was no increase in the lung and liver wet/dry weight ratio. At 4 weeks PMI duration, an increase in LVEDP and a decrease in LVPSP was accompanied by an increase in lung and liver wet/dry weight ratio. Animals in the 16-week PMI group exhibited clear signs of congestive heart failure as indicated by labored breathing, lethargic behavior, a further increase in LVEDP, decrease in

LVPSP and presence of the lung and the liver congestion. Heart failure subsequent to myocardial infarction has been reported to be associated with depressed hemodynamic function both in the left and right side of the heart and during the progression of heart failure changes in the right ventricle follow changes seen in the left ventricle (Hill and Singal, 1997).

The drug treatment was started at 4 weeks post surgery duration based on the fact that the early signs of heart failure were evident at 4 weeks following the surgery. The dosage of losartan (2 mg/ml) used by us in this study has also been used by other investigators (Milavetz *et al.*, 1996). Losartan treatment normalized the increase in LVEDP and improved LVPSP. There was also no lung and liver congestion in the losartan group. Other studies have also reported a decrease in LVEDP and improvement in survival in MI rats treated with losartan (Milavetz *et al.*, 1996; Richer *et al.*, 1999). Both early and late treatment with losartan following MI has been reported to be beneficial in modifying the changes in the central venous pressure as well as in inhibiting the collagen deposition and regression of cardiac hypertrophy (Smits *et al.*, 1992). Losartan has been reported to improve hemodynamics and induce coronary angiogenesis in MI rats (Sladek *et al.*, 1996; Schieffer *et al.*, 1994). In addition to being beneficial in heart failure following MI, losartan has also been shown to exert beneficial effects in volume-overload induced (Ruzicka *et al.*, 1993) and pressure- overload induced heart failure (Crozier *et al.*, 1995). Our data, in this study, shows that the beneficial effects of AT_1 receptor blockade in addition to hemodynamic improvements may also involve an improvement in the myocardial antioxidant reserve.

3. Oxidative Stress and Heart Failure

There is significant data from patients, as well as animal experiments, supporting the argument that increased oxidative stress may be involved in the pathogenesis of heart failure (McMurray *et al.*, 1990; Sobotka *et al.*, 1993; Palace *et al.*, 1999a; Dhalla and Singal, 1994; Hill and Singal, 1997). Furthermore, there is also a strong evidence that activation of RAS and AT_1 receptors results in an increase in oxidative stress (Pueyo *et al.*, 1998; Rajagopalan *et al.*, 1996; Zhang *et al.*, 1999). In the present study, we observed an increase in oxidative stress as well as its modulation by the AT_1 receptor blockade by losartan.

3.1 Clinical studies

Markers of oxidative stress such as malondialdehyde, breath pentane and lipid peroxidation have been reported to be significantly higher in heart failure patients (McMurray *et al.*, 1990; Weitz *et al.*, 1991; Sobotka *et al.*, 1993; Belch *et al.*, 1991; Diaz-Velez *et al.*, 1996). A significant increase in free radicals and decrease in vitamin E levels have also been demonstrated in patients undergoing coronary artery bypass graft surgery (Scragg *et al.*, 1989). A decrease in plasma antioxidant enzyme activities and an increase in the markers of oxidative stress have also been reported (Ghatak *et al.*, 1996; Chandra *et al.*, 1994; Keith *et al.*, 2001). Treatment with vitamin E in these patients normalized the indices of oxidative stress (Ghatak *et al.*, 1996). Beneficial effects of antioxidant vitamins in various cardiovascular diseases has lent further support to this concept.. The Health Professional Follow-up study (Rimm *et al.*, 1993) and the Nurses Health Study (Stampfer *et al.*, 1993) found a direct correlation between vitamin E intake and reduced risk of coronary artery disease. The Cambridge Heart Antioxidant Study (CHAOS) reported a significant decrease in the incidence of non-fatal MI in patients receiving vitamin E. Conversely, data not

supporting the role of vitamin E in heart disease has also been reported (Yusuf *et al.*, 2000; Keith *et al.*, 2001). Thus, further studies are required to settle these controversies.

3.2 Animal studies

Many studies have reported the production of free radicals in isolated ischemic-reperfused hearts (Jeroudi *et al.*, 1994; Palace *et al.*, 1999c). A reduction in infarct size by SOD and catalase was observed in dogs that underwent ischemia followed by reperfusion (Jolly *et al.*, 1984). Increased oxidative stress has also been documented in catecholamine-induced (Singal *et al.*, 1983) and adriamycin-induced (Singal and Iliskovic, 1998; Li and Singal, 2000) cardiomyopathies. Depressed antioxidants and increased oxidative stress has also been observed in rats with heart failure subsequent to MI rats (Hill and Singal, 1997; Khaper and Singal, 2000). Direct evidence for an increased production of hydroxyl radical originating from superoxide anion and hydrogen peroxide in the failing myocardium has also been provided using the ESR technique (Ide *et al.*, 2000). In a recent study, in the rabbit coronary artery ligation model of heart failure, it was demonstrated that gene therapy using extracellular SOD offered cardioprotection by reducing infarct size (Li *et al.*, 2001).

The present study also demonstrates for the first time that, in addition to reducing cardiac remodeling and improving survival as has been reported by other investigators (Raya *et al.*, 1992; Sun and Weber, 1996), losartan also reduces oxidative stress and maintains myocardial endogenous antioxidants in the MI model of heart failure. In the present study, SOD, catalase and GSHPx activities were maintained at 1 week PMI duration but were significantly decreased at 4 and 16 weeks PMI duration. Our laboratory has previously reported that heart failure subsequent to MI in rats correlated with depressed antioxidant

reserve and increased oxidative stress (Hill and Singal, 1997). Diet, supplemented with vitamin E, improved endogenous levels of vitamin E and improved cardiac function establishing a close link between antioxidant deficit and cardiac dysfunction (Palace *et al.*, 1999a). Myocardial antioxidants have been reported to change in various other physiological and pathological conditions including hypertrophy (Gupta and Singal, 1989; Kirshenbaum and Singal, 1992) exercise (Kanter *et al.*, 1985) and adriamycin-induced cardiomyopathy (Singal and Iliskovic., 1998; Li and Singal, 2000). A decrease in catalase and SOD activity at 16 weeks PMI duration correlated with a decrease in mRNA abundance for these two enzymes. The protein content for MnSOD, CuZnSOD, GSHPx and catalase enzymes remained unchanged.

Treatment with losartan normalized the decrease seen in GSHPx activity. There was a slight but insignificant increase in the catalase and SOD activities following losartan treatment. Losartan treatment also resulted in an increase in the catalase activity in the control group. The upregulation of MnSOD mRNA at 4 weeks may be an adaptive response to an increase in oxidative stress. There have been very few studies to date studying the changes in antioxidant enzyme activity, mRNA abundance and protein content in heart failure. A recent study by Dieterich and colleagues, reported that in patients with end stage heart failure, the enzyme activity, mRNA and protein content for catalase was upregulated (Dieterich *et al.*, 2000). In another study, it was found that a significant decrease in catalase enzyme activity was associated with no change in mRNA and protein levels (Baumer *et al.*, 2000). It is possible that changes in antioxidants may be unique not only to the disease condition but also to the stage of myocardial dysfunction.

In the present study, vitamin A and E levels were found to be significantly decreased in the severe failure stage. Treatment with losartan resulted in a significant increase in the myocardial retinol content in the 16-week PMI group although it had no effect on the tocopherol level. In the past, it has been demonstrated that depressed cardiac function in the MI rats was associated with a decrease in vitamin E content (Hill and Singal, 1997; Palace *et al.*, 1999a).In this study it was also documented that by supplementing the diet with vitamin E, myocardial and liver concentration of vitamin E were elevated in the MI animals (Palace *et al.*, 1999a). Several epidemiological studies have reported an inverse correlation between vitamin A and E intake and the risk of cardiovascular diseases (Kushi *et al.*, 1996).

In this study, severe heart failure stage was accompanied by reduced levels of reduced glutathione (GSH) and increased levels of oxidized glutathione (GSSG). The redox ratio (GSH/GSSG) was also significantly decreased in the viable myocardium of the MI rats as compared to the control. In addition to the redox ratio, the lipid hydroperoxide content was also significantly higher in the MI rats. In a previous study we used thiobarbituric acid reactive substances (TBARS) assay to measure free radical mediated lipid peroxidation (Hill and Singal, 1996; Khaper and Singal, 1997). However, due to limited specificity with this assay we chose to use a more sensitive method for determining lipid peroxidation by quantifying lipid hydroperoxides. The increased concentration of lipid hydroperoxide in cellular membranes are indicative of actual damage mediated by oxygen radicals (Ohishi *et al.*, 1985). In this study, the significantly higher concentration of lipid hydroperoxide concentration correlated with a drop in the redox ratio. An increase in lipid hydroperoxide content has also been reported by us in right heart failure subsequent to pulmonary

hypertension (Pichardo *et al.*, 1999) and in isolated hearts subjected to global ischemia-reperfusion (Palace *et al.*, 1999c). Redox ratio is inversely related to oxidative stress and is in fact considered as a sensitive indicator of oxidative stress (Ferrari *et al.*, 1985). Increased oxidative stress has also been reported in patients with heart failure (Weitz *et al.*, 1991; Roberts *et al.*, 1990; Sobotka *et al.*, 1993; McMurray *et al.*, 1990). This increase has also been found to correlate directly with the severity of heart failure (Sobotka *et al.*, 1993; Diaz-Velez *et al.*, 1996).

