Decreased left ventricular function, myocarditis, and coronary arteriolar medial thickening following monocrotaline administration in adult rats

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Running title: Cardiac effects of monocrotaline in adult rats
ABSTRACT

Decreased right as well as left ventricular function can be associated with pulmonary hypertension (PH). Numerous investigations have examined cardiac function following induction of pulmonary hypertension with monocrotaline (MCT) assuming that MCT has no direct cardiac effect. We tested this assumption by examining left ventricular function and histology of isolated and perfused hearts from MCT-treated rats. Experiments were performed on 50 male Sprague-Dawley rats (348±6 (SD)). Thirty-seven rats received MCT (50 mg/kg s.c.) (MCT group) while the remainder did not (Control group). Three weeks later pulmonary artery pressure was assessed echocardiographically in 20 MCT and 8 Control rats. The hearts were then excised and perfused in the constant pressure Langendorff mode to determine peak left ventricular pressure (LVP), the peak instantaneous rate of pressure increase (+dP/dt\text{max}) and decrease (-dP/dt\text{max}), as well as the rate pressure product (RPP). Histological sections were subsequently examined.

Pulmonary artery pressure was higher in the MCT treated group compared to the Control group (12.9±6 vs. 51±35.3 mmHg (P<0.01)). Left ventricular systolic function and diastolic relaxation were decreased in the MCT group compared to the Control group (+dP/dt\text{max} 4178±388 vs. 2801±503 mmHg/s, LVP 115±11 vs. 83±14 mmHg, RPP 33688±1910 vs. 23541±3858 beats/min•mmHg, -dP/dt\text{max} -3036±247 vs. -2091±389 mmHg/s; P<0.0001). The impairment of cardiac function was associated with myocarditis and coronary arteriolar medial thickening. Similarly depressed ventricular function and inflammatory infiltration was seen in 12 rats 7 days after MCT administration. Our findings appear unrelated to the degree of PH and indicate a direct cardio-toxic effect of MCT.
KEY WORDS

cardiovascular physiology, pyrrolizidine alkaloids, cardiomyopathy, coronary vessels
INTRODUCTION

Pulmonary hypertension represents a serious clinical situation that can occur either as a primary process or secondary to a variety of diseases, conditions and agents (39). Regardless of etiology, all cases of pulmonary hypertension exhibit similar structural vascular remodeling resulting in elevated pulmonary vascular resistance (55). An important aspect of the management of patients with pulmonary hypertension concerns the effects of pulmonary hypertension on cardiac function. Right ventricular dysfunction in the setting of chronic pulmonary hypertension has been well described both clinically and experimentally (8, 33, 48). Decreases in left ventricular systolic and diastolic function have also been reported with pulmonary hypertension (2, 15, 37, 43). Several mechanisms have been proposed to explain such left ventricular effects including alteration in left ventricular geometry due to leftward displacement of the interventricular septum, interstitial edema in the left ventricular wall, decrease in left ventricular preload, increase in myocardial stiffness, diastolic asynchrony of the apical and lateral walls, humoral effects originating in the right ventricle and alterations in intracellular calcium handling (see references in 15, 43).

There have been numerous animal investigations of right and left ventricular function following induction of pulmonary hypertension with the toxin monocrotaline (MCT) (10, 11, 12, 24, 57). The changes in cardiac function following administration of MCT have been attributed to the effects of the associated pulmonary hypertension.

Monocrotaline, derived from the plant *Crotalaria spectabilis*, has been used extensively to produce pulmonary hypertension in rats (51). Monocrotaline is a pyrrolizidine alkaloid that is known to be pneumotoxic as well as hepatotoxic in
mammalian species. A single dose of MCT in the rat results 14 days later in progressive and sustained pulmonary hypertension (41). The pathological features seen following MCT administration are similar to those evident in human primary and secondary pulmonary hypertension. They are characterized histologically by endothelial damage followed by pulmonary vascular remodeling including hypertrophy of medial smooth muscle cells in pulmonary arteries and arterioles and neo-muscularization of non-muscular distal pulmonary arteries (41, 50). In addition, diffuse lung interstitial mononuclear infiltration, hemorrhage and edema are found in the lungs of rats treated with MCT or its bioactive derivative MCT pyrrole (6, 47). Hemodynamically there is evidence of elevated pulmonary vascular resistance, increased pulmonary artery pressure, and subsequent right ventricular hypertrophy (41, 47, 50). These changes are accompanied by increases in right ventricular systolic and diastolic pressures and ultimately by right ventricular failure (9, 19, 27, 52). The severity of MCT-induced pulmonary hypertension appears to be correlated with the extent of the structural changes of the pulmonary vasculature (41). The precise mechanism of MCT action on the pulmonary vascular bed is unknown.

