OXIDATIVE STRESS CHANGES IN EXPERIMENTAL *COR PULMONALE*

A Thesis Presented to the University of Manitoba in Partial Fulfilment of the Requirement for the Degree of:

MASTER OF SCIENCE IN PHYSIOLOGY

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I. ABSTRACT

Although right heart failure (RHF) contributes to 30% of all cardiovascular complications, most of the information available on heart failure in general is based on the experiences with left heart failure (LHF). *Cor pulmonale* is an adaptive response by which the right ventricle (RV) responds to its hemodynamic burden by hypertrophy and RV failure occurs when hypertrophy cannot compensate for hemodynamic burden. Subcellular and molecular events that lead to heart failure appear to be multifactorial and complex. One of the mechanisms common to most of the cardiovascular complications is an increase in the oxidative stress. In fact, several studies including data from our laboratory have suggested that oxidative stress may be a major contributor in the evolution of heart failure. Evidence in support of this oxidative stress hypothesis, with respect to compensated stage of RV failure, was provided earlier from our laboratory. The objective of this study was to further characterize and define oxidative stress changes during the development of hypertrophy followed by RHF.

Male Sprague-Dawley rats weighting 180 ± 10 g were divided into two groups: CONT (control), MCT (monocrotaline). Monocrotaline was administered i.p. in a single dose of 60 mg/kg body weight. After the MCT injection, both CONT and MCT-treated animals were monitored daily for 6 weeks for their general condition, food and water intake and body weight. At different post-treatment stages of RV hypertrophy, hemodynamic function including right ventricular systolic pressure (RVSP), right ventricular end diastolic pressure (RVEDP), left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP) were recorded. Myocardial tissue was studied with respect to different

antioxidant enzyme activities [catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD)], lipid peroxidation and histology. Lung and liver weights were recorded and their histological examination was also done. Appropriate statistical tests were applied for analysis of the data.

After 1 week of post-treatment period, rats in the MCT group showed reduced weight gain and general lack of appetite. Development of pulmonary hypertension was established by clinical signs of respiratory distress and histopathologic evidence of MCT pneumotoxicity. Subsequent to this change, RV hypertrophy was manifest as increased RV weight as well as by an increase in its free wall thickness without any change in LV free wall. This was, however, associated with sustained RV function as indicated by the maintenance of RVEDP and RVSP. At this stage of RV hypertrophy, there were no significant changes in GSHPx, SOD and catalase activities. Myocardial lipid peroxidation in the MCT group was also not different from the CONT group.

At 2 weeks post MCT treatment, compensated RV pressure overload was established clinically by aggravated respiratory distress without peripheral cyanosis and paraclinically by a significant increase in RVEDP and RVSP, no congestion in lung and liver, and significant RV as well as interventricular septum (IVS) hypertrophy. Interventricular septum wall was remodelled to a flatter appearance rather than curbing into the RV. There was no functional or histopathologic alteration in LV. This stage of compensated hemodynamics of RV was associated with a significant increase in myocardial catalase and GSHPx antioxidant enzyme activities. SOD activity and lipid peroxidation did not show any significant change.

At 6 weeks post-MCT treatment, the development of decompensated RV failure was established by clinical signs including severe respiratory distress, peripheral cyanosis, congested lung and liver and depressed cardiac function manifested in decreased RVSP as well as increased RVEDP. Right ventricle dysfunction at this stage was associated with a significant decrease in GSHPx and catalase activities and a significant increase in lipid peroxidation. This severe MCT-pneumotoxicity stage was accompanied by bulging of the IVS into the LV, which appeared as part of the RV.

These characteristic hemodynamic, biochemical and histopathologic alterations demonstrate that, in early stages of MCT-induced pulmonary hypertension at 1 and 2 weeks, RV hypertrophy was accompanied by sustained hemodynamic function and an increase in antioxidant reserve. The gross appearance of IVS was normal at 1 week, flat or straight at 2 weeks and bulging into the LV at 6 weeks. In the later stage at 6 weeks, clinical RHF was associated with abnormalities of systolic and diastolic function and a decreased antioxidant reserve. Biphasic changes in RV antioxidant enzymes, increasing during hypertrophy and decreasing in failure may suggest a role of oxidative stress in the pathogenesis of ventricular dysfunction.

II. INTRODUCTION

Cor pulmonale is a term that describes the pathologic effects of lung dysfunction on the right side of the heart. In this, pulmonary hypertension is the link between right ventricular (RV) structure-function changes and lung pathology. The common denominator in each case is the RV dysfunction with subsequent profound decrease in stroke volume and cardiac output (Roskovec A et al., 1986). *Cor pulmonale* accounts for 5% to 10% of heart disease, and 15% to 20% of all cases of heart failure. It results in almost 20% of heart-related hospital admissions. Its clinical management has been a challenge for clinicians mainly because no single treatment is universally applicable.

The underlying mechanism of myocardial failure remains a very active area of investigation. Clinical as well as basic research efforts have focused on defining the pathophysiology of heart failure in different clinicopathologic states. Several defects at the cellular and the molecular levels have been identified in the hypertrophied as well as failing myocardium. The list includes defects in the production and utilization of high energy phosphates (Bing, 1983), abnormalities in excitation contraction coupling and calcium movements (Gwathmey et al., 1987), downregulation of ß-adrenergic receptors (Bristow et al., 1982; Vatner et al., 1985), alteration in ventricular geometry and architecture (Gaurdon et al., 1993; Braunwald, 1997; Mc Kay et al., 1986), free radicals and lipid peroxidation (Belch et al., 1991; Kaul et al., 1993; Hill and Singal, 1994; Siveski-Iliskovic et al., 1994) and apoptosis (Narula et al ., 1996). However, it is not clear which of these changes is primary and is responsible for the initiation and which is the consequence of the disease. In this regard, significant evidence supporting the role of reactive oxygen radicals in chronic

pathologic states of the myocardium has been presented (Kaul et al., 1993; Mc Murray et al., 1990; Sobotka et al., 1993; Dhalla et al., 1996; Khaper and Singal, 1997; Hill and Singal, 1996).

Although alterations in myocardial antioxidant reserve in the compensated stage of RV failure have been reported (Pichardo et al., 1998), oxidative stress changes with respect to RV hypertrophy and the decompensated stage of RV failure remain to be examined. In the present study, the question of antioxidant status in RV hypertrophy and RV failure was approached by comparing the antioxidant enzyme activities and oxidative stress at these stages in relation to the hemodynamic function. Monocrotaline was used to induce chronic pulmonary hypertension, RV pressure overload, RV hypertrophy and eventually RV failure. The study describes characteristic changes in the antioxidant status and oxidative stress in hypertrophy and heart failure stages. A clear correlation is seen in the sustained RV function during hypertrophy and maintained antioxidant status in early stages, while heart failure at later stages correlated with decreased antioxidant reserve. The study suggests a role of oxidative stress in the pathogenesis of right heart failure (RHF).

III. LITERATURE REVIEW

A. General Background

Congestive heart failure (CHF) is defined clinically as a constellation of symptoms and signs denoting to the congestion of the systemic and/or pulmonary venous beds and low cardiac output owing to the inability of cardiac chambers to discharge their contents adequately. In this condition, despite adequate filling of the ventricles, cardiac output is inadequate to meet the metabolic demands of the body. The impaired ventricular function is associated with diminished exercise capacity, high incidence of ventricular arrhythmias, and reduced life expectancy. The manifestations of heart failure can be attributed to three main hemodynamic changes: 1) decreased cardiac output causing inadequate perfusion of various organ systems; 2) left atrial hypertension with pulmonary venous congestion; and 3) right atrial hypertension with systemic venous congestion. Depending on the underlying mechanism, i.e. left vs. right ventricular failure, these features may be present in varying combinations. Signs and symptoms progress as CHF worsens, causing a progressive loss of functional capacity. The New York Heart Association (NYHA) functional classification is widely used to define the stage of heart failure (Braunwald et al., 1997).

To some extent, the right and left sides of the heart act as two distinct anatomic and functional units. Under various pathologic stresses, one side or even one chamber may fail. So from the clinical standpoint, left sided and right sided failure can occur independently. Nevertheless, because the vascular system is a closed circuit, failure of one side can not exist for long without eventually producing excessive strain on the other, terminating in total heart

failure. Despite this interdependency, a clearer understanding of the pathologic physiology and anatomy can be derived from consideration of failure of each side.

The RV may fail because of *Cor pulmonale* due to pulmonary lung disease, primary pulmonary hypertension, tricuspid insufficiency, RV infarction, congenital anomaly, intrinsic disease or pulmonary stenosis. In addition, it may fail as a consequence of left ventricular (LV) failure. *Cor pulmonale*, pulmonary (right-sided) hypertensive heart disease is responsible for 30% of hospital admission for RV failure (Rockville, 1984).

B. Right Ventricular (RV) Hemodynamics in Disease

Acute RV free wall injury would seemingly reduce the function of the RV cavity to that of a passive conduit in the right sided circulation (Fuerey et al., 1984). With the free wall rendered non-compliant by injury and chamber dilation (Coma et al, 1980), septal thickening and motion can reduce the RV internal dimension during systole and displace the blood into the pulmonary circulation. Thus, the RV free wall ablation experiments are consistent with the current view that the septum has an integral role in supporting RV function (Santamore et al.,1976; Santamore et al., 1979).

Selected cardiopulmonary disorders that highlight RV hemodynamic adjustment to relatively pure RV volume overload and pressure overload are congenital secundum atrial septal defect and primary pulmonary hypertension, respectively. Chronic volume overload is well tolerated by RV and does not quickly modify the normal RV hemodynamics (Libersthon et al., 1981).

In response to the chronic, progressive increase in afterload during primary pulmonary hypertension (PPH), the RV both hypertrophies and enlarges. The initial

pathologic abnormality in PPH is thought to be medial hypertrophy in the muscular pulmonary arteries and arterioles (Rich et al., 1988) which contributes to pulmonary vasoconstriction and proliferative intimal lesion resulting in a sever obliterative arteriopathy. The burden of pulmonary disease on RV function is related to the elevation in pulmonary artery pressure. With increased afterload, RV performance improves by augmenting contractility (Weber et al., 1983). For a given preload and afterload, RV stroke volume is enhanced by catecholamine stimulation (Benotti et al., 1984). With acute as well as chronic elevations in pulmonary artery pressure, reflex sympathetic nervous system activations helps maintain function.

C. *Cor pulmonale*

The concept, *Cor pulmonale,* was introduced over 200 years ago, but the exact origin of the term is uncertain. Osler (1892; Richards DW, 1966) commented that hypertrophy of the RV results from increased resistance in the pulmonary circulation, as in cirrhosis of the lung and emphysema. McGinn and White used the term of acute *Cor pulmonale* in the discussion of a case of acute, massive thromboembolism (Mc Ginn and White, 1935). *Cor pulmonale* is characterized by hypertrophy and dilation of RV secondary to the pulmonary hypertension caused by diseases of the pulmonary parenchyma and/or pulmonary vascular system between the origins of the main pulmonary artery and the entry of the pulmonary veins to the left atrium (MacNee W, 1994). The most common cause of chronic *Cor pulmonale* in North America is chronic obstructive pulmonary disease (COPD).

C.1. Remodelling of the Right Ventricle (RV)

The geometric configuration of the RV makes this chamber suitable for ejection of relatively large volume of blood, with minimal myocardial shortening (Weber K et al.,1983). Under normal circumstances, pulmonary vasculature provides low resistance to RV out flow, about one tenth the resistance to flow of the systemic bed (Grossman W, Braunwald E, 1988). The pulmonary vasculature must react to wide variation in blood flow without much change in pressure. In 1500 human hearts at autopsy, Horen et al. showed a linear relation between RV mass and free wall area. Therefore, an increase in afterload causes RV enlargement, with both dilation and hypertrophy. Hypertrophy allows normalization of wall stress and improved ejection performance, yet myocardial function could be still reduced (Belik J et al., 1989).