Treatment with losartan increased the redox ratio by significantly improving the GSH content and decreasing the GSSG content as well as decreased lipid hydroperoxide content. Losartan has also been reported to reduce lipid peroxidation in hypertensive patients (Keidar, 1998) and prevented the formation of superoxide anion in a guinea pig model of heart hypertrophy (Lang *et al.*, 2000). These observations support the hypothesis that an increase in oxidative stress may in fact be a cause of heart failure and its modulation, as seen in losartan-treated MI animals, results in improved cardiac function and survival.

There have been no studies to date reporting the effects of losartan on oxidative stress in heart failure condition. However, a recent study from atherosclerosis apo-E deficient mice documented that treatment with losartan reduced LDL peroxidation in the atherosclerosis lesion area (Keidar, 1998). An increase in Ang II in hypertensive rats was associated with increased vascular smooth muscle cell $O2^{-}$ production via NADH/NADPH oxidase activation and losartan treatment to these rats normalized $O2^{-}$ production (Rajagopalan *et al.*, 1996). In another study performed on rabbits, losartan treatment normalized vascular $O2^{-}$ production in a dose-dependent manner and prevented tolerance to nitroglycerine (Kurz *et*

al., 1999). Thus a number of studies have documented a role for angiotensin II in the production of free radicals. In particular, angiotensin II has been reported to stimulate the production of superoxide anion (Griendling *et al.*, 1994). Peroxynitrite, another potent oxidant, was also decreased by losartan in cultured rat aortic endothelial cells (Pueyo *et al.*, 1998). In this study, the endothelial cells were exposed to angiotensin II, which is known to elicit the production of nitric oxide (NO). NO has been shown to interact with superoxide anion to form peroxynitrite (Yasmin *et al.*, 1997). In a human study on vascular endothelial cells, it was reported that angiotensin II-induced the production of superoxide anion via activation of membrane bound NADH/NADPH oxidase. Losartan treatment inhibited the production $O2^{-}$ and also reduced the activity of NADH/NADPH (Zhang *et al.*, 1999). Thus, the idea that losartan may block the reported increase in free radical stress due to angiotensin II is supported by many studies.

In our previous study we demonstrated that MI rats treated with captopril not only exhibited improved hemodynamic function but also alleviate the signs and symptoms of congestive heart failure (Khaper and Singal, 1997). Captopril treatment also resulted in maintenance of endogenous antioxidant reserve and decreased oxidative stress (Khaper and Singal, 1997; Khaper *et al.*, 1998). Comparing the results of the losartan study with the previously published captopril study, it is apparent that captopril and losartan have similar beneficial effects on the hemodynamic parameters and alleviate the signs of congestive heart failure. However, there are some differences in the antioxidant enzyme activities and oxidative stress parameters. Captopril resulted in the maintenance of all the three antioxidant enzymes, however, with losartan only GSHPx was improved. Although a clear mechanism

of captopril protection is not established, it is speculated that the additional beneficial effects of captopril on the myocardial antioxidants could be attributed to the intrinsic antioxidant property of this drug (Chopra et al., 1992). Although there have been no studies to date on the role of losartan as an antioxidant, it could be possible that losartan may have some indirect antioxidant effect. In this regard, losartan treatment has been reported to excrete uric acid in patients with hypertension (Puig *et al.*, 1999). Furthermore, in diabetic cardiomyopathy in rats, increased MDA content and depressed SOD, catalase and GSHPX activities were prevented by losartan (Kedziora-Kornatowska, 1999). In patients with coronary artery disease, losartan treatment improved endothelial function by increasing the bioavailability of nitric oxide (Hornig *et al.*, 2001).

4. Conclusions

In conclusion, the present study shows that as heart failure progresses, the depressed cardiac function is associated with a decrease in enzymatic and non-enzymatic antioxidants and an increase in oxidative stress. The decrease in catalase and superoxide dismutase activity correlated with a decrease in the mRNA abundance of these two enzymes. This study also documents for the first time that inhibition of the AT_1 receptors with losartan not only improved cardiac function, but also caused an improvement in the myocardial antioxidant reserve and decreased oxidative stress in MI rats suggesting a newer role for losartan in the treatment of heart failure. The precise mechanism(s) for the decrease in oxidative stress and improvement in antioxidant reserve after losartan treatment is/are unclear at this time.

VI. REFERENCES

Afzal N, Dhalla NS. Differential changes in left and right ventricular SR calcium transport in congestive heart failure. Am J Physiol 1992;262:H868-H874.

Ambrosio G, Becker LC, Hutchins GM, Weisman HF, Weisfeldt ML. Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into the pathophysiology of reperfusion injury. Circulation 1986;74:1424-1433.

Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548-555.

Ames BN, Cathcart R, Schivears E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer. Proc Natl Acad Sci USA 1981;78:6858-6862.

Anversa P, Li P, Zhang X, Olivetti G, Capasso JM. Ischaemic myocardial injury and ventricular remodelling. Cardiovasc Res 1993;27:145-157.

Anversa P, Zhang X, Li P, Capasso JM. Chronic coronary artery constriction leads to moderate myocyte loss and left ventricular dysfunction and failure in rats. J Clin Invest 1992;89:618-29.

Arai M, Alpert NR, MacLennan DH, Barton P, Periasamy M. Alterations in sarcoplasmic reticulum gene expression in human heart failure. A possible mechanism for alterations in systolic and diastolic properties of the failing myocardium. Circ Res 1993;72:463-9.

Arroyo CM, Kramer JH, Dickens BF, Weglicki WB. Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitron DMPO. FEBS Lett 1987;221:101-104.

Arumanayagam M, Chan S, Tong S, Sanderson JE. Antioxidant properties of carvedilol and metoprolol in heart failure: a double-blind randomized controlled trial. J Cardiovasc Pharmacol 2001;37:48-54.

Awan NA, Mason DT. Direct selective blockade of the vascular angiotensin II receptors in therapy for hypertension and severe congestive heart failure. Am Heart J 1996;131:177-185.

Axford-Gately RA, Wilson GJ. Reduction of experimental myocardial infarct size by oral administration of alpha tocopherol. Cardiovasc Res 1991;25:89-92.

Baker KM, Campanile CP, Trachte GJ, Peach MJ. Identification of the rabbit Ang II myocardial receptor. Circ Res. 54: 286-293.

Baker KM, Chernin MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. Am J Physiol 1990;259:H324-H332.

Baker KM, Singer HA, Aceto JF. Angiotensin II receptor-mediated stimulation of cytosolic-free calcium and inositol phosphates in chick myocytes. J Pharmacol Exp Ther 1989;251:578-585.

Ball AM, Sole M. Oxidative stress and the pathogenesis of heart failure. Cardiology Clinics 1998;16:665-675.

Barsacchi R, Pelosi G, Maffei S, Baroni M, Salvatore L, Ursini F, Verunelli F, Biagini A. Myocardial vitamin E is consumed during cardiopulmonary bypass: indirect evidence of free radical generation in human ischemic heart. Int J Cardiol 1992;37:339-343.

Bartosz M, Kedziora J, Bartosz G. Antioxidant and prooxidant properties of captopril and enalapril. Free Radic. Biol Med 1997; 23: 729-735.

Bastounis E, Hadjinikolaou L, Pikoulis M, Tsigris C, Marinos E, Toutouzas P. Free radical related myocardial mitochondrial damage following limb ischaemia-reperfusion. Cardiovasc Res 1994;28:1868-1871.

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

Bech Laursen J, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced by not catecholamine-induced hypertension. Circulation 1997;95:588-593.

Belch JJ, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. Br Heart J 1991;65:245-248.

Bernier M, Hearse DJ, Manning AS. Reperfusion-induced arrhythmias and oxygen-derived free radicals: studies with "anti-free radical"interventions and a free radical-generating system in the isolated perfused rat heart. Circ Res 1986;58:331-340.

Bishop JE, Rhodes S, Laurent GJ, Low RB, Stirewalt WS. Increased collagen synthesis and decreased collagen degradation in right ventricular hypertrophy induced by pressure overload. Cardiovasc Res 1994;28:1581-1585.

Blum A, Miller H. Role of cytokines in heart failure. Am Heart J 1998;135:181-186.

Boheler KR, Carrier L, de la Bastie D, Allen PD, Komajda M, Mercadier JJ, Schwartz K. Skeletal actin mRNA increases in the human heart during ontogenic development and is the major isoform of control and failing adult hearts. J Clin Invest 1991;88:323-330.

Bolli R. Causative role of oxyradicals in myocardial stunning: A proven hypothesis. Basic Res Cardiol 1998;93:156-172.

Bolli R. Oxygen-derived free radicals and postischemic myocardial dysfunction ("stunned myocardium"). J Am Coll Cardiol 1988;12:239-249.

Bozkurt B, Kribbs SB, Chubb FJ Jr, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL. Pathophysiologically relevant concentrations of tumor necrosis factor-α promote progressive left ventricular dysfunction and remodelling in rats. Circulation 1998;97:1382-1391.

Braunwald E, Bristow MR. Congestive heart failure: fifty years of progress. Circulation 2000;102:IV14-23.

Brillantes AM, Allen P, Takahashi T, Izumo S, Marks AR. Differences in cardiac calcium release channel (ryanodine receptor) expression in myocardium from patients with end-stage heart failure caused by ischemic versus dilated cardiomyopathy. Circ Res 1992;71:18-26.

Bristow MR. Changes in myocardial and vascular receptors in heart failure. J Am Coll Cardiol 1993;22:61A-71A.

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB. β_1- and β_2-Adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1-receptor down-regulation in heart failure. Circ Res 1986;59:297-309.

