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

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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, Ca^{2+} 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.

Hemodynamic Measurements

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-
heze, The Netherlands), one part Dormicum (5 mg/ml midazolam; Roche, Mijdrecht, The Netherlands), and one part saline. The animals were placed on a controlled warming pad to keep body temperature constant. A tracheotomy was performed, a 25-gauge cannula was inserted, and the animals were mechanically ventilated using a pressure-controlled respirator and a mixture of air and oxygen. The animals were placed under a stereomicroscope (Zeiss, Hamburg, Germany), and a midline cervical incision was made for cannulation of the left jugular vein for infusion of hypertonic saline (10%) to determine parallel conductance (2). A midsternal thoracotomy was performed, and a combined pressure-conductance catheter (model SPR-878, Millar Instruments, Houston, TX) was introduced via the apex into the RV and positioned along the long axis of the RV. The catheter was connected to a Sigma signal processor (CD Leycom, Zoetermeer, The Netherlands), and RV pressures and volumes were recorded digitally. All data were acquired using Conduct-NT software (CD Leycom) at a sample rate of 2,000 Hz and analyzed offline by custom-made software.

**Calibration of the conductance catheter.** The volume signal of the conductance catheter was calibrated for parallel conductance and slope factor (α). The parallel conductance volume was estimated by the hypertonic saline technique (2): a 20-μl bolus of hypertonic saline (10%) was injected via the cannula in the jugular vein, and parallel conductance was calculated as previously described (38, 50). α was determined by measurement of the aortic flow using an ultrasonic flow probe (Transonic Systems, Maastricht, The Netherlands) around the ascending aorta; α was calculated as the cardiac output (CO) determined by the uncalibrated conductance catheter divided by the CO determined by aortic flow measurement.

**Hemodynamics.** RV pressure and volume signals were recorded to quantify general hemodynamic conditions. Heart rate, stroke volume, CO, RV end-diastolic volume, RV end-systolic volume, RV ejection fraction, RV end-diastolic pressure, RV peak systolic pressure, and RV end-systolic pressure were determined from pressure-volume loops. Stroke work was obtained as the area of the pressure-volume loop, and the maximal rates of RV pressure upstroke and fall (dP/dt max and dP/dt min, respectively) were calculated. The relaxation time constant (τ) was assessed as the time constant of monoexponential decay of RV pressure during isovolumic relaxation (2, 23). Effective pulmonary arterial elastance, as a measure of RV afterload, was calculated as end-systolic pressure divided by stroke volume.

**RV ESPVR and EDPVR.** RV ESPVR and EDPVR were determined from pressure-volume loops recorded during transient occlusion of the inferior vena cava by external compression of the vessel. Slopes and intercepts of these relations are considered relatively load-independent parameters of intrinsic myocardial function (34, 39, 41, 45). The slopes, end-systolic and end-diastolic elastance (Ees and Edemax, respectively), were determined by linear regression. The positions of the pressure-volume relations were quantified by calculation of the intercepts of the relations at an end-systolic pressure of 37 mmHg and an end-diastolic pressure of 5 mmHg. These pressure values were determined retrospectively as the mean end-systolic and end-diastolic pressures of all animals in the study.

**RV end-systolic, end-diastolic, and peak wall stress.** Time-varying RV wall stress [Wsi(t)] was calculated from RV cavity pressure and volume [P(t) and V(t), respectively] and RV wall volume (Vwall) according to the following formula: Wsi(t) = P(t) [1 + 3(V(t)/Vwall)] (1). RV wall volume was approximated as the sum of the RV free wall mass and 0.5 times the mass of the interventricular septum (IVS).

**Tissue Preparation**

After hemodynamic measurements, hearts and lungs were rapidly dissected and weighed. The RV, left ventricle (LV), and IVS were cut free, weighed, and fixed in 4% formalin for >24 h. Lung tissue was freeze-dried for 12 h and weighed again.

**Histochemistry**

Sirius red staining. After fixation, heart tissue was embedded in paraffin. RV blocks were embedded in the upright position to distinguish the endocardium, the midwall, and the epicardium of the RV free wall in cross sections. Tissue was cut into 4-μm-thick sections and deparaffinized in Ultraclar (Klinipath, Duiven, The Netherlands) for 5 min. The sections were rehydrated in decreasing graded alcohols (from 100% to 25%) and washed twice for 5 min each in distilled water and TBS (150 mM NaCl and 10 mM Tris-HCl, pH 8.0).