In the course of right-sided chamber enlargement in response to the chronic and progressive increase in RV, afterload can reach massive proportions and dominate the cardiac silhouette in chest radiography. During enlargement, the RV chamber can remodel to a point that it takes on the ellipsoidal geometric features of the normal LV. The LV cavity is progressively reduced in size, particularly in the septal to free wall dimension. As pulmonary pressure increases and the RV chamber dilates the normal diastolic convexity of the septum toward the RV chamber first flattens and later bows convexly into the LV chamber. In echocardiography and radionuclide angiography, septal thickening and motion have a striking visual appearance that creates the impression of septal work in support of RV function. In fact the diastolic position and shape of the septum reflect the pressure gradient across the IVS (Tanaka et al.,1980). The septum moves paradoxically during systole, i.e. anteriorly toward

the RV cavity rather than to the LV chamber. In PPH it is typically flat or convexly directed toward the LV cavity. During systole, the septum tends to remain stented in its diastolic configuration even when RV systolic pressures are less than LV.

Progressive pressure afterload has several important and predictable influences on RV systolic performance. RV ejection fraction is uniformly decreased in moderate to advanced states of PPH typically in the 30% to 40% range and lower when frank RV failure develops. Stress in hypertrophied free walls is thought to be high, reflecting an inadequate degree of RV hypertrophy and possibly associated reduced coronary blood reserve (Murray et al., 1970). By the time right-sided heart dysfunction progresses to overt right-sided heart failure, the terminal stages of the disease are at hand.

D. Signs and Symptoms of Right Heart Failure (RHF)

The symptoms and signs of RHF have their origin in systemic venous congestion and RV dilation. Systemic venous pressure increases as a result of increased blood volume in the systemic veins secondary to inadequate RV emptying during systole. Generalized vasoconstriction in response to sympathetic over activity also causes such an increase.

The dominant symptoms of RV failure are those of systemic venous congestion. In contrast to LV failure, in which symptoms of pulmonary venous congestion are predominate. Pulmonary symptoms are rare unless there is associated LV failure or unless RV failure is due to chronic lung disease. Paroxysmal nocturnal dyspnea is uncommon. Patients may complain of fatigue as cardiac out put is reduced. Patients usually note edema of the ankles and feet initially, which later spreads to to the legs and abdomen. The edema is

bilateral and pitting. In bedridden patients, edema may first develop to over the sacrum. In its advanced stage, edema becomes widespread (anasarca) with ascites.

Right upper quadrant abdominal pain occurs due to liver enlargement. Anorexia and nausea develop secondary to congestion of the gastrointestinal tract. The absorption of nutrients is impaired and in persistent systemic venous congestion, significant protein loss may result (protein losing gastroenetropathy). All these factors cause gradual weight loss and ultimately cardiac cachexia. Other symptoms include fatigue, daytime oliguria and nocturia.

E. Cellular and Molecular Mechanism of Transition From Hypertrophy to Heart Failure

Both acute and chronic forms of heart failure involve mechanical dysfunction during systole and/or diastolic phases of the cardiac cycle (Morgan et al., 1990; Dhalla et al., 1982). Functional abnormalities in excitation-contraction coupling, contractile protein function and energetics have been identified in failing animal and human myocardium. At the molecular level, alterations have been observed in the expression of several proteins that are central to normal myocardial structure and function (Thaik et al., 1995). More recently, a surge in experimental and clinical studies have begun to document and characterize the phenomenon of oxidative stress as a major mechanism in the development of decompensating myocardium. A detailed discussion of the proposed pathophysiological mechanisms that may contribute to the development of heart failure is provided below.

The general process by which the ventricular myocardium experiences changes in the structure and function is often referred to as remodelling (Cohn, 1995). Myocardial remodelling is a normal feature during maturation and is an useful adaptation to increased

demands (e.g. athletic training) in the adult. However, when this process occurs in response to pathologic stimuli (e.g. abnormal wall stress), the remodelling that ensues, although perhaps adaptive in the short term, is maladaptive in the long term, and it often eventuates in further myocardial dysfunction (Katz, 1994). The process of cardiac remodelling consists of a number of molecular and cellular changes. In general, pathologic myocardial remodelling involves an increased myocardial mass associated with hypertrophy of individual myocytes, alteration in gene expression and changes in both the quantity and quality of extracelluar matrix. Recent works suggest that pathologic remodelling also involves the death of cardiac myocytes by apoptosis.

F. Structural and Biochemical Alterations Occurring With Adaptive Hypertrophy and Evolving Failure

Immediately upon imposition of a large pressure overload, the increase in work performed by the ventricle exceeds the augmentation of cardiac mass and heart dilates (Meerson, 1996). As a consequence, a compensatory phase sets in as the ventricle hypertrophies and the contractile function returns to approximately normal level. Mitochondria proliferate and myofibrils are laid down in parallel and sarcomeres in series so that both length and cross sectional diameter of myofibers increase (Anversa et al., 1986). In what Meerson has termed the exhaustion phase, several events take place: 1) there is lysis of myofibrils; 2) lysosomes increase in number; 3) the sarcoplasmic reticulum (SR) becomes distorted (Dalen et al., 1987); 4) the surface densities of key tubular system are reduced; and 6) fibrous tissue takes the place of cardiac cells.

F.1. Increase in Myocardial Mass

The major mechanism for the increase in myocardial mass is hypertrophy of individual myocytes. One of the earliest experimental systems for the study of hypertrophy used cardiac myocytes cultured from neonatal rat hearts. In this system, norepinephrine enlarged myocyte size by increasing protein synthesis in the absence of cell division (Knowlton et al., 1993). A number of other factors have now been identified as potential causes of myocyte hypertrophy *in vivo*, including angiotensin (Sadshima et al., 1993), endothelin (Subeita et al. 1990) and inflammatory cytokines (Thaik et al ., 1995).

F.2. Apoptosis

There is evidence that pathologic remodelling also involves the death of myocyte by apoptosis or necrosis. Apoptosis is a pivotal feature of normal tissue development in the fetus and of cell replacement in certain adult tissues (e.g. thymus). In contrast to cell necrosis, apoptosis is a genetically regulated series of energy-dependent molecular and biochemical events. Unlike necrosis, apoptosis usually occurs in isolated cells in the absence of inflammation. A typical identifying feature of cells undergoing apoptosis is the presence of double stranded DNA breaks, which present as nucleosomesized fragments.

Recently, Narula et al. (1996) and Olivetti et al. (1997) provided the first evidence that apoptosis occurs in myocardium of patients with end-stage, dilated cardiomyopathy. Similarly, Sarasta (1997) showed the presence of apoptotic cells in the infarct and periinfarct regions of the myocardium of patients dying from recent MI. The findings from several *in vitro* systems and animal models have suggested that apoptosis also occurs in the myocardium in response to a variety of other insults including ischemia - reperfusion (Gottlieb et al., 1994),

MI (Kajstura et al.,1996), rapid ventricular pacing (Liu et al., 1995) and mechanical stretch. Although the evidence is persuasive, the pathophysiologic significance of apoptosis in myocardial failure is unknown.

These observations led to the thesis that progressive myocardial failure may reflect the continuing loss of viable myocytes (Colucci et al., 1991). Hemodynamic overload results in myocardial remodelling with the reexpression of a fetal gene program. Apoptosis may be the result of an aborted growth response to pathophysiologic stimuli reactivating a dormant fetal growth program in cells no longer capable of progressing through the cell cycle .

F.3. Extracellular Matrix

Collagen and other connective tissues increase during the process of hypertrophy and also following the loss of myocytes. These changes in the cardiac skeleton, which have been termed interstitial heart disease (Weber et al., 1989), can contribute to the impairment of both diastolic and systolic function in the failing myocardium (Weber et al., 1991). It may be significant that connective-tissue hypertrophy progresses more slowly than myocyte hypertrophy due to pressure loading. In addition, the collagen of the hypertrophied, pressure-overloaded myocardium differs from that of the normal myocardium. The increase in collagen not only increases myocardial stiffness but also predisposes to reentrant arrhythmia caused by abnormal electrical dispersion (Weber et al., 1989). Quantitative and qualitative changes in the connective tissue framework of the heart probably play a significant role in the ventricular remodelling process associated with various types of CHF.

F.4. Calcium Handling

It has been suggested that altered myocyte calcium handling plays an important role in the development of CHF, both in humans and in animal models of CHF (Morgan et al., 1990). Calcium transport into the SR occurs via the action of SR Ca^2 pump. This process requires the hydrolysis of one molecule of ATP for two calcium ions that are pumped against a large concentration gradient into the lumen of the SR. In human CHF, a reduction has been noted in enzyme activity of the SR Ca^2 -ATPase (Schwinger et al., 1995), and similar results have also been obtained in animal models of ventricular hypertrophy (VH) and CHF (Gupta et al., 1997). Calcium is released from the SR via the calcium-sensitive SR calcium- release channel (Fozzard et al., 1992). The channel tightly binds ryanodine and is referred to as ryanodine receptor (RYR). The level of RYR mRNA has been shown to be reduced in both human CHF (Arai et al., 1994) and animal VH (Matsui et al., 1995). Calcium enters the cardiac myocyte during the plateau phase of the action potential via the L-type calcium channels (Fozzard et al., 1992). In human CHF, decreased levels of mRNA coding for the calcium channels have been reported (Takahashi et al., 1992).

F.5. Neurohumoral Changes

F.5.a. Autonomic nervous system. A complex series of neurohumoral changes takes place consequent to the two principal hemodynamic alterations in heart failure: reduction of cardiac output and arterial hypertension. Many of these neurohumoral changes occur in response to the inadequate atrial volume characteristic of systolic heart failure. Thus in CHF, there are defects both in cardiac sympathetic and parasympathetic control systems (Viquerat et al., 1985). At relatively early stages in the evolution of CHF, augmentation of

generalized sympathetic nervous system activity is observed with a concomitant decrease in parasympathetic tone. This results in increased circulating levels of NE. At the same time, there is increased activity of the sympathetic nerves to the heart with augmented NE release. Ultimately, this results in marked decrements in myocardial NE stores and these changes may contribute to the progression of the disease. Heart failure is also associated with significant impairment of baroreceptor reflexes that control both sympathetic and parasympathetic nervous system activity to the systemic arteries and veins and to the heart (Hardy et al., 1991).

Myocardial β-adrenergic receptor density is downregulated and the production of second messenger - cAMP is decreased in heart failure (Bristow et al., 1982). These may be the result of increased circulating catecholamines that are not buffered (i.e. removed from receptor sites) by dysfunctional sympathetic nerve terminals. Indeed, in severe heart failure, norepinephrine (NE) stores in sympathetic nerve endings are depleted, and these nerves neither synthesize, store, nor normally release catecholamine. Thus, the failing myocardium becomes functionally denervated, leaving exposed $ß_1$-receptors. In the non-failing human ventricular myocardium, the total ß-receptor density is approximately 90 fmol/mg, with the $ß_1$ and the $ß_2$ proportion being 80% and 20%, respectively. In failing the myocardium, most of the decrease results from a selective decrease in $ß_1$-receptors (Bristow et al ., 1982 ; Bristow et al ., 1986). As a consequence, $ß_2$ receptors constitute about 40 percent of the total ß-receptors in the failing human myocardium. The decrease in $ß_1$ receptor density and ß- receptor downregulation probably account for much of the decrease in inotropic potential under the influence of ß- adrenergic receptors.

Although $ß_2$-receptor density is relatively well preserved, the maximal adenylate cyclase response is decreased about 30% to 35% (Bristow et al., 1986). The H_2 histamine, the A_1 adenosine, and the α-receptor pathways appear to be relatively normal in heart failure. There is evidence that vasoactive intestinal peptide receptors are decreased in heart failure but have increased receptor affinity, with a supersensitive dose responsive curve.

Heart failure is associated with altered levels of G proteins (guanine nucleotide-binding regulatory proteins) which couple a variety of receptors to effector enzymes and either stimulate or inhibit adenylate cyclase. Thus, there is evidence of a decrease in stimulatory guanine nucleotide-binding regulatory proteins (G_S) and of an increase in inhibitory guanine nucleotide-binding regulatory proteins (G_I). In terms of the changes in G_S and G_I, there are potentially important differences between patients with heart failure due to coronary artery disease and patients with failure due to idiopathic dilated cardiomyopathy (Feldman et al., 1991).

F.5.b. Renin-angiotensin system. Like norepinephrine, angiotensin has the potential to act directly on cardiac cells independently of its vascular and metabolic actions, thereby affecting myocardial remodelling. Angiotensin increases myocardial protein synthesis in cardiomyocyte and DNA synthesis in cardiac fibroblast (Sadoshima et al., 1993). In addition, angiotensin can cause apoptosis in cardiac myocytes in culture (Kajstura et al., 1997). Both effects can be blocked by an antagonist selective for AT_1-receptor.