Brunner-La Rocca HP, Vaddadi G, Esler MD. Recent insight into therapy of congestive heart failure: focus on ACE inhibition and angiotensin-II antagonism. J Am Coll Cardiol 1999;33:1163-1173.

Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, Thompson M, Giroir B. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. Circulation 1998;97:1375-1381.

Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 1990;10:357-382.

Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunology Today 1994;15:7-10.

Campbell DJ. Circulating and tissue renin-angiotensin system. J Clin Invest 1987; 79: 1-6.

Campbell DJ, Habener JF. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. J Clin Invest 1986;78:31-39.

Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. Am J Physiol 1991;260:H1057-H1061.

Ceconi C, Curello S, Albertini A, Ferrari R. Effect of lipid peroxidation on heart mitochondria oxygen consuming and calcium transporting capacities. Mol Cell Biochem 1988;81:131-135.

Chandra M, Chandra N, Agrawal R, Kumar A, Ghatak A, Pandey VC. The free radical system in ischemic heart disease. Int J Cardiol 1994;43:121-125.

Charney RH, Levy DK, Kalman J, Buchholz E. Free radical activity increased with NYHA class in congestive heart failure. J Am Coll Cardiol 1997;29:930-939.

Chatelain E, Boscoboinik DO, Bartoli GM, Kagan VE, Gey FK, Packer L, Azzi A. Inhibition of smooth muscle cell proliferation and protein kinase C activity by tocopherols and tocotrienols. Biochim Biophys Acta 1993;1176:83-89.

Chello M, Mastroroberto P, Romano R, Bevacqua E, Pantaleo D, Ascione R, Marchese AR, Spampinato N. Protection by coenzyme Q10 from myocardial reperfusion injury during coronary artery bypass grafting. Ann Thorac Surg 1994;5:1427-1432.

Chen EP, Bittner HB, Davis RD, Van Trigt P, Folz RJ. Physiologic effects of extracellular superoxide dismutase transgene overexpression on myocardial function after ischemia and reperfusion injury. J Thorac Cardiovasc Surg 1998;115:450-458.

Chen L, Haught WH, Yang B, Saldeen TGP, Parathasarathy S, Mehta JL. Preservation of endogenous antioxidant activity and inhibition of lipid peroxidation as common mechanisms of antiatherosclerotic effects of vitamin E, lovastatin and amlodipine. J Am Coll Cardiol 1997;30:569-575.

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 1979;18:5294-5299.

Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL. Identification of angiotensin II receptor subtypes: Biochem Biophys Res Commun 1989; 165: 196-203.

Chiu AT, McCall DE, Ardecky RJ. Angiotensin II receptor subtypes and their selective non-peptide ligands. Receptor 1990;1:33-40.

Chopra K, Singh M, Kaul N, Andrabi KI, Ganguly NK. Decrease of myocardial infarct size wiht desferrioxamine: possible role of oxygen free radicals in its ameliorative effect. Mol Cell Biochem 1992a;113:71-76.

Chopra M, Beswick H, Clapperton M, Dargie HJ, Smith WE, McMurray J. Antioxidant effects of angiotensin converting enzyme (ACE) inhibitors: free radical and oxidant scavenging are sulphydryl-dependent, but lipid peroxidation is inhibited by both sulphydryl and non-sulphydryl-containing ACE inhibitors. J Cardiovasc Pharmacol 1992b;19:330-340.

Clairborne A. Catalase activity In: Handbook of methods for oxygen radical research, edited by R. A. Greenwald. Boca Raton, Fl: CRC, 1985, 283-284.

Clark IA, Cowden WB, Hunt NH. Free radical-induced pathology. Medicinal Research Review 1985;5:297-332.

Cohn JN. Overview of the treatment of heart failure. Am J Cardiol 1997;80:2L-6L.

CONSENSUS Trial Study Group, The. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med 1987;316:1429-1435.

Cordis GA, Maulik N, Das DK. Detection of oxidative stress in heart by estimating the dinitrophenylhydrazine derivative of malonaldehyde. J Mol Cell Cardiol 1995;27:1645-1653.

Cowan DB, Weisel RD, Williams WG, Mickle DA. The regulation of glutathione peroxidase gene expression by oxygen tension in cultured human cardiomyocytes. J Mol Cell Cardiol 1992; 24: 423-433.

Crozier I, Ikram H, Awan N for the Losartan Hemodynamics Study Group: Losartan in heart failure: hemodynamic effects and tolerability. Circulation 1995;91:691-697.

Currello S, Ceconi C, de Giuli F, Panzali AF, Milanesi B, Calarco M, Pardini A, Marzollo P, Alfieri O, Messineo F, Ferrari R. Oxidative stress during reperfusion of human hearts: potential sources of oxygen free radicals. Cardiovasc Res 1995;29:118-125.

Dalla Libera L, Ravara B, Angelini A, Rossini K, Sandri M, Thiene G, Battista Ambrosio G, Vescovo G. Beneficial effects on skeletal muscle of the angiotensin ii type 1 receptor blocker irbesartan in experimental heart failure. Circulation 2001;103:2195-2200.

Danser AH. Local renin-angiotensin system. Mol Cell Biochem 1996; 157: 211-216.

Danser AH, Saris JJ, Schuijt MP, van Kats JP. Is there a local renin-angiotensin system in the heart? Cardiovasc Res 1999;44:252-265.

de Jong JW, Schoemaker RG, de Jonge R, Bernocchi P, Keijzer E, Harrison R, Sharma HS, Ceconi C. Enhanced expression and activity of xanthine oxidoreductase in the failing heart. J Mol Cell Cardiol. 2000;32:2083-2089.

Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996; 28: 506-514.

Dhalla AK, Singal PK. Antioxidant changes in hypertrophied and failing guinea pig hearts. Am J Physiol 1994;266: H1280-H1285.

Dhalla NS, Dixon IM, Rupp H, Barwinsky J. Experimental congestive heart failure due to myocardial infarction: sarcolemmal receptors and cation transporters. Basic Res Cardiol 1991;86:13-23.

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

Dickstein K, Gottlieb S, Fleck E, Kostis J, Levine B, DeKock M, LeJemtel T. Hemodynamic and neurohumoral effects of the angiotensin II antagonist losartan in patients with heart failure. J Hypertens Suppl 1994;12:S31-S35.

Dieterich S, Bieligk U, Beulich K, Hasenfuss G, Prestle J. Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart. Circulation 2000;101:33-39.

Dixon IM, Hao J, Reid NL, Roth JC. Effect of chronic AT(1) receptor blockade on cardiac Smad overexpression in hereditary cardiomyopathic hamsters. Cardiovasc Res 2000;46:286-297.

Dixon IM, Hata T, Dhalla NS. Sarcolemmal calcium transport in congestive heart failure due to myocardial infarction in rats. Am J Physiol 1992a;262:H1387-1394.

Dixon IM, Hata T, Dhalla NS. Sarcolemmal Na(+)-K(+)-ATPase activity in congestive heart failure due to myocardial infarction. Am J Physiol 1992b;262:C664-C671.

Dixon IM, Ju H, Jassal DS, Peterson DJ. Effect of ramipril and losartan on collagen expression in right and left heart after myocardial infarction. Mol Cell Biochem 1996;165:31-45.

Dixon IM, Kaneko M, Hata T, Panagia V, Dhalla NS. Alterations in cardiac membrane Ca^{++} transport during oxidative stress. Mol Cell Biochem 1990;99:125-133.

Doroshow JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. Cancer Res 1983;43:460-472.

Dostal DE, Baker KM. Biochemistry, Molecular Biology, and Potential Roles of the Cardiac Renin-Angiotensin System. In: The Failing Heart, edited by N.S. Dhalla, R.E. Beamish, N. Takeda and M. Nagano. Lippincott-Raven, Philadelphia, 1995, pp 275-294.

Dzau VJ. Circulating versus local renin-angiotensin system in cardiovascular homeostasis. Circulation 1988;77:I4-13.

Dzau VJ. Short- and long-term determinants of cardiovascular function and therapy: contributions of circulating and tissue renin-angiotensin systems. J Cardiovasc Pharmacol 1989;14:S1-S5.

Dzau VJ. Molecular biology of angiotensin II biosynthesis and receptors. Can J Cardiol 1995;11:21F-26F.

Dzau VJ, Packer M, Lilly LS, Swartz SL, Hollenberg NK, Williams GH. Prostaglandins in severe congestive heart failure. Relation to activation of the renin--angiotensin system and hyponatremia. N Engl J Med 1984;310(6).

Eberhardt RT, Kevak RM, Kang PM, Frishman WH. Angiotensin II receptor blockade: an innovative approach to cardiovascular pharmacotherapy. J Clin Pharmacol 1993;33:1023-1038.

Ellis GR, Anderson RA, Lang D, Blackman DJ, Morris RH, Morris-Thurgood J, McDowell IF, Jackson SK, Lewis MJ, Frenneaux MP. 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 2000;36:1474-1482.

Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. Epidemiology 1992;3:194-202.

Eze MO. Membrane fluidity, reactive oxygen species, and cell-mediated immunity: implications in nutrition and disease. Med Hypotheses 1992;37:220-224.

Feldman AM. Modulation of adrenergic receptors and G transduction proteins in failing human ventricular myocardium. Circulation 1993;87:IV 27-IV 34.

Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am J Pathol 1978; 90: 57-70.

Ferrari R, Agnoletti L, Comini L, Gata G, Bachetti T, Cargnoni A, Ceconi C, Curello S, Visioli O. Oxidative stress during myocardial ischemia and heart failure. Eur Heart J 1998;19:B2-B11.

