Characterization of right ventricular function after monocrotaline-induced pulmonary hypertension in the intact rat

Marleen H. M. Hessel, Paul Steendijk, Brigit den Adel, Cindy I. Schutte, and Arnoud van der Laarse.

Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands

Submitted 7 April 2006; accepted in final form 22 May 2006

Hessel, Marleen H. M., Paul Steendijk, Brigit den Adel, Cindy I. Schutte, and Arnoud van der Laarse. Characterization of right ventricular function after monocrotaline-induced pulmonary hypertension in the intact rat. Am J Physiol Heart Circ Physiol 291: H2424–H2430, 2006.—We characterized hemodynamics and systolic and diastolic right ventricular (RV) function in relation to structural changes in the rat model of monocrotaline (MCT)-induced pulmonary hypertension. Rats were treated with MCT at 30 mg/kg body wt (MCT30, n = 15) and 80 mg/kg body wt (MCT80, n = 16) to induce compensated RV hypertrophy and RV failure, respectively. Saline-treated rats served as control (Cont, n = 13). After 4 wk, a pressure-conductance catheter was introduced into the RV to assess pressure-volume relations. Subsequently, rats were killed, hearts and lungs were rapidly dissected, and RV, left ventricle (LV), and interventricular septum (IVS) were weighed and analyzed histochromically. RV-to-LV + IVS weight ratio was 0.29 ± 0.05 in Cont, 0.35 ± 0.05 in MCT30, and 0.49 ± 0.10 in MCT80 (P < 0.001 vs. Cont and MCT30) rats, confirming MCT-induced RV hypertrophy. RV ejection fraction was 49 ± 6% in Cont, 40 ± 12% in MCT30 (P < 0.05 vs. Cont), and 26 ± 6% in MCT80 (P < 0.05 vs. Cont and MCT30) rats. In MCT30 rats, cardiac output was maintained, but RV volumes and filling pressures were significantly increased compared with Cont (all P < 0.05), indicating RV remodeling. In MCT80 rats, RV systolic pressure, volumes, and peak wall stress were further increased, and cardiac output was significantly decreased (all P < 0.05). However, RV end-systolic and end-diastolic stiffness were unchanged, consistent with the absence of interstitial fibrosis. MCT-induced pressure overload was associated with a dose-dependent development of RV hypertrophy. The most pronounced response to MCT was an overload-dependent increase of RV end-systolic and end-diastolic volumes, even under nonfailing conditions.

right ventricular hypertrophy; right ventricular failure; pressure-volume relations

MYOCARDIAL HYPERTROPHY is a compensatory mechanism whereby cardiac tissue adapts to increased workload. Depending on the degree or duration of increased workload, ventricular hypertrophy may progress from a compensatory state to impaired systolic or diastolic function and heart failure. This transition is characterized by alterations in extracellular matrix composition, energy metabolism, β-adrenergic responsiveness, myofilament proteins, Ca2+ handling, signal transduction pathways, and gene expression profiles (6, 18, 19, 26, 35, 37, 44, 49).

A model that is frequently used for study of functional, structural, and molecular changes associated with right ventricular (RV) compensated hypertrophy and RV failure is treatment of rats with monocrotaline (MCT), a pyrrolizidine alkaloid (12, 15, 29, 30). MCT selectively injures the vascular endothelium of the lung and induces pulmonary vasculitis (49). Muscularization and hypertrophy of media in pulmonary arteries lead to increased vascular resistance and increased pulmonary arterial pressure (8, 27, 28, 33, 43). MCT-induced pulmonary hypertension is associated with development of compensated RV hypertrophy, which progresses to failure within weeks, depending on the dose of MCT and the age of the animals (6, 13, 48). The functional consequences of MCT treatment have been studied in isolated papillary muscles and trabeculae (19, 20, 46), in the isolated working heart (48), and in vivo by echocardiography (3, 7, 16, 17).