The complete renin angiotensin system (RAS) is represented in the myocardium, and several components are upregulated with myocardium remodelling or failure, including angiotensin converting enzyme activity and, the level of angiotensenogen mRNA and the

density of angiotensin receptors. Interestingly, there is evidence that myocyte angiotensin is released by the stretch of the cell and can mediate the effects of stretching on myocyte hypertrophy and gene expression (Sadoshima et al., 1993). These observations suggest that angiotensin could play an important role in pathologic myocardial remodelling, both as a circulating hormone and as an autocrine/paracrine mediator produced in response to hemodynamic overload.

G. Free Radicals, Antioxidants and Oxidative Stress

Numerous lines of investigation in humans as well as animal studies support the importance of oxidative stress in the development of pathophysiologic states in the cardiovascular system. Although the involvement of free radicals in various types of tissue injury has been known for some time, their role in cardiovascular disease was first documented by early studies dealing with catecholamine-induced cardiomyopathy (Singal et al., 1981) and ischemia-reperfusion injury (Bolli, 1988). These early studies have been followed by a series of elegant and detailed experiments which have provided incremental evidence supporting free radical involvement in cardiac injury (Kaul et al ., 1993).

G.1. Oxygen Radicals

In the simplest terms, a free radical is any atom or molecule that has an unpaired electron in their outer orbit making that atom a highly reactive species. The production of free radicals occurs either by the addition or by the removal of an electron in a reduction/oxidation reaction (Kaul et al., 1993; Singal et al., 1988). Oxygen is also the terminal acceptor of electrons for oxidative phosphorylation and this tetravalent reduction is associated with the production of high energy phosphates. However, sequential univalent

reduction results in the formation of reactive oxygen intermediates (Halliwell, 1987; Weiss, 1986; Kaul et al., 1993; Singal et al., 1988). In the univalent reduction pathway, the addition of a single electron to molecular oxygen results in the production of superoxide anion radical ($O_2^{-\cdot}$). Some of the sources of superoxide radical include activated neutrophils, xanthine oxidase and NADPH oxidase (Freeman and Crapo, 1982; Halliwell, 1987). The addition of another electron to the $O_2^{\cdot}$ results in the formation of hydrogen peroxide (H_2O_2). Hydrogen peroxide is capable of causing cell damage by interacting with transition metals such as iron. A single electron reduction of H_2O_2 results in the formation of the hydroxyl radical ($OH^{\cdot}$) which is highly reactive, has an extremely short half-life, and therefore has a very limited diffusion capacity (Kaul et al., 1993; Singal et al., 1988). The addition of a fourth electron finally results in the formation of water.

H. Free Radicals and Cellular Injury

Reactive oxygen intermediates such as $O_2^{\cdot}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$ are called activated *oxygen species* and are collectively known as partially reduced forms of oxygen (PRFO) (Kaul et al., 1993). Nitric oxide reacts with superoxide anion to form peroxynitrite which plays an important role in ischemia-reperfusion injury. These reactive species can interact with macromolecules and initiate free radical chain reactions resulting in membrane and cell damage (Kaul et al., 1993; Singal et al., 1988). These reactive oxygen molecules when produced in excess, have the potential to interact with many cellular structures including lipids, proteins and nucleic acid. Such interactions may result in dysfunction of the macromolecules and with time (especially in lipid membranes) lead to chain reactions which cause further disruption of membrane structure and function. Thus, in these chemical

reactions, there are structural as well as functional changes in the macromolecules leading to modifications/alterations in cell and organ function.

The lipid peroxidation chain reaction is initiated by the removal of a hydrogen atom from the unsaturated site in a fatty acid resulting in the production of a lipid radical. This radical can further react with other neighboring polyunsaturated fatty acids (PUFA) to propagate the reaction (Kaul et al., 1993; Singal et al., 1988). The addition of an oxygen molecule to these lipid radicals results in the formation of lipid peroxides. Free radical-induced lipid peroxidation has been suggested to alter membrane structure and function resulting in cell dysfunction and cell death (Kaul et al., 1993; Singal et al., 1988). Proteins rich in sulfhydryl groups are also highly susceptible to free radical attack (Kaul et al., 1993). In the myocardium, oxygen radicals have been shown to effect Na^{+}/Ca^{++} exchange, Na^{+}-K^{+} ATPase and Ca^{++} ATPase activities (Kaneko et al., 1990; Kramer et al., 1984; Dixon et al., 1990). Free radicals can also attack the nucleic acids by modifying deoxyribose and base moieties and producing base damage, single strand breaks, adducts and chromosomal aberrations. Mitochondrial DNA is found to be more susceptible to free radical attack than nuclear DNA because it is closer to the site of oxygen free radical production (Richter et al., 1992).

I. The Antioxidant Defense System in the Heart

Several counteracting mechanisms have evolved in mammalian cells to effectively eliminate excess in reactive oxygen radicals. In fact, a number of protective enzymatic as well as nonenzymatic antioxidant have evolved constituting an antioxidant reserve (Singal and Kirshenbaum, 1990) and a proper balance between the generation of PRFO and the

antioxidant defence system is critical for the maintenance of a normal myocardial cell structure and function (Kaul et al., 1993; Singal and Kirshenbaum, 1990).

I.1. Enzymatic Antioxidants

I.1.a. Superoxide dismutase (SOD). This enzyme is the first line of defense against free radical attack. The most common forms of SOD are CuZnSOD (molecular weight ~ 32,000) and MnSOD (molecular weight ~80,000), which are present in the cytoplasm and the mitochondria, respectively. It has been shown that superoxide dismutase activity is significantly less in the heart than in the liver.

I.1.b. Glutathione peroxidase (GSHPx). This selenium-dependent enzyme (molecular weight ~84,000) is the second line of defense against free radical-mediated damage and is present mainly in the cytoplasm. It catalyzes the reduction of hydrogen peroxides using glutathione (GSH) as a substrate. There is a relatively high concentration of this enzyme in the human heart (Kaul et al., 1993; Ferrari et al., 1985; 1998) and is therefore considered an important antioxidant enzyme in that organ. A non selenium-dependent form of GSHPx, which is not specific to H_2O_2, but which dismutates organic peroxides is also present (Lawrence and Burk 1978). Depletion of GSHPx has been reported to result in an increase in myocardial contractile function of isolated rat hearts to oxidative stress induced by hydrogen peroxide (Konz et al., 1989).

I.1.c. Catalase. This enzyme (molecular weight ~240,000) is also very important in the metabolism of hydrogen peroxide. Present at relatively low concentrations in the heart, it converts H_2O_2 to water and oxygen. However, the difference between catalase and glutathione peroxidase is that GSHPx is more effective at low concentrations of H_2O_2, i.e. in

μM range, whereas catalase is more effective at the mM concentrations of H_2O_2 (Kaul et al., 1993; Freeman and Crapo 1982).

I.2. Non-enzymatic Antioxidants

I.2.a. Vitamin E. Vitamin E is one of the major biological antioxidants and α-tocopherol is the most common type of vitamin E absorbed from the human diet (Packer 1994). Because of its lipophilicity, vitamin E offers maximum protection against free radical attack in cellular and subcellular membranes. It reacts with free radicals, yielding lipid hydroperoxides which can be removed by the GSHPx enzyme system. This effectively terminates lipid peroxide-mediated chain reactions and is therefore called a chain breaking antioxidant (Singal et al., 1998; Kaul et al., 1993, Packer, 1991). Vitamin E also functions synergistically with ascorbic acid to terminate free radical chain reactions. Recent studies have reported a new role for vitamin E that is independent of its antioxidant property. These include inhibition of smooth muscle cell (SMC) proliferation and growth during atherosclerosis, occurring mainly as a result of its ability to activate the release of TGF-ß (Ozer et al., 1995).

I.2.b. Vitamin C. Vitamin C is a water-soluble molecule which quenches reactive oxygen metabolites directly, leading to the formation of dehydroascorbate. Vitamin C also plays an important role in the regeneration of tocopherol.

I.2.c. Carotene. Carotene, a precursor of vitamin A, is known to quench active oxygen species. Epidemiological studies have shown that increased levels of ß-carotene and other carotenoids are associated with a decreased risk of cardiovascular diseases (Riemersma, 1994; Palace et al., 1999).

I.2.d. Glutathione. Glutathione is a tripeptide which is present in high concentrations in the majority of the eukaryotic cells. One of its important functions is to protect cells against peroxides generated during aerobic metabolism. It undergoes redox cycling between the reduced (GSH) and oxidized (GSSG) forms (Kaul et al., 1993; Clark et al., 1985). In the heart, glutathione is predominantly (>95%) in the GSH form. It also acts as a cosubstrate for GSHPx and plays an important role in defending against free radical-mediated lipid peroxidation. The redox ratio, which is the ratio of reduced to oxidized glutathione (GSH/GSSG), is used as a sensitive index of oxidative stress (Singal et al., 1998; Kaul et al., 1993; Singal et al., 1988).

J. Oxidative Stress and Heart Failure

There is increasing evidence for the role of oxidative stress in the pathogenesis of heart failure. This concept is gaining more acceptance due to the fact that, during heart failure, changes in different neurohormones, cytokines, nitric oxide and activated inflammatory cells are closely linked to oxidative stress at the cellular and molecular level.

J.1. Oxidative Stress and Heart Failure (Clinical Implications)

The correlation between heart failure and oxidative stress status in humans was provided by studies that carefully monitored diverse indices of oxidative stress in the circulation of heart failure patients (McMurray et al., 1990). Plasma malondialdehyde-like activity (MDA), a marker of lipid peroxidation, is high in patients with ischemic and non-ischemic dilated cardiomyopathy, and seems to correlate with the severity and chronicity of symptoms, and inversely with ejection fraction and exercise capacity (Diaz-Velez et al., 1996; Keith et al., 1998). 8-ISO-PGF2a, which has recently been shown to be a specific,

quantitative index of oxidative stress *in vivo* (Mallat et al., 1998), is raised in pericardial fluid obtained at the time of surgery in patients with heart failure and the value correlates with functional class and echocardiographic measures of LV dilation (Mallet et al., 1998).

Increased oxidative stress may be related to deficiencies in the antioxidant system, or may also indicate an enhanced production of reactive oxygen species (ROS). In fact, consistent with observations from animal studies of our lab, Keith and colleagues (1998) showed reduced antioxidant activity and vitamin C concentrations in plasma from patients with chronic heart failure.

Cheng et al. (1995) have shown that stretching a papillary muscle for 3 hours caused a 2-fold increase in the generation of ROS. Importantly, this effect was associated with the induction of apoptosis in both myocytes and fibroblasts, as well as induction of fos protein, a molecule associated with apoptosis pathways. This experiment suggests that mechanical stress on the myocardium can increase oxidative stress in the tissue and lead to apoptosis of myocytes.

Another potentially important stimulus for increased oxidative stress in the myocardium is exposure to inflammatory cytokines, such as tumor necrosis factor-α, which can increase in the failing myocardium (Levine et al., 1990; Torre-Amione et al., 1996) and have the ability to stimulate free radical production (Lopez et al., 1997). Increasing evidence from both animal (Bozkurt et al., 1998) and human studies (Levine et al., 1990) have reported elevated levels of cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 α (IL-1a) and atrial naturietic factor in MI and failure conditions. Increased concentrations of these cytokines were shown to have a direct relation to the severity of failure (Ferrari et al., 1998;

Levine et al., 1990; Givertz et al., 1998). Recent studies suggest that the deleterious effect of TNF-α is receptor-mediated and it also directly activates nitric oxide, which is cytotoxic to myocardial cells by virtue of its ability to produce free radicals and cause apoptosis (Blum and Miller 1998). The pathological potential of TNF-α is demonstrated in a study where transgenic mice overexpressing TNF-α in the heart developed cardiomyopathy associated with apoptosis (Bryant et al., 1998). In a recent study, it was demonstrated that TNF-α and Ang II induce hypertrophy in cultured neonatal cardiac myocytes by virtue of producing free radicals and antioxidants such as BHA, vitamin E and catalase inhibited this effect of TNF-α and Ang II by scavenging the radicals (Nakamura et al., 1998). TNF-α not only produces PRFO, but is also activated by hydrogen peroxide in the presence of a P38MAP kinase (Meldrum et al., 1998).