Ferrari R, Alfieri O, Currello S, Ceconi C, Cargnoni A, Marzollo P, Pardini A, Caradonna E, Visioli O. Occurrence of oxidative stress during reperfusion of the human heart. Circulation 1990;81:201-211.

Ferrari R, Ceconi C, Curello S, Guarnieri C, Calderera CM, Albertini A, Visioli O. Oxygen-mediated myocardial damage during ischemia and reperfusion: role of the cellular defenses against oxygen toxicity. J Mol Cell Cardiol 1985;17:937-945.

Ferrari R, Curello S, Boffa GM, Condorelli E, Pasini E, Guarnieri G, Albertini A. Oxygen free radical-mediated heart injury in animal models and during bypass surgery in humans. Effects of alpha-tocopherol. Ann N Y Acad Sci 1989;570:237-253.

Feuerstein G, Ruffolo RR Jr. Beta-blockers in congestive heart failure: the pharmacology of carvedilol, a vasodilating beta-blocker and antioxidant, and its therapeutic utility in congestive heart failure. Adv Pharmacol 1998;42:611-615.

Flamigni F, Guarnieri C, Toni R, Caldarera CM. Effect of oxygen radicals on heart mitochondrial function in alpha-tocopherol deficient rabbits. Int J Vitam Nutr Res 1982;52:402-406.

Foote CS, Denny RW. Chemistry of singlet oxygen VII. Quenching by β-carotene. J Am Chem Soc 1968;90:6233-6235.

Forrest VJ, Kang YH, McClain DE, Robinson DH, Ramakrishnan N. Oxidative stress-induced apoptosis prevented by Trolox. Free Radic Biol Med 1994;16:675-684.

Forsmark P, Aberg F, Norling B, Nordenbrand K, Dallner G, Ernster L. Inhibition of lipid peroxidation by ubiquinol in submitochondrial particles in the absence of vitamin E. FEBS Lett 1991;285:39-43.

Francis GS, Goldsmith SR, Levine TB, Olivari MT, Cohn JN. The neurohumoral axis in congestive heart failure. Ann Intern Med 1984;101:370-377.

Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. Lab Invest 1982;47:412-425.

Frei B, Stocker R, England L, Ames BN. Ascorbate: the most effective antioxidant in human blood plasma. Adv Exp Med Biol 1990;264:155-163.

Fridovich I. The biology of oxygen radicals. Science 1978;201:875-880.

Frimm CC, Sun Y, Weber KT. Angiotensin II receptor blockade and myocardial fibrosis of the infarcted rat heart. J Lab Clin Med 1997;129:439-446.

Garlick PB, Davies MJ, Hearse DJ, Slater TF. Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circ Res 1987;61:757-760.

Gerschman R, Gilbert DL, Nye SW, Swyer P, Fenn WO. Oxygen poisoning and X-irradiation: A mechanism in common. Science 1954;119:623-626.

Gey KF. Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. Br Med Bull 1993b;49:679-699.

Gey KF, Stahelin HB, Eichholzer M. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study. Clin Investig 1993a;71:3-6.

Ghatak A, Brar MJ, Agarwal A, Goel N, Rastogi AK, Vaish AK, Sircar AR, Chandra M. Oxy free radical system in heart failure and therapeutic role of oral vitamin E. Int J Cardiol 1996;57:119-127.

Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? Metabolism 1995;44:363-368.

Givertz MM, Colucci WS. New targets for heart failure therapy: endothelin, inflammatory cytokines, and oxidative stress. Lancet 1998;352: 34-38.

Gohlke P, Linz W, Scholkens BA, Kuwer I, Bartenbach S, Schnell A, Unger T. Angiotensin-converting enzyme inhibition improves cardiac function: role of bradykinin. Hypertension 1994; 23:411-418.

Gomberg M. An instance of trivalent carbon: triphenylmethyl. J Am Chem Soc 1900;22:757-761.

Goodfriend TL, Elliott ME, Catt KJ. Angiotensin receptors and their antagonists. N Engl J Med 1996;334:1649-1654.

Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994;94:1621-1628.

Gottlieb SS, Dickstein K, Fleck E, Kostis J, Levine TB, LeJemtel T, DeKock M. Hemodynamic and neurohormonal effects of the angiotensin II antagonist losartan in patients with congestive heart failure. Circulation 1993;88:1602-1609.

Grossman W, Lorell BH. Hemodynamic aspects of left ventricular remodeling after myocardial infarction. Circulation 1993;87:VII 28-VII 30.

Gupta M, Singal PK. Higher antioxidative capacity during a chronic stable heart hypertrophy. Circ Res 1989;64:398-406.

Haendeler J, Zeiher AM, Dimmeler S. Vitamin C and E prevent lipopolysaccharide-induced apoptosis in human endothelial cells by modulation of Bcl-2 and Bax. Eur J Pharmacol 1996;317:407-411.

Halliwell B. Oxidants and human disease: some new concepts. FASEB J 1987;1:358-364.

Halliwell B. Antioxidant characterization. Methodology and mechanism. Biochem Pharmacol 1995;49:1341-1348.

Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1984;1396-1397.

Hayashida W, Horiuchi M, Dzau VJ. Intracellular third loop domain of angiotensin II type-2 receptor. Role in mediating signal transduction and cellular function. J Biol Chem 1996;271:21985-21992.

Hedner T, Oparil S, Rasmussen K, Rapelli A, Gatlin M, Kobi P, Sullivan J, Oddou-Stock P. A comparison of the angiotensin II antagonists valsartan and losartan in the treatment of essential hypertension. Am J Hypertens 1999;12:414-417.

Heller R, Munscher-Paulig F, Grabner R, Till U. L-Ascorbic acid potentiates nitric oxide synthesis in endothelial cells. J Biol Chem 1999;274:8254-8260.

Hense HW, Stender M, Bors W, Keil V. Lack of an association between serum vitamin E and myocardial infarction in a population with high vitamin E levels. Atherosclerosis 1993;103:21-28.

Hess ML, Krause S, Kontos HA. Mediation of sarcoplasmic reticulum disruption in the ischemic myocardium: proposed mechanism by the interaction of hydrogen ions and oxygen free radicals. Adv Exp Med Biol 1983;161:377-389.

Hess ML, Kukreja RC. Free radicals, calcium homeostasis, heat shock proteins, and myocardial stunning. Ann Thorac Surg 1995;60:760-766.

Hill MF, Singal PK. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. Am J Pathol 1996;148:291-300.

Hill MF, Singal PK. Right and left myocardial antioxidant responses during heart failure subsequent to myocardial infarction. Circulation 1997;96:2414-2420.

Hockenbery DM, Oltavi ZN, Yin XM, Milliman CL, Korsmeyer ST. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell 1993;75:241-251.

Hornig B, Landmesser U, Kohler C, Ahlersmann D, Spiekermann S, Christoph A, Tatge H, Drexler H. Comparative effects of ACE inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. Circulation 2001; 103; 799-805.

Hurt J, Hsu JL, Dougall WC, Visner GA, Burr IM, Nick HS. Multiple mRNA species generated by alternate polyadenylation from the rat manganese superoxide dismutase gene. Nucleic Acids Res 1992;20:2985-2990.

Hutchins GM, Bulkley BH. Infarct expansion versus extension: two different complications of acute myocardial infarction. Am J Cardiol 1978; 41: 1127-1132.

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

Inagami T, Yamano Y, Bardhan S, Chaki S, Guo DF, Ohyama K, Kambayashi Y, Takahashi K, Ichiki T, Tsuzuki S, et al. Cloning, expression and regulation of angiotensin II receptors. Adv Exp Med Biol 1995;377:311-317.

Ingwall JS. Is cardiac failure a consequence of decreased energy reserve? Circulation. 1993;87:VII 58-VII 62.

Janero DR, Burghardt B.Oxidative injury to myocardial membrane: direct modulation by endogenous alpha-tocopherol. J Mol Cell Cardiol 1989;21:1111-1124.

Jeroudi MO, Hartely CJ, Bolli R. Myocardial reperfusion injury: role of oxygen free radicals and potential therapy with antioxidants. Am J Cardiol 1994;73:2B-7B.

Johns TNP and Olson BJ. Experimental myocardial infarction: I. A method of coronary occlusion in small animals. Ann Surg 1954;140:675-682.

Johnston CI. Angiotensin receptor antagonists: focus on losartan. Lancet. 1995;346:1403-1407.

Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR. Canine myocardial reperfusion injury: its reduction by the combined administration of superoxide dismutase and catalase. Circ Res 1984;54:277-285.

Ju H, Zhao S, Jassal DS, Dixon IMC. Effect of AT_1 receptor blockade on cardiac collagen remodeling after myocardial infarction. Cardiovasc Res 1997;35:223-232.

Kahonen M, Tolvanen JP, Kalliovalkama J, Wu X, Karjala K, Makynen H, Porsti I. Losartan and enalapril therapies enhance vasodilatation in the mesenteric artery of spontaneously hypertensive rats. Eur J Pharmacol 1999;368:213-222.

Kajstura J, Liu Y, Baldini A, Li B, Olivetti G, Leri A, Anversa P. Coronary artery constriction in rats: necrotic and apoptotic myocyte death. Am J Cardiol 1998;82:30K-41K.

Kako KJ. Free radical effects on membrane protein in myocardial ischemia/reperfusion injury. J Mol Cell Cardiol 1987;19:209-211.

Kane DJ, Ord T, Anton R, Bredesen DE. Expression of bcl-2 inhibits necrotic neural cell death. J Neurosci Res 1995;40:269-275.