Previous studies have shown selective induction of RV hypertrophy or RV failure after 4 wk of treatment with a low dose (30 mg/kg body wt) and a high dose (80 mg/kg body wt) of MCT, respectively (6). The purpose of this study was to accurately determine RV function in normal rats and rats treated with a low dose (30 mg/kg body wt) or a high dose (80 mg/kg body wt) of MCT in vivo with use of a combined pressure-conductance catheter. RV systolic and diastolic characteristics were quantified in the three groups of rats. The advantages of this technique are the rapid acquisition time of time-dependent pressure and volume data in the RV in vivo and the acquisition of pressure-volume loops during preload alterations to record RV end-systolic and end-diastolic pressure-volume relations (ESPVR and EDPVR) as load-independent measures of intrinsic ventricular function.

MATERIALS AND METHODS

Animal Model

All animals were treated in accordance with the national guidelines and with permission of the Animal Experiments Committee of the Leiden University Medical Center. A total of 44 Wistar male rats (200–250 g body wt; Harlan, Zeist, The Netherlands) was randomly assigned to three groups. The animals received a single subcutaneous injection of MCT (Sigma, Zwijndrecht, The Netherlands) diluted in PBS in a low dose (MCT30, 30 mg/kg body wt, n = 15) or a high dose (MCT80, 80 mg/kg body wt, n = 16). Control (Cont) rats (n = 13) were injected with an equal volume of PBS. The animals were housed, five animals per cage, with a 12:12-h light-dark cycle and an unrestricted food supply. The rats were weighed three times a week. After 4 wk, RV function was assessed by pressure-conductance catheter, and the rats were killed.

Instrumentation. The rats were sedated by inhalation of a mixture of isoflurane (4%) and oxygen. Subsequently, general anesthesia was administered by injection of fentanyl-fluanisone-midazolam (0.25 ml/100 g body wt ip). The mixture consisted of two parts Hypnorm (0.315 mg/ml fentanyl + 10 mg/ml fluanisone; VitalPharma, Maar-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
RV FUNCTION AFTER MCT-INDUCED PULMONARY HYPERTENSION
pared with Cont (both /H11001 increased RV filling pressure (all /H11001 increased RV end-diastolic and end-systolic volumes and in-
maintained, indicating a compensatory state at the expense of peak pressure was significantly increased ( /H11001 rats ( /H11001 rats and was significantly higher in MCT80 rats (+69% compared with Cont and +40% compared with MCT30, both /P < 0.001). Corresponding results were obtained for RV wall thickness (Table 1).

RV Function

RV function was determined by recording pressure-volume loops. Results are summarized in Table 2. In MCT30 rats, RV peak pressure was significantly increased (+30%), whereas RV ejection fraction was significantly reduced (−18%), compared with Cont (both /P < 0.05). CO and stroke volume were maintained, indicating a compensatory state at the expense of increased RV end-diastolic and end-systolic volumes and increased RV filling pressure (all /P < 0.05 vs. Cont).

In MCT80 rats, RV peak pressure was further increased (+69%, /P < 0.05 vs. Cont), consistent with the increased pulmonary arterial elastance, which was twice as high as that in Cont and MCT30 rats (both /P < 0.01). RV ejection fraction decreased further (−47% compared with Cont and −35% compared with MCT30, both /P < 0.05) and CO was significantly reduced by 31% compared with MCT30 rats ( /P < 0.05). RV dilatation was evident from progressive increases of RV end-systolic and end-diastolic volumes (roughly twice the corresponding values of Cont). τ was significantly increased in MCT80 rats compared with Cont and MCT30 rats, indicating prolonged early relaxation. RV peak wall stress of MCT80 rats was increased by 90% ( /P < 0.05) compared with Cont rats and by 29% compared with MCT30 rats.

