Akhavein F, Jean St-Michel E, Seifert E, Rohlicek CV. Decreased left ventricular function, myocarditis, and coronary arteriolar medial thickening following monochrotaline administration in adult rats. J Appl Physiol 103: 287–295, 2007. First published April 5, 2007; doi:10.1152/japplphysiol.01509.2005.—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 monochrotaline (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 g (SD)]. Thirty-seven rats received MCT (50 mg/kg sc; 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 max) and decrease (−dP/dt 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 with 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 with the Control group (+dP/dt max 4,178 ± 388 vs. 2,801 ± 503 mmHg/s, LVP 115 ± 11 vs. 83 ± 14 mmHg, RPP 33,688 ± 1,910 vs. 23,541 ± 3,858 beats·min⁻¹·mmHg⁻¹, −dP/dt max −3,036 ± 247 vs. −2,091 ± 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 cardiotoxic effect of MCT.
cardiac effects of MCT. For this purpose, we assessed the left ventricular systolic function and diastolic relaxation in MCT-treated rats in the isolated perfused heart preparation. In addition, we conducted histological studies of the ventricular myocardium and intramural 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 ± 6 g (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 wk after assignment to the MCT or Control group for any signs or symptoms of cutaneous, respiratory, or cardiac problems. They were weighed weekly. At 3 wk, PAP was assessed by echocardiography and left ventricular function was 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.

**MCT Preparation**

MCT (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 24 mg/ml.

**PAP**

After light sedation with an intraperitoneal injection of pentobarbital sodium (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 PAP was calculated using the equation: PAP = 137.2–3.3(AT) (30).

**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 ip), 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 PAP determination. The hearts were perfused at a constant pressure of 100 cmH2O with modified Krebs-Ringer buffer at 37°C consisting (in mM) of 119 NaCl, 4.8 KCl, 1.3 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3 as well as 15 glucose maintained at 37°C and aerated with 95% O2 at a constant pressure of 100 cmH2O with modified Krebs-Ringer buffer at 37°C and aerated with 95% O2. Following 10 min of stabilization heart rate (HR), peak left ventricular pressure (LVP), the maximum rate of pressure increase (+dP/dt_max), the maximum rate of pressure decrease (−dP/dt_max), and the rate-pressure product (RPP; RPP = HR·LVP) were determined over five cardiac cycles. Completion of the entire procedure required no more than 15 min.

**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 left ventricle or papillary muscles. Sixteen 5-μm-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, intramural coronary arteries, and ventricular wall thickness. Intramural coronary artery area, lumen area, wall area, and arteriolar wall thickness were measured in five 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 five control and five 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 means ± 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**

MCT-treated rats showed significantly less weight gain than the Control group over a period of 21 days (51 ± 10 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.

**PAP**

MCT administration caused pulmonary hypertension. Pulmonary arterial systolic pressure estimated from AT (see METHODS) was significantly higher in the MCT-treated group compared with the Control group [12.9 ± 6 vs. 51 ± 35.3 mmHg (P < 0.01); see Fig. 1]. In 7 of 20 of the MCT group PAP was <20 mmHg (10 ± 7), in 5 of 20, PAP was >20 mmHg and <60 mmHg (47 ± 7), while in 8 of 20, PAP was >60 mmHg (89 ± 9).

**Isolated Heart Perfusion**

The hearts of 17 of 20 MCT-treated and 8 of 8 control rats that underwent echocardiography were successfully excised and perfused. We were unable to obtain perfusion data in one animal with PAP <20 mmHg, one animal with PAP between 20 and 60 mmHg, and one animal with PAP >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 with 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 with the Control group with \( \frac{dP}{dt_{\text{max}}} \), LVP, and RPP all significantly less in the MCT group compared with the Control group. Left ventricular diastolic relaxation was also decreased in the MCT rats with \( \frac{dP}{dt_{\text{max}}} \) significantly less than in Control group (see Fig. 2). The depressed systolic function and diastolic relaxation were unrelated to the degree of preexisting pulmonary hypertension with no statistical correlation (see METHODS) among \( +\frac{dP}{dt_{\text{max}}} \), \( -\frac{dP}{dt_{\text{max}}} \), LVP, or RPP, and PAP level (see Fig. 3).

**Histology**

_Ventricular wall thickness._ The left ventricular posterior wall, interventricular 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, interventricular 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 (Fig. 4). Cell types seen included mononuclear cells (lymphocytes, monocytes, and macrophages) as well as occasional polymorphonuclear cells. In each section, there were six to eight scattered foci of dense mononuclear and polymorphonuclear cell accumulation predominantly in the left

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**Fig. 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.