Kaneko M, Singal PK, Dhalla NS. Alterations in heart sarcolemmal Ca^{2+} binding activities due to oxygen radicals. Basic Res Cardiol 1990;85:45-54.

Kanter MM, Hamlin RL, Unverferth DV, Davis HW, Merola AJ. Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. J Appl Physiol 1985;59:1298-1303.

Kappus H. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In: Oxidative Stress, edited by H. Sies. Academic Press, London, 1985, pp 273-303.

Kaul N, Siveski-Iliskovic N, Hill M, Slezak J, Singal PK. Free radicals and the heart. J Pharmacol Toxicol Meth 1993;30:55-67.

Kaul N, Siveski-Iliskovic N, Thomas TP, Hill M, Singh N, Singal PK. Probucol improves antioxidant activity and modulates development of diabetic cardiomyopathy. Nutrition 1995;11:551-554.

Kedziora-Kornatowska K. Effects of angiotensin convertase inhibitors and AT_1 angiotensin receptor antagonists on the development of oxidative stress in the kidney of diabetic rats. Clin Chim Acta 1999; 287: 19-27

Keidar S. Angiotensin, LDL peroxidation and atherosclerosis. Life Sci 1998;63:1-11.

Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, Jeejeebhoy KN. Increased oxidative stress in patients with congestive heart failure. J Am Coll Cardiol 1998;31:1352-1356.

Keith ME, Jeejeebhoy KN, Langer A, Kurian R, Barr A, O'Kelly B, Sole MJ. A controlled clinical trial of vitamin E supplementation in patients with congestive heart failure. Am J Clin Nutr 2001;73:219-224.

Khaper N, Hill MF, Pichardo J, Singal PK. Effects of captopril on myocardial oxidative stress changes in post-MI rats. Angiotensin II Blockade, edited by N.S. Dhalla, P. Zahradka, I.M.C. Dixon and R.E. Beamish. Kluwer Academic Publishers, Boston, 1998, pp 527-536.

Khaper N, Rigatto C, Seneviratne C, Li T, Singal PK. Chronic treatment with propranolol induces antioxidant changes and protects against ischemia-reperfusion injury. J Mol Cell Cardiol 1997;29:3335-3344.

Khaper N, Singal PK. Effects of afterload reducing drugs on the pathogenesis of antioxidant changes and congestive heart failure in rats. J Am Coll Cardiol 1997;29:856-861.

Khaper N, Singal PK. Modulation of oxidative stress by a selective inhibition of angiotensin II type 1 receptors in MI rats. J Am Coll Cardiol 2001;37:1461-6.

Kirshenbaum LA, Gupta M, Thomas TP, Singal PK. Antioxidant protection against adrenaline-induced arrhythmias in rats with chronic heart hypertrophy. Can J Cardiol 1990; 6:71-74.

Kirshenbaum LA, Hill M, Singal PK. Endogenous antioxidants in isolated hypertrophied cardiac myocytes and hypoxia-reoxygenation injury. J Mol Cell Cardiol 1995;27:263-272.

Kirshenbaum LA, Singal PK. Changes in antioxidant enzymes in isolated cardiac myocytes subjected to hypoxia-reoxygenation. Lab Invest 1992; 67: 796-806.

Kirshenbaum LA, Singal PK. Increase in endogenous antioxidant enzymes protects hearts against reperfusion injury. Am J Physiol 1993;2265:H484-H497.

Kirshenbaum LA, de Moissac D. The bcl-2 gene product prevents programmed cell death of ventricular myocytes. Circulation 1997;96:1580-1585.

Kitamoto S, Egashira K, Kataoka C, Usui M, Koyanagi M, Takemoto M, Takeshita A. Chronic inhibition of nitric oxide synthesis in rats increases aortic superoxide anion production via the action of angiotensin II. J Hypertens 2000;18:1795-1800.

Klein HH, Pich S, Lindert S, Nebendahl K, Niedman P. Failure of chronic high dose oral vitamin E treatment to protect the ischemic-reperfused porcine heart. J Mol Cell Cardiol 1993;25:103-112.

Klein HH, Pich S, Lindert S, Nibendahl K, Niedman P, Kreuzer H. Combined treatment with vitamins E and C in experimental myocardial infarction in pigs. Am Heart J 1989;118:667-673.

Klein HH, Pich S, Nebendahl K, Niedmann P, Schuff-Werner P. Acute treatment with vitamin E does not protect the regionally ischemic, reperfused porcine heart. Basic Res Cardiol 1991;86:32-39.

Kok FJ, de Bruijn AM, Vermeeren R, Hofman A, van Laar A, de Bruin M, Hermus RJ, Valkenburg HA. Serum selenium, vitamin antioxidants and cardiovascular mortality: a nine year follow-up study in the Netherlands. Am J Clin Nutr 1987;45:462-468.

Kramer JH, Mak IT, Weglicki WB. Differential sensitivity of canine cardiac sarcolemmal and microsomal enzymes to inhibition by free radical induced lipid peroxidation. Circ Res 1984;55:120-124.

Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, Glembotski CC, Quintana PJ, Sabbadini RA. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest 1996;98:2854-2865.

Kukreja RC, Nayeem MA, Aian YZ, Hess ML. Evolution and adaptation of free radical scavengers and the potential for therapeutic intervention. In: Adaptation Biology and Medicine (Vol. 1), edited by B.K. Sharma, N Takeda, NK Ganguly and PK Singal. Narosa Publishing, New Delhi, India, 1997, pp 383-395.

Kumar D, Kirshenbaum L, Li T, Danelisen I, Singal P. Apoptosis in isolated adult cardiomyocytes exposed to adriamycin. Ann N Y Acad Sci 1999;874:156-168.

Kurz S, Hink U, Nickenig G, Borthayre AB, Harrison DG, Munzel T. Evidence for a causal role of the renin-angiotensin system in nitrate tolerance. Circulation 1999; 99:3181-3187.

Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N Engl J Med 1996;334:1156-1162.

Lacourciere Y. The angiotensin II receptor antagonist Losartan: a new advance in the treatment of hypertension. Can J Cardiol; 11: 45F-48F, 1995.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-685.

Lang RM, Elkayam U, Yellen LG, Krauss D, McKelvie RS, Vaughan DE, Ney DE, Makris L, Chang PI. Comparative effects of losartan and enalapril on exercise capacity and clinical status in patients with heart failure. The Losartan Pilot Exercise Study Investigators. J Am Coll Cardiol 1997;30:983-991.

Lavoisier A-L. Alterations qu'eprouve l'air resire. Recueil des memoires de Lavoisier. 1785, Read to the Societe de Medicine. Reprinted as part of "Memoires sur la Respiration et al Transpiration des Animaux" in "Les Maitres de la Pensee Scientifique." Gauthier-Villaus et cie , editors, Paris, 1920.

Lawrence RA, Burk RE. Species, tissue and subcellular distribution of non-selenium dependent glutathione peroxidase activity. J Nutr 1978;108:211-215.

Lebovitz RM, Zhang H, Vogel H, Cartwright J Jr, Dionne L, Lu N, Huang S, Matzuk MM. Neurodegeneration, myocardial injury and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci 1996;93:9782-9787.

Lechat P. Prevention of heart failure progression: current approaches. Eur Heart J 1998;19:B12-18.

Levine TB, Franciosa JA, Cohn JN. Acute and long-term response to an oral converting-enzyme inhibitor, captopril, in congestive heart failure. Circulation 1980;62:35-41.

Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in congestive heart failure. N Eng J Med 1990;323:236-241.

Levy Y, Bartha P, Ben-Amotz A, Brook JG, Danker G, Lin S, Hammerman H. Plasma antioxidants and lipid peroxidation in acute myocardial infarction and thrombolysis. J Am Coll Nutr 1998;17:373-341.

Li Q, Bolli R, Qiu Y, Tang XL, Guo Y, French BA. Gene therapy with extracellular superoxide dismutase protects conscious rabbits against myocardial infarction. Circulation 2001;103:1893-1898.

Li Q, Bolli R, Qiu Y, Tang XL, Murphee SS, French BA. Gene therapy with extracellular superoxide dismutase attenuates myocardial stunning in conscious rabbits. Circulation 1998;98:1438-1448.

Li T, Singal PK. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. Circulation 2000;102:2105-2110.

Lindenmayer GE, Harigaya S, Bajusz E, Schwartz A. Oxidative phosphorylation and calcium transport of mitochondria isolated from cardiomyopathic hamster hearts. J Mol Cell Cardiol 1970;1:249-259.

Linz W, Scholkens BA. A specific B2-bradykinin receptor antagonist HOE 140 abolishes the antihypertrophic effect of ramipril. Br J Pharmacol 1992:105:771-772.

Liu Z, Hilbelink DR, Crockett WB, Gerdes AM. Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas. 1. Developing and established hypertrophy. Circ Res 1991;69:52-58.

Lo MW, Goldberg MR, McCrea JB et al Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. Clin Pharmacol Ther 1995;58:641-649.

Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. Am J Clin Nutr. 1996 Aug;64(2):190-6.

Lowry OH, Rosenbrough NT, Farr AL and Randall AT. Protein measurements with the Folin phenol reagent. J Biol Chem 1951;193:265-275.

Mak IT, Arroyo CM, Weglicki WB. Inhibition of sarcolemmal carbon-centered free radical formation by propranolol. Circ Res 1989;65:1151-1156.

Mak IT, Freedman AM, Dickens BF, Weglicki WB. Protective effects of sulfhydryl-containing angiotensin converting enzyme inhibitors against free radical injury in endothelial cells. Biochem Pharmacol 1990;40:2169-2175.

