EFFECTS OF AFTERLOAD REDUCING DRUGS ON THE PATHOGENESIS OF HEART FAILURE IN RATS

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By

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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I. ABSTRACT

Despite significant advances in the field, congestive heart failure subsequent to myocardial infarction remains a serious health problem with grave prognosis. Loss of myocardial tissue due to a lack of blood supply results in chronically increased workload on the remaining viable myocardium. Cardiac hypertrophy occurs in response to the increase in workload and also to neurohumoral adjustments. If the infarct is relatively small, the surviving myocardium is able to compensate for the loss of heart muscle. However, if the infarct is relatively large, heart function rapidly declines, and this decompensated hypertrophied state is manifest as heart failure. Failing heart may be characterized by a number of metabolic alterations including altered energy production and utilization, excitation-contraction coupling, contractile protein and sympathetic support, no consensus as to the exact cause of failure has been reached.

The use of vasodilators in the treatment of heart failure is common, and has been shown to attenuate left ventricular dilation and improve survival rate. Recently, congestive heart failure subsequent to myocardial infarction has been shown to be associated with a decrease in endogenous antioxidants and an increase in oxidative stress. In the present study, we examined the effects of two afterload reducing drugs; i) captopril, an angiotensin converting enzyme inhibitor and ii) prazosin, an α_1 blocker on the pathogenesis of heart failure. It was reasoned that if the reported decrease in endogenous antioxidants and an increase in oxidative stress have a causal relationship with the pathogenesis of heart failure, then improved prognosis with vasodilatory therapy should also be accompanied by a decrease in oxidative stress and an increase in endogenous antioxidants.

In order to characterize the effects of vasodilator therapy on the antioxidant and oxidative stress changes in the heart at different stages of hypertrophy and heart failure subsequent to myocardial infarction, the left anterior descending coronary artery was ligated in rats. After hemodynamic assessments, hearts were removed for a study of the myocardial endogenous antioxidants and lipid peroxidation (an index of oxidative stress) at 1, 4 and 16 weeks after the surgery. In addition, body weight, heart weight and degree of lung and liver congestion were also examined at 1, 4 and 16 weeks. Animals in both sham operated control and infarcted groups, at 4-weeks post infarction, were randomly assigned to two separate sub-groups and treated with captopril (2g/L in drinking water, daily) and prazosin (0.2 mg/Kg S.C. daily) for up to 16 weeks post-infarction. These animals were hemodynamically assessed at 16 weeks and sacrificed for different studies.

Hemodynamic assessment of control and experimental animals revealed no change in left ventricular end diastolic pressure (LVEDP) at 1 week post-myocardial infarction (PMI) compared to control. However, there was a significant increase in LVEDP at 4 and 16 weeks. Left ventricular peak systolic pressure (LVPSP) in 1 week PMI group remained unchanged. In 4- and 16-week PMI groups, LVPSP was reduced compared to control and the difference was significant at 16 weeks. Aortic systolic pressure (ASP) was also significantly depressed at 16 weeks. The rise in LVEDP in captopril treated PMI animals at 16 weeks was attenuated with respect to untreated experimental animals, while the treated group exhibited significant improvement in the LVPSP and ASP parameters compared to the non-treated PMI group. Prazosin treatment of the PMI animals also modulated LVEDP, LVPSP and ASP changes.

The lung and liver wet/dry weight ratios in the untreated PMI group remained unchanged at 1 week as compared to the respective control. However, this ratio for lung as well as liver was significantly increased at 16 weeks. Both captopril and prazosin modulated this increase in wet/dry weight ratios in these tissues. The scar mass at 4 and 16 weeks was significantly increased as compared to 1 week PMI group. This gain in scar mass in the captopril and prazosin treated groups, when compared to 16 week PMI untreated group, was significantly less.

Superoxide dismutase activity in the PMI group was unchanged at 1 week followed by a significant decrease at 4 and 16 weeks compared to control values. Glutathione peroxidase remained unchanged at 1 week, but at 4 and 16 weeks the activity was significantly depressed. A similar trend was seen with respect to catalase activity. Treatment with captopril and prazosin resulted in improvement in the SOD activity compared to the untreated PMI group. GSHPx and catalase activities in captopril treated PMI group were significantly higher compared to the untreated PMI group. However, with the prazosin treatment these activities remained unchanged compared to the untreated PMI group. Lipid peroxidation as indicated by thiobarbituric acid reacting substances (TBARS), remained unchanged at 1 week compared to control. At 4 and 16 weeks TBARS were higher with a significant increase seen at 16 weeks. TBARS in the captopril and prazosin treated PMI groups were significantly lower compared to their respective untreated PMI groups.

As both drugs improved the hemodynamic function and reduced oxidative stress, it is suggested that reduced wall stress or other humoral changes, subsequent to a treatment

with afterload reducing drugs, may be important in modulating subcellular, cellular and cardiac remodelling due to MI. Preservation of tissue antioxidant levels (activities) with captopril may also involve some direct effects of the drug at the tissue level. This may include tissue level changes in the renin-angiotensin system as well as improved coronary flow upon inhibition of the angiotensin converting enzyme. In any case, the present study provides evidence that heart failure subsequent to myocardial infarction is associated with an antioxidant deficit and increase in oxidative stress. The study also shows that treatment with vasodilators not only improved hemodynamic parameters but was also associated with an increase in antioxidants and a decrease in oxidative stress. Although the molecular basis of antioxidant changes remains to be understood, the study suggests the potential benefits of augmenting myocardial endogenous antioxidants in MI patients by pharmacological and/or other approaches.

II. INTRODUCTION

Heart failure is a clinical state, whereby the heart is unable to provide sufficient blood and nutrients for metabolic needs of the body. Congestive heart failure (CHF) is defined by excessive retention and accumulation of fluid in body tissues. Although the clinical syndrome has long been recognized and significant advances have been made in the management of heart failure patients, pathogenesis of the congestive heart failure condition remains to be understood. The failure may be caused by a primary defect in the cardiac muscle or it could be secondary to a chronic increase in cardiac workload which can occur due to valvular defects, hypertension, atherosclerosis etc. Irrespective of the inciting stimulus, the ultimate inability of the heart to maintain flow may result either due to a loss of the myocardial cells and/or subtle subcellular biochemical defects. Myocardial cell damage in myocarditis or myocardial infarction with subsequent loss of heart function leads to acute failure of the heart as a pump and can be ascribed to an inadequate number of normally contracting cardiac myocytes. However, it is the loss of contractile function *per se* in the chronic state without any further loss of myocytes, that remains to be described at the subcellular level.

Several biochemical processes including the production and utilization of high energy phosphates, excitation-contraction coupling and calcium metabolism have been reported to be defective in heart failure. Whether any or all of these changes are the result of the heart failure condition or if in fact these may be causal, remains unanswered. Recently it has been shown that in models of congestive heart failure in rats subsequent to myocardial infarction and in guineapigs subjected to chronic pressure overload that a decrease in

myocardial endogenous antioxidants and an increase in oxidative stress occurs. The latter has been suggested to cause myocardial cell damage and loss of contractile function, and may also explain some of the subcellular changes seen in heart failure.

An increase in cardiac work is accomplished by adjustments in preload, contractility, heart rate and afterload. In conditions of a chronic increase in work load, heart hypertrophy and other compensatory neurohumoral changes also take place. In heart failure, some of these adaptations not only become inadequate but they may in fact contribute to a further increase in cardiac workload, thus worsening the heart failure condition. One such change is an increase in vascular resistance which is also a major determinant of the afterload and ventricular wall stress. Reducing vascular resistance has been shown to decrease intracardiac pressure, increase stroke volume and improve the condition of heart failure in patients.

Captopril, an inhibitor of angiotensin converting enzyme (ACE), blocks the conversion of angiotensin I to angiotensin II. The latter is not only a powerful vasoconstrictor but it is also known to have many other effects at the cardiac cell level. In addition, ACE is important in the breakdown of bradykinin which is also a potent vasodilator. Inhibition of ACE is reported to improve coronary flow. As captopril may influence several processes, interpretation of the data from any study involving this drug should be done with a caution. Prazosin, an α-adrenergic receptor blocker, inhibits the vasoconstrictory effect of catecholamines which are generally present in high concentrations in heart failure patients. Both captopril and prazosin have also been shown to improve the

hemodynamic function and prolong life in rats with congestive heart failure subsequent to myocardial infarction.

In the present study, we reasoned that if the myocardial antioxidant deficit and increased oxidative stress subsequent to myocardial infarction in rats are events intimately linked to congestive heart failure, then improved prognosis with vasodilatory therapy should be associated with a modulation of these changes. The present study was therefore designed to test the hypothesis that improved hemodynamic function with an afterload reduction in the treatment of congestive heart failure may also involve improvement in the myocardial endogenous antioxidant status.

Heart failure subsequent to myocardial infarction was achieved by surgical occlusion the left descending coronary artery in rats. At mild failure stage (4 weeks), the animals were treated with captopril and prazosin. These animals were monitored for body mass, as well as heart, lung and liver weight; hemodynamic function; myocardial antioxidant enzyme activities and oxidative stress. The present study shows that the use of vasodilators mitigates the pathogenesis of heart failure. Drug treatment was not only accompanied by an improved hemodynamic function but it was also associated with an improvement in the endogenous antioxidants and a decrease in oxidative stress.

III. LITERATURE REVIEW

A. General Background:

Heart failure is a common clinical problem known to affect 3 million people in North America including approximately 200,000 in Canada. Acute heart failure occurs when underlying conditions precipitate loss of pump function relatively quickly (i.e. in a few seconds to days) and approximately 35% of individuals die suddenly. The remaining 65% escape such a fate either spontaneously or due to appropriate medical help. On the other hand, a slow progression of underlying causes of heart dysfunction results in what is generally known as chronic heart failure. In these patients a sudden increase in cardiac demand, a failure of therapy and/or a sudden progression of the underlying heart disease can also result in acute heart failure. Heart failure is not a disease in itself but it is a consequence of the myocardial cell damage and/or abnormalities of electrical conduction secondary to other factors. In this regard, there are three basic causes of heart failure: 1) intrinsic abnormalities such as coronary artery disease, cardiomyopathy and myocarditis; 2) increased workload secondary to valvular defects or hypertension; and 3) iatrogenic myocardial damage. However, left ventricular failure secondary to coronary artery disease is the most common cause of acute as well as chronic heart failure.

Loss of contracting myocardium due to myocardial infarction (MI) results in a chronic increase in workload of the remaining viable myocardium in the surviving patients. In addition to neurohumoral adjustments, heart responds by an increase in muscle mass and this process of heart hypertrophy represents a fundamental compensatory mechanism that permits the ventricle to sustain normal perfusion pressure (Cohen and Shah, 1974; Maron

and Ferrans, 1978; Meerson, 1969; Wikman-Coffelt et al., 1979). However, if this increased workload on the left ventricle is allowed to continue for a prolonged period, despite the presence of hypertrophy, cardiac pumping action becomes inadequate and heart failure supervenes. This left heart hypofunction has two major consequences: a) pulmonary changes including pulmonary congestion, right heart abnormalities and edema manifesting congestive heart failure; b) a drop in renal blood flow (ischemia), cerebral hypoxia and related complications. This congestive heart failure (CHF) following MI is a common clinical problem with grave prognosis (Braunwald, 1982). It is the transition of compensated hypertrophy to heart failure subsequent to MI which remains to be explained and is the subject of present study.

An adverse consequence of hypertrophy is the development of diastolic dysfunction which may even precede the transition to heart failure (Katz, 1990; Anversa et al., 1992). A number of subcellular biochemical alterations in terms of free radical and antioxidants, energy production and utilization, Ca^{2+} metabolism and contractile proteins, have been identified in the failing, hemodynamically overloaded heart subsequent to the development of hypertrophy (Bing, 1983; Cohen and Shah, 1974; Dhalla et al., 1983; Dhalla et al.,1978; Katz, 1990; Dhalla and Singal, 1994; Hill and Singal, 1995). However, there is no consensus with respect to which of the changes is compensatory, and which, if any, represents the primary defect in heart failure. It may be possible that pathways leading to heart failure may have a variety of origins.

It has been reported that in some patients with coronary artery disease, there is a down-regulation of the mechanical performance in the remaining viable myocardium

otherwise receiving a normal coronary flow at rest (Helfant et al., 1974; Cohn et al., 1975; Rahimtoola, 1985). The defect has been attributed to a so called "flow-metabolic mismatch phenomena" (Marshall et al., 1983; Vanoverschelde et al.,1992). Such a depression in function occurring in short term may be compensated and masked by other adaptive changes in the heart (e.g. hypertrophy) as well as other neurohumoral adjustments. In this regard, upregulation of Renin-Angiotensin System (RAS) as well as sympathetic system has been well documented.

In a classical view of RAS function and origin, angiotensinogen produced in liver is cleaved by renin released from kidney to produce a decapeptide known as angiotensin I. This is then converted by angiotensin converting enzyme to angiotensin II, an octapeptide with very strong vasopressor character. However, recent studies have provided evidence for the presence of different components of the RAS system in several tissues including heart (Campbell and Habener, 1986; Dzau and Re, 1987; Dzau, 1988). In addition to its strong vasopressor effect, angiotensin II has been shown to play a role in cardiac hypertrophy (Khairallah et al., 1972; Dzau, 1987; Baker, 1990). These findings have opened a totally new perspective for the role of RAS in heart under normal as well as in different heart failure conditions.

B. <u>Biochemical Basis of Heart Failure</u>:

Although intensive research thus far in this field has not identified the precise biochemical mechanism of heart failure, several subcellular, cellular and system level abnormalities have been well characterized.

B.1. Contractile proteins in heart failure:

The contractile machinery of the myocardium consists of thick and thin filaments. There are three myosin isozymes depending on the composition of the myosin. V_1 isozyme is composed of two α myosin chains and possess the highest ATPase activity. V_2 is composed of one α and one β myosin chain and has intermediate ATPase activity. V_3 is composed of two β myosin chains and V_3 has the lowest ATPase activity (Swynghedauw et al., 1983). The V_1/V_3 ratio is directly proportional to the velocity of muscle shortening (Alpert and Mulieri, 1982). In a normal heart, this ratio has been reported to remain unchanged whereas during chronic pressure overload this ratio has been shown to decrease (Mercadier et al., 1981). V_1 is the isozyme expressed predominantly in normal ventricles, but in heart failure conditions, it is V_3 that is predominant (Mercadier et al., 1981). A severe depression in myosin ATPase activity in the failing human heart was considered to be a crucial feature in the development of end-stage heart failure (Pagani et al., 1988).

Studies on hemodynamically overloaded rat heart have shown activation of β-myosin heavy chain (MHC) and deactivation of α-MHC at the transcriptional level, which may have accounted for decreased ATPase activity and thus decreased velocity of contraction in these hypertrophied hearts (Schwartz et al., 1986). There was also an increase in mRNA abundance for α-skeletal actin in the hypertrophied heart. Alteration of contractile proteins is not restricted to MHC, as changes in myosin light chain have also been reported in human heart subjected to mechanical stress (Hirzel et al., 1985). Schwartz and associates (1993) studied the mRNA expression of α-cardiac actin from the heart failure patients and found that the mRNA abundance for this actin remained unchanged compared to control, thus

suggesting that myosin and actin are independently regulated in human heart during failure. All these studies provide the evidence that there are alterations in the contractile proteins both during hypertrophy and heart failure. Whether these changes could account for the transition from compensated hypertrophy to heart failure is not known.

B.2. Energy metabolism and calcium homeostasis in heart failure:

A number of studies have delineated defects in oxidative phosphorylation, high energy phosphate metabolism and calcium supply to myofilaments in heart failure. However, there are conflicting reports about whether oxidative phosphorylation in hypertrophy and heart failure is normal or altered. In this regard, mitochondrial energy production was found to either increase or remain unchanged during hypertrophy stage (Chidsey et al., 1966 and Meerson et al. 1964). Meanwhile another group of investigators reported decreased mitochondrial energy production (Schwartz and Lee, 1962). In severe end-stage failure, mitochondrial respiratory function was depressed whereas the compensatory hypertrophy stage was accompanied by either normal or slightly depressed mitochondrial respiratory function (Newman, 1983). In a guinea pig model of heart failure, decreased ATP and creatine phosphate stores were reported (Feinstein, 1962). The high energy phosphates were reported to be at control level during hypertrophy stage but were below control level in the failure stage (Alpert and Hamrell, 1976; Bittl and Ingwall, 1987). It is likely that these differences are due to the differences in the stage of failure or in the animal model used in a given study.

Calcium plays a very important role in the contractile function of the heart (Dhalla et al., 1982). Numerous studies in the past have shown that there is an impairment in

intracellular calcium handling, thereby accounting for impaired contraction process in the heart (Dhalla et al., 1983). Cellular structures such as sarcolemma and the sarcoplasmic reticulum are important sites for intracellular calcium regulation. Alteration in their structure and activity result in reduced calcium uptake thereby resulting in increased intracellular calcium, ultimately affecting the excitation-contraction-relaxation cycle (Dhalla et al., 1983; Carafoli and Bing, 1988; Gwathmey and Morgan, 1985). Depressed rate of sarcoplasmic reticular calcium uptake was demonstrated in failing heart muscle obtained from humans (Harigaya and Schwartz, 1971). Impairment in calcium handling in human heart failure has also been reported by others (Gwathmey et al., 1987). It is clear that both high energy phosphate and calcium metabolism are altered in heart failure, but their precise contribution in the pathogenesis remains to be defined.