Table 1. General and cardiac characteristics of Cont, MCT30, and MCT80 animals

<table>
<thead>
<tr>
<th></th>
<th>Cont</th>
<th>MCT30</th>
<th>MCT80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 13)</td>
<td>(n = 15)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>379±31</td>
<td>362±21</td>
<td>300±31†‡</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>1.35±0.13</td>
<td>1.40±0.22</td>
<td>1.44±0.20</td>
</tr>
<tr>
<td>Heart wt/body wt, mg/g</td>
<td>3.53±0.38</td>
<td>3.88±0.47</td>
<td>5.00±1.29‡</td>
</tr>
<tr>
<td>RV wt, g</td>
<td>0.24±0.03</td>
<td>0.29±0.05</td>
<td>0.37±0.08§</td>
</tr>
<tr>
<td>LV wt, g</td>
<td>0.50±0.07</td>
<td>0.50±0.04</td>
<td>0.49±0.07</td>
</tr>
<tr>
<td>IVS wt, g</td>
<td>0.31±0.06</td>
<td>0.30±0.04</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>IVS/(LV + IVS) wt</td>
<td>0.29±0.05</td>
<td>0.35±0.05</td>
<td>0.49±0.10§</td>
</tr>
<tr>
<td>RV wt/body wt, mg/g</td>
<td>0.63±0.01</td>
<td>0.74±0.47</td>
<td>1.24±0.23§</td>
</tr>
<tr>
<td>RV thickness, mm</td>
<td>0.98±0.26</td>
<td>1.26±0.23</td>
<td>1.43±0.41*</td>
</tr>
<tr>
<td>Lang wt, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td>1.32±0.21</td>
<td>1.66±0.77</td>
<td>2.25±0.49†</td>
</tr>
<tr>
<td>Dry</td>
<td>0.27±0.05</td>
<td>0.34±0.15</td>
<td>0.55±0.13§</td>
</tr>
<tr>
<td>Lung dry wt/lung wet</td>
<td>0.20±0.04</td>
<td>0.21±0.04</td>
<td>0.24±0.04</td>
</tr>
<tr>
<td>Lung wet wt/body wt, mg/g</td>
<td>3.47±0.44</td>
<td>4.58±2.02</td>
<td>7.08±2.62‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; /n, number of rats. Cont, control; MCT30 and MCT80, monocrotaline at 30 and 80 mg/kg body wt; RV, right ventricle; LV, left ventricle; IVS, interventricular septum. * /P < 0.01; † /P < 0.001 vs. Cont. ‡ /P < 0.05; § /P < 0.001 vs. MCT30.

Table 2. Cardiac and RV function of Cont, MCT30, and MCT80 animals

<table>
<thead>
<tr>
<th></th>
<th>Cont</th>
<th>MCT30</th>
<th>MCT80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 15)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>399±30</td>
<td>380±48</td>
<td>320±85†‡</td>
</tr>
<tr>
<td>SV, μl</td>
<td>208±74</td>
<td>237±82</td>
<td>196±66</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>82±27</td>
<td>89±27</td>
<td>61±222</td>
</tr>
<tr>
<td>VED, μl</td>
<td>245±92</td>
<td>441±270*</td>
<td>648±325*</td>
</tr>
<tr>
<td>VES, μl</td>
<td>431±152</td>
<td>649±323*</td>
<td>806±368*</td>
</tr>
<tr>
<td>Pp, mmHg</td>
<td>33±6</td>
<td>42±15*</td>
<td>55±20*</td>
</tr>
<tr>
<td>Peds, mmHg</td>
<td>29±7</td>
<td>35±14</td>
<td>50±17*‡</td>
</tr>
<tr>
<td>Pw max, mmHg/s</td>
<td>2.709±790</td>
<td>2.473±776</td>
<td>2.332±903</td>
</tr>
<tr>
<td>Pw max, mmHg/s</td>
<td>1.668±428</td>
<td>1.781±675</td>
<td>1.959±881</td>
</tr>
<tr>
<td>Ees, %</td>
<td>49±6</td>
<td>40±12*</td>
<td>26±6*‡</td>
</tr>
<tr>
<td>SW, mmHg demanding</td>
<td>5.381±2.323</td>
<td>7.384±4.423</td>
<td>7.901±3.189*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>14±4</td>
<td>12±3</td>
<td>27±12*‡</td>
</tr>
<tr>
<td>Ees, mmHg demanding</td>
<td>0.15±0.06</td>
<td>0.16±0.05</td>
<td>0.28±0.15§</td>
</tr>
<tr>
<td>WsDemand, mmHg</td>
<td>144±53</td>
<td>213±112</td>
<td>274±111*</td>
</tr>
<tr>
<td>WsDemand, mmHg</td>
<td>14±11</td>
<td>36±26</td>
<td>31±16</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; SV, stroke volume; CO, cardiac output; VED, RV end-systolic volume; VES, RV end-diastolic volume; Pp, RV peak pressure; Peds, RV end-systolic pressure; Pw max, RV end-diastolic pressure; EF, RV ejection fraction; SW, RV stroke work; dPw max, maximal rate of pressure increase; dPw max, maximal rate of pressure decline; τ, relaxation time constant; Ees, effective arterial elastance; WsDemand, RV peak wall stress; WsDemand, RV end-diastolic wall stress. * /P < 0.05; † /P < 0.01 vs. Cont. ‡ /P < 0.05; § /P < 0.01 vs. MCT30.
that in Cont rats (data not shown). Perivascular fibrosis in MCT-treated rats did not differ from interstitial or replacement fibrosis in MCT-treated rats. Similarly, cant differences between groups (Fig. 4), indicating no inter-