Mak IT, Weglicki WB. Protection by beta-blocking agents against free radical-mediated sarcolemmal lipid peroxidation. Circ Res 1988;63:262-266.

Makino N, Panagia V, Gupta MP, Dhalla NS. Defects in sarcolemmal Ca2+ transport in hearts due to induction of calcium paradox. Circ Res 1988;63:313-321.

Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. 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 1998;97:1536-1539.

Mankad S, d'Amato TA, Reichek N, McGregor WE, Lin J, Singh D, Rogers WJ, Kramer CM. Combined an Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. 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 1998;97:1536-1539.

Mankad S, d'Amato TA, Reichek N, McGregor WE, Lin J, Singh D, Rogers WJ, Kramer CM. Combined angiotensin II receptor antagonism and angiotensin-converting enzyme inhibition further attenuates postinfarction left ventricular remodeling. Circulation 2001;103:2845-2850.

Marklund S L. Pyrogallol autooxidation. In: Handbook of methods for oxygen radical research, edited by R. A. Greenwald. Boca Raton, Fl, CRC, 1985, pp 243-247.

Martorana PA, Linz W, Scholkens BA. Does bradykinin play a role in the cardiac antiischemic effect of the ACE-inhibitors? Basic Res Cardiol. 1991;86:293-296.

Massey KD, Burton KP. Alpha-tocopherol attenuates myocardial membrane-related alterations resulting from ischemia-reperfusion. Am J Physiol 1989;256:H1192-1199.

Maulik N, Yoshida T. Oxidative stress developed during open heart surgery induces apoptosis: reduction of apoptotic cell death by ebselen, a glutathione peroxidase mimic. J Cardiovasc Pharmacol 2000;36:601-608.

McAlpine HM, Cobbe SM. Neuroendocrine changes in acute myocardial infarction. Am J Med 1988;84:61-66.

McCord JM. Free radicals and myocardial ischemia: overview and outlook. Free Radic Biol Med 1988;4:9-14.

McCord JM, Fridovich I. Superoxide dismutase. An enzymatic function for erythrocuprein (hemecuprein). J Biol Chem 1969;244:6049-6055.

McKay RG, Pfeffer MA, Pasternak RC, Markis JE, Come PC, Nakao S, Alderman JD, Ferguson JJ, Safian RD, Grossman W. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. Circulation 1986;74:693-702.

McMurray J, Mclay J, Chopra M, Bridges A, Belch JJF. Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol 1990;65:1261-1262.

Meerson FZ, Kagan VE, Kozlov YuP, Belkina LM, Arkhipenko YuV The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart. Basic Res Cardiol 1982;77:465-485.

Mehta JL, Nichols WW, Donnelly WH, Lawson DL, Thompson L, ter Riet M, Saldeen TG. Protection by superoxide dismutase from myocardial dysfunction and attenuation of vasodilator reserve after coronary occlusion and reperfusion in dog. Circ Res 1989;65:1283-1295.

Meldrum DR, Dinarello CA, Cleveland JC Jr, Cain BS, Shames BD, Meng X, Harken AH. Hydrogen peroxide induces tumor necrosis factor α-mediated cardiac injury by a p38 nitrogen-activated protein kinase-dependent mechanism. Surgery 1998;124:291-296.

Mercadier JJ, Lompre AM, Duc P, Boheler KR, Fraysse JB, Wisnewsky C, Allen PD, Komajda M, Schwartz K. Altered sarcoplasmic reticulum Ca2(+)-ATPase gene expression in the human ventricle during end-stage heart failure. J Clin Invest 1990;85:305-309.

Michorowski B, Ceremuzynski L. The renin-angiotensin--aldosterone system and the clinical course of acute myocardial infarction. Eur Heart J 1983;4:259-264.

Mickle DA, Li RK, Weisel RD, Birnbaum PL, Wu TW, Jackowski G, Madonik MM, Burton GW, Ingold KU. Myocardial salvage with trolox and ascorbic acid for an acute evolving infarction. Ann Thorac Surg 1989b;47:553-557.

Milavetz JJ, Raya TE, Johnson CS, Morkin E, Goldman S. Survival after myocardial infarction in rats: captopril versus losartan. J Am Coll Cardiol 1996;27:714-719.

Morris TE, Sulakhe PV. Sarcoplasmic reticulum Ca^{2+}-pump dysfunction in rat cardiomyocytes briefly exposed to hydroxyl radicals. Free Radic Biol Med 1997;22:37-47.

Moser M. Angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and calcium channel blocking agents: a review of potential benefits and possible adverse reactions. J Am Coll Cardiol. 1997;29: 1414-1421.

Moudgil R, Menon V, Xu Y, Musat-Marcu S, Kumar D, Jugdutt BI. Postischemic apoptosis and functional recovery after angiotensin II type 1 receptor blockade in isolated working rat hearts. J Hypertens 2001;19:1121-1129.

Murohara Y, Yui Y, Hattori R, Kawai C. Effects of superoxide dismutase on reperfusion arrhythmias and left ventricular function in patients undergoing thrombolysis for anterior wall acute myocardial infarction. Am J Cardiol 1991;67:765-767.

Muskowitz R, Kukin M. Oxidative stress and congestive heart failure. Congestive Heart Failure 1999;5:153-163.

Myers CE, McGuire WP, Liss RH, Infirm I, Grutzinger K, Young RC. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumour response. Science 1977;19:165-167.

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

Nakao K, Minobe W, Roden R, Bristow MR, Leinwand LA. Myosin heavy chain gene expression in human heart failure. J Clin Invest 1997;100:2362-2370.

Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996;335:1182-1189.

Naslund U, Haggmark S, Johansson G, Marklund SL, Reiz S, Oberg A. Superoxide dismutase and catalase reduce infarct size in a porcine myocardial occlusion-reperfusion model. J Mol Cell Cardiol 1986;18:1077-1084.

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

Nohl H, Hegner D. Evidence for the existence of catalase in the matrix space of rat-heart mitochondria. FEBS Lett 1978;89:126-130.

Ohishi N, Ohkawa H, Miike A, Tatano T, Yagi K. A new assay for lipid peroxides using a methylene blue derivative. Biochem Internat 1985;10:205-211.

Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, DeLoreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. N Engl J Med 1997;336:1131-1141.

Olivetti G, Capasso JM, Sonnenblick EH, Anversa P. Side-to-side slippage of myocytes participates in ventricular wall remodeling acutely after myocardial infarction in rats. Circ Res 1990;67:23-34.

Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S. Cytokine gene expression after myocardial infarction in rat hearts: possible implication in left ventricular remodeling. 1998;98:149-156.

Oparil S, Aurup P, Snavely D, Goldberg A. Efficacy and safety of losartan/ hydrochlorothiazide in patients with severe hypertension. Am J Cardiol 2001;87:721-726.

Oskarsson HJ, Heistad DD. Oxidative stress produced by angiotensin too. Implications for hypertension and vascular injury. Circulation 1997;95:557-559.

Ozer NK, Boscoboinik A, Azzi A. New roles of low density lipoproteins and vitamin E in the pathogenesis of atherosclerosis. Biochem Mol Bio Int 1995;35:117-124.

Packer JE, Slater TF, Watson RL. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature 1979;278:737-738.

Packer L. Protective role of vitamin E in biological systems. Am J Clin Nutr 1991;53:1050-1055.

Packer L. Vitamin E is nature's master antioxidant. Scientific American Science and Medicine 1994; 54-63.

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

Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-169.

Palace VP, Brown SB. HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (Salvelinus namaycuh) exposed to coplanar 3,3,4,4,5-paentachlorobiphenyl. Env Tox Chem 1994;13:473-476.

Palace VP, Hill MF, Farahmand F, Singal PK. Mobilization of antioxidant vitamin pools and hemodynamic function following myocardial infarction. Circulation. 1999a;99:121-126.

Palace VP, Khaper N, Qin Q, Singal PK. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Rad Biol Med 1999b;26:746-761.

Palace VP, Kumar D, Hill MF, Khaper N, Singal PK. Regional differences in non-enzymatic antioxidants in the heart under control and oxidative stress conditions. J Mol Cell Cardiol 1999c;31: 193-202.

Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radic Biol Med 1999;27:42-50.

Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacological doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am J Clin Nutr 1993;57:650-656.

Pelouch V, Dixon IM, Sethi R, Dhalla NS. Alteration of collagenous protein profile in congestive heart failure secondary to myocardial infarction. Mol Cell Biochem 1993;129:121-131.

Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Progressive ventricular remodeling in rat with myocardial infarction. Am J Physiol 1991;260:H1406-H1414.

Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, et al. on behalf of the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement trial (SAVE). N Engl J Med 1992;327:669-677.

Pfeffer MA, Pfeffer JM, Gervasio AL. Development and prevention of congestive heart failure following myocardial infarction. Circulation 1993;87:120-125.

Pichardo J, Palace V, Farahmand F, Singal PK. Myocardial oxidative stress changes during compensated right heart failure in rats. Mol Cell Biochem 1999;196:51-57.

Pitt B, Segal R, Martinez FA, Meurers G, Cowley AJ, Thomas I, Deedwania PC, Ney DE, Snavely DB, Chang PI. on behalf of ELITE Investigators. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study, ELITE). Lancet 1997;349:747-752.

Prasad K, Gupta JB, Kalra J, Lee P, Mantha SV, Bharddwaj B. Oxidative stress as a mechanism of cardiac failure in chronic volume overload in canine model. J Mol Cell Cardiol 1996;28:375-385.

Przyklenk K, Kloner RA. Superoxide dismutase plus catalase improve contractile function in the canine model of the"stunned myocardium". Circ Res 1986;58:148-156.