K. Experimental Models of Right Heart Failure (RHF)

K.1. Changing Atmospheric Conditions

One of the earliest models for experimentally-induced RHF was exposing animals to anoxemic conditions, in a steel respiratory chamber kept at lower pressure (Van Liere, 1974). Under these circumstances, the heart weight/body weight ratio of rats increased by about 150% of the normal value and the difference was primarily due to hypertrophy of the RV. Guinea pigs subjected to simulated atmospheric conditions of 18,000 feet in a steel tank showed RV hypertrophy (Valdivia, 1957). In another model, rats breathing at a low oxygen pressure showed RV hypertrophy as indicated by an increase of 80% in the RV (LV+S) ratio (Roberts et al., 1995). In response to chronic hypoxia, there was pulmonary vessel wall smooth cell hypertrophy and or/hyperplasia.

K.2. Surgical Approaches

The induction of pressure overload by banding pulmonary artery is one of the most common models of experimental RV hypertrophy (NG et al., 1995; Roberts et al., 1995).

K.3. Chemically Induced Right Ventricle (RV) Failure

The monocrotaline (MCT) model of RV failure has been used since 1961 (Lalich and Merkow, 1961). MCT, which is derived from crotalaria seeds, is converted by the liver to the dehydromonocrotaline (monocrotaline pyrole) (Mattocks et al., 1970). It damages the pulmonary vasculature producing medial hypertrophy of muscular pulmonary arteries (Smith et al., 1985), increases the impedance to blood flow and causes pulmonary hypertension. Such hemodynamic loading, in turn, leads to RV hypertrophy and eventually failure (Chesey et al., 1973). Thus, it is a noninvasive model of RVH with minimal changes to the left side of the heart. The histologic lesions in the lungs of MCT-treated rats bear similarity to those observed in people with primary pulmonary hypertension. In the rats, the endothelium appears to be the site of initial injury (Reid et al., 1986). Endothelial cell hypertrophy, platelet and fibrin thromboemboli, fibrosis, vasculitis, and vascular occlusion have been observed (Walcott et al ., 1970; Watanabe & Ogata, 1976; Reindel et al., 1990). Pulmonary vascular remodelling, characterized by medial hypertrophy of pulmonary arteriole and extension of smooth muscle to normally nonmuscular pulmonary arterioles, also occurs.

There are also numerous similarities between the pathophysiologic alterations in adult respiratory distress syndrome in people and MCT-induced pneumotoxicity in rats. Both have two rather distinct phases of pulmonary response. The early phase of adult respiratory distress syndrome (ARMS) and the early period of pneuomotoxicity due to MCT are

characterized by increase in vascular permeability and subsequent pulmonary edema (Plestina & Stoner, 1972). Quite large doses of MCT administered intraventricularly may produce a severely and rapidly developing pulmonary edema, which like ARMS, is fatal between 1-2 d (Plestina & Stoner, 1972). People with ARMS and rats treated with MCT have endothelial cell lesions and decreased ability to remove biogenic amines from the circulation (Morel et al., 1985).

L. Monocrotaline

L.1. General Information

The term pyrrolizidine alkaloid describes a group of a structurally related compounds of plant origin that are characterized by pyrrolizidine nucleus to which is attached a variety of functional groups (Mattocks, 1986). The pyrrolizidine nucleus (Fig. 1) consists of two fused, five-membered rings that share a common nitrogen atom at the center or bridge head of the molecule (Schoental, 1968 ; Huxtable, 1979). MCT is a macromolecule diester of the necine base, retronecine, to which a dicarboxylic acid, monocrotalic acid, is esterified at position 1 and 7. The chemical structure of MCT was first identified by Adams and Rogers (1939) and has been confirmed by nuclear magnetic resonance and infrared and mass spectroscopic techniques (Culvenor & Dal Bon, 1964). When purified, MCT is a white, crystalline powder with a molecular weight of 325.3. It has a melting point of 202-203 C°.

Approximately 370 different pyrrolizidine alkaloids have been isolated and identified by various chromatographic and spectroscopic techniques. MCT or 12ß, 13ß,- dehydroxy-12a, 13a, 14a-trimethyl-crotal-1-enine is a bitter-tasting pyrrolizidine alkaloid that is well

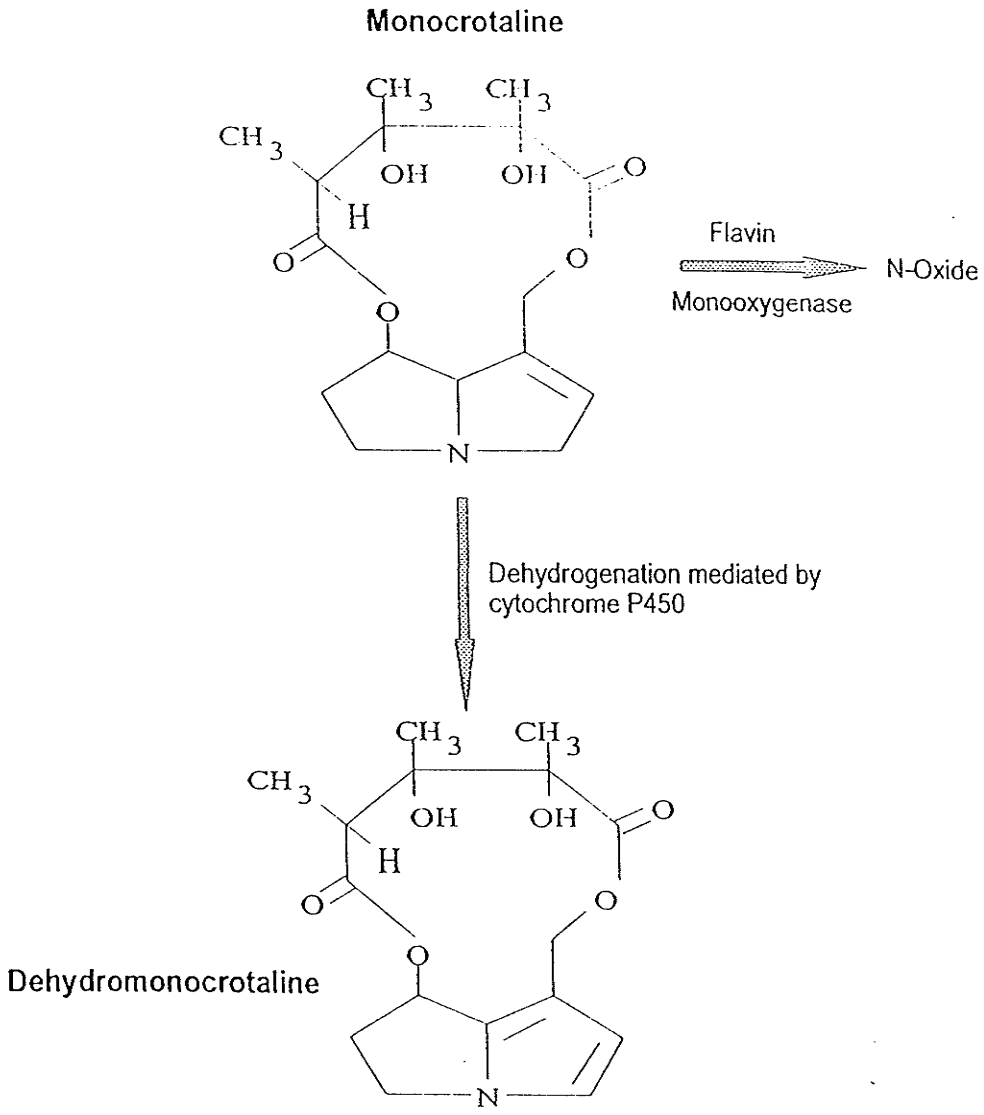

Figure 1: Monocrotaline metabolism in the liver.

known for its hepatic and cardiopulmonary toxicity in animals. Numerous plants of the Crotalaria genus produce MCT in their seeds and leaves (Mattocks, 1986).

L.2. Metabolism and Bioactivation

Pyrrolic derivatives, formed by dehydrogenation within the smooth endoplasmic reticulum of the liver, appear to be the responsible for the hepatic and pulmonary toxicity of MCT (Mattocks, 1986). MCT pyrrole (MCTP), also called dehydromonocrotaline, is a toxic metabolite of MCT formed by cytochrome 450 monooxygenase system in the liver (Mattocks and White, 1970). It is a reactive electrophile that is capable of causing tissue injury. Depending on the site of intravenous administration, chemically synthesized MCTP results in lung (Butler, 1970) or liver lesions in rats (Newberene et al., 1971) similar to those caused by parent compound MCT. Chloramphenicol and SKF-525 A, chemicals that inhibit cytochrome P-450 monooxygenase activity, decrease MCT-induced organ toxicity (Mattocks and White, 1970) and augment the toxicity of MCT (Mattocks, 1972).

L.3. Pharmacokinetics

Early pharmacokinetic studies of pyrrolizidine alkaloids were limited due to inability to detect accurately tissue levels of metabolites using radio-labeled pyrrolizidine alkaloids that lack high specific activity. The original studies using ^{3}H-MCT-administered at a dose of 60 mg/kg to rats revealed that approximately 70% of the MCT was recovered unmetabolized from the urine within 3 h (Hayashi, 1966). Most of the remaining label was detected in the bile as metabolites. Measurements of Pyrrosia activity using the Ehrlich assays (Mattocks and White, 1970) indicate that pyrrole derivatives accumulate in the lungs, liver and kidneys`

within minutes after administration of MCT. Peak activity occurs between 25 and 90 min and then decreases to low levels by 48 h (Allen et al ., 1972).

Estep and colleagues (1991) treated rats with 60/mg ^{14}C-MCT /kg and found that 90% of the radioactivity was present in the urine and bile by 7 h. Although the concentration of radioactivity in the plasma decreased dramatically at 7 h, the content of radioactivity in erythrocytes decreased much less, indicating that MCT is retained in erythrocytes. These data suggest that erythrocytes may serve as carriers of MCT metabolites and may involved in pulmonary toxicity that occurs after administration. Recently, Lame and colleagues (1997) demonstrated that administration of radio-labeled MCTP to rats caused extensive labeling of erythrocytes of which most of the radioactivity was associated with ß-globin chains. This study provides evidence for the potential of hemoglobin or other erythrocyte proteins to serve as carriers for active Pyrrosia metabolites.

L.4. Cardiac Toxicity

Rats chronically exposed to MCT in drinking water or food (Lafranconi et al., 1984; Huxtable et al., 1977) or to spectabilis seeds in food develop RV hypertrophy. A single s.c. administration of MCT (Hayashi & Lalich, 1967) or a single i.v. injection of MCTP given to rats also results in right heart enlargement. There is general agreement that the cardiac lesions occur as a physiologic response to an increased workload that results from a sustained elevation in pulmonary arterial pressure. Lesions are limited to the RV and IVS. No major lesions in the LV or atria have been reported.

The macroscopic evidence of the right heart enlargement is accompanied by an increased rate of RV protein synthesis (Huxtable et al., 1977), an increase in total collagen

content of the RV (Lafranconi et al., 1984), a marked decrease in ratio of RV DNA: RNA ratio but no change in ventricular lipid content (Lafranconi et al., 1984) and an increase in 1,2-diacylglycerol content (Okumura et al., 1992). Werchan and colleagues (1989) observed marked increases in both cross sectional area and cell length in myocytes from the RV of rats treated with MCT. A similar increase in RV wet weight and the diameter of cardiac myocytes was detected in guinea pigs treated with large dose of MCT 7 d previously (Tatebe et al., 1996). Physiologic changes in electrical potential of the cardiac muscle occur concurrently with cardiac hypertrophy and a right shift in the mean electrical axis of the electrocardiogram was observed (Brunei et al., 1983). Pump failure may occur late in the disease development, after prolonged increases in pulmonary vascular resistance that results in excessive cardiac afterload of rats treated with MCT.