B.3. Downregulation of the adrenergic system:

During congestive heart failure, there is increased levels of plasma catecholamines associated with decreased tissue catecholamine levels. Chronic exposure to such catecholamines leads to decreased myocardial responsiveness. The decrease in the responsiveness could be due to a downregulation of β-receptors or some other alteration in the components of the β-adrenergic signalling pathway. Numerous studies have shown alterations in the different components of the adrenergic system during cardiac hypertrophy. Depletion of catecholamines in both clinical and experimental hypertrophy was demonstrated (Chidsey et al., 1964). A reduction in the cAMP as well as norepinephrine content of the pressure overloaded ventricle has also been reported (Mason et al., 1971; Stewart et al., 1978). In addition to changes in catecholamine levels, decreased

responsiveness to the adrenergic stimulation in experimental heart failure has also been reported (Covell et al., 1966; Tong et al., 1991). A decrease in responsiveness may be due to the defect or downregulation of the β_1-receptors (Denniss et al., 1989; Bristow et al., 1982). Cardiac G_i proteins have also been shown to be increased in hearts of patients with idiopathic dilated cardiomyopathy, both at the cellular and molecular levels (Feldman et al., 1988, 1989).

In addition to β-receptor alteration, changes in α-receptors have also been studied but not as extensively as β-receptors. The α_1 adrenergic receptors were shown to be increased in guinea pig hypertrophied hearts (Karliner et al., 1980). No change in the density of these receptors was reported in the failing hearts (Bristow et al., 1988).

C. <u>Management Strategies for Heart Failure</u>:

As the severity of heart failure ranges from mild pump dysfunction to an urgent threat to life, the treatments for heart failure also vary from a calm non-urgent approach to emergency measures (Braunwald et al., 1982). As a result of the harmful consequence of ischemic heart disease, different surgical procedures and use of different pharmacological agents have been introduced over the past many years and these measures certainly have made a difference in managing the pathogenesis of the heart disease. Reperfusion of the ischemic myocardium, proved to be of great importance in the treatment of ischemic heart disease (Danforth et al., 1960). Coronary bypass surgery which reinstitutes perfusion and oxygen delivery to the ischemic region of the myocardium has emerged as a fundamental strategy in the treatment of ischemic heart disease (Hearse and Bolli, 1992). It is also clear that reperfusion, though essential and initially important, may exacerbate the injury

sustained during ischemia (Jennings et al., 1960) and causes "reperfusion injury" (Jennings et al., 1960). Thrombolytic agents such as streptokinase and urokinase have been shown to reduce myocardial infarction (Rentrop et al., 1981). Some studies have suggested that the benefit of thrombolytic therapy is greater if administered in the early phase of reperfusion (Kirshenbaum, 1992).

The clinical syndrome of congestive heart failure subsequent to MI has long been recognized but its management remains one of the major problems in clinical cardiology (Pfeffer et al., 1993; Chopra et al., 1989; Braunwald and Grossman, 1992; Sharpe et al., 1990). Intensive research geared towards preventing heart failure has resulted in the use of different pharmacological agents, aiming to reduce the severity and incidence of this clinical condition. Some of these agents include angiotensin converting enzyme inhibitors, other vasodilators, diuretics, digitalis and β-adrenergic blockers.

C.1. Angiotensin converting enzyme inhibitors:

Afterload reduction or vasodilator therapy has now become an important form of treatment both for acute and chronic heart failure (Franciosa et al., 1972; Chatterjee et al., 1973; Awan et al., 1977; Toher and Francis, 1993). The recognition that patients with CHF often have increased peripheral vascular resistance due to vasoconstriction and an accumulation of extravascular fluid ("congestion") associated with salt and water retention (Francis et al., 1984; Francis, 1988) has led to the use of vasodilator therapy (Furberg and Yusuf, 1985; Braunwald and Colucci, 1984; Packer, 1983). The predominant action of the vasodilators on the heart is to reduce afterload and impedance.

There is now an overwhelming evidence that the renin-angiotensin system (RAS) is activated in patients with severe, decompensated heart failure (Merrill et al., 1946; Genest et al., 1968; Kawaguchi and Kitabatake, 1995). Large scale clinical trials have suggested that the activation of the RAS is linked to an adverse outcome in heart disease. This has led to the use of various agents that antagonize this system (Braunwald, 1991; Riegger, 1993). In chronic heart failure, decreased contractility of the left ventricle leads to reduced cardiac output which ultimately results in systemic arterial and venous vasoconstriction (Cannon et al.,1983; Cohn et al., 1979). This vasoconstriction appears to be mediated, in part, by the renin-angiotensin system (Brown et al., 1970; Genest et al., 1968). The role of renin-angiotensin system and the use of ACE inhibitors in heart failure has been a subject of many reviews (Kawaguchi et al., 1995; Watkins et al., 1976; Dzau et al., 1981; Curtiss et al., 1978). Studies in the past have examined the role of this system in different stages of heart failure. It has been shown that at the onset or in moderate heart failure, the RAS system serves as a compensatory mechanism and when the blood pressure is normalized, this system gets deactivated after which plasma-renin activity and angiotensin levels are back to normal. However during severe heart failure, the RAS system remains activated, resulting in increased system vascular resistance thereby reducing stroke volume (Dzau et al., 1981; Miller et al., 1982).

Angiotensin II is also responsible for increasing the preload via sodium retention and an increase in workload via systemic vasoconstriction (Laragh, 1962, Curtis et al., 1978). The normal cardiac response to an increase in afterload by increasing its contractility while the failing heart is less able to do so as a result of which there is decreased cardiac output

eventually leading to heart failure (Furberg and Yusuf, 1985). The initial activation of the RAS system is an attempt to maintain cardiac output. However over a period of time, this system further deteriorates cardiac function, thus worsening the condition. Therefore angiotensin converting enzyme inhibitors such as captopril, enalapril and many others have been used in an attempt to improve cardiac function (Hirsh et al., 1991; Konstam et al., 1992; Pfeffer, 1993; Pfeffer et al., 1993; Sharpe et al., 1991). In a ventricular-pacing model of chronic heart failure, ACE inhibition resulted in retardation of the progression of pump failure by decreasing peripheral vascular resistance (Riegger et al., 1984).

ACE inhibitors have been classified as: ACE inhibitors from natural products, sulphydryl (SH) containing ACE inhibitors, non-SH ACE inhibitors and other miscellaneous inhibitors (Juggi et al.,1993). Captopril was the first potent SH containing ACE inhibitor synthesized. This short acting ACE inhibitor is used frequently for the treatment of congestive heart failure (Romankiewicz et al. 1983; Giles, 1991; Jugdutt et al., 1995; Levine et al., 1980). Beneficial effects of long term therapy with captopril were demonstrated not only to improve left ventricular function but also to improve survival (Pfeffer et al, 1985b, 1992; Captopril Multicentre Research Group, 1983). Early treatment with captopril in patients with left ventricular dysfunction was shown to improve exercise tolerance and prevent progression of ventricular dilation and development of clinical heart failure (Pfeffer et al., 1985a, 1988; Cannon et al., 1983; Jugdutt et al., 1992; Oldroyd et al., 1991).

Prevention of ventricular dilation by captopril was also demonstrated in genetically induced heart failure in rats (Pfeffer et al., 1982). Several studies in patients with left ventricular dysfunction following myocardial infarction have shown that ventricular

enlargement was favourably altered by treatment with captopril (Pfeffer et al., 1988). In a high-risk group of patients with baseline distortion of left ventricular shape, those who received captopril had fewer clinically apparent manifestations of heart failure (Lamas et al., 1989).

Because of the presence of a sulphydryl group in its molecular structure, captopril has also been found to be a free radical scavenger (Chopra et al., 1989). Thus the beneficial effects of captopril may also involve an anti-free radical effect in addition to its vasodilatory action. Since ACE is known to enzymatically inactivate bradykinin, a known vasodilator, ACE inhibition may enhance availability of bradykinin independent of its effects on angiotensin II levels. Reduction in angiotensin facilitated release of catecholamines could be another consequence of ACE inhibition. Thus any interpretation of the data obtained from the use of ACE inhibitors must take these other aspects into consideration. In addition to ACE inhibitors, a number of studies have demonstrated that blocking the angiotensin I receptor also improved hemodynamic function (Sweet and Rucinska, 1994; Crozier et al., 1995). Studies by Burnier et al. (1994) have shown that the beneficial effects of ACE inhibitors and AT_1 antagonists are similar in terms of improving systemic and regional hemodynamic parameters. Dickstein et al. (1994) studied the effects of losartan (AT_1 antagonist) in patients with heart failure and found that losartan caused favourable vasodilation with no side effects. Thus the use of AT_1 receptor antagonist may prove useful in designing more precise experiments for defining the role of angiotensin II in heart failure.

C.2. Other Vasodilators:

In addition to ACE inhibitors, a wide spectrum of drugs are available including: direct-acting vasodilators such as organic nitrates (e.g. hydralazine); α-blocker (e.g. prazosin); Ca^{2+}-antagonists (e.g. nifedipine and diltiazem) and many more (Furberg and Yusuf, 1985; Packer and Le Jemtel, 1982). Several studies have clearly established that vasodilators improve left ventricular performance and reduce symptoms of left ventricular failure (Franciosa, 1982). Nitrates and nitroglycerin were among the earliest agents evaluated for chronic vasodilator therapy (Steward, 1988). Hydralazine has been an effective antihypertensive agent for many years in the management of left ventricular failure. In patients with left ventricular failure due to ischemic or idiopathic cardiomyopathy, oral hydralazine increased cardiac output by 56% and discontinuation of the drug resulted in significant reduction of cardiac output (Chatterjee et al., 1980).

Another widely used antihypertensive agent prazosin has been shown to improve exercise tolerance and enhance left ventricular performance (Goldman et al.,1980, Colucci et al., 1980; Schirger and Sheps, 1977). Unlike hydralazine, prazosin is an indirect acting agent, its action being receptor mediated. Prazosin does, however, resemble hydralazine in acting primarily on arterioles than arteries (Miller et al., 1982). It has also been proposed that prazosin may have some direct effect on vascular smooth muscle although this issue is debatable. Prazosin which reduces both preload and afterload has clinically been used for the treatment of heart failure (Awan et al. 1978; Miller et al., 1977; Bertel et al., 1981; Feldman et al., 1981; Goldman et al., 1980). This dual action of the drug is suggested to relate closely to the beneficial effects it has on the heart. Increase in stroke volume is due

to the afterload reducing property of the drug and decrease in the elevated left ventricular filling pressures is by decreasing the preload (Miller et al., 1977; Awan et al., 1978). Awan and associates (1977, 1978) have demonstrated that both preload and afterload reduction by prazosin not only resulted in increased cardiac output but also reduced evidence of pulmonary venous congestion and enhanced exercise tolerance in patients with heart failure. Packer et al., (1979a) have shown that the hemodynamic effects of initial prazosin doses i.e. reduced systemic arterial pressure, along with increased cardiac output, are no longer evident following repeated doses of the drug. Increasing the prazosin dosage also did not help maintain the hemodynamic responsiveness. Thus one of the drawbacks with this agent is the development of tolerance.

C.3. Positive inotropic agents and beta-blockers:

Positive inotropic agents increase contractile force of the myocardium. Digoxin is one of the most commonly used inotropic agents. Indeed recent trials have provided support for the effectiveness of digoxin in relieving symptoms, increasing ejection fraction and improving exercise tolerance in patients with heart failure (Packer et al., 1993, Captopril Digoxin Multicenter Research Group, 1988). In patients with atrial fibrillation, digitalis glycosides are very useful in controlling heart function. Agents such as dopamine and isoproterenol act on the β-adrenergic receptors, leading to stimulation of these receptors and thereby causing increased intracellular calcium concentrations via increase in cyclic AMP content.

Although heart failure is accompanied by decreased contractility and beta-adrenergic agonists such as dobutamine, dopamine etc. increase contractility at least initially, chronic

treatment with these agents has been shown to be deleterious. Therefore beta-adrenergic antagonists or β-blockers have been utilized in patients with heart failure (Swedberg et al., 1980). Metoprolol is one of the most commonly used β-blockers. Propranolol, atenolol and timolol are some of the other β-blockers used in patients with acute myocardial infarction and have been shown to reduce mortality and morbidity (Hjalmarson et al., 1981). In all of the above listed pharmacological approaches, the choice of drug as well as dosage would be determined by the type and stage of heart failure.

D. Oxidative Stress and Cardiac Injury:

More recently an increase in oxidative stress and a relative decrease in "antioxidant reserve" have been suggested to be involved in myocardial dysfunction and also in heart failure in chronic conditions (Singal and Kirshenbaum, 1990; Gupta and Singal, 1989a; Dhalla and Singal, 1994; Hill and Singal, 1995). In fact, the idea of oxidative stress and its role in cardiac injury has currently become a hot issue.

D.1. Free radicals:

A free radical is any atom or molecule that has an unpaired electron in its outermost orbit thus making these species highly reactive. Endogenous sources of free radicals include enzyme and non-enzyme systems located in the subcellular membranes, plasma membrane and blood cell elements. These radicals can be produced in the cells and tissues by various processes and reactions required for the maintenance of normal metabolism as well as energy production (Kaul et al., 1983; Singal et al., 1988). Free radical production can occur either by the addition of an electron or by its removal in a reduction/oxidation reaction. Because of the parallel spin of electrons in its outermost orbit, oxygen is also viewed

as a diradical. This unique electronic configuration of oxygen leads it to an univalent reduction pathway in which different reactive oxygen species are produced which are potentially toxic to all biological materials. Singlet oxygen ($^1O_2$) is the first excited state of oxygen. Singlet oxygen can initiate different oxygen radical chain reactions to form reactive oxygen intermediates. Addition of a single electron results in the production of superoxide anion radical ($O_2^{-\bullet}$) and its protonated form $HO_2^{\bullet}$. Addition of another electron to superoxide radical results in the formation of peroxide anion, which protonates to form hydrogen peroxide (H_2O_2). The reduction of H_2O_2 leads to the production of hydroxyl radical ($OH^{\bullet}$) and hydroxyl anion (OH^-). $^1O_2$, $O_2^{-\bullet}$, $HO_2^{\bullet}$, H_2O_2, $OH^{\bullet}$ and OH^- are called activated oxygen species and are collectively termed as partially reduced forms of oxygen (PRFO) (Kaul et al., 1993). By definition, H_2O_2 is not a radical because all the electrons in its outermost orbit are paired. However, H_2O_2 is a strong oxidizing agent and is capable of causing cell damage.

Although by definition only $HO_2^{\bullet}$, $O_2^{-\bullet}$ and $OH^{\bullet}$ are free radicals, usually all of the reactive oxygen intermediates are referred to as free radicals. Different species of PRFO possess different reactivities and have different half lives. $O_2^{-\bullet}$ is a highly reactive radical and has a very short half life. H_2O_2 on the other hand has a relatively longer half life and therefore its site of damage can be different than its site of production. $OH^{\bullet}$ has the shortest half life and is the most reactive of all the species. Under normal physiological conditions, the tissue concentration of free radicals is limited because of the presence of the antioxidant defense systems and the balance between free radicals and antioxidants is maintained. Any time this balance is shifted in favour of a relative increase in free radicals,

an oxidative stress condition occurs which can cause cell injury (Clark et al., 1985; Rao et al., 1983; Kaul et al., 1993).

D.2. Free radical mediated cell injury:

Free radicals are significantly important in the maintenance of normal physiological function. However, an increased production of these toxic chemicals is potentially lethal. Ample evidence suggesting that oxygen radicals cause damage both at the cellular and molecular level is now available. Biomolecules such as lipids, protein or DNA are the target of free radical injury (Singal et al., 1988a; Clark et al., 1985).

Lipid peroxidation is one way by which free radicals cause injury to the cell structure. Free radicals interact with the polyunsaturated lipids on the membrane and cause oxidation of these lipids resulting in the production of a lipid radical. These lipid radicals interact with the neighbouring lipids to form lipid peroxides (Kaul et al., 1993; Halliwell, 1987). Lipid peroxidation can be detected by a variety of ways. Thiobarbituric acid (TBA) reaction (Lee et al., 1991), the detection of conjugated dienes (Dormandy and Wickens, 1987) and the measurement of fluorescent products formed by the interaction of lipid peroxides and other tissue contents are some of the most practically used methods (Kaul et al.,1993; Aust, 1985). Of these, the TBA method is the most widely used.

Oxidation of both structural and functional proteins, important for the normal functioning of the cell is another site of free radical attack. Proteins rich in sulphydryl-group are much more susceptible to this free radical attack. Evidence suggests that the free radicals through oxidization modify the protein structure and/or function and these oxidized proteins undergo proteolysis (Weiss, 1986). Free radicals attacking the enzymes involved

in the signal transduction pathway have also been reported. In this regard, alteration in the redox state of thiol groups associated with phospholipase C and phospholipase D by free radicals have been shown to effect the enzyme activity (Dai et al., 1992; Meij et al., 1994).