For determination of the amount of interstitial, perivascular, and/or replacement fibrosis, RV sections were stained with sirius red (42) and photographed using a microscope (Nikon Eclipse, Nikon Europe, Badhoevedorp, The Netherlands) equipped with a ×40 objective and a digital camera (model DXM1800, Nikon). Quantitative image analysis was performed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD).

The extent of fibrosis was quantified as the sirius red-stained percentage of the total area, measured per image. In each section, four images were acquired and analyzed to compensate for variations within a section. A total of 132 images of 33 animals (11 Cont, 12 MCT30, and 10 MCT80) were quantified.

**Results**

**Body, Lung, and Heart Weights**

The time curves of body weights of the three experimental groups over a 4-wk period after MCT injection indicate a slightly reduced weight gain in MCT30 rats (Fig. 1). The MCT80 animals showed a more pronounced growth retardation, resulting in a significantly lower (P < 0.05) body weight after day 11, than Cont rats. The MCT80 animals started to lose weight at around day 25, which is a sign of heart failure comparable to cachexia in patients with chronic heart failure. In contrast to Cont and MCT30 animals, MCT80 rats showed an obvious lack of physical activity.

Table 1 summarizes characteristics of the animals on the day they were killed. Body weight of MCT80 rats was significantly lower than that of Cont and MCT30 rats (P < 0.001 vs. Cont and MCT30). Wet and dry lung weights were significantly higher in MCT80 than in Cont and MCT30 rats, confirming the presence of an extensive proliferative pulmonary response in MCT80 rats. These increases were not related to pulmonary edema, because dry-to-wet weight ratios of lung tissue did not differ significantly between the groups.

**RV weight of MCT80 animals was significantly higher than that of Cont and MCT30 rats (P < 0.001 vs. Cont and MCT30). Wet and dry lung weights were significantly higher in MCT80 than in Cont and MCT30 rats, confirming the presence of an extensive proliferative pulmonary response in MCT80 rats. These increases were not related to pulmonary edema, because dry-to-wet weight ratios of lung tissue did not differ significantly between the groups.**

**RV weight of MCT80 animals was significantly higher than that of Cont and MCT30 rats, whereas LV and IVS weights were unaffected by MCT treatment. The degree of RV hypertrophy was determined as RV-to-(LV + IVS) weight ratio (Fig. 2). This hypertrophy parameter tended to increase in...
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). $\tau$ 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 2. Cardiac and RV function of Cont, MCT30, and MCT80 animals