Pueyo ME, Arnal JF, Rami J, Michel JB. Angiotensin II stimulates the production of NO and peroxynitrite in endothelial cells. Am J Physiol 1998;274:C214-C220.

Puig JG, Mateos F, Buno A, Ortega R, Rodriguez F, Dal-Re R. Effects of eprosartan and losartan on uric acid metabolism in patients with essential hypertension. J Hypertension 1999; 17: 1033-1039.

Quan F, Korneluk RG, Tropak MB, Gravel RA. Isolation and characterization of the human catalase gene. Nucleic Acids Res 1986;14:5321-5335.

Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. 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 1996;97:1916-1923.

Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol-induced oxidative stress in rat tissues. Pharmacol Res 1998;38:297-303.

Raya TE, Fonken SJ, Lee RW, Daugherty S. Goldman S, Wong PC, Timmermans PB, Morkin E. Hemodynamic effects of direct angiotensin II blockade compared to converting enzyme inhibition in rat model of heart failure. Am J Hypertens 1991;4:334S-340S.

Reed DJ. Glutathione depletion and susceptibility. Pharmacol Rev 1984;36:35S-33S.

Reed DJ. Glutathione: toxicological implications. Ann Rev Pharmacol Toxicol 1990;30:603-631.

Reeves JP, Bailey CA, Hale CC. Redox modification of sodium-calcium exchange activity in cardiac sarcolemmal vesicles. J Biol Chem 1986;201:4948-4955.

Reilly MP, Delanty N, Roy L, Rokach J, Callaghan PO, Crean P, Lawson JA, FitzGerald GA. Increased formation of the isoprostanes IPF2alpha-I and 8-epi-prostaglandin F2alpha in acute coronary angioplasty: evidence for oxidant stress during coronary reperfusion in humans. Circulation 1997;96:3314-3320.

Richer C, Gervais M, Fornes P, Giudicelli JF. Combined selective angiotensin II AT1-receptor blockade and angiotensin I-converting enzyme inhibition on coronary flow reserve in postischemic heart failure in rats. J Cardiovasc Pharmacol 1999; 34: 772-781.

Riemersma RA. Epidemiology and the role of antioxidants in preventing coronary heart disease: a brief overview. Proc Nutr Soc 1994;53:59-65.

Riemersma RA, Wood DA, Macintyre CC, Elton RA, Gey KF, Oliver MF. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. Lancet 1991;337:1-5.

Rojas C, Cadenas S, Lopez-Torres M, Perez-Campo R, Barja G. Increase in heart glutathione redox ratio and total antioxidant capacity and decrease in lipid peroxidation after vitamin E dietary supplementation in guinea pigs. Free Radic Biol Med 1996;21:907-915.

Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary disease in men. N Engl J Med 1993;328:1450-1456.

Rump LC, Oberhauser V, Schwertfeger E, Schollmeyer P. Experimental evidence to support ELITE. Lancet 1998;351:644-645.

Rush JE, Rajfer SI. Theoretical basis for the use of angiotensin II antagonists in the treatment of heart failure. J Hypertension 1993;11:69S-71S.

Ruzicka M, Yuan B, Harmsen E, Leenen FH. The renin-angiotensin system and volume overload-induced cardiac hypertrophy in rats. Effects of angiotensin converting enzyme inhibitor versus angiotensin II receptor blocker. Circulation 1993;87:921-930.

Sadoshima J, Qui Z, Morgan JP, Izumo S. Angiotensin II and other hypertrophic stimuli mediated by G-protein coupled receptors activated tyrosine kinase, mitogen activated protein kinase, and 90KD S6 kinase in cardiac myocytes. Circ Res 1995;76:1-15.

Saran M, Michel C, Bors W. Reactions of NO with $O_2^{\bullet-}$. Implications for the action of endothelium-derived relaxing factor. Free Radical Res Commun 1989;10:221-226..

Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Voipo-Pulkki L-M. Apoptosis in human acute myocardial infarction. Circulation 1997;95:320-323.

Schieffer B, Wirgner A, Meybrunn M, Seitz S, Holtz J, Riede UN, Drexler H. Comparative effects of chronic angiotensin converting enzyme inhibition and angiotensin

II type 1 receptor blockade on cardiac remodelling after myocardial infarction in the rat. Circulation 1994;88:2273-2282.

Schror K. Role of prostaglandins in the cardiovascular effects of bradykinin and angiotensin converting enzyme inhibitors in heart failure. J Cardiovascular Pharmacol 1992;20:68-73.

Schwartz K, Chassagne C, Boheler KR. The molecular biology of heart failure. J Am Coll Cardiol 1993;22:30A-33A.

Scragg R, Jackson R, Holdaway I, Woollard G, Woollard D. Changes in plasma vitamin levels in the first 48 hours after onset of acute myocardial infarction. Am J Cardiol 19891;64:971-974.

Sebbag L, Forrat R, Canet E, Renaud S, Delaye J, de Lorgeril M. Effects of dietary supplementation with alpha-tocopherol on myocardial infarct size and ventricular arrhythmias in a dog model of ischemia-reperfusion. J Am Coll Cardiol 1994;24:1580-1585.

Selye H, Bajusz E, Grasso S, Mendell P. Simple technique for the surgical occlusion of coronary vessels in the rat. Angiology 1960;11:398-407.

Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ. bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 1991;67:879-888.

Sies H, ed. Oxidative Stress, Oxidants and Antioxidants. Academic Press, London and New York, 1991.

Singal PK, Beamish RE, Dhalla NS. Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv Exp Med Biol 1983;161:391-401.

Singal PK, Deally CM, Weinberg LE. Subcellular effects of adriamycin in the heart: a concise review. J Mol Cell Cardiol 1987;19:817-828.

Singal PK, Ghani RA-A, Khaper N, Palace V, Hill MF. Antioxidant adaptations and cardiac dysfunction. Involvement of vitamin E. J Adapt Med 1997a;1:5-15.

Singal PK, Hill MF, Ganguly NK, Khaper N, Kirshenbaum LA, Pichardo J. Role of oxidative stress in heart failure subsequent to myocardial infarction. L'Information Cardiologique. 1996;20:343-362.

Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Eng J Med 1998;339:900-905.

Singal PK, Iliskovic N, Li T, Kumar D. Adriamycin cardiomyopathy: pathophysiology and prevention. FASEB J 1997b;11:931-936.

Singal PK, Kapur N, Beamish RE, Das PK, Dhalla NS. Antioxidant protection against epinephrine-induced arrhythmias. In: Stress and Heart Disease, edited by R.E. Beamish, P.K. Singal, and N.S. Dhalla. Martinus Nyhoff Publications, Boston, 1985, pp 190-201.

Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS. Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 1982;60:1390-1397.

Singal PK, Khaper N, Farahmand F, Bello-Klein A. Oxidative stress in congestive heart failure. Curr Cardiol Rep 2000;2:206-211.

Singal PK, Khaper N, Palace V, Kumar D. The role of oxidative stress in the genesis of heart disease. Cardiovasc Res 1998;40; 426-432.

Singal PK, Kirshenbaum LA. A relative deficit in antioxidant reserve may contribute in cardiac failure. Can J Cardiol 1990;6:47-49.

Singal PK, Petkau A, Gerrard JM, Hrushovetz S, Foerster J. Free radicals in health and disease. Mol Cell Biochem 1988;84:121-122.

Singal PK, Tong J. Vitamin E deficiency accentuates adriamycin-induced cardiomyopathy and cell surface changes. Mol Cell Biochem 1988;84:163-171.

Singh N, Dhalla AK, Seneviratne CK, Singal PK. Oxidative stress and heart failure. Mol Cell Biochem 1995;147:77-81.

Singh RB, Niaz MA, Rastogi SS , Rastogi S. Usefulness of antioxidant vitamins in suspected acute myocardial infarction (The Indian Experiment of Infarct Survival-3). Am J Cardiol 1996;77:232-236.

Siveski-Iliskovic N, Hill M, Chow DA, Singal PK. Probucol protects against adriamycin cardiomyopathy without interfering with its antitumor effect. Circulation 1995;91:10-15.

Siveski-Iliskovic N, Kaul N, Singal PK. Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 1994;89:2829-2835.

Skidgel RA, Erdos EG. The broad substrate specificity of human angiotensin I converting enzyme. Clin Exp Hypertens A. 1987;9:243-259.

Sladek T, Sladkova J, Kolar F, Papousek F, Cicutti N, Korecky B, Rakusan K. The effect of AT1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. Cardiovasc Res 1996; 31: 568-576.

Smith JL. Pathological effects due to increase of oxygen tension in air breathed. J Physiol 1899;24:19-35.

Smits JF, van Krimpen C, Schoemaker RG, Cleutjens JP, Daemen MJ. Angiotensin II receptor blockade after myocardial infarction in rats: effects on hemodynamics, myocardial DNA synthesis, and interstitial collagen content. J Cardiovasc Pharmacol 1992;20:772-778.

Sobotka PA, Brottman MD, Weitz Z, Birnbaum AJ, Skosey JL, Zarling EJ. Elevated breath pentane in heart failure reduced by free radical scavenger. Free Rad Biol Med 1993;14:643-647.

Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 1993;328:1444-1449.

Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 1996;347:781-786.

Sullivan MJ. Congestive heart failure: trends in epidemiology and therapy. In: Congestive Heart Failure: Current Clinical Issues, edited by G.T. Kennedy, M.H. Crawford, Futura Publishing Company Inc., Armonk, New York, 1994, pp 111-115.

Sun Y, Weber KT. Angiotensin II receptor binding following myocardial infarction in the rat. Cardiovasc Res 1994; 28: 1623-1628.