It has been generally assumed that MCT has no effect on the myocardium or coronary vessels per se. Of interest in this regard is seldom-cited older work describing myocarditis and diffuse coronary artery changes following MCT administration in rats (3, 7). There is limited contemporary data on the direct cardiac effects of MCT. For this purpose, we have assessed the left ventricular systolic function and diastolic relaxation in MCT-treated rats in the isolated perfused heart preparation. In addition we have
conducted histological studies of the ventricular myocardium and intra-mural coronary arteries. Our findings indicate that there are direct deleterious cardiac effects of MCT.
METHODS AND MATERIALS

Animals and experimental protocol:

Experiments were conducted on 50 male Sprague-Dawley rats, weighing 348±6g (Charles River; Montreal, Canada). Twenty-five rats were randomly selected to receive an injection of MCT (50 mg/kg) subcutaneously over the dorsum of the neck (MCT group). Thirteen rats did not receive any MCT and acted as controls (Control group). These two sets of animals were observed daily for 3 weeks after assignment to the MCT or Control group for any signs or symptoms of cutaneous, respiratory or cardiac problems. They were weighed weekly. At three weeks pulmonary artery pressure was assessed by echocardiography and left ventricular function studied by isolated heart perfusion. Histological studies were subsequently performed on these hearts as well as on hearts that did not undergo isolated perfusion. In 12 additional rats left ventricular function and cardiac histology were examined 7 days following MCT injection. The experimental protocol was approved by the McGill University Animal Care Committee.

Monocrotaline preparation:

Monocrotaline (Sigma) was dissolved in 1N HCl, neutralized with 1N NaOH, buffered to pH 7.38, and diluted with 0.9% saline to achieve a final concentration of 24mg/ml.

Pulmonary artery pressure:

After light sedation with an intraperitoneal injection of sodium pentobarbital (20 mg/kg) 20 MCT treated and 8 control rats underwent pulsed wave Doppler transthoracic
echocardiographic interrogation in the pulmonary artery with two-dimensional guidance using a commercially available echocardiography machine (Acuson 128XP) and a 7.5 MHz short focus, phased-array transducer. Pulmonary artery acceleration time (AT) was measured from the onset to the peak velocity of forward systolic pulmonary artery flow. Peak systolic pulmonary artery pressure (PAP) was calculated using the equation: 

\[
PAP = 137.2 - 3.3(\text{AT})
\]  

Isolated heart perfusion:

The hearts of the 28 animals studied echocardiographically and those of the 12 rats treated with MCT 7 days earlier were isolated and perfused in the constant pressure Langendorff mode. The rats were anesthetized (total pentobarbital dose 50 mg/kg i.p.), their hearts exposed, chilled with cold saline, and cannulated in situ by way of the aorta. In the rats studied echocardiographically this was done immediately following pulmonary artery pressure determination. The hearts were perfused at a constant pressure of 100 cm H\text{2}O with modified Krebs-Ringer buffer at 37°C consisting (in mM) of 119 NaCl, 4.8 KCl, 1.3 CaCl\text{2}, 1.2 KH\text{2}PO\text{4}, 1.2 MgSO\text{4}, 25 NaHCO\text{3} as well as 15 glucose maintained at 37°C and aerated with 95% O\text{2} + 5% CO\text{2}. Retrograde aortic flow was measured using an ultrasonic flow probe (Transonic). Electrocardiographic recordings were made from three fine needle electrodes inserted into the right ventricle and atria (Powerlab). A small balloon was inserted in the left ventricle by way of the mitral valve. This balloon was connected to a pressure transducer and its volume adjusted to achieve a diastolic pressure of 10 mmHg at the beginning of the experiment and the volume kept constant for the duration of the study. The natural resonant frequency (48 Hz) and the
damping ratio (0.12) of our pressure recording system were determined (22) and found to be within the range required for accurate measurement of left ventricular pressure and its first derivative using fluid filled catheters (18). The pressure signal, retrograde aortic flow signal and electrocardiogram were recorded digitally using Powerlab instrumentation and software. Following ten minutes of stabilization heart rate (HR), peak left ventricular pressure (LVP), the maximum rate of pressure increase (+dP/dt\textsubscript{max}), the maximum rate of pressure decrease (-dP/dt\textsubscript{max}), and the rate–pressure product (RPP; RPP=HR\times LVP) were determined over five cardiac cycles. Completion of the entire procedure required no more than 15 minutes.