<table>
<thead>
<tr>
<th></th>
<th>Cont ($n = 12$)</th>
<th>MCT30 ($n = 15$)</th>
<th>MCT80 ($n = 9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>399 ± 30</td>
<td>381 ± 48</td>
<td>320 ± 58*‡</td>
</tr>
<tr>
<td>SV, $\mu$L</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>$V_{ES}$, $\mu$L</td>
<td>245 ± 92</td>
<td>441 ± 270*</td>
<td>634 ± 325*</td>
</tr>
<tr>
<td>$V_{ED}$, $\mu$L</td>
<td>431 ± 152</td>
<td>649 ± 323*</td>
<td>804 ± 368*</td>
</tr>
<tr>
<td>$P_{peak}$, mmHg</td>
<td>33 ± 6</td>
<td>42 ± 15*</td>
<td>55 ± 20*</td>
</tr>
<tr>
<td>$P_{ES}$, mmHg</td>
<td>29 ± 7</td>
<td>35 ± 14</td>
<td>50 ± 17*‡</td>
</tr>
<tr>
<td>$P_{ED}$, mmHg</td>
<td>3.9 ± 2.5</td>
<td>6.1 ± 2.4</td>
<td>5.4 ± 2.4</td>
</tr>
<tr>
<td>$dP/dt_{Max}$, mmHg/s</td>
<td>2,709 ± 790</td>
<td>2,473 ± 776</td>
<td>2,332 ± 903</td>
</tr>
<tr>
<td>$dP/dA_{Max}$, mmHg/s</td>
<td>1,668 ± 428</td>
<td>1,781 ± 675</td>
<td>1,959 ± 881</td>
</tr>
<tr>
<td>$E_a$, %</td>
<td>49 ± 6</td>
<td>40 ± 12*</td>
<td>26 ± 6*‡</td>
</tr>
<tr>
<td>$SW$, mmHg/µl</td>
<td>5,381 ± 2,323</td>
<td>7,384 ± 4,423</td>
<td>7,901 ± 3,189*</td>
</tr>
<tr>
<td>$E_a$, mmHg/µl</td>
<td>14.5 ± 0.05</td>
<td>12.3 ± 14</td>
<td>27.12*‡</td>
</tr>
<tr>
<td>$E_{max}$, mmHg/µl</td>
<td>0.15 ± 0.06</td>
<td>0.16 ± 0.05</td>
<td>0.28 ± 0.15*§</td>
</tr>
<tr>
<td>$WS_{max}$, mmHg</td>
<td>144 ± 53</td>
<td>213 ± 112</td>
<td>274 ± 111*</td>
</tr>
<tr>
<td>$WS_{ES}$, 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; $V_{ES}$, RV end-systolic volume; $V_{ED}$, RV end-diastolic volume; $P_{peak}$, RV peak pressure; $P_{ES}$, RV end-systolic pressure; $P_{ED}$, RV end-diastolic pressure; $EF$, RV ejection fraction; SW, RV stroke work; $dP/dA_{max}$, maximal rate of pressure increase; $dP/dA_{max}$, maximal rate of pressure decline; $\tau$, relaxation time constant; $E_a$, effective arterial elastance; $WS_{max}$, RV peak wall stress; $WS_{ED}$, RV end-diastolic wall stress. *$P < 0.05$; †$P < 0.01$ vs. Cont. ‡$P < 0.001$ vs. Cont. §$P < 0.001$ vs. MCT80.
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, no differences between groups (Fig. 4), indicating no interstitial, perivascular, and replacement fibrosis. Quantification of volume percentages showed no significant differences between groups for the normalized parameters. Both relations were shifted toward larger volumes in MCT30 rats and were 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.

### 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 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 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).

### 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 hypertension 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

### Table 3. Pressure-volume indexes in Cont, MCT30, and MCT80 animals

<table>
<thead>
<tr>
<th></th>
<th>Cont (n = 12)</th>
<th>MCT30 (n = 15)</th>
<th>MCT80 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{ES}$, mmHg/µl</td>
<td>0.082±0.053</td>
<td>0.100±0.084</td>
<td>0.094±0.087</td>
</tr>
<tr>
<td>$E_{ED}$, mmHg/µl</td>
<td>0.093±0.20</td>
<td>0.082±0.015</td>
<td>0.079±0.022</td>
</tr>
<tr>
<td>$E_{ES}$, mmHg/µl</td>
<td>0.0074±0.0091</td>
<td>0.0062±0.0028</td>
<td>0.0067±0.0028</td>
</tr>
<tr>
<td>$E_{ED}$, mmHg/µl</td>
<td>0.0082±0.0019</td>
<td>0.0061±0.0014</td>
<td>0.0055±0.0021</td>
</tr>
<tr>
<td>$V_{ES,37}$, µl</td>
<td>391±185</td>
<td>478±321</td>
<td>722±550</td>
</tr>
<tr>
<td>$V_{ED,5}$, µl</td>
<td>541±344</td>
<td>614±377</td>
<td>1,025±386†</td>
</tr>
</tbody>
</table>

Values are means ± SD. $E_{ES}$, end-systolic elastance; $E_{ED}$, end-diastolic stiffness; $E_{ES}^N$, normalized $E_{ES}$; $E_{ED}^N$, 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.
that this progressive dilatation causes a gradual increase in wall stress, with the highest values in the MCT80 group, despite the increased muscle mass, illustrating decompen-
sation 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 limit-
ing 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\text{max} and +dP/dt\text{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 ($E_{\text{Es}}$) (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 $\beta$-adrenergic receptor system. Rats with MCT-induced RV hypertrophy showed a reduction in RV $\beta$-adrenoceptor density due to a downregulation of the $\beta_1$-adrenoceptor and showed $\beta_1$-adrenoceptor-G protein-adenylyl cyclase system desensitization (24, 25). This reduced $\beta_1$-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 $\beta_1$-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 hypertension, 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.


44. Van der Laarse A. Hypothesis: troponin degradation is one of the factors responsible for deterioration of left ventricular function in heart failure. *Cardiovasc Res* 56: 8–14, 2002.