Swedberg K, Kjekshus J, Snapinn S. Long-term survival in severe heart failure in patients treated with enalapril. Ten year follow-up of CONSENSUS I. Eur Heart J 1999;20:136-139.

Tappia PS, Hata T, Hozaima L, Sandhu MS, Panagia V, Dhalla NS. Role of oxidative stress in catecholamine-induced changes in cardiac sarcolemmal Ca2+transport. Arch Biochem Biophys 2001;387:85-92.

Teiger E, Than VD, Richard L, Wisnewsky C, Tea BS, Gaboury L, Tremblay J, Schwartz K, Hamet P. Apoptosis in pressure-overload induced heart hypertrophy in the rat. J Clin Invest 1996;97: 2891-2897.

Thai HM, Van HT, Gaballa MA, Goldman S, Raya TE. Effects of AT1 receptor blockade after myocardial infarct on myocardial fibrosis, stiffness, and contractility. Am J Physiol 1999;276:H873-H880.

Tiemeier DC, Tilghman SM, Leder P. Purification and cloning of a mouse ribosomal gene fragment in coliphage lambda. Gene 1977; 2: 173-191.

Timmermans PB. Pharmacological properties of angiotensin II receptor antagonists. Can J Cardiol 1999;15:26F-28F.

Timmermans PB, Chiu AT, Herblin WF, Wong PC, Smith RD. Angiotensin II receptor subtypes. Am J Hypertens 1992;5:406-410

Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev 1993;45:205-251.

Timmermans PB, Smith RD. Angiotensin II receptor subtypes: selective antagonists and functional correlates. Eur Heart J 1994;15:79-87.

Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704-711.

Tsunoda K, Abe K, Hagino T, Omata K, Misawa S, Imai Y, Yoshinaga K. Hypotensive effect of losartan, a nonpeptide angiotensin II receptor antagonist, in essential hypertension. Am J Hypertens 1993;6:28-32.

Tsutsui H, Ide T, Hayashidani S, Suematsu N, Utsumi H, Nakamura R, Egashira K, Takeshita A. Greater susceptibility of failing cardiac myocytes to oxygen free radical-mediated injury. Cardiovasc Res 2001;49:103-109.

Tsuyuki RT, Yusuf S, Rouleau JL, Maggioni AP, McKelvie RS, Wiecek EM, Wang Y, Pogue J, Teo KK, White M, Avezum A Jr, Latini R, Held P, Lindgren E, Probstfield J. Combination neurohormonal blockade with ACE inhibitors, angiotensin II antagonists and beta-blockers in patients with congestive heart failure: design of the Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) Pilot Study. Can J Cardiol 1997;13:1166-1174.

Tzanidis A, Lim S, Hannan RD, See F, Ugoni AM, Krum H. Combined angiotensin and endothelin receptor blockade attenuates adverse cardiac remodeling post-myocardial infarction in the rat: possible role of transforming growth factor beta(1). J Mol Cell Cardiol 2001;33:969-981.

Vaage J, Antonelli M, Bufi M, Irtun O, DeBlasi RA, Corbucci GG, Gasparetto A, Semb AG. Exogenous reactive oxygen species deplete the isolated rat heart of antioxidants. Free Radic Biol Med 1997;22:85-92.

Varo N, Etayo JC, Zalba G, Beaumont J, Iraburu MJ, Montiel C, Gil MJ, Monreal I, Diez J. Losartan inhibits the post-transcriptional synthesis of collagen type I and reverses left ventricular fibrosis in spontaneously hypertensive rats. J Hypertens 1999;17:107-114.

Varo N, Iraburu MJ, Varela M, Lopez B, Etayo JC, Diez J. Chronic AT(1) blockade stimulates extracellular collagen type I degradation and reverses myocardial fibrosis in spontaneously hypertensive rats. Hypertension 2000;35:1197-1202.

Verma A, Hill M, Bhayana S, Pichardo J, Singal PK. Role of glutathione in acute myocardial adaptation. In: Adaptation Biology and Medicine: Subcellular Basis, Edited by Sharma BK, Takeda N, Ganguly NK, Singal PK. Narosa Publishers, New Delhi, India, 1997, pp 399-408.

Watkins L Jr, Burton JA, Haber E, Cant JR, Smith FW, Barger AC. The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. J Clin Invest 1976;57:1606-1617.

Weglicki WB, Mak IT, Dickens BF, Kramer JH. Beta-blockers, calcium channel blockers and the sulfhydryl-ACE inhibitors demonstrate protection against free-radical-mediated injury of cardiovascular cells and membranes. Rev Port Cardiol 1992;11:1009-1011.

Weglicki WB, Mak IT, Simic MG. Mechanisms of cardiovascular drugs as antioxidants. J Mol Cell Cardiol 1990;22:1199-1208.

Weisman HF, Bush DE, Mannisi JA, Weisfeldt ML, Healy B. Cellular mechanisms of myocardial infarct expansion. Circulation 1988; 78: 186-201.

Weiss SJ. Oxygen, ischemia and inflammation. Acta Physiol Scand 1986;548:9-31.

Weitz ZW, Birnbaum AJ, Sobotka S, Zarling EJ, Skosey JL. High breath concentration during acute myocardial infarction. Lancet 1991:337:933-935.

Werns SW, Lucchesi BR. Leukocytes, oxygen radicals, and myocardial injury due to ischemia and reperfusion. Free Radic Biol Med 1988;4:31-37.

Westlin W, Mullane K. Does captopril attenuate reperfusion-induced myocardial dysfunction by scavenging free radicals? Circulation 1988;77:I30-139.

Wharton J, Morgan K, Rutherford RA, Catravas JD, Chester A, Whitehead BF, De Leval MR, Yacoub MH, Polak JM. Differential distribution of angiotensin AT2 receptors in the normal and failing human heart. J Pharmacol Exp Ther 1998;284:323-336.

Williams JF. Evolving concepts in congestive heart failure. Part I. Modern Concepts Cardiovasc Dis 1990;59:43-47.

Wohaieb SA, Godin DV. Alterations in free radicals tissue-defense mechanisms in streptozotocin-induced diabetes in rat. Effects of insulin treatment. Diabetes 1987;36:1014-1018.

Wong PC, Hart SD, Zaspel AM, Chiu AT, Ardecky RJ, Smith RD, Timmermans PB. Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: DuP 753 (AII-1) and PD123177 (AII-2). J Pharmacol Exp Ther 1990;255:584-592.

Xiang K, Cox NJ, Hallewell RA, Bell GI. Multiple Taq I RFLPs at the human manganese superoxide dismutase (SOD2) locus on chromosome 6. Nucleic Acids Res 1987;15:7654.

Yasmin W, Strynadka KD, Schulz R. Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. Cardiovascular Res 1997;33:422-432.

Ye Y, Gong G, Ochiai K, Liu J, Zhang J. High-energy phosphate metabolism and creatine kinase in failing hearts: a new porcine model. Circulation 2001;103:1570-1576.

Yeo HC, Helbock HJ, Chyu DW, Ames BN. Assay of malondialdehyde in biological fluids by gas chromatography-mass spectrometry. Anal Biochem 1994;220:391-396.

Yoshida T, Watanabe M, Engelman DT, Engelman RM, Schley JA, Maulik N, Ho YS, Oberley TD, Das DK. Transgenic mice overexpressing glutathione peroxidase are resistant to myocardial ischemia reperfusion injury. J Mol Cell Cardiol 1996;28:1759-1767.

Yoshida T, Maulik N, Engelman RM, Ho YS, Magnenat JL, Rousou JA, Flack JE 3rd, Deaton D, Das DK. Glutathione peroxidase knockout mice are susceptible to myocardial ischemia reperfusion injury. Circulation 1997;96:II-216-220.

Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, Lysko KA, Davis LL, Feuerstein G. Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J Pharmacol Exp Ther 1992;263:92-98.

Yue TL, Wang X, Gu JL, Ruffolo RR Jr, Feuerstein GZ. Carvedilol, a new vasodilating beta-adrenoceptor blocker, inhibits oxidation of low-density lipoproteins by vascular smooth muscle cells and prevents leukocyte adhesion to smooth muscle cells. J Pharmacol Exp Ther 1995;273:1442-1449.

Yucel D, Aydogdu S, Cehreli S, Saydam G, Canatan H, Senes M, Cigdem Topkaya B, Nebioglu S. Increased oxidative stress in dilated cardiomyopathic heart failure. Clin Chem 1998;44:148-154.

Yusuf S, Pepine CJ, Garces C, Pouleur H, Salem D, Kostis J, Benedict C, Rousseau M, Bourassa M, Pitt B. Effect of enalapril on myocardial infarction and unstable angina in patients with low ejection fractions. Lancet 1992;340:1173-1178.

Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of angiotensin converting enzyme-inhibitor, ramipril, on cardiovascular events in high risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000; 342: 145-153.

Zhang H, Schmeisser A, Garlichs CD, Plotze K, Damme U, Mugge A, Daniel WG. Angiotensin II-induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH-/NADPH-oxidases. Cardiovasc Res 1999;44:215-22.

Zile MR, Mukherjee R, Clayton C, Kato S, Spinale FG. Effects of chronic supraventricular pacing tachycardia on relaxation rate in isolated cardiac muscle cells. Am J Physiol 1995;268:H2104-H2113.

Zweier JL, Kuppusamy P, Williams R, Rayburn BK, Smith D, Weisfeldt ML, Flaherty JT. Measurement and characterization of postischemic free radical generation in the isolated perfused heart. J Biol Chem 1989;264:18890-18895.