Olhlenschlager and associates (1969) treated rats with a single sc injection of MCT and noted a time-dependent cardioventricular hypertrophy. At 21 and 45 d, MCT-treated rats had significant increases in immunoreactive natriureticpeptide in the ventricle. Analysis of cardiac mRNA indicated that significant increases in both atrial and ventricular atrial natriuretic peptide (ANP) mRNA had occured suggesting that cardiopulmonary lesions induced by MCT caused a compensatory extension in cardiac endocrine activity. Although the exact mechanism for ventricular production of atrial natriuretic peptide during pulmonary hypertension is unknown, a close association between ANP and RV hypertrophy in MCT-treated rats has been reported (Ohie et al., 1993). The vast majority of immunoreactive ANP originated from the RV free wall and ventricular septum.

The potential relationship between cardiac remodelling and endothelin-1 has also been investigated in rats treated with MCT. Rats that received FR139317, an endothelin-A receptor antagonist did not develop as large an increase in RV/LV weight ratio compared to rats that received MCT alone. The isoform ratio of beta-myosin heavy chain protein and the isoform ratio of beta-myosin heavy chain mRNA were also less in rats that were treated with the MCT only. These results indicate that inhibition of the action of endothelin-1 via receptor blockade attenuates cardiac hypertrophy in this model (Ishikawa et al., 1996).

L.5. Pulmonary Toxicity

Clinical signs are usually not evident immediately after a single exposure of rats to doses of MCT that result in pulmonary hypertension. Within 3-7 days, rats show signs of illness including anorexia, failure to weight gain, tachypnea. As lung injury and vascular remodelling progresses, animals develop variable degrees of dyspnea, weakness and diarrhea, and peripheral cyanosis (Schoental & Head, 1995; Turner & Lalich, 1965). Macroscopic lesions induced by exposure of rats to MCT (P) are numerous, complex and progress with time. Both the vasculature and epithelium are inured in a delayed and progressive way (Hayashi et al., 1984). At necroscopy, rats may have various degrees of pleural effusion (Chesney et al., 1974), congestion and hemorrhage (Schoental & Head, 1995). Endothelial cells particularly those of alveolar capillaries and small arterioles, are among the first cells to develop the lesions. With time, vascular injury progresses in severity and extends to all levels of vessels. Significant paranchymal lesions include alveolar epithelial metaplasia, edema, and infiltration of numerous inflammatory cells.

IV. MATERIALS AND METHOD

A. Animals and Treatment Protocol

Male Sprague-Dawley rats weighting 180 ± 10 g were used in this study. Animals were purchased from Central Animal Care Services at the University of Manitoba. All animals used in this study were maintained and treated in accordance with the policies and procedures of the Canadian Council on Animal Care (CCAC). Animals were given food and water *ad libitum*, and divided into two groups: CONT (control), MCT (monocrotaline-treated).

Animals in the MCT group received a single, intraperitoneal injection (60 mg/kg body weight) of monocrotaline (Sigma Chemical Co., St Louis, MO) in 1N HCL buffered to pH 7.0 with 1N NaOH. In the CONT group, animals received the same volume of buffer. Animals were kept one in each cage to allow measurement of individual food and water intake. All animals were observed for 6 weeks after MCT treatment for general appearance including signs and symptoms of respiratory distress, behaviour, body weight and mortality. The animals in the CONT and MCT groups were hemodynamically assessed at 1, 2 and 6 weeks and utilized in further analysis.

B. Hemodynamic Studies

For hemodynamic assessment, animals were anesthetized with ketamine and xylasin (60 mg/kg and 10 mg/kg, respectively, i.p.). A catheter with a miniature pressure transducer at its tip (Millar Microtip) was inserted into the right carotid artery, aorta and then advanced into the LV. LVEDP and LVSP were recorded. For recording of RV pressures the transducer was inserted into the right jugular vein and then advanced to the RV. After

hemodynamic recordings, the animals were killed and the hearts and other organs were removed for further studies.

C. Tissue Weights

Pieces of tissue from the lung and the liver were removed and weighted to obtain the wet/dry weight ratio. For recording the dry weight, pre-weighed tissue was chopped into smaller pieces and was placed in an oven at 65 °C until a constant weight was reached (generally after 24 hours). Atria and great vessels were trimmed from the hearts. The RV, LV and septum were dissected away and weighted. Wet to dry weight ratios for different regions of the heart as well as with body weight were recorded.

D. Biochemical Studies

D.1. Catalase

The RV was homogenized in 9 vol. of 0.05 M potassium phosphate buffer (pH 7.4) and centrifuged at 40,000 x g for 30 minutes. Supernatant, 50 μL was added to the cuvette containing 2.95 ml of 19 mM H_2O_2 solution prepared in potassium phosphate buffer (Claiborne, 1985). The color was read at 240 nm on a Spectronic 601 Spectrophotometer every min for 5 min. Commercially available catalase was used as a standard. Specific activity of the enzyme was expressed as units per milligram protein.

D.2. Glutathione Peroxidase (GSHPx)

GSHPx activity was expressed as nanomoles of reduced nicotinamide adenine dinucleotide phosphate (NADP) converted to oxidized nicotinamide adenine dinucleotide phosphate (NADP) per minute per milligram protein, with a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6 (Paglia and Valentine, 1967). Tissue was homogenized

1:10 in 75 mmol/1 phosphate buffer, pH 7.0. Homogenate was centrifuged at 18000 g for 45 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 then were 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 mmol/L Na_2 EDTA, 100 µl of 3.0 mmol/L NADPH, and 100 µl of cytosolic fraction. The reaction was started by addition of 100 µl of 7.5 mM H_2O_2 and the conversion of NADPH to NADP was monitored by a continuous recording of change of absorbance at 340 nm at 1-min intervals for 5 minute.

D.3. Superoxide Dismutase (SOD)

Supernatant (20,000 g for 20 min) was assayed for SOD activity by following the inhibition of pyragallol autooxidation (Marklund, 1985). Pyrogallol (24 mM) was prepared in 10 mM HCl and kept at 4°C before use. Catalase (30 µM stock solution)was added to Tris HCL buffer containing 25 µl pyragallol and 10 µl catalase. The final 3 ml was made up with the same buffer. Changes in absorbance at 420 nm were recorded at 1-min intervals for 5 min. SOD activity was determined from a standard curve of percentage inhibition of Pyrogallo autooxidation with a known SOD activity. Data were expressed as SOD units per millligram protein as compared with the standard.

D.4. Lipid Peroxidation

Measurement of lipid peroxidation in the myocardium was done by determining thiobarbituric acid reactive substances (TBARS). The assay was performed using a method described previously (Singal and Pierce, 1986). Hearts were quickly excised and washed.

After removing the atria, extraneous fat and connective tissue, the ventricles 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 in a water bath. A 2 ml aliquot was withdrawn from the incubation mixture and pipetted into a 12 ml Corning culture tube. This was followed by the addition of 2.0 ml of 40% trichloroacetic acid and 1.0 ml of 0.2% thiobarbituric acid (TBA). In order to minimize peroxidation during assay procedure, 100 µl of 2% butylated hydroxy-toluene was added to 50 ml TBA reagent mixture. Tubes were then boiled for 15 minutes and cooled on ice for 20 min. Two ml of 70% trichloroacetic acid were added and tubes were allowed to stand for 20 min, at which time the tubes were centrifuged at 800 g for 20 min. The developed colour in the supernatant was read at 532 nm on a spectrophotometer. Commercially available malondialdehyde was used as the standard.

E. Histopathology

For histological studies, tissues were harvested at different post-treatment durations. Hearts, lungs and liver were excised and fixed in a formalin-calcium solution and processed for paraffin embedding using a routine procedure. The ventricles as well as lungs in paraffin were sectioned transmurally at a thickness of 5 µm. The sections were stained with either hematoxylin and eosin or Massons Trichrome.

F. Proteins and Statistical Analysis

Proteins were determined by the method describe elsewhere (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 ANOVA followed by Bonferroni's test was used to identify differences between groups. Values of $p<0.05$ were considered significant.

V. RESULTS

A. General Observations

Following the administration of monocrotaline (MCT), the animals were observed daily for six weeks for their food and water intake, body weight, general behaviour and presence of any clinical signs of respiratory distress as well as heart failure. There was a nominal decrease in the food of MCT group as well as water consumption after five days as compared to the CONT group. This condition of lower food and water intake in the MCT group was observed during the entire period of study.

Animals in the MCT group demonstrated a persistent growth depression and nominal weight gain. Lower weight gain due to a MCT injection was significant in all MCT-treated rats (Fig. 2). The difference in body weights between MCT-treated and CONT rats became apparent within days and was significant 2 weeks after the MCT treatment. In spite of some gain of body weight in the MCT-treated rats during the post-treatment period, the body weights of these rats remained significantly lower than those in the CONT group.

One week after treatment, the MCT-treated rats showed signs of hypoactivity and fatigue. The most noticeable characteristic of rats in the MCT-treated group was the development of respiratory distress which was apparent one week after MCT treatment as well as engorgement of the jugular vein at three weeks associated with peripheral cyanosis. These signs persisted and aggravated in the following weeks of the post-treatment period.

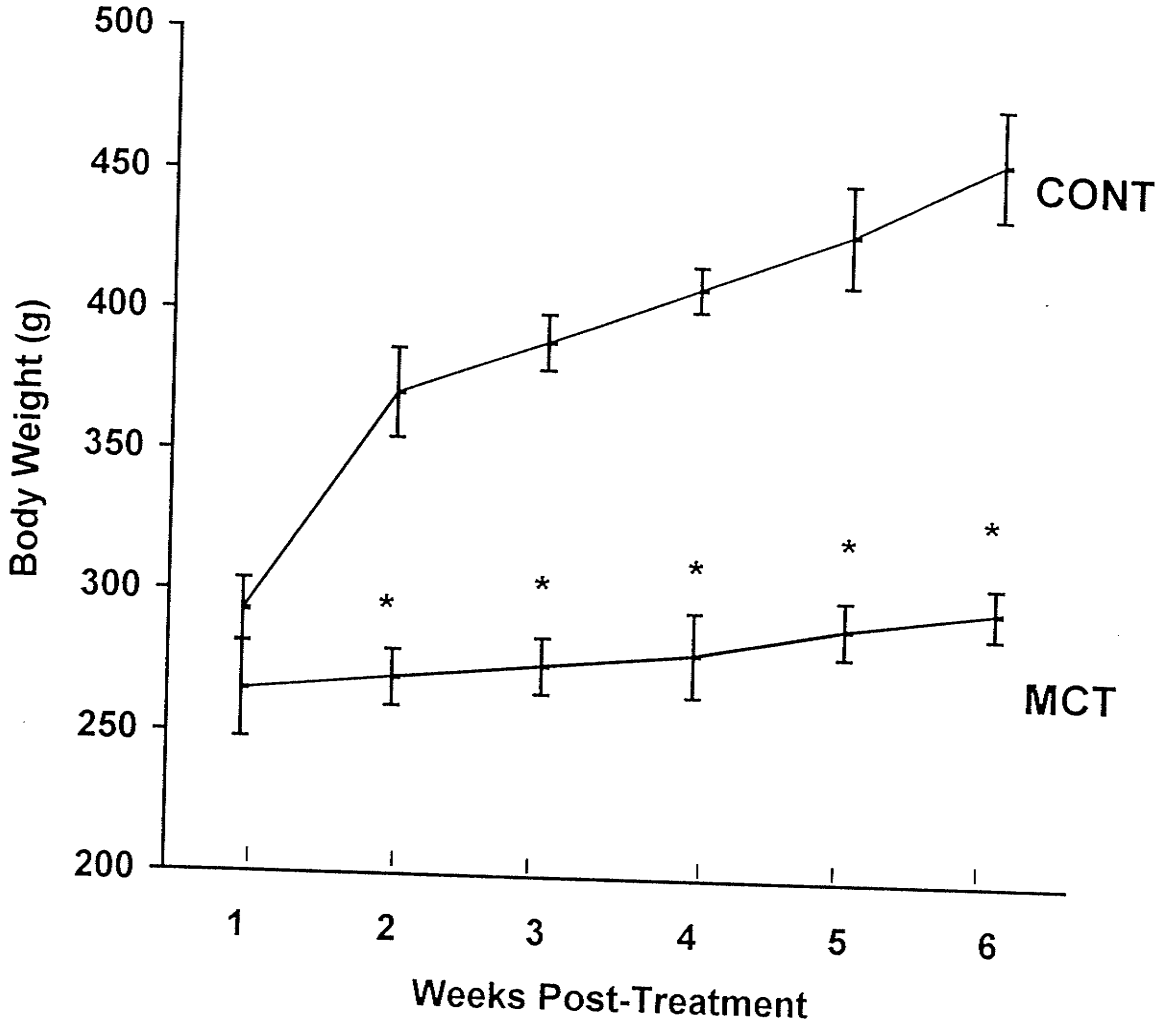

Figure 2: Effects of monocrotaline (MCT) on body weight as compared to controls (CONT). Data presented are mean ± SEM for 6-7 rats. *) $p<0.05$.