**Histology:**

Following the isolated heart perfusion experiments the hearts were placed in 10% buffered formalin. Ten days later the hearts were transversally sectioned at the mid-level of the LV papillary muscles. Sixteen 5\(\mu\)m thick sections per heart were stained with Hematoxylin and Eosin (H&E) or Mason’s trichrome stain. The right ventricular free wall, interventricular septum and left ventricular posterior wall were examined by light microscopy on the H&E slides for myocytes, interstitium, intra-mural coronary arteries and ventricular wall thickness. Intramural coronary artery area (Aa), lumen area (La), wall area (Wa), and arteriolar wall thickness (Wt) were measured in 5 coronary arterioles of the right ventricular free wall, interventricular septum and left ventricular free wall respectively per section from digitized images using ImageJ software (1). Mason’s trichrome stained slides were used to evaluate the extent of collagen deposition. The
hearts of 5 control and 5 rats treated with MCT 21 days earlier which did not undergo isolated heart perfusion were also fixed, stained and examined as described above.

**Statistical analysis:**

All data are expressed as mean ± SD. Comparison of mean values between the groups was performed using an independent t-test. Correlations were analyzed using Pearson’s correlation coefficient. The null hypothesis of no effect was rejected at \( P < 0.05 \).
RESULTS:

Clinical observations:

Monocrotaline-treated rats showed significantly less weight gain than the Control group over a period of 21 days (51±10 g vs. 90±8 g). None of the animals showed signs of illness related to right heart failure such as dyspnea or peripheral edema and none exhibited ascites on direct examination of the abdominal cavity at the time of cardiac excision. There was no mortality in either group.

Pulmonary artery pressure:

Monocrotaline administration caused pulmonary hypertension. Pulmonary arterial systolic pressure estimated from AT (see METHODS) was significantly higher in the MCT treated group compared to the Control group (12.9±6 vs. 51±35.3 mmHg (P<0.01)) (see Figure 1). In 7/20 of the MCT group pulmonary arterial pressure was less than 20 mmHg (10±7), in 5/20 pulmonary arterial pressure was greater than 20 mmHg and less than 60 mmHg (47±7), while in 8/20 pulmonary arterial pressure was greater than 60 mmHg (89±9).

Isolated heart perfusion:

The hearts of 17/20 MCT treated and 8/8 control rats that underwent echocardiography were successfully excised and perfused. We were unable to obtain perfusion data in one animal with PAP less than 20 mmHg, one animal with PAP between 20-60 mmHg, and one animal with PAP greater than 60 mmHg. There was no difference in heart rate between the MCT and Control groups (298±37 vs. 295±21
beats/min). Coronary flow was significantly less in the MCT group compared to the Control group (13.2±1.7 vs. 15.9±1.6 ml/min). Left ventricular systolic function was decreased in the MCT group compared to the Control group with +dP/dt max, LVP, and RPP all significantly less in the MCT group compared to the Control group. Left ventricular diastolic relaxation was also decreased in the MCT rats with -dP/dt max significantly less than in Control group (see Figure 2). The depressed systolic function and diastolic relaxation were unrelated to the degree of pre-existing pulmonary hypertension with no statistical correlation (see Methods) between +dP/dt max, -dP/dt max, LVP or RPP and pulmonary artery pressure level (see Figure 3).