Free radicals have also been reported to damage the DNA, by producing base damage, single-strand breaks and chromosomal abberations (Schraufstatter et al., 1986). Such modifications have been shown to result in cellular abnormalities (Shamberger et al., 1974).

D.3. Antioxidant defense system:

There are both enzymatic and non-enzymatic defense systems to protect the cell from the deleterious effects of free radicals.

D.3.a Enzymatic antioxidants: Enzymatic defense system includes superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT). Non-enzymatic antioxidants are tocopherols, glutathione, carotenes and ascorbic acid (Kaul et al., 1993, Singal and Kirshenbaum, 1990).

Superoxide dismutase (SOD): SOD is the first line of defense against oxidative damage. SOD catalyzes the dismutation of O_2 reduced to H_2O_2. SOD is found both in the cytosol and mitochondria of mammalian cells. There are different forms of SOD, depending on where it is localized. The CuZn SOD found in the cytosol has a molecular weight of about 32,000 daltons. MnSOD primarily localized in the mitochondrial matrix has a molecular weight of about 95,000 daltons (Fridovich, 1975). Various other forms of SOD have recently been identified carrying different metals at its active site (Marklund, 1984; Kaul et al., 1993).

Glutathione peroxidase (GSHPx): This enzyme represents the second line of defense against free radical mediated damage. It catalyzes the reduction of hydroperoxides using glutathione (GSH) as a substrate to form oxidized glutathione (GSSG). Glutathione peroxidase is a tetrameric protein with a molecular weight of about 85,000 daltons (Lawrence and Burk, 1978). This enzyme is present in relatively high concentrations in cardiac muscle. For a continuous functioning of this enzyme, oxidized glutathione must be reduced back to GSH (reduced form).

Catalase (CAT): Located primarily in peroxisomes, CAT is responsible for converting H_2O_2 to oxygen and water, thus preventing excessive accumulation of H_2O_2. It is a heme enzyme with a molecular weight of about 240,000 daltons. The concentration of catalase is reported to be low in the cardiac muscle (Doroshow et al.,1980). Both catalase and glutathione peroxidase regulate H_2O_2 concentration within the heart but the difference between these two enzymes is that GSHPx is more effective at lower concentrations of H_2O_2, whereas catalase is more effective at higher concentrations of H_2O_2.

D.3.b. Non-enzymatic antioxidant defense system: Among the non-enzymatic antioxidants, Vit E (α tocopherol), Vit C (ascorbic acid) and glutathione have been most frequently studied.

Vitamin E: Vitamin E is known as a chain breaking antioxidant. Either alone or in synergism with Vit C, Vit E terminates free-radical reaction. It is a determinant of the peroxidative status of the heart tissue (Janero and Burghardt, 1989). Numerous in vitro and in vivo studies have shown that deficiency of Vit E results in increased susceptibility of

myocardial cell membranes to free radical mediated damage (Singal et al., 1983; Singal and Tong, 1988b), suggesting that Vit E is an important antioxidant (Singal et al., 1983, Packer, 1994).

Glutathione: Is present in high concentrations in most eukaryotic cells, mostly as GSH (reduced glutathione) with little of GSSG (oxidized glutathione). It acts as a co-substrate of GSHPx, to remove H_2O_2 or other lipid peroxides and result in the formation of water and GSSG (Ross et al., 1985).

Vitamin C: Ascorbic acid may directly reduce free radicals with the concomitant formation of dehydroascorbate. It also plays an important role in the regeneration of Vit E (Packer et al., 1979b). It is known both to quench excited species and also to react directly with PRFO.

D.4. Free-radical induced myocardial dysfunction:

The list of pathological conditions where oxygen free radicals are involved continues to grow rapidly. Ischemia-reperfusion, hypoxia reoxygenation, inflammation, aging, cardiomyopathy, heart failure subsequent to pressure and volume overload and drug toxicity are some of the conditions where involvement of free radicals has been documented (Freeman and Crapo, 1982; Thompson and Hess, 1986; Weiss, 1986; Ferrari et al., 1991; Kaul et al., 1993, Singal et al., 1993; Cross, 1987; Singal et al., 1982). Myocardial endogenous antioxidants have also been reported to change under these various physiological and pathological conditions (Gupta and Singal, 1989a; Siveski-Iliskovic et al., 1994, 1995; Hill and Singal, 1995; Dhalla and Singal, 1994; Kaul et al., 1993; Singal et al.,

1995). Thus the injury may be a combined effect of a reduction in endogenous antioxidants and an increase in free-radical production.

Ischemia - reperfusion: Endogenous antioxidant enzymes have been reported to be depressed during ischemia (Ferrari et al., 1985, Guarnieri, 1980) as well as under hypoxic conditions (Fox et al., 1983; Dhaliwal et al., 1991). These changes in the enzymes correlated with poor recovery of function upon reperfusion/reoxygenation. Fuji et al., (1992) showed that in hypoxia-reoxygenation condition, mRNA abundance for manganese superoxide dismutase was decreased during hypoxia and was increased upon subsequent reoxygenation. Another study demonstrated significant increase in SOD and GSHPx activity in hypertrophied hearts after subjected to hypoxia-reoxygenation (Kirshenbaum and Singal., 1992).

Ageing: Age-related changes in antioxidant enzyme activities have been reported. Gupta and Singal, (1989b), showed that there was an age-dependent increase in SOD activity in rats for up to about 6 months of age. In another study, SOD and catalase activities were found to be depressed in rat hearts that were 72 weeks old (Sohal et al., 1990). Also, superoxide anion and hydrogen peroxide were reported to be increased with age in these rat hearts (Sohal et al., 1990). These data show an increase (Gupta and Singal, 1989a) as well as decrease in antioxidant enzyme activities, depending on the age of the animal (Sohal et al., 1990, Singal et al., 1995).

Exercise: Strenuous physical exercise increases oxidative metabolism, which results in increased free radical production (Singal et al., 1995). In this regard, increased free radical production was found in the skeletal muscle and the liver of exercising rats (Davies

et al., 1982). In another study, Kanter et al., (1985) showed that exercise trained mice exhibited increased levels of SOD, catalase and GSHPx in their liver and blood and also these animals showed reduced mortality due to doxorubicin (Kanter et al., 1985), a drug known to cause cardiomyopathy by producing free radicals (Singal et al., 1987).

Hypertrophy and heart failure: In cardiac hypertrophy subsequent to chronic pressure overload induced by banding of the abdominal aorta, an increase in myocardial antioxidants and decrease in lipid peroxidation was observed (Gupta and Singal, 1989a). Furthermore, these animals exhibited better recovery of contractile function and reduced incidence of arrhythmias upon reperfusion (Kirshenbaum and Singal, 1993). In a guinea pig model of heart hypertrophy subsequent to a chronic pressure overload, there was an increase in the antioxidant reserve at 10 weeks. At the 20 week failure stage, the hearts showed decreased antioxidant reserve accompanied by poor hemodynamic function (Dhalla and Singal, 1994; Randhawa and Singal, 1992).

In a recent study on rat hearts at different stages of heart failure subsequent to myocardial infarction, a significant decrease in antioxidant reserve and an increase in lipid peroxidation was seen at the severe heart failure stage (Hill and Singal, 1995). Furthermore, oxidative stress at 1 week after the MI was significantly less and hemodynamic function at this stage in these animals was maintained. However, at longer post-MI intervals oxidative stress increased while heart functions decreased with animals showing clinical signs and symptoms of heart failure. Although a strong correlation between endogenous antioxidant decrease and occurrence of heart failure was observed, cause and effect relationship remains

to be determined. In this regard, an afterload reduction therapy has been reported to decrease mortality and improve prognosis in rats subjected to coronary ligation (Pfeffer et al., 1985a,b).

The present study was undertaken to further examine the relationship among myocardial endogenous antioxidants, lipid peroxidation, hemodynamic function and stage of heart failure in MI rats subjected to vasodilatory therapy with captopril and prazosin. A comparative analysis was also done to distinguish whether the beneficial effects were secondary to afterload reduction or due to some direct myocardial consequences of angiotensin II effects.

IV. MATERIALS AND METHODS

A. Animals:

Male Sprague Dawley rats weighing 150 ± 10g were purchased from the Central Animal Care Unit, University of Manitoba and maintained in the animal holding facility of the St. Boniface General Hospital Research Centre. Animals were provided with standard rat chow and water ad libitum unless mentioned otherwise.

B. Experimental Model and Study Groups:

Myocardial infarction was produced via occlusion of the left anterior descending coronary artery according to the procedure first described by Johns and Olson (1954) and later modified by Selye et al. (1960). 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 proximal to 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. Following ligation, the heart was gently repositioned in the chest. Excess air was drawn into a syringe, and the chest was closed. Closure of the incision was accomplished by a purse-string suture. The rats were maintained on a positive pressure ventilation, delivering 2% isoflurane at the rate of 2 L/min throughout the surgery. The entire surgical procedure was carried out in sterile conditions. Control animals were handled in a similar fashion with the exception that the suture around the coronary artery was not tied and the thread was only passed through the muscle.

Following the operation, animals were allowed to recover on the table. Animals were monitored for their weight, behavioral pattern, mortality, etc.

In all there were five study groups with their respective age matched controls. These groups were as follows: 1) 1 week post myocardial infarction (PMI) and 1 week control; 2) 4-week PMI and 4-week control; 3) 16-week PMI and 16-week control; 4) Captopril treated group: 16-week PMI and 16-week control and in each group Captopril treatment was started 4 weeks after the surgery and continued up to 16 weeks; 5) Prazosin treated group: 16-week PMI and 16-week control and in each group, Prazosin treatment was started 4 weeks after the surgery and was continued up to 16 weeks.

C. Hemodynamic Studies:

Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A miniature pressure transducer catheter (Millar Micro-Tip, model PR 249, Houston, Texas) was inserted into the right carotid artery and then advanced into the left ventricle. Left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), aortic diastolic pressure (ADP) and aortic systolic pressure (ASP) were recorded on a computer for an on line analysis (Axotape acquisition data program and Acqknowledge 3.0). After hemodynamic assessment, the rats were sacrificed and the heart and other organs were removed for further studies.

D. Tissue Weight Determination:

The heart, in case of infarction, was first weighed with the scar and then the scar tissue was cut out and the heart was weighed again, from which weight of scar tissue was obtained. For ventricular weight in sham control group, both right and left ventricles were

included. In the PMI group, right and viable left ventricle were taken. In order to obtain the wet/dry weight ratio of the lungs and liver, the organs were freed from adhering tissues. The sample tissues were weighed, chopped into smaller pieces and placed in the oven at 65°C until a constant weight was obtained, which was usually after 24 hrs.

E. Antioxidant Enzyme Assays:

E.1. Superoxide Dismutase (SOD) Assay

Superoxide dismutase activity in the hearts was determined by the method previously described by Marklund (1985). Hearts were homogenized (1:10) in 50 mmol/L Tris-HCl, pH 8.20, containing 1 mmol/L diethylenetriamine pentaacetic acid. The homogenate was centrifuged at 20,000 x g for 20 minutes. The supernatant was aspirated and assayed for total superoxide dismutase activity by following the inhibition of pyrogallol auto-oxidation. Pyrogallol (24 mmol/L) was prepared in 10 mmol/L HCl and kept at 4°C prior to use. Catalase, 30 μmol/L stock solution was prepared 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. Changes in absorbance at 420 nm were recorded at 1 min intervals for 5 min. Data are expressed as total SOD Units per milligram protein derived from an SOD standard curve of pyrogallol autoxidation with commercially available SOD.

E.2. Glutathione Peroxidase (GSHPx) Assay

GSHPx activity was determined in whole heart by the method previously described by Paglia and Valentine (1967). Hearts were homogenized (1:10) in 75 mmol/L phosphate buffer, pH 7.0. Homogenate was centrifuged at 20,000 g for 25 minutes and the supernatant was aspirated and assayed for total cytosolic GSHPx activity. GSHPx activity was assayed

in a 3-ml cuvette containing 2.0 mL of 75 mmol/L phosphate buffer, pH 7.0. The following solutions were then added: 50 μL of 60 mmol/L glutathione, 100 μL glutathione reductase solution (30 U/mL), 50 μL of 0.12 mol/L NaN_3, 100 μL of 15 mmol/L Na_2 EDTA, 100 μL of 3.0 mmol/L NADPH, and 100 μL of cytosolic fraction. The reaction was started by the addition of 100 μL of 7.5 mmol/L H_2O_2 and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbance at 340 nm at one minute intervals for 5 min. GSHPx activity was expressed as nanomoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized to nicotinamide adenine dinucleotide phosphate (NADP) per min per milligram protein, with a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6.

E.3. Catalase Assay

Catalase activity in the hearts was determined by the method previously described by Clairborne (1985). Hearts were homogenized in (1:10) 50 mmol/L potassium phosphate buffer, pH 7.4. Homogenate was centrifuged at 40,000 g for 30 min. 50 μL of supernatant was added to a 3-mL cuvette which contained 2.95 mL of 19 mmol/L hydrogen peroxide in 50 mmol/L potassium phosphate buffer, pH 7.4. Changes in absorbance at 240 nm were continuously followed for 5 min. Catalase activity was expressed as U/mg protein.

F. Thiobarbituric Acid Reactive Substances Assay

Lipid peroxide content in hearts was determined by measuring the thiobarbituric acid reactive substances (TBARS) by the method as described previously (Singal and Pierce, 1986). Hearts were homogenized in (10% wt/vol) 0.2 Mol/L Tris-0.16 Mol/L KCl buffer, pH 7.4 and incubated at 37°C for 1 hour. After one hour, a 2 mL aliquot was collected from

the incubation mixture and poured into a Corning culture tube. To this tube, 2.0 ml of 40% trichloroacetic acid and 1.0 ml of 0.2% thiobarbituric acid (TBA) were added. 100 μL of 2% butylated hydroxy-toluene was added to the TBA reagent mixture in order to minimize peroxidation during the assay procedure. The mixture was then boiled for 15 min and then allowed to cool on ice for 5 min. Following that, 2 ml of 70% trichloroacetic acid was added, and tubes were allowed to stand for 20 min, and after 2 min, the sample was centrifuged at 800 g for 20 min. The developed colour was read at 532 nm. Commercially available malondialdehyde was used as a standard.

G. Vasodilator Therapy:

For studying the effects of vasodilator therapy on the pathogenetic changes subsequent to myocardial infarction, animals in two separate experiments were treated with Captopril and Prazosin. Captopril, [25]-1-[3-mercapto-2-methylpropienylprotine]L, was given to 4 week control as well as 4 week post myocardial infarction animals (Pfeffer et al., 1985b). Captopril (2 g) was dissolved in 1 L of drinking water and equal amount of the water was given to each rat, placed in separate cages (Pfeffer et al., 1985b). The amount of water consumed daily by each rat was monitored. The animals were also monitored for their body weight and behaviour during the entire course of the treatment. The treatment was continued up to 16 weeks of post myocardial infarction. The animals at the end of 16 weeks PMI were assessed for hemodynamic function and hearts and other tissue were studied as described above.

Another vasodilator, Prazosin hydrochloride, an α adrenergic blocker, was used for comparison (Miller et al., 1977). Prazosin obtained from SIGMA, was given subcutaneously

(0.2 mg/kg), both to 4 week control and 4 week PMI rats daily for up to 16 weeks post-surgery duration.

H. Proteins and Statistical Analysis:

Proteins were determined by the method described by Lowry et al. (1951). Data were expressed as the mean ± SEM. For a statistical analysis of the data, group means were compared by one-way ANOVA and Bonferroni's test was used to identify differences between groups. Values of $p<0.05$ were considered significant.

V. RESULTS

A. General Characteristics, Mortality and Body Weight:

All sham control and coronary ligated animals were monitored daily for their general behaviour and body weight. Different biometric, biochemical and hemodynamic parameters were studied at 1, 4 and 16 weeks after the surgery. Animals in sham control group as well as coronary ligated group were either given captopril or prazosin at 4 weeks after the surgery as described in the "Materials and Methods".

In terms of general appearance and behaviour of animals, nothing unusual was noted in any of the sham control, experimental and drug treated groups. Mortality in the coronary ligated animals during or immediately after the surgery was about 20%. Another 12% of the animals died within 24 hrs following the surgery. Body weight gain in animals in all coronary ligated groups was slightly lower than their respective sham control groups (Table 1 and Fig 1). Body weight gain in 16-week PMI group treated with captopril was significantly less than the untreated 16-week PMI group (Table 1) as well as captopril treated sham control group (Fig 1). Prazosin treatment did not influence the body weight gain in any of the groups (Fig 1).

B. Ventricular Weight and Ventricular to Body Weight Ratio:

For ventricular weight, both the atria and extraneous fat were removed. For the sham control group, both right and left ventricles were included and for the PMI group, both right and the viable left ventricle were included. These data are also shown in Table 1 and Figure 2. Ventricular weight in 1- and 4-week PMI animals was comparable to

Table 1: Body weight, ventricular weight and ventricular to body weight ratio in sham control and myocardial infarction groups at 1, 4 and 16 weeks.