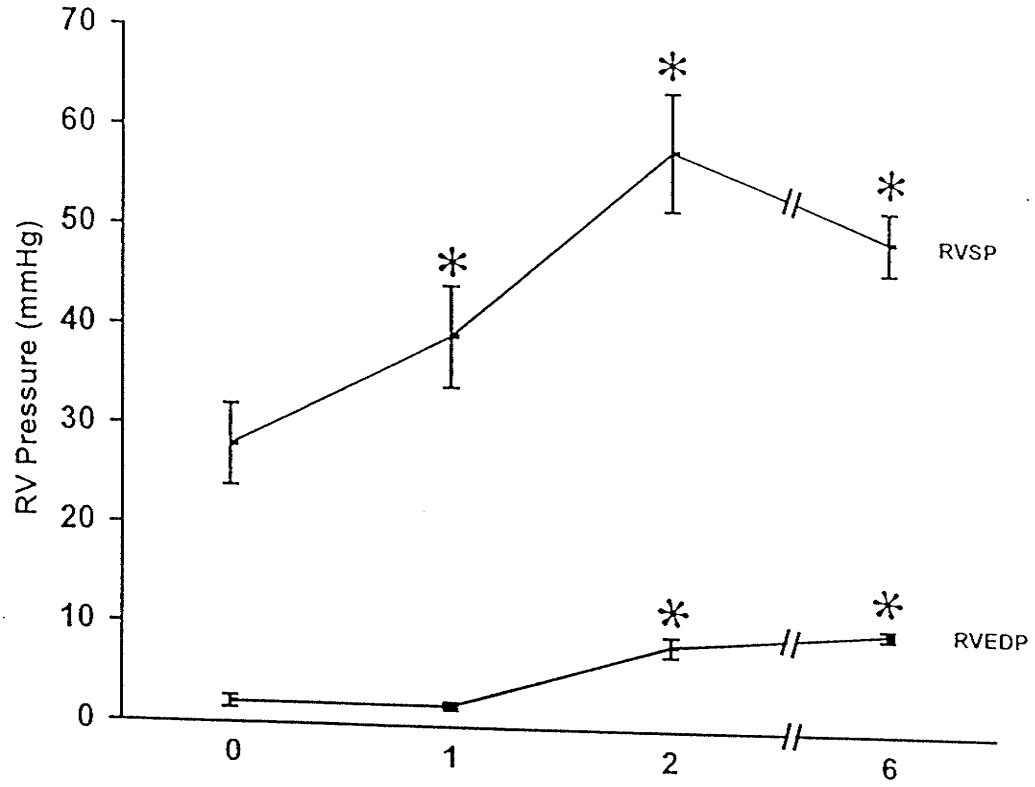

Figure 3: Effects of monocrotaline (MCT) on RVSP and RVEDP in the 1st, 2nd and 6th weeks. Data are mean ± SEM for 5-6 hearts. *) $p<0.05$ compared to CONT.

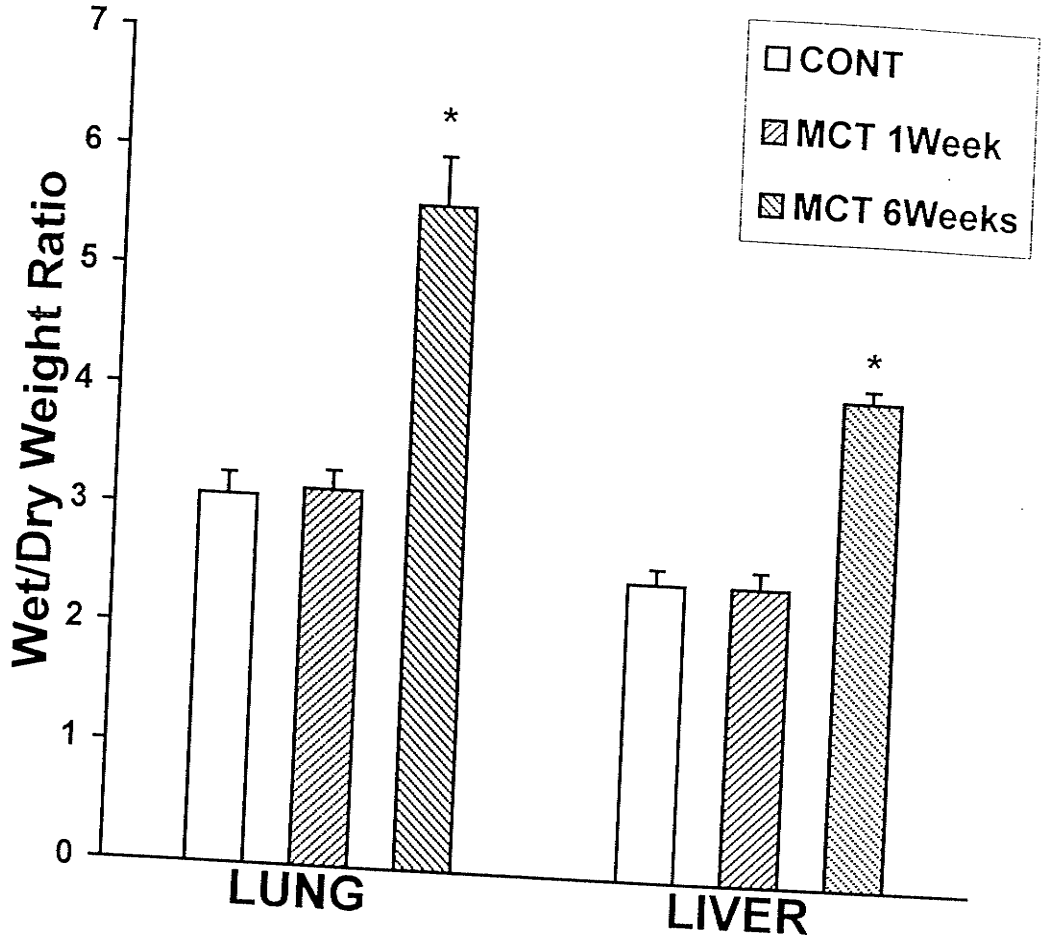

Figure 4: Effects of monocrotaline (MCT) on the lung and liver wet/dry weight ratio in the 1st and 6th weeks. Data presented are mean ± SEM for 6-7 rats. *) $p<0.05$ as compared to the control and 1-week group.

Table 1: Effect of monocrotaline on regional heart weight and body weight ratios.

	Control	MCT		
		1 Week	2 Weeks	6 Weeks
RV/(LV+IVS) mg/mg	0.24 ± 0.03	0.35 ± 0.04	0.39 ± 0.09	0.62 ± 0.06*
RV/BW mg/g	0.54 ± 0.09	1.18 ± 0.10	0.97 ± 0.21*	1.67 ± 0.29*
IVS/BW mg/g	1.10 ± 0.10	1.28 ± 0.12	1.05 ± 0.11*	1.19 ± 0.15*
LV/BW mg/g	1.33 ± 0.15	1.43 ± 0.13	1.39 ± 0.15	1.25 ± 0.21
(LV+IVS)/BW mg/g	2.36 ± 0.12	2.56 ± 0.16	2.42 ± 0.16	2.19 ± 0.22

MCT, monocrotaline (60 mg/kg). Data are mean ± 5-6 animals. *) $p<0.05$ compared to control. There was not significant change within six weeks in these parameters.

compared to their relative control. There was no significant change in LV weight or LV to body weight ratio in the MCT group in comparison with CONT group.

E. Myocardial Endogenous Antioxidant Enzymes

The endogenous antioxidant enzyme activities, glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase were examined in hearts at 1, 2 and 6 weeks post-MCT injection and compared with their respective controls. These data are shown in Fig. 5.

E.1. Glutathione Peroxidase (GSHPx)

At 1 week of MCT post-treatment period, glutathione peroxidase activity was not changed while in 2 weeks after MCT injection there was a significant increase and at 6 weeks there was a significant decrease in GSHPx activity compared to the CONT group.

E.2. Catalase

MCT administration caused a significant increase in catalase activity in the 2-week MCT group compared to the CONT group, while in 6 weeks a significant decrease was observed in the MCT group.

E.3. Superoxide Dismutase (SOD)

The activity of superoxide dismutase in MCT-treated rats slightly increased in the 2-week group and in the 6-week group there was some decline. These changes, however, were not significantly different in the MCT group compared to the control.

F. Lipid Peroxidation

The amount of lipid peroxidation was determined by evaluating myocardial thiobarbituric acid reactive substances (TBARS) and these data are shown in Fig. 6. At 1

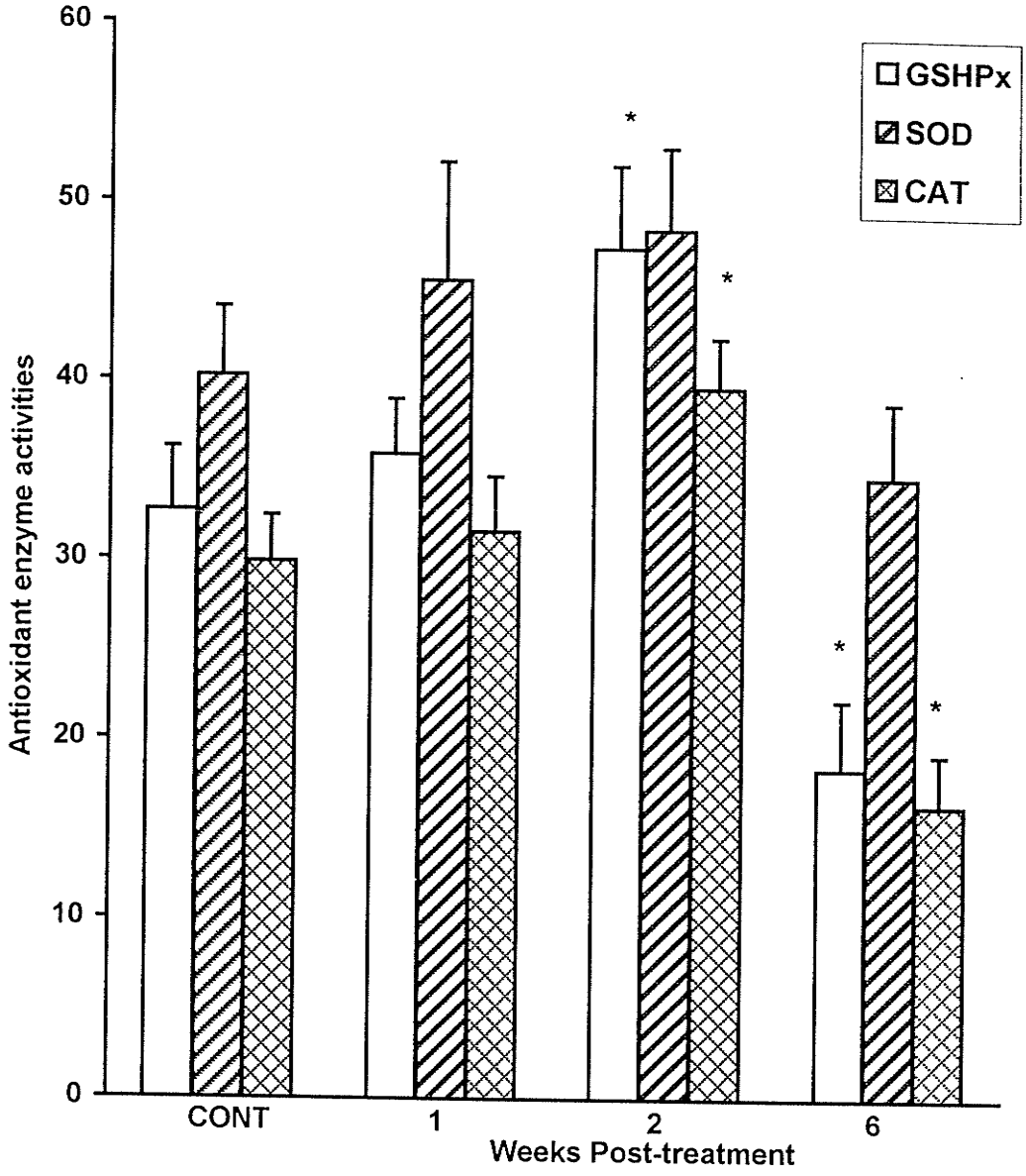

Figure 5: Effects of monocrotaline (MCT) on myocardial glutathione peroxidase (GSHPx, nmol/mg prot), superoxide dismutase (SOD, U/mg prot) and catalase (CAT, U/mg prot) activities. Data presented are mean ± SEM for 6-7 rast. *) $p<0.05$ as compared to the value in the CONT group.