**Histology:**

*Ventricular wall thickness:* The left ventricular posterior wall, inter-ventricular septum and right ventricular free wall in the hearts of the isolated and perfused MCT treated animals were significantly thicker than in the hearts of the isolated and perfused Control group (2.15±0.2 vs 1.7±0.08 mm, P<0.0005; 1.97±0.19 vs 1.72±0.05 mm, P<0.01; 0.85±0.1 vs 0.53±0.06 mm, P<0.0001).

*Myocardium:* Widening and distension of the interstitial space was seen diffusely and to the same extent throughout the myocardium of the isolated and perfused hearts of both groups as has been reported previously in the myocardium of hearts following perfusion with electrolyte solution (58). Examination of right ventricular free wall, inter-ventricular septum, and left ventricular posterior wall in the Control group revealed normal myocytes without evidence of inflammatory cells or collagen deposition. In
contrast, in MCT-treated rats a diffuse interstitial inflammatory infiltration was evident throughout the myocardium (Figure 4). Cell types seen included mononuclear cells (lymphocytes, monocytes, and macrophages) as well as occasional polymorphonuclear cells. In each section, there were 6-8 scattered foci of dense mononuclear and polymorphonuclear cell accumulation predominantly in the left ventricular posterior wall. Myocytes in and around these foci showed degenerative changes, fragmentation, coagulative myocytolysis and necrosis. There was no fibrosis or collagen deposition in these foci. In some hearts, there were small areas of hemorrhage separate from these necrotic sites. In 3 rats, we found features of subendocardial necrosis, coagulative myocytolysis with well-developed fibrosis and extensive collagen deposition in the left ventricular free wall and left ventricular papillary muscles. There was no correlation between the extent or severity of inflammatory infiltration, necrosis, myocytolysis or fibrosis and the severity of pulmonary hypertension or right ventricular free wall thickness.

**Intramural coronary arterioles:** The coronary vessels of the Control group appeared normal. However, in the MCT treated rats a diffuse intramural coronary arteriolar medial thickening and decreased coronary arteriolar luminal area were seen throughout the left ventricular posterior wall, inter-ventricular septum and right ventricular free wall (Figure 5). The external diameter of the intramural coronary arteries and their overall area were similar in the Control group and MCT group. However, the internal diameter in the MCT group was significantly less than in the Control group. Both arterial wall thickness and area in the MCT group were significantly greater than that in the Control group and the
luminal area in the MCT group was markedly less than in the Control group (see Table 1). Examination of the media of the intramural coronary arteries revealed enlarged and hypertrophied smooth muscle cells. The nuclei of these cells were large with 1-2 nucleoli and extended far into the intima sometimes extending all the way to the adventitia. In some cases there was extensive disarray of the smooth muscle cells in the media with intercellular fibrosis and collagen deposition. Similar changes were seen in small conductive arteries (larger than 0.170 mm). There was no correlation between these changes and severity of pulmonary hypertension or right ventricular free wall thickness.

**Histology without prior isolated heart perfusion:** Histological examination of the hearts of 5 MCT treated rats that did not undergo isolated perfusion revealed the same inflammatory and degenerative myocardial changes as well as medial arteriolar thickening as described above without widening and distension of the interstitial space. The myocardium and coronary vessels of 5 control hearts that did not undergo isolated perfusion were normal.

**Left ventricular performance and histology 7 days after MCT administration:**

Similar changes in left ventricular function and myocardial histology were seen in the hearts of the 12 rats treated with MCT 7 days earlier compared to those treated 21 days earlier. Of note is that previous work has shown that pulmonary artery pressure does not increase until at least 10 days following MCT administration in the rat (21,25). Left ventricular systolic function and diastolic relaxation were significantly decreased in the MCT treated group compared to the Control group (LVP 94±22 vs. 115±11 mmHg,
+dP/dt_{max} 3044±713 vs. 4178±388 mmHg/s, RPP 20790±4621 vs. 33688±1910 beats/min•mmHg, -dP/dt_{max} 1613±414 vs. 3035±247 mmHg/s, P<0.05). Interstitial inflammatory infiltration and myocyte degeneration were seen as is described above 21 days following MCT administration (Figure 6). In contrast to our findings 21 days following MCT administration the coronary arterioles appeared normal without evident medial thickening or decreased luminal area compared to the Control hearts (0.007±0.002 vs. 0.006±0.001 mm, 0.004±0.002 vs. 0.005±0.002 mm²).
DISCUSSION