sis. Quantification of volume percentages showed no signifi-

ance, only the shift in the EDPVR for the MCT80 group 

despite the clear trends, because of large within-group vari-

ability, even more pronounced in the MCT80 rats (Fig. 3). However, despite the clear trends, because of large within-group variability, only the shift in the EDPVR for the MCT80 group reached statistical significance.

Myocardial Fibrosis

RV myocardial sections were stained with sirius red for assessment of interstitial, perivascular, and replacement fibrosis. Quantification of volume percentages showed no significant differences between groups (Fig. 4), indicating no interstitial or replacement fibrosis in MCT-treated rats. Similarly, perivascular fibrosis in MCT-treated rats did not differ from that in Cont rats (data not shown).

Pressure-Volume Relations

Slopes of ESPVR and EDPVR, $E_{ES}$ and $E_{ED}$, were not significantly different between Cont, MCT30, and MCT80 rats (Table 3). To correct for the influence of RV muscle mass, we also performed a univariate ANOVA with RV muscle mass as a cofactor; the results did not show significant differences between groups for the normalized parameters. Both relations were shifted toward larger volumes in MCT30 rats and were significantly different between Cont, MCT30, and MCT80 rats.

Myocardial Fibrosis

RV myocardial sections were stained with sirius red for assessment of interstitial, perivascular, and replacement fibrosis. Quantification of volume percentages showed no significant differences between groups (Fig. 4), indicating no interstitial or replacement fibrosis in MCT-treated rats. Similarly, perivascular fibrosis in MCT-treated rats did not differ from that in Cont rats (data not shown).

Valves are months $\pm$ SD. $E_{ES}$, end-systolic elastance; $E_{ES}^N$, normalized $E_{ES}$; $E_{ED}$, end-diastolic stiffness; $E_{ED}^N$, normalized $E_{ED}$; $V_{ES,37}$, end-systolic volume at 37 mmHg mean end-systolic pressure (position of end-systolic pressure-volume relation); $V_{ED,5}$, end-diastolic volume at 5 mmHg mean end-diastolic pressure (position of end-diastolic pressure-volume relation). $E_{ES}^N$ and $E_{ED}^N$ were obtained by adding RV muscle mass as a cofactor in univariate analysis of variance and evaluating $E_{ES}$ and $E_{ED}$ in the 3 groups at a common RV muscle mass (440 mg). *$P < 0.05$ vs. Cont. †$P < 0.05$ vs. MCT30.

Relations Between RV Performance and Myocardial Characteristics

In studies on the cellular/molecular transition from compensated hypertrophy to failure using the MCT model, MCT is supposed to induce a dose-dependent increase in pulmonary vascular resistance with subsequent RV pressure overload and increased wall stress, leading to compensatory hypertrophy and, ultimately, RV dilatation and pump failure. However, this process has not been studied in the intact animal using detailed invasive hemodynamic measurements. Data reported in Tables 1–3 generally support these assumptions but also show substantial variability in the various parameters within groups. To provide a better insight into the underlying mechanisms, we investigated the relations between the various indexes of RV pressure overload, hypertrophy, wall stress, and RV function.