Post Surgery Duration	Body Wt (g)		VW (g)		VW/BW (Ratio X 1000)	
	SHAM	PMI	SHAM	PMI	SHAM	PMI
1 Week	336.2 ± 3.7	320.0 ± 7.7	0.76 ± 0.2	0.70 ± 0.6	2.26 ± 0.4	2.2 ± 0.2
4 Weeks	473.2 ± 13.2	435 ± 5.6	0.99 ± 0.2	1.0 ± 0.3	2.1 ± 0.8	2.3 ± 0.1
16 Weeks	697.8 ± 14.4	630 ± 13	1.7 ± 0.4	1.9 ± 0.7	2.4 ± 1.2	3.0 ± 0.2*

Values are mean ± S.E. of 10-12 animals. VW, Ventricle weight; BW, Body weight. For the ventricle weight in the infarcted (PMI) group, viable left myocardium and right ventricle were included. *) Significantly higher ($P<0.05$) than the 1 and 4 week PMI groups.

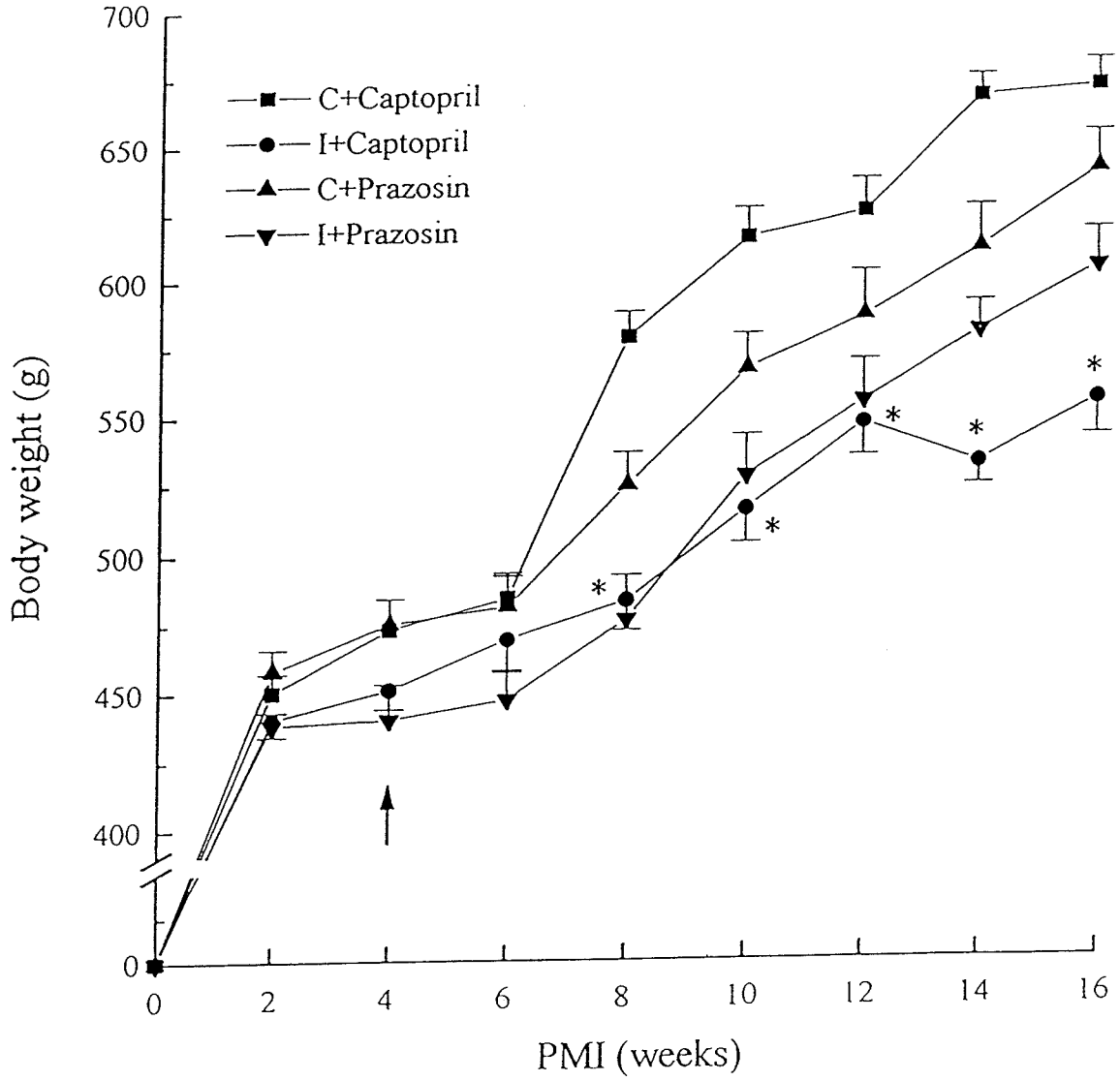

FIG 1: Effects of captopril and prazosin on body weight subsequent to myocardial infarction (PMI). Values are mean ± S.E. of 10-12 animals. C) Sham control; I) Infarcted group. Arrow indicates the time at which the drug treatment was started. *) Significantly different($P<0.05$) from non-infarcted sham control animals treated with captopril. Body weight gain in sham control without the drug treatment was not different from the animals receiving either of the drugs (data not shown).

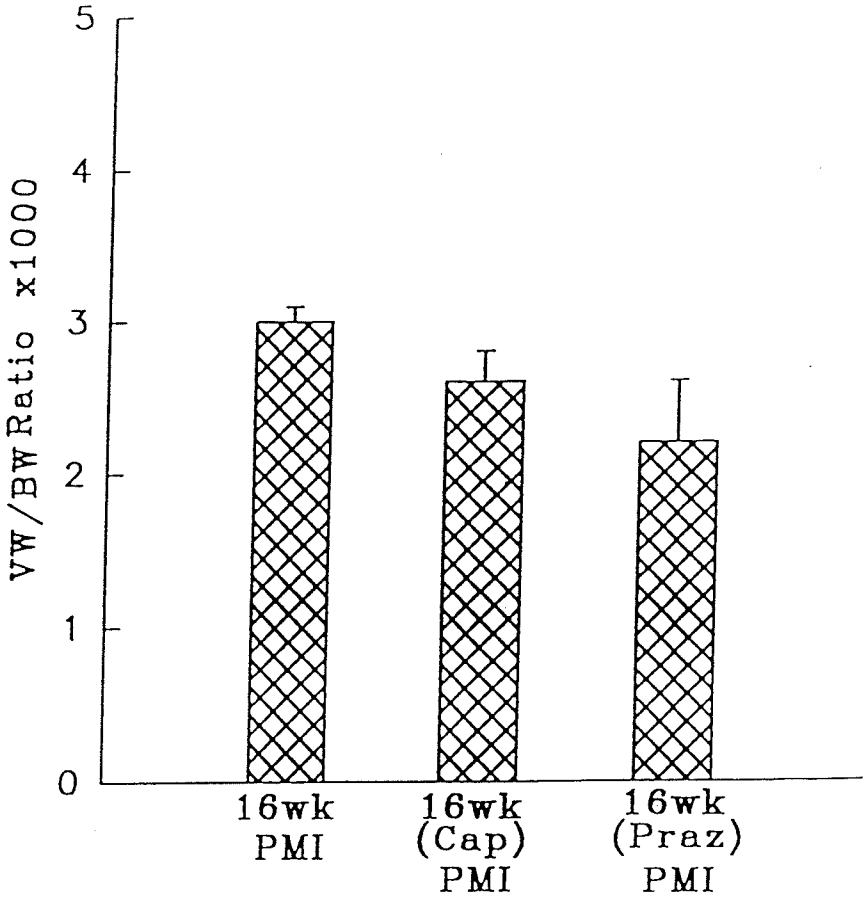

FIG 2: Ventricle/Body weight ratio in rats at 16 weeks PMI. For the ventricle weight, viable left myocardium and right ventricle were included. Effects of captopril (Cap) and prazosin (Praz) treatment on this ratio at 16 weeks are also shown. Data are mean ± S.E. of 6-8 experiments.

the weights in their respective sham controls. In the 16-week PMI group, ventricular weight was ~12% increased when compared sham control but this difference was not statistically significant.

Ventricular to body weight ratio in the 16 week sham group was not different from 1- and 4-week sham controls. However, this ratio in the 16-week PMI group was significantly higher than the 1- and 4-week PMI groups (Table 1). In animals treated with captopril or prazosin, this ratio at 16 weeks was less than PMI untreated group but these differences were statistically not significant due to a large scatter around the mean values (Fig 2).

C. Scar Weight:

Scar tissue weight was examined in 1-, 4- and 16-week untreated PMI groups (Fig 3) as well as in 16-week PMI animals treated with captopril or prazosin (Fig 4). The scar tissue weight in the 4- and 16-week untreated PMI groups was significantly higher as compared to the 1 week group. This gain in scar tissue weight was significantly less in the 16 week PMI animals treated with captopril or prazosin (Fig 4).

D. Lung and Liver Wet/Dry Weight Ratio:

The wet to dry weight ratio for lungs as well as liver did not change in 1-, 4- and 16-week sham control animals (Table 2). In the untreated PMI group at 1 and 4 weeks, there was no change in this ratio for both organs. At 16 weeks, however, the ratio for the liver was about 25% higher and for the lungs 33% higher than 16-week sham group and these differences were significant (Table 2).

In the PMI group, captopril treatment significantly attenuated wet to dry weight ratio both for the lungs (Fig 5A) and the liver (Fig 5B). Similarly prazosin treatment also in the

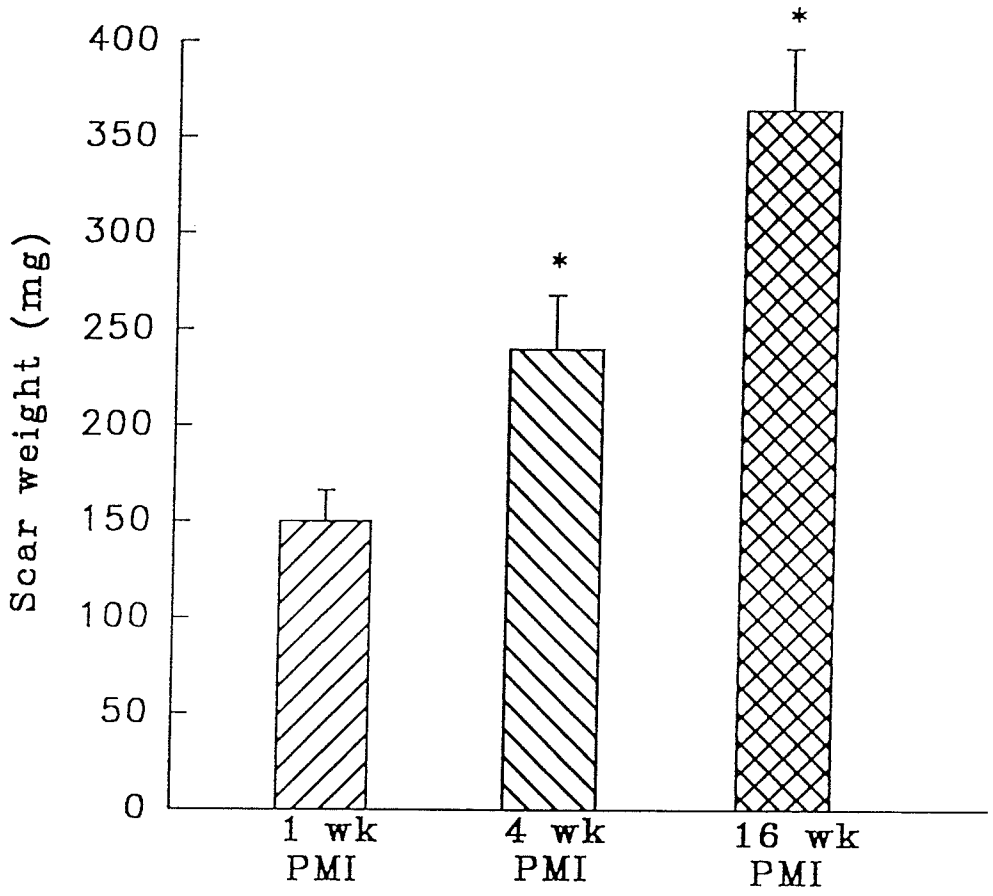

FIG 3: Scar weight at 1, 4 and 16 weeks post-surgery durations. Data are mean ± S.E. of 6-8 hearts. *) Significantly different ($P<0.05$) from 1 week group (ANOVA followed by Bonferroni test). PMI) Post myocardial infarction.

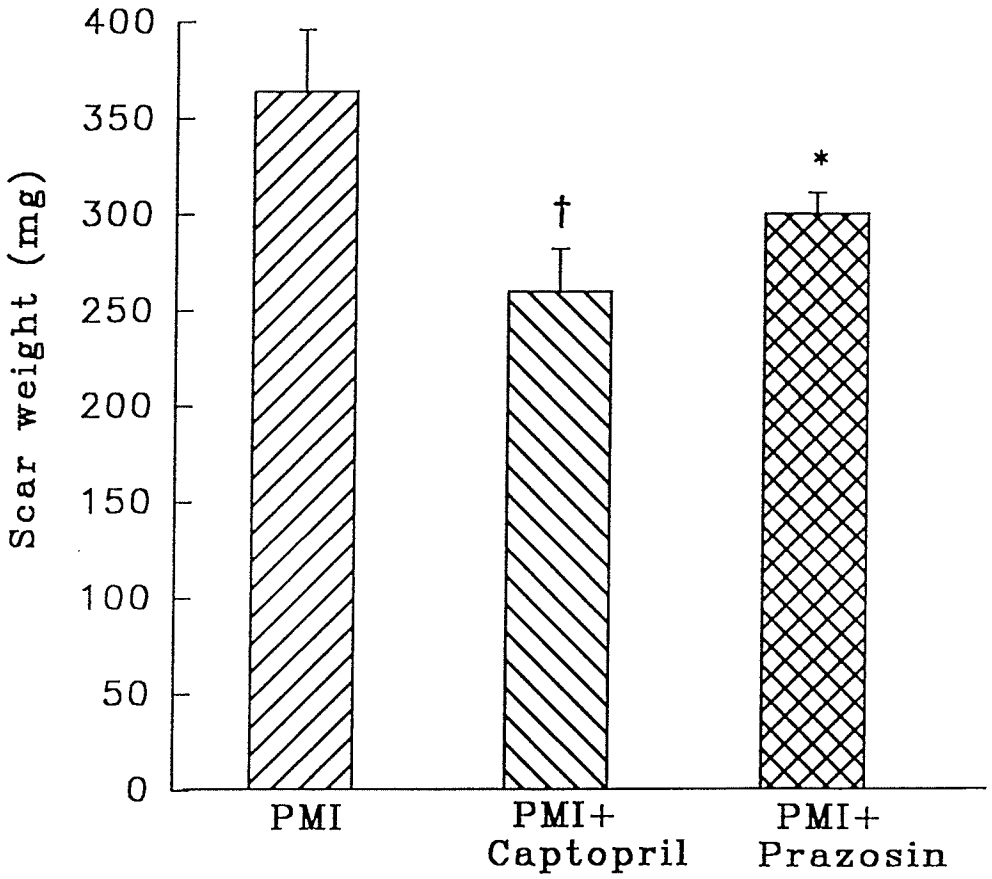

FIG 4: Effects of captopril and prazosin on the scar weight at 16 weeks. Data are mean ± S.E. of 6-8 hearts. *) Significantly different from the untreated PMI group ($P<0.05$) by ANOVA; †) ANOVA followed by Bonferroni test.

Table 2: Lung and liver wet/dry weight ratios in animals at 1, 4 and 16-week post-surgical durations.

Post Surgery Duration	Lung		Liver	
	SHAM	PMI	SHAM	PMI
1 Week	4.76 ± 0.19	4.78 ± 0.22	3.19 ± 0.02	3.20 ± 0.51
4 Weeks	4.66 ± 0.27	4.97 ± 0.58	3.21 ± 0.54	3.42 ± 0.11
16 Weeks	4.64 ± 0.19	6.20 ± 0.32*	3.22 ± 0.17	4.04 ± 0.28†

Values are mean ± S.E. of 5-8 experiments. Significantly different ($p<0.05$) from respective sham control groups. †) ANOVA and *) ANOVA followed by Bonferroni test.