We have found an impairment of left ventricular systolic function and diastolic relaxation in isolated and perfused hearts of adult rats treated with MCT. This impairment of cardiac function is associated with a diffuse myocarditis as well as coronary arteriolar medial thickening. The effects of MCT on cardiac function as well as myocardial and coronary arteriolar histology appear to be independent of the pulmonary hypertension caused by MCT administration. Our results indicate that MCT administration has a significant direct effect on the heart.

There have been previous reports concerning cardiac histological changes following prolonged ingestion of *Crotalaria spectabilis* or its derivative MCT by rats. Blaustein et al. (7) noted that prolonged ingestion of MCT by adult Wistar rats resulted in coronary angiopathy and myocarditis. These authors describe lipid infiltration of the wall of right and left ventricular coronary arteries with some animals exhibiting subintimal or intimal coronary arterial hyperplasia. In addition they show a histological figure demonstrating extensive myocardial destruction with infiltration of monocytes and lymphocytes. It is not clear whether the latter section was taken from the right or left ventricle. The presence of such myocardial changes in the right ventricle could be explained as a consequence of myocardial injury resulting from pulmonary hypertension. It is more difficult to attribute such changes in the left ventricle to pulmonary hypertension. Allen et al. (3) describe the effects of prolonged ingestion by adult Sprague-Dawley rats of *Crotalaria spectabilis* on various organs as well as on multiple levels of the systemic and pulmonary vasculatures. In particular they describe myocardial inflammation, degeneration and fibrosis as well as coronary arterial
endothelial fragmentation and medial disruption “particularly in the right ventricle”. More recent investigations in the rat have noted right ventricular myocardial inflammation, degeneration and fibrosis between 2 and 5 weeks following a single dose of subcutaneous MCT that has been attributed to myocardial injury consequent to pulmonary hypertension (13, 26, 27, 44). In these newer reports coronary vessel morphology is commented on only in the paper of Hirokawa et al. (26) and left ventricular myocardial histology is specifically commented on only in the work of Chen et al. (13). In contrast to earlier work and our own findings Hirokawa et al. (26) found no noticeable changes in the intramural coronary arteries of MCT-treated rats while Chen et al. (13) specifically state that no left ventricular changes were seen after MCT administration. The reason for these discrepancies is unclear.

The cardiac histological changes that we have seen 21 days following a single dose of MCT share features with those that have been described in the lung. The latter have been extensively reviewed by Schultze (51). MCT administration causes pulmonary parenchymal edema and inflammatory cell infiltration. In addition a diffuse pulmonary arterial angiopathy is particularly evident in smaller vessels following MCT administration. An important feature of this angiopathy is medial hypertrophy, hyperplasia and collagen deposition. Of interest is that the increased pulmonary arterial medial wall thickness is not evident in the first week following monocrotaline administration as we have observed here for the coronary arterioles. The precise mechanisms producing these changes are not known.

We have found increased thickness of the right ventricular free wall in rats treated with MCT. This is expected given the pulmonary hypertension produced by such
treatment and is consistent with previous investigations (50). In addition we have found increased inter-ventricular septal and left ventricular posterior wall thickness in the MCT treated rats. Of note is that several prior animal studies have demonstrated septal as well as left ventricular posterior wall hypertrophy associated with right ventricular hypertension produced by pulmonary arterial banding or emphysema (4, 5, 23, 34, 35, 54).

Coronary arteriolar medial muscle thickness was increased and luminal area was decreased on histological examination of the myocardium of the rats treated with MCT 21 days earlier. These structural effects as well as MCT induced depression of endothelial dependent relaxation (45) may have increased coronary arteriolar vasoreactivity and vascular resistance. It is difficult to precisely correlate coronary artery flow with coronary arteriolar cross-sectional area in fixed histological specimens. However, in our experiments coronary flow at the same perfusion pressure was decreased in the isolated hearts of the MCT treated animals compared to the Control group. This effect may have been more marked in vivo and have produced myocardial ischemia leading to some of the myocardial fibrosis observed.