Figure 5A shows RV hypertrophy as a function of peak RV pressure. The significant overall correlation supports the concept of gradual pressure overload-induced RV hypertrophy. The functional consequences are reflected in Fig. 5B, which shows a gradual decrease in ejection fraction, with the lowest values in the MCT80 group, despite substantially higher hypertrophy in this group. The drop in ejection fraction is caused largely by increased end-diastolic volume, because stroke volume was reasonably maintained (Table 2). However, we did not find a significant correlation between RV end-diastolic volume and severity of hypertrophy. Finally, Fig. 5C shows...
that this progressive dilatation causes a gradual increase in wall stress, with the highest values in the MCT80 group, despite the compensatory increase in muscle mass, illustrating decompensation in these animals. The significant negative correlation within the MCT80 group indicates that the MCT80 animals showing the highest RV hypertrophy had the least wall stress. Therefore, it appears that at least part of the variability within the MCT80 group is caused by the ability to adequately increase RV weight, thereby opposing RV dilatation and limiting excessive RV wall stress.

**DISCUSSION**

This study was conducted to characterize changes in RV structure and function after MCT treatment in the intact rat model. Apart from RV hypertrophy, the primary response to MCT treatment was RV dilatation, i.e., increases of RV end-systolic and end-diastolic volumes and, consequently, decreases of RV ejection fraction.

The dose-dependent RV hypertrophy strongly correlated with MCT-induced pressure overload, but, despite this increased muscle mass, RV wall stress gradually increased, ultimately leading to RV decompensation. Interestingly, RV $E_{ES}$ and end-diastolic stiffness did not change significantly, even when we corrected for myocardial muscle mass, suggesting that intrinsic myocardial function was not importantly altered. The unchanged diastolic stiffness was consistent with the absence of changes in fibrosis and the fact that filling pressures remained relatively normal. However, ESPVR and EDPVR showed a tendency to be shifted toward larger volumes, suggesting myocyte slippage as a potential mechanism for dilatation. In addition, early active relaxation, as reflected by $\tau$, was severely depressed in the MCT80 group, consistent with severe RV hypertrophy. Moreover, a recent study demonstrated reduced protein levels of sarcoplasmic reticulum Ca$^{2+}$-ATPase in the RV of MCT-treated animals, which should result in a slower decline of intracellular Ca$^{2+}$ concentration during relaxation and may partially explain the prolonged $\tau$ (19).

In studies in which a single dose of 40–60 mg MCT/kg body wt was administered, up to 50% of the animals progressed to RV failure after 4 wk, whereas the remaining animals developed stable compensatory RV hypertrophy without signs of heart failure (5, 16, 36, 47). The failing animals showed a significantly greater degree of RV hypertrophy, reflecting a higher pulmonary arterial pressure (16, 47). Recently, selective induction of compensated RV hypertrophy or RV failure after 4 wk was demonstrated by using a low dose (30 mg/kg body wt) or a high dose (80 mg/kg body wt) of MCT, respectively (6). In the present study, MCT30 rats developed a phenotype without clinical signs of cardiac failure, whereas MCT80 rats showed progressive body weight loss (cachexia), an increased respiratory rate, and a lack of physical activity starting at day 25. In this latter group, hemodynamic data could be obtained in only 9 of the 16 animals. The remaining seven animals died shortly before the measurements ($n = 2$), during induction of anesthesia ($n = 2$), or during instrumentation ($n = 3$), presumably due to very poor cardiac function. In the MCT30 group, no animals died before or during the hemodynamic studies, whereas one animal in the Cont group died during the instrumentation phase. Thus the hemodynamic data of the MCT80 group reported in our study probably reflect the animals with relatively preserved cardiac function, which may partly explain the substantial overlap of functional parameters between the groups.