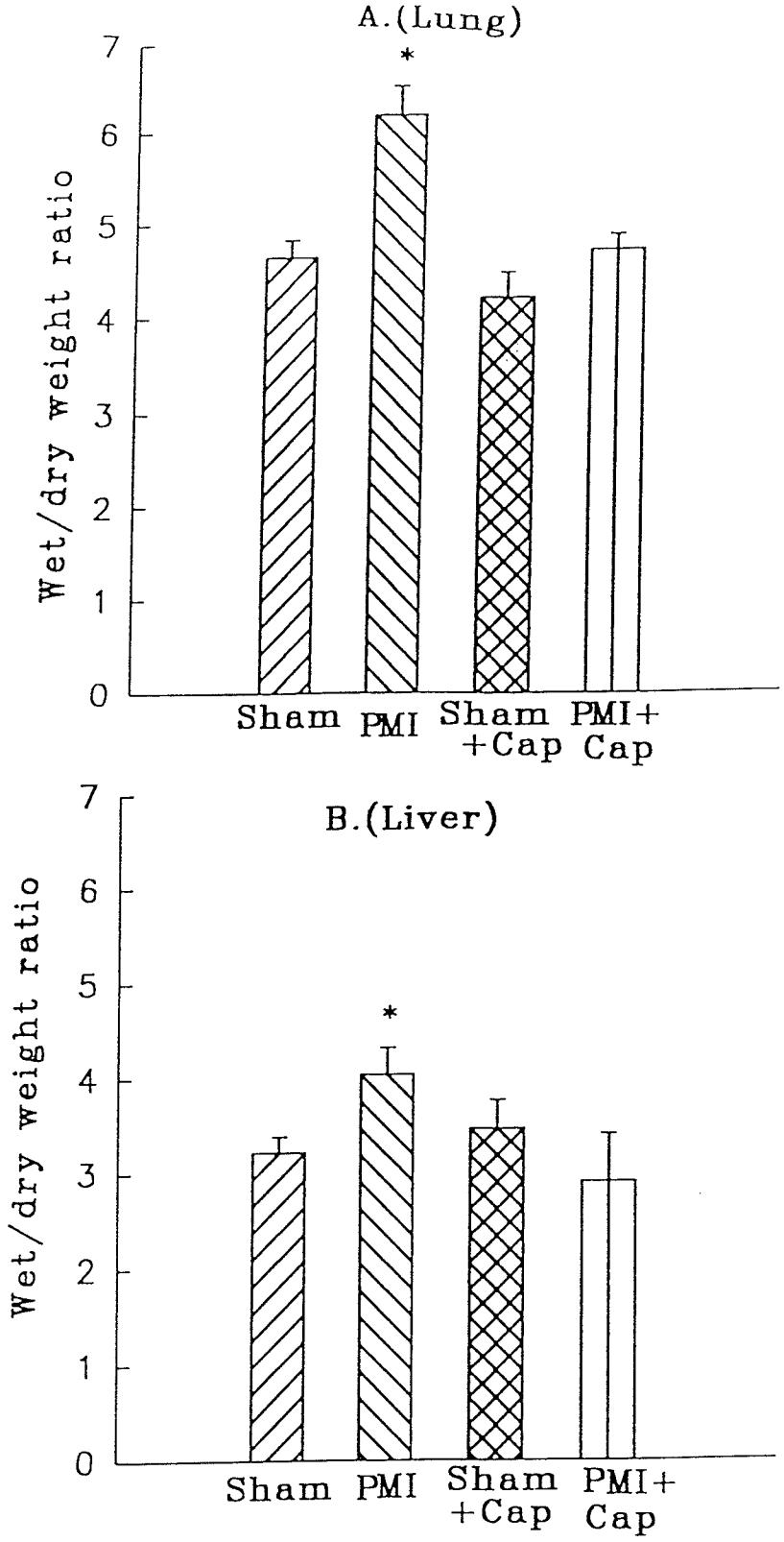

FIG 5A & B: Effects of captopril on the lung (A) and liver (B) weight ratios at 16-weeks PMI. Values are mean ± S.E. of 10-12 animals. *) Significantly different ($P<0.05$) from all other groups.

PMI group, significantly modulated the increase in wet to dry weight ratio for both the lungs (Fig 6A) and the liver (Fig 6B). Neither of the drug treatment had an effect on this ratio in any of the sham controls (Fig 5 and 6).

E. Hemodynamics:

Hemodynamic function in the sham control and PMI animals was assessed at 1, 4 and 16 weeks. Peak systolic pressure in the left ventricle (LVPSP) in the PMI group did not change at 1 week. In the 4-week PMI group, there was a marginal reduction in the LVPSP, and this parameter was significantly depressed at 16 weeks (Table 3). Left ventricular end diastolic pressure (LVEDP) in the PMI group was unchanged at 1 week, but was significantly elevated in the 4 and 16 weeks. Left ventricular end diastolic pressure in the PMI animals was significantly elevated at 4 and 16 weeks (Table 3).

Captopril treated PMI animals at 16 weeks was associated with an attenuated rise in their LVEDP and had a significantly higher LVPSP compared to the non-treated PMI group (Table 4). Both LVEDP and LVPSP were also significantly different from sham control captopril treated animals. Prazosin treatment of the PMI animals also modulated LVEDP and LVPSP changes in the infarcted animals. Only LVEDP was still higher than its sham control prazosin treated animals, while LVPSP in the 16-week treated PMI animals was no longer different from their 16 week sham control animals treated with this drug.

Both aortic systolic (ASP) and aortic diastolic pressures (ADP) were also monitored and these data are shown in Table 5. ASP in the PMI group was significantly decreased at 16 weeks while ADP in these animals was significantly elevated at 4 and 16 weeks as compared to their respective sham controls. These differences in the ASP and ADP in the

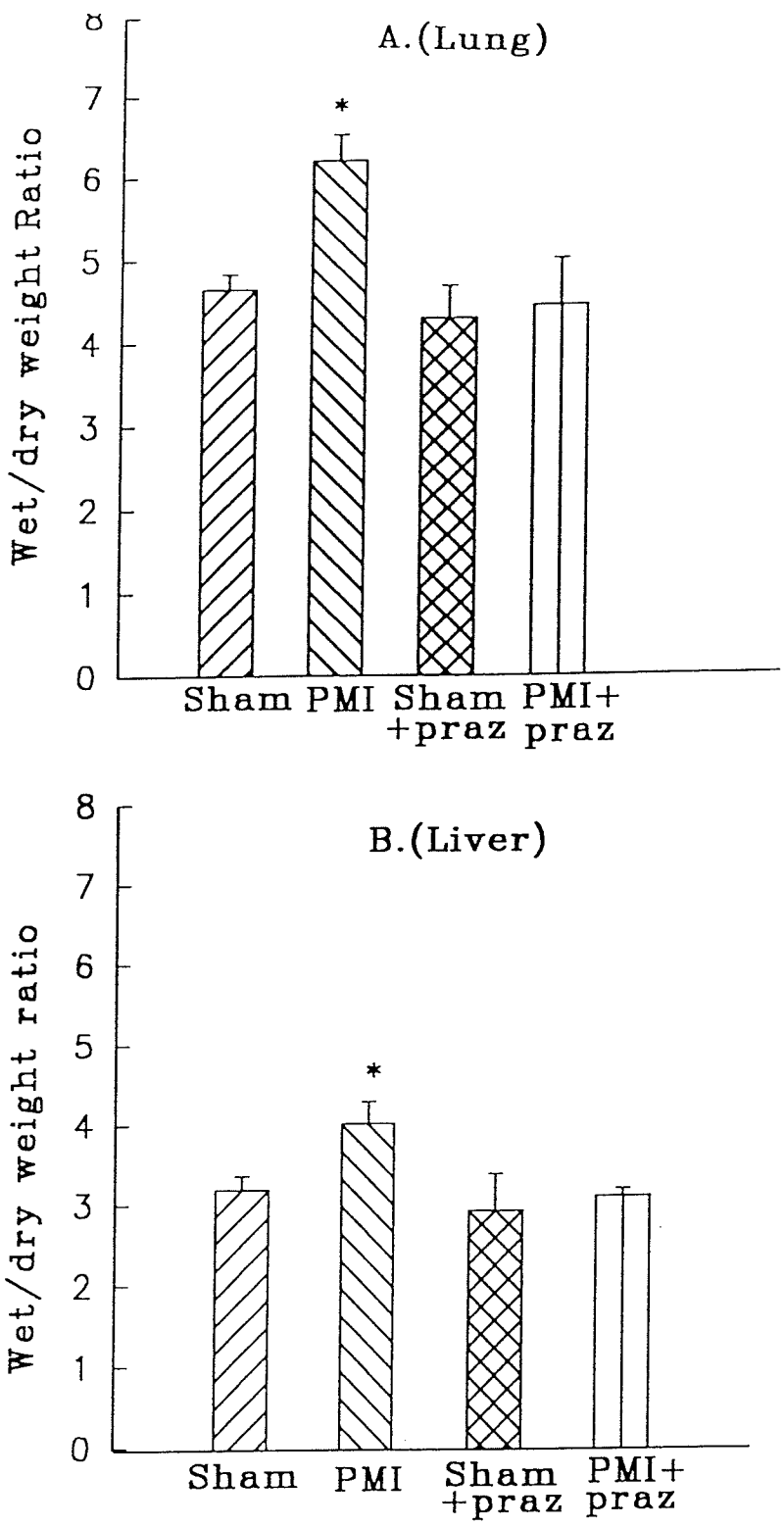

FIG 6A & B: Effect of prazosin on the lung (A) and liver (B) weight ratios at 16-weeks PMI. Values are mean ± S.E. of 10-12 animals. *) Significantly different ($P<0.05$) from all other groups.

Table 3: Ventricular pressures of rats at 1, 4 and 16 weeks post-surgery durations as compared to sham.

Post-surgical durations	LVEDP (mmHg)		LVPSP (mmHg)	
	SHAM	PMI	SHAM	PMI
1 Week	2.0 ± 0.28	2.2 ± 1.4	125.7 ± 6.7	130.7 ± 5.9
4 Weeks	2.6 ± 0.63	6.5 ± 0.9†	124.5 ± 8.3	109.5 ± 1.7
16 Weeks	3.3 ± 0.60	27.5 ± 1.2*	128.7 ± 4.6	88.6 ± 2.8*

Values are mean ± S.E. of 6-8 animals. LVEDP - left ventricular end-diastolic pressure. LVPSP - left ventricular peak systolic pressure. †) Significantly different ($P<0.05$) from respective sham control group by using ANOVA and *) ANOVA followed by Bonferroni test.

Table 4: Ventricular pressures in rats at 16 weeks post-surgery duration, with and without captopril and prazosin treatment.

Treatment	LVEDP (mmHg)		LVPSP (mmHg)	
	SHAM	PMI	SHAM	PMI
Untreated	3.3 ± 0.6	27.5 ± 1.2*	128.7 ± 4.6	88.6 ± 2.8*
Captopril	3.2 ± 0.4	10.8 ± 0.2†ø	129.3 ± 6.2	103.2 ± 4.7†ø
Prazosin	2.8 ± 0.5	8.2 ± 1.6†ø	125.2 ± 6.2	115.6 ± 4.6ø

Values are mean ± S.E. of 10-12 animals. LVEDP - left ventricular end diastolic pressure. LVPSP - left ventricular peak systolic pressure. †) Significantly different ($P<0.05$) from respective control group by using ANOVA and *) ANOVA followed by Bonferroni test. ø) Significantly different ($P<0.05$) from untreated PMI group.

Table 5: Aortic pressures of rats at 1, 4 and 16 weeks post-surgery durations.

Post-surgical durations	ADP (mmHg)		ASP (mmHg)	
	SHAM	PMI	SHAM	PMI
1 Week	63.6 ± 3.17	65.2 ± 1.7	114.4 ± 2.1	101.4 ± 2.2
4 Weeks	61.8 ± 2.0	72.3 ± 1.3†	106.6 ± 3.2	97.5 ± 4.5
16 Weeks	63.7 ± 4.1	75.3 ± 1.1†	102.8 ± 3.7	93.6 ± 3.9†

Values are mean ± S.E. of 6-8 animals. ADP - aortic diastolic pressure. ASP - aortic systolic pressure. †) Significantly different P(<0.05) from their respective sham controls (ANOVA).

PMI animals with respect to their control values were not noticed in animals treated with either captopril or prazosin (Table 6).

F. Antioxidant Enzymes:

Myocardial activities of endogenous antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT) were examined at 1, 4 and 16 weeks of post-myocardial infarction in all groups and these data are shown in Table 7. SOD activity was unchanged at 1 week in the PMI group as compared to sham control. There was about 19% and 45% decrease in the SOD activity in the 4 and 16 week PMI groups respectively compared to their sham controls and these changes were statistically significant. GSHPx activity at 1 week was unchanged or slightly higher in 1 week PMI group. However at 4 and 16 weeks, this enzyme activity was depressed by 30% and 37% respectively relative to their control values. A similar trend was seen with respect to CAT activity. At 1 week there was no change in catalase activity in the PMI group followed by a significant decrease at 4 and 16 weeks by about 27% and 40% respectively, as compared to their control values.

Treatment with captopril resulted in improvement in SOD activity, compared to nontreated PMI group (Table 8). Prazosin treatment also significantly improved the SOD activity in the PMI group. GSHPx activity in captopril treated infarcted group was higher compared to 16-week untreated PMI group. However in prazosin treated PMI group, the activity remained unchanged compared to the untreated PMI group (Table 8). Catalase activity was significantly higher in captopril group compared to the untreated PMI group. This enzyme activity in the prazosin treated group did not change much, compared to untreated PMI group (Table 8).

Table 6: Aortic pressures of rats at 16 weeks post-surgery durations, with and without captopril and prazosin treatments.

Treatment	ADP (mmHg)		ASP (mmHg)	
	SHAM	PMI	SHAM	PMI
Untreated	63.7 ± 4.1	75.3 ± 1.1†	102.8 ± 3.7	93.6 ± 3.9†
Captopril	65.6 ± 2.8	69.2 ± 1.8	105.7 ± 2.3	100.2 ± 3.9
Prazosin	62.4 ± 2.1	64.8 ± 3.8	120.1 ± 7.6	109.2 ± 9.1

Values are mean ± S.E. of 6-8 animals. ADP - aortic diastolic pressure. ASP - aortic systolic pressure. †) Significantly different ($P<0.05$) from respective control group by using ANOVA.

Table 7: Myocardial endogenous antioxidant enzyme activities in hearts of 1, 4 and 16 week sham and PMI rats.

Post-surgical durations	SOD (U/mg protein)		GSHPx (nmoles/mg protein)		CAT (U/mg protein)	
	SHAM	PMI	SHAM	PMI	SHAM	PMI
1 Week	35.2 ± 3.1	38.1 ± 2.7	86.4 ± 1.9	96.6 ± 7.1	26.9 ± 1.7	29.4 ± 2.8
4 Weeks	34.8 ± 5.1	28.2 ± 4.2†	84.6 ± 1.3	60.3 ± 1.9†	30.8 ± 2.6	22.3 ± 1.7†
16 Weeks	33.1 ± 5.3	18.1 ± 4.7*	78.6 ± 2.8	49.5 ± 3.6†	29.7 ± 2.3	18.2 ± 3.4†

Data are mean ± S.E. of 6-8 experiments. †) Significantly different ($P<0.05$) from sham control by using ANOVA; *) ANOVA followed by Bonferroni test; SOD - superoxide dismutase; GSHPx - glutathione peroxidase; CAT - catalase.

Table 8: Effects of captopril and prazosin treatment on myocardial endogenous antioxidant enzyme activities at 16 weeks PMI.

Treatment	SOD (U/mg protein)		GSHPx (nmoles/mg protein)		CAT (U/mg protein)	
	SHAM	PMI	SHAM	PMI	SHAM	PMI
Untreated	33.1 ± 5.3	18.1 ± 4.7*	78.6 ± 2.8	49.5 ± 3.6†	29.7 ± 2.3	18.2 ± 3.4†
Captopril	35.1 ± 2.7	24.2 ± 10.5†	76.1 ± 6.7	69.2 ± 0.5ø	32.3 ± 3.8	38.5 ± 2.5ø
Prazosin	35.2 ± 10.3	29.2 ± 7.6ø	82.9 ± 2.5	46.3 ± 4.7	29.1 ± 1.9	22.4 ± 1.7†

Data are mean of 6-8 experiments. †) Significantly different ($P<0.05$) from the respective sham control by using ANOVA; *) ANOVA followed by Bonferroni test. ø) Significantly different ($P<0.05$) from the untreated PMI group. SOD - superoxide dismutase; GSHPx - glutathione peroxidase; CAT - catalase.

G. Lipid Peroxidation:

Lipid peroxidation, which is an index of oxidative stress, was assessed in 1-, 4- and 16-week sham and PMI groups by evaluating myocardial thiobarbituric acid reacting substances (TBARS) and these data are shown in Table 9. TBARS in 1 week PMI remained unchanged compared to its respective control. TBARS in the 4- and 16-week PMI groups increased by 12% and 48% respectively as compared to their sham controls. The increase in 16-week PMI group was statistically significant (Table 9).

TBARS in sham controls treated with captopril or prazosin remained unchanged compared to the untreated group (Figs 7 and 8). However in the captopril treated group, TBARS were significantly lower as compared to its respective untreated PMI group (Fig 7). TBARS in the prazosin treated PMI group was also lower than the untreated PMI group (Fig 8).

Table 9: Myocardial lipid peroxidation (TBARS) at 1, 4 and 16 weeks post-surgery durations.