As noted in the INTRODUCTION decreases in left ventricular function have been described in association with pulmonary hypertension. However, it appears that the decrease in left ventricular diastolic relaxation and systolic function that we have observed was independent of increases in pulmonary artery pressure and was more likely due to a direct effect of MCT reflected in the myocardial and coronary artery changes evident histologically. Several finding support this contention. The decrease in left ventricular function in the hearts of rats treated with MCT 21 days earlier was
independent of the pre-existing pulmonary arterial pressure and was evident even in the hearts of animals without pulmonary hypertension or an increase in RV wall thickness. In addition we have observed a decrease in left ventricular function and left ventricular inflammatory changes as early as seven days following MCT administration. In this regard previous investigators have shown that pulmonary hypertension does not develop until after 10 days following MCT administration to rats (21,25). Finally the possibility of a mechanical interaction on the left ventricle of a hypertensive right ventricle was avoided as left ventricular function was measured in an isolated heart model with an empty right ventricle.

Our finding of decreased left ventricular systolic function and relaxation following MCT administration is supported by the recently published work of Lourenco et al (36). These authors have noted lower left ventricular systolic pressure and dP/dt as well as a longer time constant of isovolumetric relaxation (\(\tau\)) in anesthetized rats 6 weeks after MCT administration compared to untreated animals. Although the latter findings could be attributed to the severe pulmonary hypertension in their animals they have also demonstrated a negative force frequency relationship of isolated left ventricular muscle strips indicating an inherently reduced contractile reserve. In contrast to our own work these authors observed no inflammatory changes or increased fibrosis of the left ventricular myocardium. This discrepancy may be due to the small number of MCT treated hearts examined histologically (n=4) by these authors as well as the survival of only less effected rats to six weeks in their work. It is also possible that the absence of inflammatory changes in their work reflects the longer post exposure period of six weeks that could have allowed for resolution of such changes as is seen in viral myocarditis.
These authors also found an increase in endothelin-1 mRNA in the left ventricular myocardium of MCT treated rats with an improvement of the force frequency relationship of \textit{in vitro} muscle strips treated with an endothelin blocker. This is not contradictory to our own work as increased endothelin-1 expression as well as improved contractile function with endothelin antagonism have been demonstrated in the failing left ventricle (42).

The mechanisms of MCT action are not entirely clear. It is known that MCT is metabolized in the liver to bioactive pyrrollic derivatives including monocrotaline pyrrole by P-450 3A (32). Following a single dose of subcutaneous MCT significant concentrations of MCT and/or its derivatives are found in various tissues including the liver, lungs, heart and kidneys (17). Monocrotaline pyrrole has been shown to interfere with normal DNA and protein synthesis (28). Whether these are the cause of the endothelial and inflammatory effects of MCT is not known. Investigation of the time course over which MCT produces pulmonary hypertension indicates that both in anesthetized (21) and awake rats (25) pulmonary artery pressure remains unchanged until at least 10 days following MCT administration with a gradual increase thereafter. Direct measurement of pulmonary artery pressure in anaesthetized rats 21 days following MCT administration by various investigators (14, 25, 40) has shown an elevation in systolic pulmonary artery pressure to a similar degree and with a comparable variability as we have found echocardiographically in lightly sedated rats at the same interval following MCT administration.

As outlined in the Introduction numerous studies have utilized the rat monocrotaline model to study cardiac function in the setting of pulmonary hypertension.
Our work indicates that this model may not be suited to such investigation. While alternative animal models exist they each have their own limitations and disadvantages. A number of investigators have created right ventricular hypertension by placement of a pulmonary artery band in pigs, dogs, sheep, rabbits and rats (16,29,35,46,59). However, this method requires operative intervention and the degree of right ventricular hypertension can be variable. Chronic hypoxia can be used to cause pulmonary hypertension in various mammals (53). However, chronic hypoxia also has effects on cardiac function. For instance chronic hypoxia has been shown to decrease left ventricular adrenergic receptor content (31, 38) while we have recently shown that transient hypoxic exposure in early life decreases the left ventricular response to inotropic stimulation at maturity (49). Rodman and co-workers (56) have reported the development of a transgenic mouse in which the postnatal activation of a bone morphogenic protein receptor (BMPRII) mutation results in increased pulmonary artery muscularization and right ventricular hypertrophy. Although this transgenic model is of interest it remains to be established that there are no associated cardiac effects of the postnatal activation of this mutation.