Fig. 6

and 2 weeks, no change was observed in the amount of TBARS in the MCT-treated rats relative to respective controls. At 6 weeks, a significant increase in lipid peroxidation was noticed (Fig. 6).

G. Histopathology

Since significant functional changes were seen at 2 and 6 weeks, a detailed histological examination of different tissues was done at these time points. At 1 week, there no major changes in the gross histology.

G.1. Heart

At 2 weeks, in the MCT group, there was some hypertrophy of the RV wall as well as IVS. The RV cavity was enlarged. There was no change in the LV. The endocardium appeared normal and no lesion was found in the pulmonary valve (Fig. 7). In microscopic sections, muscle fiber branches and anastomosis were normal. The interstices spaces were filled with light staining connective tissue. There was no myocardial degeneration.

At six weeks in the macroscopic section, the RV dilated and the RV wall thickness significantly increased at the pulmonary conus and posterior wall of the RV as compared with that at anterior wall of RV. Leftward displacement of the IVS was quite apparent. Hypertrophy of the IVS was also apparent. There was no significant hypertrophy of the LV macroscopically.

G.2. Lung

At 2 weeks in the MCT group in the macroscopic section, hemorrhagic foci were evident. The lungs were not edematous or congested (Fig. 8). In the microscopic section, no abnormalities were seen in vessels identifiable as veins. The capillaries were congested

CONTROL

2w Treatment

6w Treatment

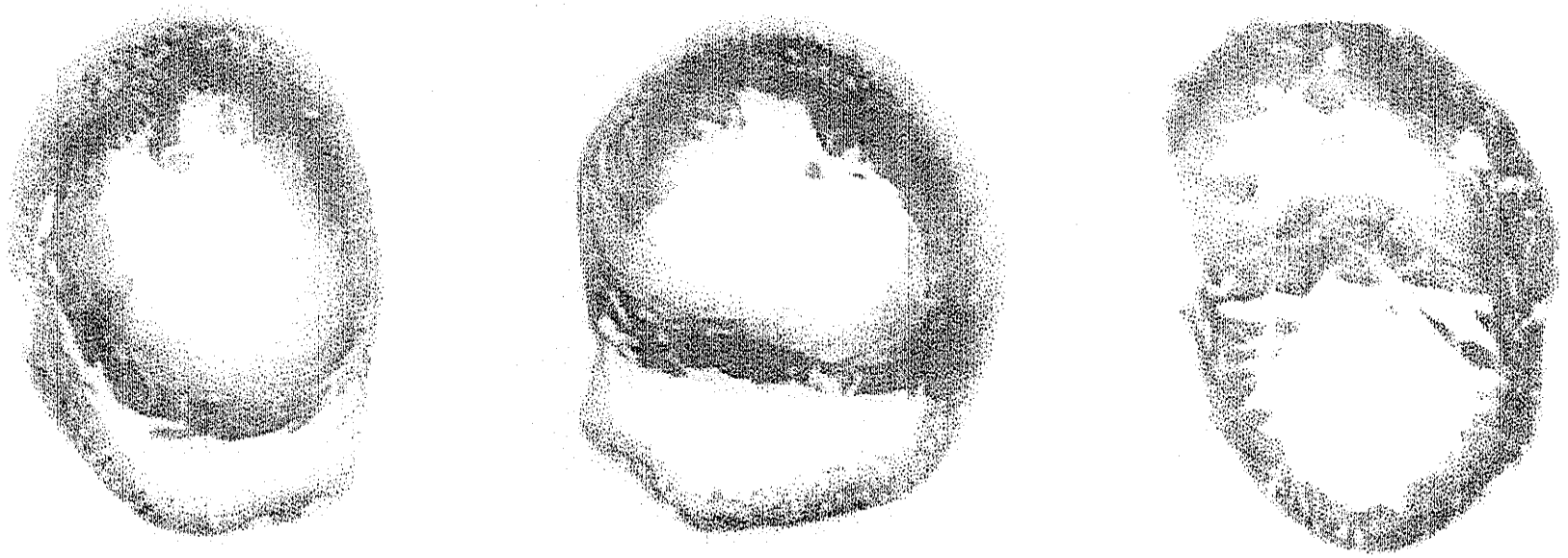

Figure 7

HEART CROSS SECTIONS: From control and 2-week and 6-week MCT-treated animals. At 2 weeks there was right ventricle (RV) and septal (IVS) hypertrophy associated with some dilation and leftward displacement of IVS without any changes in LV free wall. At 6 weeks, there was significant RV hypertrophy and dilation associated with IVS bulging toward the left ventricle.

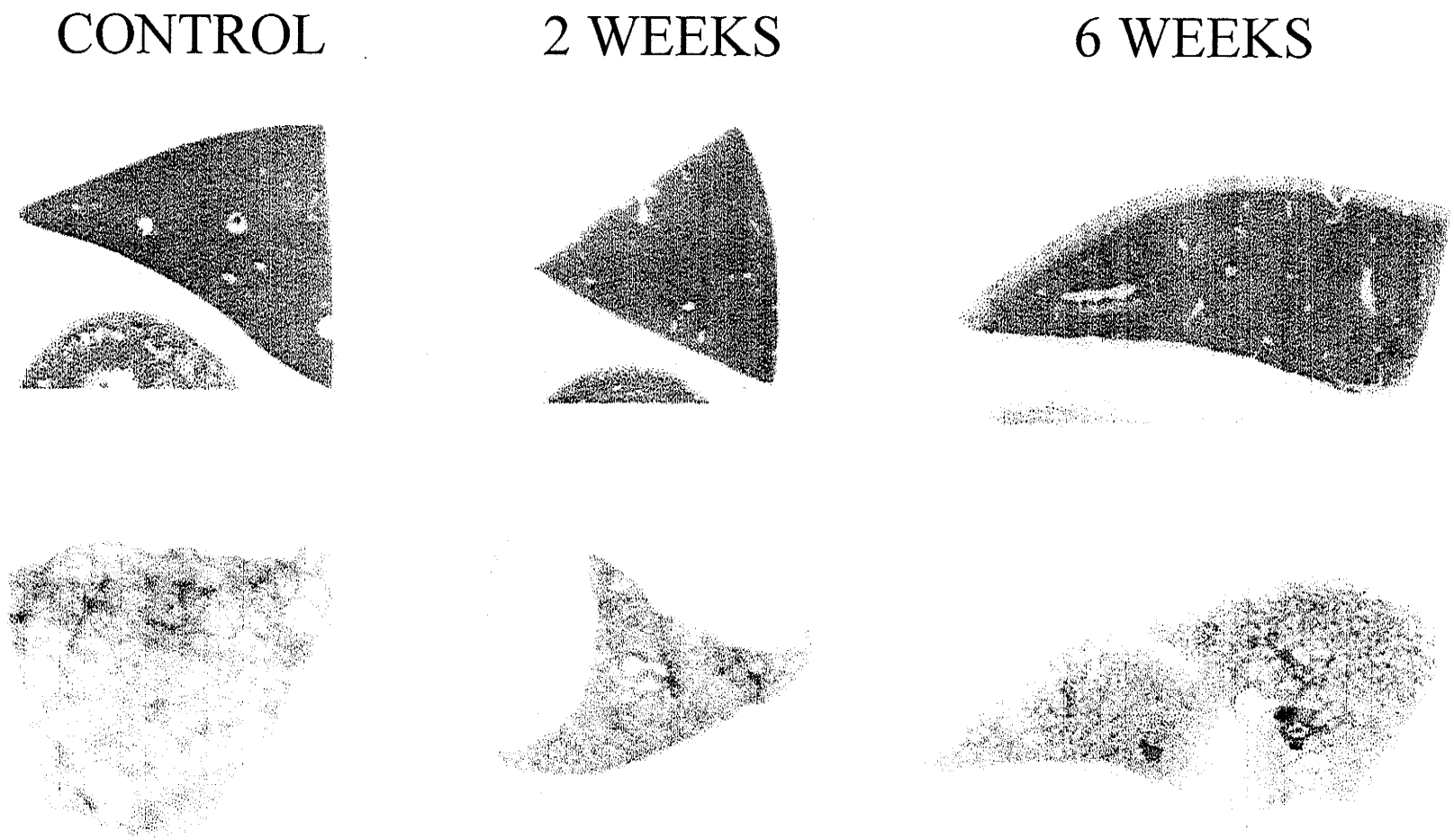

Figure 8

UPPER PANEL: Liver sections from control, 2- and 6-week MCT-treated animals. In control, the liver was not congested and not turgid, while at 6 weeks, the liver was dark in appearance, congested and firm in palpation.

LOWER PANEL: Lung sections. Hemorrhegic foci were evident at 2 weeks. At 6 weeks, the lungs were edematous and congested with multiple foci of hemorrhage.

and in some areas had ruptured into the alveolar sacs where free erythrocytes were present. Alveolar capillary thrombosis associated with edema, hemorrhage and focal necrosis appeared in some areas. An acute inflammatory infiltration surrounded some capillaries. The cells forming the alveolar wall are pleomorphic. The walls of the arteries were thicker than controls. There was no epithelialization of the alveoli and pulmonary arteritis.

At 6 weeks, the lungs in the MCT group were edematous and congested and multifocal, petechial hemorrhages were evident. The most prominent were multifocal, irregularly shaped, red-brown foci of induration. The dorsal, anterior regions of the right caudal lung lobe were affected more severely in comparison with other parts of the lung, although the hilar regions of the right caudal lung lobe were also affected. The vessels of arteriolar caliber were prominent and thickened due to presence of layers of smooth muscle cells. In a few instances, endothelial cell proliferation had resulted in a complete obstruction of the arterioles. Thrombi and intimal lesions caused significant luminal obstruction in damaged arteries.

G.3. Liver

At 2 weeks, the liver was not congested and was not firm in palpation. In the microscopic section, there was no evidence of centrilobular vein occlusions. No focal regeneration was observed (Fig. 8). At six weeks, the liver was dark in appearance, congested and firm in palpation. There was diffuse congestion of parenchyma. Hemorrhage zones of necrosis and infarction were evident. The central vein appeared normal but in some places the wall of the portal vein was necrotic, with an associated thrombosis. The bile duct and arteries were not affected. There was scattered mononuclear infiltration.

VI. DISCUSSION

Heart failure is a serious and growing health problem. The disease accounts for a large number of hospital admissions and in spite of remarkable improvements in therapy, morbidity and mortality due to heart failure continue to increase. Medical costs are also a substantial burden on health budgets of the nations. Clinicians have long appreciated that myocardial dysfunction is a progressive condition. Although the initial clinical event (e.g. MI) may be so mild that there is little or no immediate reduction in overall pump function, there is a relentless deterioration in both the structure and the function of the ventricle which is often referred to as remodelling. Myocardial remodelling is a normal feature during development and a useful adaptation to meet increased physiological demands (e.g.athletic training). However, when it occurs in response to pathologic stimuli (e.g. abnormal wall stresses), though adaptive in the short term, is maladaptive in the long run and often eventuates in further myocardial dysfunction and failure which even in survived patients results in decreased life expectancy, and lowers quality of life (Katz et al., 1994).

Since CHF is a pathophysiologic state that evolves with time, one of the solutions lies in identifying early changes and subcellular pathways that lead to heart failure. This will allow to design specific therapeutic strategies that will moderate the progression of the disease. Although abnormalities of the right heart form a significant percentage of overall cardiac events, its pathophysiology is not understood.