Post-surgery Duration	TBARS (nmoles/g wet wt)	
	SHAM	PMI
1 Week	74.7 ± 5.3	72.1 ± 0.8
4 Weeks	70.2 ± 2.9	78.1 ± 4.5
16 Weeks	71.1 ± 3.1	104.1 ± 7.5†

Data are mean of 6-8 experiments. †) Significantly different ($P<0.05$) from respective sham control (ANOVA); TBARS - thiobarbituric acid reactive substances.

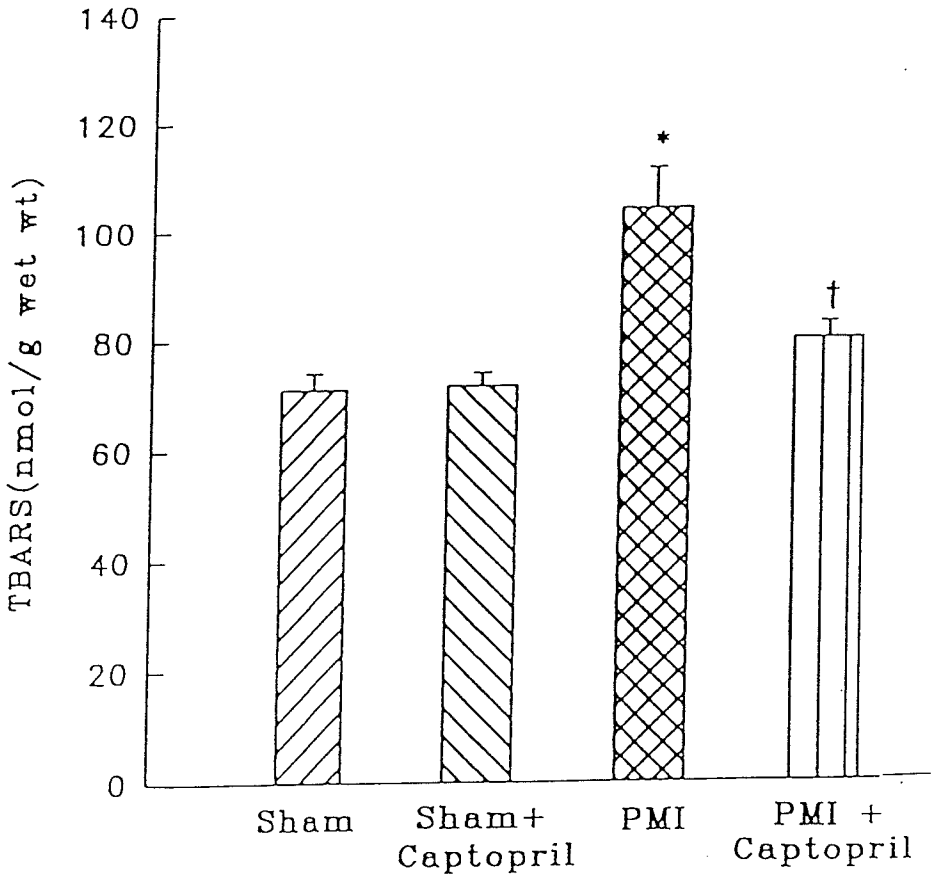

FIG 7: Effects of captopril on myocardial lipid peroxidation at 16 weeks post-surgical duration. Values are mean ± S.E. of 6-8 animals. *) Significantly different ($P<0.05$) from all the groups; †) Significantly different ($P<0.05$) from 16 week PMI group.

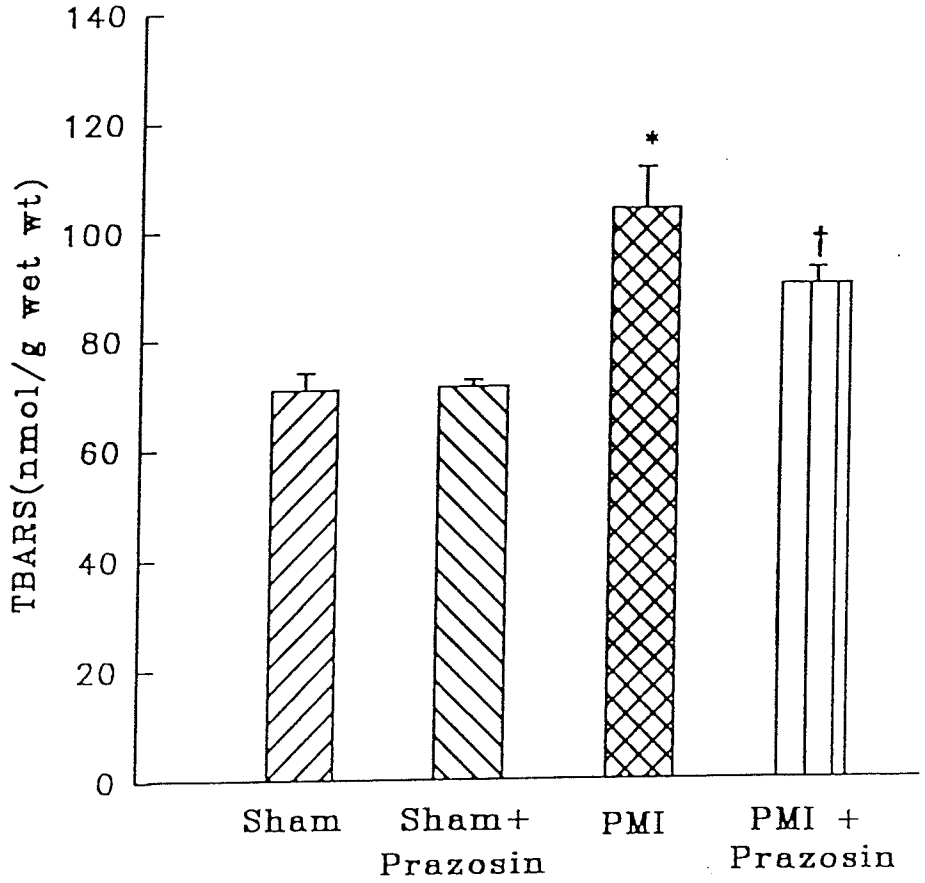

FIG 8: Effects of prazosin on myocardial lipid peroxidation at 16 weeks PMI. Values are mean ± S.E. of 6-8 animals. *) Significantly different ($P<0.05$) from all other groups (ANOVA followed by Bonferroni test). †)

VI. DISCUSSION

Despite major advances in the management strategies of this clinical condition, heart failure subsequent to myocardial infarction remains a major health problem . It is generally characterized by depressed cardiac function with associated abnormalities in the neuroendocrine and different metabolic systems. One of the major humoral changes is the activation of the renin-angiotensin system (RAS) which is suggested to play an important role in the pathogenesis of congestive heart failure (Francis et al., 1984; McAlpine et al., 1988). In addition to these mechanisms, deficit in the myocardial antioxidant system is also suggested to be an important factor that may be contributing in the development of heart failure (Hill and Singal, 1995; Gupta and Singal, 1989a; Dhalla and Singal, 1994). In fact, role of oxidative stress in the pathogenesis of heart failure in humans has also been demonstrated in some studies (McMurray et al., 1990). Present study demonstrates for the first time that improved hemodynamic function subsequent to afterload reduction therapy in MI rats was associated with an increase in the antioxidants in the heart. The study supports the hypothesis that antioxidant deficit may have a major role in the pathogenesis of heart failure subsequent to MI.

We employed the coronary artery ligation procedure to develop heart failure in rats. This method was originally introduced by Johns and Olson (1954) later modified by Selye et al. (1960). In this model, different degrees of failure are achieved at different post-myocardial infarction (PMI) durations. We studied changes in myocardial antioxidants and oxidative stress in hemodynamically assessed animals at different stages of the PMI (1, 4 and 16 weeks) with and without afterload reduction therapy using captopril and prazosin.

In untreated animals at 1 week PMI, there was no change in left ventricular peak systolic (LVPSP), left ventricular end diastolic (LVEDP), aortic systolic (ASP) and aortic diastolic (ADP) pressures. Signs of lung and liver congestion were also not apparent indicating that this is "prefailure" stage. However, at 4 weeks PMI, LVEDP was elevated, whereas LVPSP was slightly depressed, but the change was not significant. Pulmonary edema and liver congestion were also absent in this experimental group. Thus, it was considered as a mild failure and a functionally compensated stage. At 16 weeks PMI, LVEDP rose along with a significant depression in LVPSP as well as ASP. Lung and liver wet to dry weight ratios were significantly higher. An increase in LVEDP may have caused the retrograde circulatory stasis resulting in an accumulation of fluid in the lung and liver. All these features suggested that these animals were in a severe failure stage. The increase in LVEDP, congestion of lung and liver seen in our study at 16 weeks have also been previously reported by others in this animal model (Pfeffer et al., 1984; Dixon et al., 1990; Hill and Singal, 1995). Thus prefailure stage at 1 week, mild failure stage at 4 weeks and a severe failure stage at 16 weeks were reproduced. It is pointed out that this classification is arbitrary and is adopted for a comprehensive description of the state of the experimental animals, in order to establish a relationship among these and other parameters.

There are still differing opinions about the changes in scar weight and percent left ventricular area it occupies during the post-infarction period. In a dog model, infarct expansion, thinning and ventricular enlargement has been reported to occur between 2 days and 6 weeks after the coronary ligation (Jugdutt, 1995). In previous studies on dogs, infarcted tissue was reported to occupy 20% of the left ventricle on day 1 and 10% at 6

weeks (Jugdutt and Khan, 1994; Jugdutt and Amy, 1986). However, under chronic conditions left ventricle dilation may also contribute to the percent reduction in scar area reported in several studies (Jugdutt and Amy, 1986; Jugdutt and Khan, 1994). In patients, infarct expansion/extension has also been reported (Hutchins and Bulkley, 1978; Eaton et al., 1979; Shuster and Bulkley, 1979), and has been associated with increased mortality. It may also be important in the formation of aneurism (McKay et al., 1986). These infarct expansions may in fact be very early or acute changes. In a rat model of myocardial infarction, the scar weight was reported to remain unchanged (Dixon, et al., 1990).

In our study, scar size was assessed indirectly by weighing the ventricles including the scar and after removal of the scar. It is pointed out that this approach of obtaining the scar tissue weight as well as using it as a measure of the scar size may not be very precise. Nevertheless, the scar tissue weight in our study was significantly reduced in both captopril and prazosin group, which may partly explain the reduced mortality and improved prognosis with captopril (Pfeffer et al., 1985a,b). The ventricle to body weight ratio was increased from 1 to 16 weeks. Increase in this ratio in animals treated with captopril was less than the untreated group. Pfeffer and associates (1985a) also have reported decrease in this ratio in the captopril treated animals. ACE inhibition using captopril was shown to decrease infarct expansion and thinning, progressive ventricle dilation and infarct collagen levels (Jugdutt, 1995). Thus drug-induced reduction in scar expansion as well as ventricle to body weight ratio in MI rats clearly indicate a moderation of the process of myocardium remodelling.

The beneficial effects of captopril have been studied extensively both in animals and humans. Our data on animals in the captopril group demonstrated not only improved hemodynamic function at 16 weeks PMI but any signs of lung and liver congestion were also absent in these animals. Pfeffer and associates (1985a) showed that a prolonged treatment with captopril resulted in normalization of hemodynamic parameters in the infarcted groups. We also noted that the increase seen in LVEDP in the 16 week infarcted group was less in the captopril group. The volume reducing effect of captopril probably is due to its preload and afterload reducing properties, leading to a decrease in the load, opposing shortening and thereby permitting the ejection of a normal stroke volume from a less dilated ventricle (Pfeffer et al., 1985a; Pfeffer et al., 1988). In "SAVE" trial in humans with left ventricular dysfunction, captopril was reported to improve survival and reduce morbidity and mortality (Pfeffer et al. 1982). In another study by Sharpe et al., (1988) in patient with symptom-less left ventricular dysfunction after myocardial infarction, treatment with captopril attenuated ventricular enlargement and prevented further deterioration of ventricular performance.

Myocardial antioxidant enzymes including SOD, GSHPx and CAT have been reported to change in various physiological and pathophysiological conditions (Gupta and Singal, 1989a; Kanter et al., 1985; Rao et al. 1983; Dhalla and Singal, 1994). These antioxidants have also been reported to change under various oxidative stress conditions (Dhaliwal et al., 1991, Ferrari et al., 1985). Based on all these observations, it has been suggested that "antioxidant status of the heart is a dynamic function adjusting to the physiological and/or pathophysiological conditions imposed" (Gupta and Singal, 1989a).

In this model of heart failure, characteristic changes in superoxide dismutase, glutathione peroxidase and catalase were seen at different PMI durations. At 1 week, no change or a slight increase in antioxidants was observed. At 4 weeks, there was significant depression in SOD, GSHPx and CAT activities and at 16 weeks the decrease in these antioxidant enzymes was even more drastic. Antioxidant decrease in heart failure has been documented before (Hill and Singal, 1995). Lipid peroxidation was higher at 4 and 16 weeks. Increase in lipid peroxidation in heart failure due to pressure overload (Dhalla and Singal, 1994), subsequent to myocardial infarction (Hill and Singal, 1995), and in case of cardiomyopathic hamsters (Kobayashi et al. 1987) has also been reported. Increased lipid peroxidation measured by breath pentane content is known to occur in heart failure patients (Weitz et al., 1991; Sobotka et al., 1993). The increase in oxidative stress in the failing stages may be conducive to causing oxidant-induced cell damage by lipid peroxidation and by oxidation of protein thiol groups (Ferrari et al., 1991). At any rate, the study demonstrates that decrease in antioxidants and increase in lipid peroxidation at different PMI durations correlate with poor cardiac function or heart failure.

Since captopril and prazosin have been shown to reduce the symptoms of congestive heart failure as well as improve functional capacity of the heart (Pfeffer et al., 1992; Cohn et al., 1986; Feldman et al., 1981), the other objective of the present study was to study the effects of these two vasodilatory drugs on the myocardial antioxidants and lipid peroxidation. Because the early signs of heart failure were evident at 4 weeks, the vasodilatory therapy was started at 4 weeks PMI duration. The present study demonstrated for the first time that chronic treatment with captopril, not only improved the hemodynamic function, but also

resulted in better maintenance of the antioxidant enzymes as compared to the untreated group. As a result, oxidative stress in the captopril and prazosin treated groups was significantly less compared to the untreated group. This was also evidenced by a decrease in myocardial lipid peroxidation in the drug-treated groups. Thus our data as well as the findings reported by others suggest that a relative deficit in antioxidants and an increase in oxidative stress may play an important role in the pathogenesis of heart failure.

Because of the other humoral and tissue level effects of ACE and angiotensin II, the exact mechanism by which ACE inhibition with captopril treatment improves left ventricular function is not yet defined. In this regard, the presence of sulphydryl group in captopril can also act as a free radical scavenger (Chopra et al., 1989; Sobotka et al. 1993). Furthermore, inhibition of ACE results in an increase in bradykinin, nitric oxide (Palmer et al., 1987), prostaglandin E_2 and prostacyclin (Needleman et al., 1975) thereby reducing the afterload albeit using a different mechanism.

In addition to peripheral effects of ACE inhibition by captopril, direct effect on the coronary circulation (Daly et al., 1985; Margrini et al., 1987) as well as the myocardial tissue effects (Dzau and Re, 1987; Dzau, 1987) have also been reported. In this regard, increases in the ventricular angiotensinogen mRNA levels in the viable myocardium subsequent to MI have been reported (Lindpainter et al., 1993). Cardiac ACE mRNA levels in experimental heart failure were also found to be increased (Hirsch et al., 1991). Angiotensinogen gene expression in the left ventricle was shown to be suppressed by ACE inhibition (Dzau, 1988). These data suggest that angiotensin II in heart failure subsequent to MI influences expression of different components of RAS at the tissue level. Thus

ventricular remodelling in MI may involve distension of the scar tissue as well as altered gene expression in the viable myocardium. These changes could be due to both increased wall stress subsequent to the increased afterload and direct effects of angiotensin II on cardiac myocytes.

Effects of ACE, other than modulating angiotensin II levels, can be better characterized by using agents or antagonists that can specifically block angiotensin II receptor. Losartan, a specific angiotensin II receptor antagonist, has been shown to cause favourable vasodilation effect evident from decreased mean arterial pressure and systemic vascular resistance in heart failure patients (Dickstein et al., 1994). These hemodynamic changes were comparable with ACE inhibitors (Burnier et al., 1994). However, the decrease in oxidative stress reported in our study suggests that the free radical scavenging effect of captopril may also be important. Captopril has been shown to have a beneficial effect in ischemia and reperfusion injury which is known to involve free radical mechanisms (Daly et al., 1985). Recent study by Sobotka et al. (1993) showed that in patients with congestive heart failure, the breath pentane content was high. But with captopril therapy in these patients, there was reduced breath pentane. Captopril has also been shown to protect against free radical mediated injury in isolated working rat hearts (Pi and Chen, 1989; Westlin and Mullane, 1988). It is possible that some component of protection may come from a direct antioxidant effect on the drug.