To our knowledge, this study is the first to evaluate dose-dependent effects of MCT injection using invasive RV hemodynamics in the intact rat model. Previous studies in MCT-treated animals in vivo have used echocardiography and
showed progressive development of pulmonary hypertension and RV hypertrophy consistent with our findings (3, 7, 16, 17), but no volumetric data have been reported. More detailed functional parameters were obtained by Werchan et al. (48) in the model of the isolated perfused heart with working RV: increased RV peak systolic pressures were found 3 wk after MCT injection (60 mg/kg body wt), but considerable variability was present. Consistent with our findings, the authors reported significantly increased RV systolic pressure only for animals with severe RV hypertrophy (>0.5), whereas +dP/dt max and dP/dt min were unchanged with mild hypertrophy and increased with severe hypertrophy. Similar to our study, diastolic RV pressure was not importantly altered.

In vitro studies with RV papillary muscles and trabeculae isolated from rats 3 and 4 wk after MCT treatment (40 mg/kg body wt) demonstrated a negative force-frequency relation and reduced maximum force compared with controls (20, 46).

Interestingly, our findings indicate substantial differences in RV function adaptation between the MCT model and the pulmonary artery banding (PAB) model, which is frequently used in RV pressure-overload research (4, 9, 14, 22, 23, 26, 31). Depending on the degree/severity of pulmonary artery stenosis, most PAB studies report an almost twofold increase in RV muscle mass, comparable to our findings, but despite the even higher RV systolic pressures in most PAB studies, generally no clear signs of cardiac failure and almost no RV dilatation have been reported (14, 22, 23, 26). A recent study in rats with instrumentation similar to that used in the present study showed that 6 wk of PAB resulted in an RV systolic pressure of ~60 mmHg and a twofold increase in RV mass, comparable to our study. However, the PAB rats had no signs of heart failure, unchanged systemic hemodynamics and RV volumes, and increased RV contractility (Ees) (10). A comparison with the MCT-treated rats in our study suggests that effects of pressure overload and the mechanisms underlying contractility and RV dilatation are substantially different between the two models. These differences could be related to model-specific changes of the β-adrenergic receptor system. Rats with MCT-induced RV hypertrophy showed a reduction in RV β-adrenoceptor density due to a downregulation of the β1-adrenoceptor and showed β-adrenoceptor-G protein-adenyl cyclase system desensitization (24, 25). This reduced β-adrenoceptor responsiveness may explain why the hypertrophied RV cannot increase contractility, which is necessary to maintain cardiac function in MCT-induced pulmonary hypertension. In contrast, animals with pulmonary hypertension induced by PAB showed a two- to threefold increase of RV contractility in the absence of a changed β-adrenoceptor system (10, 21).

We hypothesize that MCT-induced RV remodeling is caused by cardiomyocyte slippage due to diminished adhesion of cardiomyocytes to the extracellular matrix (11, 51). However, future studies are needed to investigate this hypothesis. An important issue for clinicians caring for patients with pulmonary hypertension is determination of the optimal timing for surgical or catheter-based interventions. Studies indicate that RV failure may develop in patients with pulmonary hypertensive heart diseases, even in the absence of impaired contractility (40). Although we did not perform a longitudinal study, our findings suggest that cardiac failure was preceded by gradual cardiac dilatation and increased wall stress, despite myocardial hypertrophy. The animals failed to adequately increase contractility and showed prolonged relaxation, ultimately leading to a decrease in CO. MCT-induced pulmonary hypertension is a useful animal model for study of the transition from compensatory RV hypertrophy to RV failure, which has important clinical relevance.

In conclusion, we have characterized the chronic effects of MCT injection on myocardial structure, RV function, and hemodynamics in the intact rat model. Our findings show a dose-dependent increase in RV systolic pressure, RV myocardial mass, and RV volumes. In high-dose MCT, this leads to significantly elevated wall stress, severely reduced ejection fraction, and decreased pump function. Future studies are required to investigate the molecular mechanisms.

**REFERENCES**