In this study, we focused on the right heart failure and employed a model of chemically-induced RHF using MCT. This model has some strong points and some limitations. The rat model of MCT-induced CPH is a noninvasive, slowly developing,

reproducible and hemodynamically relevant model for pulmonary hypertension which mimics *Cor pulmonale* in a faithful fashion leading to RV hypertrophy and eventually heart failure (Werchan et al., 1989). There are some distinct similarities between the pathophysiology of MCT-induced chronic pulmonary hypertension (CPH) in humans and that seen in the rat model. It mimics many structural and functional features of RV response to CPH in humans (Meyrick et al., 1987). There are also some similarities between the pathophysiologic alterations in adult respiratory distress syndrome in people and MCT-induced pneumotoxicity in rats. Both have two rather distinct phases of pulmonary response. The early phase of adult respiratory distress syndrome (ARDS) and the early period of pneuomotoxicity due to MCT are characterized by an increase in vascular permeability and subsequent pulmonary edema (Plastina & Stoner, 1972). Quite large doses of MCT administered i.v. may produce a severely and rapidly developing pulmonary edema, which like ARDS, is fatal between 1-2 d (Plastina & Stoner, 1972). People with ARDS and rats treated with MCT have endothelial cell lesions and decreased ability to remove biogenic amines from the circulation (Morel et al., 1985).

In the present study, the presence of chronic pulmonary hypertension was indicated clinically by respiratory distress, hemodynamically by an increase in RVEDP and RVSP, and histopathalogically by evidence of MCT pneumatoxicity. Similar findings in MCT-treated rats have been reported before (Werchan et al., 1989). These data as well as congested lung and liver in the late stage of post-MCT period confirmed the existence of the RV failure and decompensated stage of *Cor pulmonale*, subsequent to the failure of cardiac and non-cardiac compensatory mechanisms. An increase in the sympathetic tone in the early stages of failure

becomes inadequate in the later stages (Tong et al., 1991). Ultrastructure damage is massive and refractoriness of the heart failure to therapeutic procedures is suggested to be due to depressed contractile function at the cardiac myofibril level.

In this study, the stages of RV hypertrophy and heart failure were produced at 2 and 6 weeks respectively. Maintenance of RVSP and RVEDP in the 1 week post MCT-treatment indicated sustained RV functioning in these animals. This stability in cardiac performance has been confirmed by a lack of clinical as well as paraclinical signs of heart failure in the MCT group at this point of study. Thus, these animals were considered to be at the non-failure stage and because of the presence of RV hypertrophy they can be considered to be at the early hypertrophic phase of the disease.

At 2 weeks post-MCT treatment, RVEDP and RVSP increased. The absence of lung or liver congestion clinically and paraclinically indicated that this MCT-group had some functional abnormalities without any effect on tissue perfusion. This stage was designed as compensated RV failure. At 6 weeks post-MCT treatment, a decrease in RVSP as well as increased RVEDP was associated with clinical and paraclinical signs of lung and liver congestion including peripheral cyanosis, tacypnea, respiratory distress and an increase in the wet/dry weight ratios of lung and liver. Thus, the 6 week post MCT-treated group was considered to be in a stage of decompensated RV failure. At 2 weeks post MCT period, RV pressure overload was associated with flattening of IVS and at 6 weeks IVS displaced towards LV. The morphological change of the RV free wall and IVS under conditions of RV pressure overload has already been reported (Cohen et al., 1976). Cohen et al. studied patients with moderate to severe pulmonary disease, documented cases of pulmonary

hypertension and demonstrated hypertrophy of RV free wall. Ohsuz et al. (1980) showed that the thickness of the hypertrophied RV wall had a significant correlation with pulmonary artery resistence in patients with RV pressure overload. These findings indicated that the thickness of the RV free wall increased according to the degree of RV pressure overload by the same mechanism as LV wall hypertrophy occurred under LV pressure overload.

This proposed categorization of experimental animals into hypertrophy, compensated and decompensated stags of heart failure, at 1, 2 and 6 weeks respectively, is being adapted here for more objective comparison of the hemodynamic function with the biochemical changes in antioxidant and oxidative stress at each stage.

Shape and motion alterations of IVS under RV pressure overload have been reported by Bemis et al. (1974) in the experiments with the isolated supported canine heart, and by Brinker et al. (1980) with the Muller maneuver physiologically. These reports suggested that increased RV pressure caused a IVS leftward shift. Regarding the mechanism of the diastolic IVS septal disformation, Kingma et al. (1983) noted that the end diastolic RV pressure gradient played an important role in this phenomenon. In primary pulmonary hypertension or congenital heart disease with pulmonary hypertension which involved the chronic RV pressure overloading condition, Louie et al. (1986), after echocardiographic examinations, showed that the IVS septum was flattened.

We found a significant correlation between RVEDP and IVS configuration at different stages of post-MCT treatment. Our study indicated that in post-MCT period at 2 weeks, RV pressure overload was associated with flattening of IVS which with increasing the RV hypertrophy and abnormal increase in RVEDP at 6 weeks shifted towards LV.

Although it has been reported from our lab that the compensated stage of MCT-induced RV failure is associated with an increase in the antioxidant reserve (Pichardo et al., 1999), the present study demonstrates for the first time the antioxidant state of RV myocardium in primary RV dysfunction (not secondary to LV dysfunction), with respect to different stages of RV hypertrophy and decompensated stage of RV failure. Furthermore, the antioxidant changes correlated with the RV dysfunction in the decompensated stage of RV failure.

It should be emphasized that the MCT-treated rat model has some limitations including certain species differences in the pattern of vascular remodelling, lung architecture, and routine aging changes that admix with toxic pyrrolizudine alkaloids and people with chronic pulmonary hypertension (Heath , 1992). Rats treated with MCT lack migration of smooth muscle cells into the vascular intima and the proliferation of these cells within the intimal layer as is typical with chronic pulmonary hypertension.

Another potential pitfall in interpreting results from these animal studies has been that effects of such agents may be protected in an unintended manner by interference with the metabolic bioactivation of MCT to MCTP. Since in our study, definite changes were seen within 2 weeks, there may not have been any problems with bioconversion. A characteristic of the MCT model is the dietary restriction that results in retarded body weight gain, which may provide protection against the pnemotoxic effects (Hayashi et al., 1985). Thus, studies that employ pharmacologic intervention as a tool to explore the mechanisms, but do not show that body weight gain is unaffected by the drug(s) employed, should be interpreted with caution. In our study, the MCT-group gained less weight than controls and the growth rate

of the MCT group, such a finding has been also reported by others (Ceconi et al., 1989 and Comini et al, 1995). This effect is probably due to gastrointestinal system disturbences as well as direct effects of MCT on the liver, where the drug produces venoocclusive disease which may have an adverse effect on the growth.

Significant research has been devoted to understanding the pathogenesis of cardiac dysfunction and progression to overt heart failure. These studies have resulted in several possible mechanisms including abnormalities in the production and the utilization of high energy phosphates, excitation contraction coupling and calcium metabolism defects (Being, 1983), release of vasoactive amines (Bristow et al., 1980), ventricular remodelling (Gaurdron et al., 1993a), free radical formation and lipid peroxidation (Dhalla and Singal, 1994; Siveski-Iliskovic et al., 1994; Belch et al., 1991; McMurray et al., 1990) and apoptosis (Narula et al., 1996).

Oxidative stress, an imbalance between oxidant production and antioxidant defenses in favor of the former, leads to tissue injury and is thought to contribute to the pathophysiology of heart failure in humans (Mc Murray et al., 1993; Diaz-Valez et al., 1996) as well as in experimental animals (Dhalla and Singal, 1996; Hill and Singal, 1996). These studies have included papillary muscle preparations, isolated perfused hearts or septal preparations and *in vivo* whole animal studies. Left ventricular dysfunction has also been induced by ischemia-reperfusion, hypoxia-reoxygenation, induction of adriamycin, aortic handling in intact guinea pigs and coronary ligation to produce infarction. Oxidative stress has been assessed by the direct detection of oxygen free radicals, the measurement of tissue levels of malondialdehyde or thiobarbituric acid reacting substances, detection of altered free

fatty acids content resulting from membrane phospholipid oxidation and assessment of the redox state (reduced glutathione:oxidized glutathione). This ratio is used as an index of oxidative stress. In some studies, the cause and effect relationship between oxidative stress and the LV dysfunction has not been established. That oxidative stress leads to heart failure is strongly supported by the observations: 1) the introduction of exogenously generated oxidant species diminishes myocardial function (Blaustein et al., 1986); 2) the degree of heart failure correlates with a progressive increase in lipid peroxidation (Hill and Singal, 1996); 3) the decrease in oxidative injury associated with supplements of antioxidants correlates with the improved function (Singal et al., 1995); and finally 4) vitamin E slows or prevents increase in the ratio of reduced:oxidized glutathione and less lipid peroxidation in an aortic banding model (Dhalla and Singal, 1996). However, the hemodynamic benefit from vitamin E in ischemia- reperfusion, for example, has not been a universal observation, probably due to differences in experimental design and the dosing schedule for vitamin E (Klein et al., 1993).

In the present study , 1 week after MCT treatment, sustained RV function was accompanied with the maintenance of antioxidants. An increase in myocardial antioxidant status was more clearly evident at 2 weeks post-MCT treatment, indicating reduced oxidative stress in the RV (Kaul et al., 1993). An increase in myocardial redox state has been reported in various conditions affecting the heart and the change is associated with maintained or improved hemodynamic function (Dhalla et al., 1996; Gupta and Singal, 1989).

Decompensated-stage of RV failure was accompanied by significant depression of catalase and GSHPx activity. An increase in oxidative stress at this stage was evidenced by a significant increase in TBARS. An increase in TBARS has been reported in heart failure

secondary to adriamycin toxicity (Siveski-Iliskovic et al., 1994) and chronic pressure overload of the heart (Dhalla and Singal, 1994). These findings suggest that a relative deficit of myocardial enedogenous antioxidant and higher oxidative stress may play a pathophysiologic role in RV failure subsequent to CPH. Furthermore, the increase in oxidative stress precedes the depressed RV malfunction. Thus, these data provide strong evidence of a close correlation between the myocardial antioxidant status and cardiac function/dysfunction in the RV.

Molecular mechanisms for the depressed activities of these antoxidants are not known. Antioxidant enzymes have been shown to be inactivated under oxidative stress (Reddy and Tappel, 1974; Kimball et al., 1976). Sympathectomy (Tolekis and Godin, 1995) and subchronic β-blockade (Khaper et al., 1997) have also been shown to modify myocardial antioxidant enzyme activities. Alterations in antioxidant enzymes under a wide range of physiological and pathological conditions such as age (Nohl and Henger, 1979), exercise (Kanter et al., 1985), β-thalassamia (Gerli et al., 1980), cardiac hypertrophy (Gupta and Singal, 1989; Dhalla and Singal, 1994), heart hypertrophy (Dhalla and Singal, 1994) have been reported.

In our study, RV pressure overload at 2 weeks was associated with significant RV hypertrophy, increased wall thickness and severe pulmonary hypertension indicated by severe MCT pneumotoxicity. It is suggested that the grade of RV pressure overload was the most important factor in the determination of RV diastolic changes, and that RV diastolic impairment was caused by decreased RV compliance due to hypertrophy of the RV free wall under RV pressure overload.

Cardiac lesions in MCT-induced progressive pneumotoxicity occur in response to an increase in workload and are limited to the RV and IVS. RV hypertrophy at 2 weeks was indicated by sustained hemodynamic function and it was associated with an increase in antioxidants. The decompensated stage of RV failure at 6 weeks indicated by increased RVEDP, depressed RVSP and RV dilation coupled with a significant increase in lung and liver wet/dry weight ratio. At this stage, there was a decrease in antioxidants and an increase in oxidative stress.

In conclusion, a gradual worsening of RV function in the early stages of the MCT-induced CPH was accompanied by hypertrophy and an increase in the antioxidant reserve, and in the later stage, RV failure was associated with decreased antioxidant reserve. Thus, the findings suggest a strong correlation between these changes. It is proposed that oxidative stress may play a role in mediating pathogenesis of RV dysfunction and failure.

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