Use of prazosin in the treatment of heart failure patients have shown that this drug produces sustained reduction in both preload and impedance with a resultant increase in cardiac output. There was also a reduced clinical evidence of pulmonary venous congestion

and enhanced exercise tolerance (Awan et al., 1977, 1978; Goldman et al.,1980; Colucci et al., 1980). However, unlike captopril, prazosin does not improve survival in heart failure patients (Cohn et al., 1986). In order to delineate the free radical scavenging effect of captopril, another vasodilator, prazosin, with no known antioxidant property, was used for comparison. In our study, like captopril, prazosin treatment was also started at 4 weeks PMI duration and continued until 16 week PMI. The scar tissue weight in the prazosin group was significantly less compared to the untreated group but was slightly higher than the captopril group. The degree of lung and liver congestion was significantly reduced in the prazosin treated group. Similar findings using prazosin have been reported earlier by others (Awan et al., 1977, 1978). The increase in LVEDP seen at severe failure stage was significantly reduced in the prazosin group. LVPSP and ASP were maintained near control levels. These data clearly indicate that the peripheral vasodilatory effects , thus wall stress, may also play a dominant role in these beneficial effects.

In terms of the antioxidant enzymes, prazosin treatment did not cause any change in GSHPx and catalase activities as compared to the untreated group. However, SOD activity was found to be improved in the prazosin group. TBARS were also found to be significantly less in the prazosin treated PMI group compared to the untreated group. These data suggest that prazosin offered beneficial hemodynamic effects and reduced the signs of lung and liver congestion which were accompanied by a reduction in oxidative stress as indicated by a reduction in lipid peroxidation.

Since protection was seen with both drugs with a common feature of vasodilation, it is likely that the prime beneficial effect may be due to an afterload reduction or reduction in wall stress. Captopril was found to be more effective and this may have to do with the

added antioxidant effect of this drug. Thus our study demonstrated for the first time that a decrease in afterload reduction was not only accompanied by improvement in the hemodynamics but also by an increase in the antioxidants and a decrease in oxidative stress. An understanding of the molecular basis of these antioxidant changes may lead to the use of early and targeted gene therapy which can play an important role in preventing heart failure.

VII. REFERENCES

Alpert NR, Hamrell BB. Cardiac hypertrophy: A compensatory and anti-compensatory response to stress. In: Cardiac Physiology for the Clinician. Vassale M (ed.), New York, Academic Press, pp 173-201, 1976.

Alpert NR, Mulieri LA. Increased myothermal economy of isometric force generation in compensated cardiac hypertrophy induced by pulmonary artery constriction in the rabbit. Circ Res. 50: 491-500, 1982.

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. 89: 618-629, 1992.

Aust SD. Lipid peroxidation. CRC Handbook of Methods for Oxygen Radical Research. CRC Press. Boca Raton, Florida, 203-207, 1985.

Awan NA, Miller RR, DeMaria AN et al. Efficacy of ambulatory systemic vasodilator therapy with oral prazosin in chronic refractory heart failure. Concomitant relief of pulmonary congestion and elevation of pump output demonstrated by improvements in symptamatology, exericise tolerance, hemodynamics and echocardiography. Circulation. 56(3): 346-354, 1977.

Awan NA, Miller RR, Miller MP. Clinical pharmacology and therapeutic application of prazosin in acute and chronic refractory congestive heart failure. Balanced systemic venous and arterial dilation improving pulmonary congestion and cardiac output. Am J Med. 6: 146-149, 1978.

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

Bertel O, Burkart F, Buhler FR. Sustained effectiveness of chronic prazosin therapy in severe chronic congestive heart failure. Am Heart J. 101: 529-533, 1981.

Bing RJ. The biochemical basis of myocardial failure. Hosp Pract Off Ed. 18(9): 93-7, 101-7, 110-2, 1983.

Bittl JA, Ingwall JS. Intracellular high energy phosphate transfer in normal and hypertrophied myocardium. Circulation. 75(Supp I): I96-I101, 1987.

Braunwald E, Mock MB, Watson J. Congestive heart failure. In: Current Research and Clinical Applications. Grune and Stratton (eds.), Orlando, Florida, pp 132-139, 1982.

Braunwald E, Colucci WS. Vasodilator therapy of heart failure: Has the promissory note been paid? New Eng J Med. 310: 459-461, 1984.

Braunwald E. ACE inhibitors: a cornerstone of the treatment of heart failure. N Eng J Med. 325: 351-353, 1991.

Braunwald E, Grossman W. Clinical aspects of heart failure. In: Heart Disease: A Textbook of Cardiovascular medicine. Braunwald E (ed.), Philadelphia, PA, W.B. Saunders Company, Vol 1: 444-463, 1992.

Bristow MR, Ginsburg R, Minobe WA, Cubicciotti RS, SAGeman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenergic receptor density in failing human hearts. N Engl J Med. 307: 205-211, 1982.

Bristow MR, Minobe W, Rasmussen R, Hershberger RE, Hoffman BB. Alpha-1 adrenergic receptors in the nonfailing and failing heart. J Pharmacol Exp Ther. 247: 1039-1045, 1988.

Brown JJ, Davies DL, Johnson VW, Lever AF, Robertson JS. Renin relationships in congestive cardiac failure treated and untreated. Am Heart J. 80: 329-342, 1970.

Burnier M, Waeber B, Brunner HR. The advantages of angiotensin II antagonism. J Hypertens. 12(2): S7-15, 1994.

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

Cannon PJ, Powers Er, Reison DS. A placebo-controlled trial of captropril in refractory chronic congestive heart failure. JACC. 2(4): 755-763, 1983.

Captopril Digoxin Multicenter Research Group. Comparative effects of therapy with captopril and digoxin in patients with mild moderate heart failure. JAMA. 259: 539-544, 1988.

Captopril Multicenter Research Group. A placebo controlled trial of captopril in refractory chronic congestive heart failure. J Am Coll Cardiol. 2: 755-801, 1983.

Carafoli E, Bing RJ. Myocardial failure. J Appl Cardiol. 3: 3-18, 1988.

Chatterjee K, Parmley WW, Ganz W, Forrester J, Walkinsky P, Crenells C, Swan HJC. Hemodynamics and metabolic responses to vasodilator therapy in acute myocardial infarction. Circulation. 48: 1183-1187, 1973.

Chatterjee K, Ports TA, Brundage BH, Massie B, Holly AN, Parmley WW. Oral hydralazine in chronic heart failure: sustained beneficial hemodynamic effects. Ann Intern Med. 92: 600, 1980.

Chidsey CA, Koiser GA, Sonnenblick EH, Spann JF, Braunwald E. Cardiac norepinephrine stores in experimental heart failure in the dog. J Clin Invest. 43: 3286-3293, 1964.

Chidsey CA, Weinbach EC, Pool PE, Morrow AG. Biochemical studies of energy production in the failing human heart. J Clin Invest. 45: 40-50, 1966.

Chopra M, Scott N, McMurray J, Mclay J, Bridges A, Smith WE, Belch JJF. Captopril: a free radical scavenger. Br J Clin Pharmac. 27: 396-399,1989.

Clairborne, A. Catalase activity. In: Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (ed). Boca Raton, Florida: CRC Press, 1985, pp 283-284.

Clark IA, Cowden WB, Hunt NH. Free radical-induced pathology. Medicinal Research Reviews. 5(3): 297-332, 1985.

Cohen J, Shah PM. Role of endocrine factors in the pathogenesis of cardiac hypertrophy. Circ Res. 35: 49-57, 1974.

Cohn JN, Mashiro I, Levine TB, Mehta J: Role of vasoconstrictor mechanisms in the control of left ventricular performance of the normal and damaged heart. Am J Cardiol. 44: 1019-1022, 1979.

Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH et al. Effects of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Coopertive Study. N Engl J Med. 314: 1547-1552, 1986.

Cohn PF, Gorlin R, Herman MV, Sonnenblick ED, Horn HR, Cohn GH, Collins JJ. Relation between contractile reserve and prognosis in patients with coronary artery disease and a depressed ejection fraction. Circulation. 51(3): 414-420, 1975.

Colucci WS, Wynee J, Holman BL, Braunwald E. Long term therapy of heart failure with prazosin: a randomized double blind trial. Am J Cardiol. 45: 337-344, 1980.

Covell JW, Chidsey CA, Braunwald E. Reduction in the cardiac response to postganglionic sympathetic nerve stimulation in experimental heart failure. Circ Res. 19: 51-66, 1966.

Cross CE. Oxygen radicals and human disease. Davis Conference. Ann Inter Med. 107: 545-562, 1987.

Crozier I, Ikram H, Awan N, Cleland J, Stephen N, Dickstein K, Frey M. Losartan in heart failure. Hemodynamic effects and tolerability. Losartan Study Group. Circulation. 91: 691-697, 1995.

Curtiss C, Cohn JN, Vrobel T, Franciosa JA. Role of the renin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. Circulation. 58: 763-770, 1978.

Dai J, Meij JA, Padua R, Panagia V. Depression of cardiac sarcolemmal phospholipase activity by oxidant-induced thiol modification. Circ Res. 71: 970-977, 1992.

Daly P, Mettauer B, Rouleau JL, Cousineau D, Burgess JH. Lack of reflex increase in myocardial sympathetic tone after captopril: potential antianginal effect. Circulation. 71: 317-325, 1985.

Danforth WA, Naegle S, Bing RJ. Effect of ischemia and reoxygenation on glycolytic reactions and adenosine triphosphate in heart muscle. Circ Res. 8: 965-971, 1960.

Davies KJA, Quintanilha T, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Comun. 107: 1178-1205, 1982.

Denniss AR, Colucci WS, Allen PD, Marsh JD. Distribution and function of human ventricular beta adrenergic receptors in congestive heart failure. J Mol Cell Cardiol. 21: 651-660, 1989.

Dhaliwal H, Kirshenbaum LA, Randhawa AK , Singal PK. Correlation between antioxidant changes during hypoxia and recovery upon reoxygenation. Am J Physiol. 261: H632-H638, 1991.

Dhalla NS, Das PK, Sharma GM. Subcellular basis of cardiac contractile failure. J Mol Cell Cardiol. 10: 363-385, 1978.

Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE. Calcium movements in relation to heart function. Basic Res Cardiol. 77: 117-139, 1982.

Dhalla NS, Singh JN, McNamara DB, Bernatsky A, Singh A, Harrow JAC. Energy production and utilization in contractile failure due to intracellular calcium overload. In: Myocardial Injury, Spitzer JJ (ed.), New York: Plenum Publishing Corp. pp 305-316, 1983.

Dhalla AK, Singal PK. Antioxidant changes in hypertrophied and failing guinea pig hearts. Am J Physiol. 266 (Heart Circ Physiol 35): H1280-H1285, 1994.

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 Hypert. 12: S31-35, 1994.

Dixon IMC, Lee S-L, Dhalla NS. Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Res. 66: 782-788, 1990.

Dormandy TL, Wickens DG. The experimental and clinical pathology of diene conjugation. Chem Phys Lipids. 45: 353-364, 1987.

Doroshow JH, Locker GY, Myers CE. Enzymatic defenses of the mouse against reactive oxygen metabolites. Alterations produced by doxorubicin. J Clin Invest. 65: 128-135 1980.

Dzau VJ, Colucci WS, Hollenberg NK, Williams GH. Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation. 63: 645-651, 1981.

Dzau VJ. Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am J Cardiol. 59: 59A-65A, 1987.

Dzau VJ, Re RN. Evidence for the existence of renin in the heart. Circulation. 75(Suppl I): 1134-1136, 1987.

Dzau VJ. Cardiac renin-angiotensin system: molecular and functional aspect. Am J Med. 84: 22-27, 1988.

Eaton LW, WEiss JL, Bulkley BH, Garrison JB, Weisfeldt ML. Regional cardiac dilatation after acute myocardial infarction. N Engl J Med. 33: 57, 1979.

Feinstein BB. Effects of experimental congestive heart failure, oubain and asphyxia on the high energy phosphate and creatine content of guinea pig heart. Circ Res. 10: 333-346, 1962.

Feldman RC, Ball RM, Winchester MA, Jailon P, Kates R, Harrison D. Beneficial hemodynamic response to chronic prazosin therapy in congestive heart failure. Am Heart J. 101: 534-540, 1981.

Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, Baumgartner WA, Van Cop C. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest. 82: 189-197, 1988.

Feldman AM, Cates AE, Bristow MR, Van Dop C. Altered expression of alpha-subunits of G proteins in failing human hearts. J Mol Cell Cardiol. 21: 359-365, 1989.

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

Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O. Oxygen free radicals and myocardial damage. Protective role of thiol containing agents. Am J Med. 91: 3C, 955-1055, 1991.

Fox RB, Harada RN, Tate RM, Repine JE. Prevention of thiol-induced pulmonary edema by hydroxyl-radical scavengers. J Appl Physiol. 55: 1456-1459, 1983.

Franciosa JA, Guiha NH, Limas CJ, Rodriquera E, Cohn JN. Improved ventricular function during nitroprusside infusion in acute myocardial infarction. Lancet. 1: 650-656, 1972.

Franciosa JA. Effectiveness of long-term vasodilator administration in the treatment of chronic left ventricular failure. Prog Cardiovasc Disease. 24: 319-330, 1982.

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

Francis GS. Neuroendocrine manifestation of congestive heart failure. Am J Cardiol. 62: 9-13, 1988.

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

Fridovich I. Superoxide dismutases. Ann Rev Biochem. 44: 147-159, 1975.

Fuji H, Kuzuya T, Hoshida S, Yamashita N, De H, Taniguchi N, Tada M. Reoxygenation induced synthesis of mitochondrial manganese-superoxide dismutase in rat neonatal myocardial cells. J Mol Cell Cardiol. 24: S-168, 1992.

Furberg CD, Yusuf S. Effect of vasodilators on survival in chronic congestive heart failure. Am J Cardiol. 55: 1110-1113, 1985.

Genest J, Granger P, de Champlain J, Boucher R. Endocrine factors in congestive heart failure. Am J Cardiol. 22: 35-42, 1968.

Giles TD. Benefits of prolonged angiotensin-converting enzyme inhibition in congestive heart failure. Cardiology. 79(1) 16-21, 1991.

Goldman SA, Johnson LL, Escala E, Cannon PJ, Weiss NB. Improved exercise ejection fraction with long term prazosin therapy in patients with heart failure. Am J Med. 68: 36-42, 1980.

Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in cellular damage induced by reoxygenation of hypoxic heart. J Mol Cell Cardiol. 12: 797-808, 1980.

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

Gupta M, Singal PK. Time course of structure, function and metabolic changes due to an exogenous source of oxygen metabolites in rat heart. Can J Physiol Pharmacol. 267: 1549-1559, 1989b.

Gwathmey JK, Morgan JP. Altered calcium handling in experimental pressure overload hypertrophy in the ferret. Circ Res. 57: 836-843, 1985.

Gwathmey JK, Capelas L, Mackinnon R, Schoen, Feldman MD, Grossman W, Morgan JP. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res. 61: 70-76, 1987.

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

Harigaya S, Schwartz A. Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Circ Res. 25: 781-794, 1971.

Hearse DJ, Bolli R. Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. Cardiovasc Res. 26: 101-108, 1992.

Helfant RH, Pine R, Meister SG, Feldman MS, Trout RG, Banka VS. Nitroglycerin to unmask reversible asynergy: correlation with post coronary bypass ventriculography. Circulation. 50: 108-113, 1974.

Hill M, Singal PK. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. Am. J. Pathol., 1995 (In Press).

Hirsh AT, Talsness CE, Schunkert H, Paul M, Dzau VJ. Tissue-specific activation of angiotensin converting enzyme in experimental heart failure. Circ Res. 69: 475-482, 1991.

Hirzel HO, Tuchschmid CR, Schneider J, Krayenbriehl HP, Schub M. Relationship between myosin isoenzyme composition, hemodynamics and myocardial structure in various forms of human cardiac hypertrophy. Circ Res. 57: 729-740, 1985.

Hjalmarson A, Herlitz J, Malek I, Ryden L, Vedin A, Waldenstrom A, Wedel H, Elmfeld P, Holmberg S, Nyberg G, Swedberg K, Waagstein F, Waldenstrom J, Wilhelmsen L. Effect on mortality of metoprolol in acute myocardial infarction: a double blind randomized trial. Lancet. II: 823-827, 1981.

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

Janero DR, Burghardt B. Cardiac membrane vitamin E and malondialdehyde levels in heart muscle of normotensive and spontaneously-hypertensive rats. Lipids. 24: 33-38, 1989.

Jennings RB, Sommers HM, Smyth GA, et al. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol. 70: 68-78, 1960.

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

Jugdutt BI, Amy RWM. Healing after myocardial infarction in the dog: changes in infarct hydroxyproline and topography. J Am Coll Cardiol. 7: 91-102, 1986.

Jugdutt BI, Humen DP, Khan MI, Schwarz-Manowski BL. Effect of left ventricular unloading with captopril on remodelling and function during healing of anterior transmural myocardial infarction in the dog. Can J Cariol. 8: 151-163, 1992.

Jugdutt BI, Khan MI. Effect of prolonged nitrate therapy on left ventricular remodeling after canine acute myocardial infarction. Circulation. 89: 2297-2307, 1994.