**Fig. 2.** Indexes of cardiac function in isolated and perfused hearts of control (solid bars) and of rats treated with monocrotaline 21 days earlier (shaded bars). A: peak left ventricular pressure (LVP); B: rate pressure product (RPP); C: maximum rate of pressure increase (\( +\frac{dP}{dt_{\text{max}}} \)); D: maximum rate of pressure decrease (\( -\frac{dP}{dt_{\text{max}}} \)). Error bars ± SD; *P < 0.0001.
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 three 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, interventricular septum, and right ventricular free wall (Fig. 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 one to two 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 (>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 five MCT-treated rats that did not undergo isolated perfusion revealed the same inflammatory and degenerative myocardial changes as described above without widening and distension of the interstitial space. The myocardium and coronary vessels of five 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 with those treated 21 days earlier. Of note is that previous work showed that PAP 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 with the Control group (LVP 94 ± 22 vs.
115 ± 11 mmHg, +dP/dt max 3,044 ± 713 vs. 4,178 ± 388 mmHg/s, RPP 20,790 ± 4,621 vs. 33,688 ± 1,910 beats·min⁻¹·mmHg⁻¹, −dP/dt max 1,613 ± 414 vs. 3,035 ± 247 mmHg/s, P < 0.05). Interstitial inflammatory infiltration and myocyte degeneration were seen as described above 21 days following MCT administration (Fig. 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 with 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 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 myocardi
d 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 and Carstens (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 noted right ventricular myocardial inflammation, degeneration, and fibrosis between 2 and 5 wk following a single dose of subcutaneous MCT 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 and Roth (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 MCT administration as we have observed here for the coronary arterioles. The precise mechanisms producing these changes are not known.

We 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 found increased interventricular septal and left ventricular posterior wall thickness in the MCT-treated rats. Of note is that several prior animal studies 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

Table 1. Left ventricular intramural coronary arteriolar dimensions in adult control and monocrotaline-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Monocrotaline</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>External diameter, mm</td>
<td>0.132±0.02</td>
<td>0.119±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Overall area, mm²</td>
<td>0.008±0.002</td>
<td>0.008±0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Internal diameter, mm</td>
<td>0.117±0.02</td>
<td>0.070±0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td>0.006±0.001</td>
<td>0.018±0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wall area, mm²</td>
<td>0.002±0.0006</td>
<td>0.004±0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lumenal area, mm²</td>
<td>0.005±0.002</td>
<td>0.003±0.002</td>
<td>&lt;0.0003</td>
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</tbody>
</table>

Values are reported as means ± SD in mm or mm². NS, not significant. Statistical comparison between groups was made by independent t-test.

Fig. 5. Left ventricular coronary arterioles from: A: control rat; B: rat 3 wk following monocrotaline administration showing medial thickening with smooth muscle hypertrophy and decreased luminal area. Hematoxylin and eosin stain, calibration bar = 20 μm.

Fig. 6. Left ventricular myocardium stained with hematoxylin and eosin 1 wk following monocrotaline administration showing extensive mononuclear infiltration. Calibration bar = 30 μm.
the myocardium of the rats treated with MCT 21 days earlier. These structural effects as well as MCT-mediated 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 with 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 observed was independent of increases in PAP and was more likely due to a direct effect of MCT reflected in the myocardial and coronary artery changes evident histologically. Several findings support this contention. The decrease in left ventricular function in the hearts of rats treated with MCT 21 days earlier was independent of the preexisting 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 observed a decrease in left ventricular function and left ventricular inflammatory changes as early as 7 days following MCT administration. In this regard previous investigators showed that pulmonary hypertension does not develop until after 10 days following MCT administration. In this regard previous investigators showed 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 Lourenço et al. (36). These authors noted lower left ventricular systolic pressure and dP/dt as well as a longer time constant of isovolumetric relaxation in anesthetized rats 6 wk after MCT administration compared with untreated animals. Although the latter findings could be attributed to the severe pulmonary hypertension in their animals, they 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 affected rats to 6 wk in their work. It is also possible that the absence of inflammatory changes in their work reflects the longer postexposure period of 6 wk that could have allowed for resolution of such changes as is seen in viral myocarditis (20). 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 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 MCT 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). MCT 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), PAP remains unchanged until at least 10 days following MCT administration with a gradual increase thereafter. Direct measurement of PAP in anesthetized rats 21 days following MCT administration by various investigators (14, 25, 40) has shown an elevation in systolic PAP to a similar degree and with a comparable variability as we found echocardiographically in lightly sedated rats at the same interval following MCT administration.

As outlined in the Introduction, numerous studies used the rat MCT 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 (33). 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 recently showed that transient hypoxic exposure in early life decreases the left ventricular response to inotropic stimulation at maturity (49). Rodman and coworkers (56) 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 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**

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