In conclusion, we have found an impairment of left ventricular systolic function and diastolic relaxation associated with a diffuse myocarditis as well as coronary arteriolar medial thickening that are independent of the severity of pulmonary hypertension and that likely results from a direct toxic effect of MCT on the myocardium. Our results call into question the use of the MCT induced pulmonary hypertension model to study the cardiac consequences of pulmonary hypertension.
GRANTS

Supported by an operating grant from the Canadian Institutes for Health Research (to CVR) and a fellowship from the Montreal Children’s Hospital Research Institute Fellowship (to ELS).
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**FIGURE & TABLE LEGENDS**

Figure 1. Pulmonary artery systolic pressure (PAP) measured echocardiographically in sedated control rats (n=8) and in rats treated with monocrotaline 21 days earlier (n=17). Error bars ±SD, * P<0.01.

Figure 2. Indices of cardiac function in isolated and perfused hearts of control (black columns) and of rats treated with monocrotaline 21 days earlier (grey columns). a) Peak left ventricular pressure (LVP), b) Rate pressure product (RPP), c) Maximum rate of pressure increase (+dP/dt\text{max}), d) Maximum rate of pressure decrease (-dP/dt\text{max}). Error bars ±SD, * P<0.0001.

Figure 3. Indices of left ventricular systolic function and diastolic relaxation determined in isolated and perfused hearts of 17 rats treated with monocrotaline 21 days earlier plotted against pulmonary artery systolic pressure (PAP) measured echocardiographically just prior to cardiac excision. a) Peak left ventricular pressure (LVP), b) Rate pressure product (RPP), c) Maximum rate of pressure increase (+dP/dt\text{max}), d) Maximum rate of pressure decrease (-dP/dt\text{max}). There was no statistical correlation between PAP and LVP, RPP, +dP/dt\text{max}, and -dP/dt\text{max}.

Figure 4. Left ventricular myocardium stained with Hematoxylin and Eosin 3 weeks following monocrotaline administration (a, b, and c) and in a control rat (d). a)
Subendocardial myocardium exhibiting diffuse inflammatory changes. b) A region showing extensive mononuclear infiltration. c) A region showing mononuclear infiltration associated with myocardial cell degeneration. d) Control left ventricular myocardium.

Figure 5. Left ventricular coronary arterioles from: a) Control rat. b) Rat 3 weeks following monocrotaline administration showing medial thickening with smooth muscle hypertrophy and decreased luminal area. Hematoxylin and Eosin stain, calibration bar = 20 µm.

Figure 6. Left ventricular myocardium stained with Hematoxylin and Eosin 1 week following monocrotaline administration showing extensive mononuclear infiltration. Calibration bar = 30 µm.

Table 1. Left ventricular intramural coronary arteriolar dimensions in adult control and monocrotaline treated rats. Measurements are reported in mm or mm² ± SD. Statistical comparison between groups was made by independent t-test.
Figure 1. Pulmonary artery pressure (PAP) measured echocardiographically in sedated control rats (n=8) and in rats treated with monocrotaline 21 days earlier (n=17). Error bars ± SD, * P<0.01.
Figure 2. Indices of cardiac function in isolated and perfused hearts of control rats (black columns) and of rats treated with monocrotaline 21 days earlier (grey columns). a) Peak left ventricular pressure (LVP), b) Rate pressure product (RPP), c) Maximum rate of pressure increase (+dP/dtmax), d) Maximum rate of pressure decrease (-dP/dtmax). Error bars ± SD, * p<0.0001.
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Figure 6. Left ventricular myocardium stained with Hematoxylin and Eosin 1 week following monocrotaline administration showing extensive mononuclear infiltration. Calibration bar = 30 μm.
### Left ventricular intramural coronary arteriolar dimensions

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<th>Control</th>
<th>Monocrotaline</th>
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<td>External diameter (mm)</td>
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