Jugdutt BI. Effect of captopril and enalapril on left ventricular geometry, function and collagen during healing after anterior and inferior myocardial infarction in a dog model. J Am Coll Cardiol. 25: 1718-1725, 1995.

Jugdutt BI, Khan M, Jugdutt SJ, Blinston GE. Combined captopril and isosorbide dinitrate during healing after myocardial infarction. Effect on ventricular remodeling function, mass and collagen. J Am Coll Cardiol. 25(2): 1089-1096, 1995.

Juggi JS, Berard GK, Van Gilst WH. Cardioprotection by angiotensin-converting enzyme (ACE) inhibitors. Can J Cardiology. 9(4): 336-352, 1993.

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

Karliner JS, Barnes P, Brown M, Dollery C. Chronic heart failure in the guinea-pig increases cardiac α-$_1$- and β-adrenergic adrenoceptors. Eur J Pharmacol 67: 115-118, 1980.

Katz AM. Cardiomyopathy of overload: a major determinant of prognosis in congestive heart failure. N Engl Med. 322: 100-110, 1990.

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

Kawaguchi H, Kitabatake K. Renin-angiotensin system in failing heart. J Mol Cell Cardiol. 27: 201-209, 1995.

Khairallah PA, Robertson AL, Davila D. Effects of angiotensin II on DNA, mRNA and protein synthesis. In: Hypertension '72. Genest J, Koin E (eds.), Springer-Verlag, New York, NY, pp 212-220, 1972.

Kirshenbaum JM. Therapy for acute myocardial infarction: an update. Heart Disease and Stroke. 1: 211-217, 1992.

Kirshenbaum LA, Singal PK. Antioxidant changes in heart hypertrophy: significance during hypoxia-reoxygenation injury. Can J Physiol Pharmacol. 70: 1330-1335, 1992.

Kirshenbaum LA, Singal PK. Increase in endogenous antioxidant enzymes protects hearts against reperfusion injury. Am J Physiol. 265(Heart Circ Physiol. 34): H484-H493, 1993.

Kobayashi A, Yamashita T, Kaneko M, Nishiyama T, Hayashi H, Yamazaki N. Effects of verapamil on experimental cardiomyopathy in the bio 14.6 Syrian hamster. J Am Coll Cardiol. 10: 1128-1134, 1987.

Konstam MA, Rousseau MF, Kronenberg MW, Udelson JE, Melin J, Stewart D, Dolan N, Edens TR, Ann S, Kinan D, Howe DM, Kilcoyne L, Metherall J, Benedict C, Yusuf S, Pouleur H, for the SOLVD Investigators. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. Circulation. 86: 431-438, 1992.

Lamas GA, Vaughn DE, Parisi AF, Pfeffer MA. Effects of left ventricular shape and captopril therapy on exercise capacity after anterior wall acute myocardial infarction. Am J Cardiol. 63: 1167-1173, 1989.

Laragh JH. Hormones and the pathogenesis of congestive heart failure: vasopressin aldosterone and angiotensin II. Circulation. 25: 1015-1023, 1962.

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

Lee V, Randhawa AK, Singal PK. Adriamycin-induced myocardial dysfunction in vitro is mediated by free radicals. Am J Physiol. 261: H985-H989, 1991.

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

Lindpainter K, Wenyan Lu, Niedermajer N, Schieffer B, Just H, Ganten D, Drexler H. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. J Mol Cell Cardiol. 25: 133-143, 1993.

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

Margrini F, Shimizu M, Roberts N, Fouad FM, Tarazi RC, Zanchetti A. Converting-enzyme inhibition and coronary blood flow. Circulation. 75(Suppl I): 1168-1174, 1987.

Marklund SL. Extracellular superoxide dismutase in human tissue and human cell lines. J Clin Invest. 74: 1398-1403, 1984.

Marklund SL. Pyrogallol autooxidation. In: Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (ed). Boca Raton, Florida: CRC Press, pp 243-247, 1985.

Maron BJ, Ferrans VJ. Ultrastructure features of hypertrophied human ventricular myocardium. Prog Cardiovasc Dis. 21: 207-238, 1978.

Marshall RC, Tillisch JH, Phelps ME, Huang SC, Carson R, Henze E, Scheller HR. Identification and differentiation of resting myocardial ischemia and infarction in man with positron computed tomography, ^{18}F-labeled fluorodeoxyglucose and N-13 ammonia. Circulation. 67: 766-778, 1983.

Mason DT, Zelis R, Amsterdam EA. Role of the sympathetic nervous system in congestive heart failure. In: Cardiovascular Regulation in Health and Disease, Bortorelli C, Zanchetti A (eds.), Cardiovascular Research Institute, pp159-172, 1971.

McAlpine HM, Morton JJ, Leckie B, Rumley A, Gillen G, Dargie HJ: Neuroendocrine activation after acute myocardial infarction. Br Heart J. 60: 117-124, 1988.

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 coronary to infarct expansion. Circulation. 74: 693-702, 1986.

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. 65: 1261-1262, 1990.

Meerson FZ, Zaletayeva TA, Lagutchev SS, Pshennikova MG. Structure and mass of mitochondria in the process of compensatory hyperfunction and hypertrophy of the heart. Exp Cell Res. 568-578, 1964.

Meerson FZ. Transformation of energy in the myocardium in hyperfunction, hypertrophy and heart failure. Circ Res. 24-25(Suppl 3): II55-II81, 1969.

Meij JTA, Suzuki S, Panagia V, Dhalla NS. Oxidative stress modifies the activity of cardiac sarcolemmal phosphase C. Biochim Biophys Acta. 1199: 6-12, 1994.

Mercadier JJ, Bouveret P, Gorza L, Schiaffion C, Clark WA, Zak R, Swynghedauw B, Schwartz K. Myosin isoenzymes in normal and hypertrophied ventricular myocardium. Circ Res. 53: 52-62, 1981.

Merrill AJ, Morrison JL, Brannon ES. Concentration of renin in renal venous blood in patients with chronic heart failure. Am J Med. 1: 468-472, 1946.

Miller RR, Awan NA, Maxwell KS, Mason DT. Sustained reduction of cardiac impedence and preload in congestive heart failure with the antihypertensive vasodilator prazosin. N Eng J Med. 297: 303-307, 1977.

Miller RR, Fennell WH, Young JB, Palomo AR, Quinones MA. Differential systemic arterial and venous actions and consequent cardiac effects of vasodilator drugs. Prog Cardiovasc Disease. 24: 353-374, 1982.

Needleman P, Marshall GR, Sobel BE. Hormone interactions in the isolated rabbit heart: synthesis and coronary vasomotor effects of prostaglandins, angiotensin and bradykinin. Circ Res. 37: 802-808, 1975.

Newman WH. Biochemical structural and mechanical defects of the failing myocardium. Pharmac Ther. 22: 215-247, 1983.

Oldroyd KG, Pye MP, Ray SG, Christie J, Ford I, Cobbe SM, Dargie HJ. Effects of early captopril administration on infarct expansion, left ventricular remodeling and exercise capacity after acute myocardial infarction. Am J Cardiol. 68: 713-718, 1991.

Packer M, Meller J, Gorlin R, Herman MV. Hemodynamic and clinical tachyphylaxis to prazosin-mediated afterload reduction in severe congestive heart failure. Circulation. 59: 531-539, 1979a.

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

Packer M, Le Jemtel TH. Physiologic and pharmacologic determinants of vasodilator response: a conceptual framework for rational drug therapy for chronic heart failure. Prog in Cardiovasc Disease. 24: 275-292, 1982.

Packer M. Vasodilators and inotropic therapy for congestive heart failure: passion and skepticism. JACC. 2: 841-852, 1983.

Packer M, Gheorghiade M, Young JB, Constantini PJ, Adams KF, Cody RJ. Withdrawal of digoxin from patients with chronic heart failure treated with angiotensin-converting enzyme inhibitors. N Eng J Med. 329: 1-7, 1993.

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

Pagani ED, Alousi AA, Grant AM, Older TM, Dziuban SW Jr, Allen PD. Changes in myofibrillar content and Mg-ATPase activity in ventricular tissues from patients withheart failure caused by coronary artery disease, cardiomyopathy, or mitral valve insufficiency. Circ Res. 63: 380-385, 1988.

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

Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature. 327: 524-536, 1987.

Pfeffer JM, Pfeffer MA, Mirsky I, Braunwald E. Regression of left ventricular hypertrophy and prevention of ventricular dysfunction by captopril in the spontaneously hypertensive rat. Proc Natl Acad Sci USA. 79: 3310-3314, 1982.

Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Ventricular performance in rats with myocardial infarction and failure. Am J Med. 76: 99-103, 1984.

Pfeffer JM, Pfeffer MA, Braunwald E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res. 57: 84-95, 1985a.

Pfeffer MA, Pfeffer JM, Steinberg C, Jinn P. Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. Circulation. 72: 406-412, 1985b.

Pfeffer MA, Gervasio L, Vaughan De, Pavasi AF, Braunwald E. Effect of captopril on progressin ventricular dilation after anterior myocardial infarction. N Eng J Med. 319: 80-86, 1988.

Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ Jr., Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, Klein M, Lamas GA, Packer M, Rouleau J, Rouleau JL,

Rutherford J, Wertheimer JH, Hawkins CM, on behalf of the SAVE Investigators. Effect of captopril on mortality and morbidity in patients with left ventricular dyfunction after myocardial infarction: results of the survival and ventricular enlargment trial. N Engl J Med. 327: 669-677, 1992.

Pfeffer MA. Angiotensin converting enzyme inhibition in congestive heart failure: benefit and perspective. Am Heart Journal. 126(3): 789-793, 1993.

Pfeffer MA, Pfeffer JM, Lamas GA. Development and prevention of congestive heart failure following myocardial infarction. Circulation. 87 (Supp IV): IV-120-IV-125, 1993.

Pfeffer JM, Fischer TA, Pfeffer MA. Angiotensin-converting enzyme inhibition and ventricular remodeling after myocardial infarction. Annu Rev Physiol. 57: 805-826, 1995

Pi X-J, Chen X. Captopril and ramipril protect against free radical injury in isolated working rat hearts. J Mol Cell Cardiol. 21: 1261-1271, 1989.

Rahimtoola SH. A perspective on the three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina. Circulation. 72 (Suppl V): V-123-125, 1985.

Randhawa AK, Singal PK. Pressure overload-induced cardiac hypertrophy with and without dilation. J Am Coll Cardiol. 20: 1569-1575, 1992.

Rao PS, Cohen MV, Mueller S. Production of free radicals and lipid peroxidation in early experimental ischemia. J Mol Cell Cardiol. 15: 713--716, 1983.

Rentrop P, Blanke H, Karsch KR, Kaiser H, Kostering H, Leitz K. Selective intracoronary thrombolysis in acute myocardial infarction and unstable angina pectoris. Circulation. 63: 307-317, 1981.

Riegger GAJ, Liebau G, Halzschuh M, Witkowski D, Steilner H, Kochsiek K. Role of the renin-angiotensin system in the development of congestive heart failure in the dog assessed by chronic converting-enzyme blockade. Am J Cardiol. 53: 614-618, 1984.

Riegger GA: ACE inhibitors in early stages of heart failure. Circulation. 87 (Supp IV): IV-117-IV-119, 1993.

Romankiewicz JA, Brogden RN, Heel Rc, Speight TM, Avery GS. Captopril: an update review of its pharmacological properties and therapeutic efficacy in congestive heart failure. Drugs. 25: 6-40, 1983.

Ross D, Norbeck N, Moldens P. The generation and subsequent fate of glutathionyl radicals in biological systems. J Biol Chem. 260: 15028-15032, 1985.

Schirger A, Sheps SG. Prazosin -- new hypertensive agent. JAMA. 237: 989-991, 1977.

Schraufstatter IU, Hinshaw DB, Hyslop RPA, Spragg RG, Cochrane CG. Oxidant injury of cell. DNA strand breaks activate polyadenosine disphosphate-ribose polymerse and lead to depletion of nicotinamide adenine dinucleotide. J Clin Invest. 77: 1312-1316, 1986.

Schwartz A, Lee KS. Study of heart mitochondria and glycolytic metabolism in experimentally induced heart failure. Circ Res. 10: 321-332, 1962.

Schwartz K, de La Bastie D, Bouveret P, Oliviero P, Alonso S, Buckingham ME. α-skeletal muscle actin mRNA's accumulate in hypertrophied adult rat hearts. Circ Res. 59: 551-555, 1986.

Schwartz K, Carrier L, Mercadier JJ, AM, Boheler K. Molecular phenotype of the hypertrophied and failing myocardium. 87: V11-5-V11-10, 1993.

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

Shamberger RJ, Andreone TL, Willis CE. Antioxidants and cancer. IV Initiating activity of melonaldehyde as a carcinogen. J Nath Cancer Inst. 53: 1771-1774, 1974.

Sharpe N, Smith H, Murphy J, Hannan S. Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. Lancet. I: 255-259, 1988.

Sharpe N, Murphy J, Smith H, Hannan S. Preventive treatment of asymptomatic left ventricular dysfunction following myocardial infarction. Eur Heart J. 11: 147-156, 1990.

Sharpe N, Smith H, Murphy J, Greaves S, Hart H, Gamble G. Early prevention of left ventricular dysfunction after myocardial infarction with angiotensin-converting enzyme inhibition. Lancet 337: 872-876, 1991.

Shuster EH, Bulkley BH. Expansion of transmural myocardial infarction: a pathophysiologic factor in cardiac rupture. Circulation. 60: 1532, 1979.

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

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

Singal PK, Pierce GN. Adriamycin stimulates low affinity Ca^{2+}-binding and lipid peroxidation but depresses myocardial function. Am. J. Physiol. 250:H419-H425, 1986.

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

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

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

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

Singal PK, Dhalla AK, Hill M, Thomas TP. Endogenous antioxidant changes in myocardium in response to acute and chronic stress conditions. Mol Cell Biochem. 129: 179-186, 1993.

Singal, P.K., Dhalla, A.K., Singh, N., Hill, M., Thomas, T.P. and Seneviratne, C.K. Antioxidants and the heart. In Pathophysiology of Heart Failure, Eds. N.S. Dhalla, Singal, P.K., Takeda, N. and Beamish, R.E. Kluwer Academic Publishers, Boston, 1995 (In Press).

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

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

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

Sohal RS, Arnold LA, Sohal BH. Age-related changes in antioxidant enzymes and prooxidant generation in tissues of the rat with special reference to parameters in two insect species. Free Rad Biol & Med. 10: 495-500, 1990.

Steward DD. Remarkable tolerance to nitroglycerine. Philiadelphia Polyclinic. 172-184, 1988.

Stewart D, Mason DT, Wikman-Coffelt J. Changes in cAMP concentration during chronic hypertrophy. Basic Res Cardiol. 73: 648-655, 1978.

Swedberg H, Hjalmarson A, Waagstein F, Wallentin I. Beneficial effects of long term beta-blockade in congestive cardiomyopathy. Br Heart J. 44: 117-133, 1980.

Sweet CS, Rucinska EJ. Losartan in heart failure: preclinical experiences and initial clinical outcomes. Eur Heart J. 15(Suppl D): 139-144, 1994.

Swynghedauw B, Moalic TM, Tecarpentier Y, Rey A, Mercadier J, Aumont MC, Schwartz K. Adaptational change of contractile proteins in chronic cardiac overload: structure and rate of synthesis. In: Perspectives in Cardiovascular Research: Myocardial Hypertrophy and Failure. Alpert NR (ed.), New York, Raven Press, Vol 7, pp 465-476, 1983.

Thompson JA, Hess ML. The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. Prog Cardiovasc Res. 28: 449-462, 1986.

Toher CA, Francis GS. The use of angiotensin-converting enzyme inhibitors in the treatment and prevention of congestive heart failure. Coronary Artery Disease. 4: 37-43, 1993.

Tong J, Ganguly PK, Singal PK. Myocardial adrenergic changes at two stages of heart failure due to adriamycin treatment in rats. Am J Physiol. 260(Heart Circ Physiol 29): H909-H916, 1991.

Vanoverschelde JL, Melin JA, Bol A, Vanbutsele R, Cogneau M, Labar D, Robert A, Michel C, Wijns W. Regional oxidative metabolism in patients after recovery from reperfused anterior myocardial infarction: relations to regional blood flow and glucose uptake. Circulation. 85: 9-12, 1992.

Watkins L Jr., Burton JA, Haber E et al. The renin-angiotensin-aldosterone system in congestive heart failure in conscious dogs. J Clin Invest. 57: 1606 - 1608, 1976.

Weiss JJ. Oxygen ischemia and inflammation. Acta Physiol Scandl. 548 (Supp 1): 9-37, 1986.

Weitz Z, Birnbaum AJ, Sobotka PA, Zarling EJ, Skosey JL. Elevated pentane levels during acute myocardial infarction. Lancet. 337: 933-935, 1991.

Westlin W, Mullane K. Does captopril attenuate reperfusion-induced myocardial dysfunction by scavenging free radicals? Circulation. 77 (Suppl I): I-30, 1988.

Wikman-Coffelt J, Parmley WW, Mason DT. The cardiac hypertrophy process. Analysis of factors determining pathological vs physiological development. Circ Res. 45: 697-707, 1979.