Right Ventricular Function Evaluation by Echocardiography in the Monocrotaline-Induced Pulmonary Arterial Hypertension Rat Model

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INTRODUCTION

PAH (pulmonary arterial hypertension) is a progressive disease characterized by increased pulmonary vascular resistance and consequently, right ventricular remodeling, hypertrophy and failure. Despite the advances in treatment for PAH during the past few decades, there is still a poor 5-year survival rate for newly diagnosed patients. In recent years, emerging therapeutic modalities have focused on disease-modifying agents that can impact the multifactorial pathophysiology of the vascular remodeling occurring in PAH in the hope of disrupting the disease and improving long term survival.

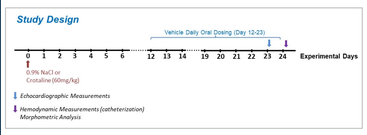
The efficacy of new treatments often centers around the terminal assessment of a test compound on right ventricle (RV) pressure without considering the evaluation of RV morphology and function. The use of noninvasive and non-terminal imaging techniques is crucial for a comprehensive examination of disease progression in animal models. Transthoracic echocardiography remains the standard approach to evaluating heart morphology and function in animal models due to its low cost and ease of use compared to other imaging modalities such as magnetic resonance imaging.

Animal models of PAH are invaluable tools in assessing the efficacy of new drug treatments. The monocrotaline (MCT)-induced PAH rat model is characterized by remodeling of the pulmonary arterial vessels, which in turn leads to increased pulmonary vascular resistance and right ventricular hypertrophy. Therefore, in this study we used echocardiography and RV catheterization to compare the functional and structural RV changes in response to MCT in Sprague Dawley rats.

OBJECTIVE

The objective of this study was to assess the use of transthoracic echocardiography in the evaluation of RV function in anesthetized male Sprague Dawley rats with MCT-induced PAH.

METHODS



On Study Day 0, Sprague Dawley rats (0.216–0.243 kg) were administered either a subcutaneous dose of MCT to induce PAH (3 mL/kg; 60 mg/kg in deionized water, pH 7.4 ± 0.2; n=10) or vehicle (deionized water, pH 7.4; n = 8). From Study Days 12 to 24, all rats received vehicle (deionized water) daily via oral gavage. Cage side observations for general health and appearance were performed once daily.

Echocardiographic measurements: On Study Day 23, RV structure and function was assessed by echocardiography under isoflurane (1–2%) anesthesia. Transthoracic echocardiography was performed using a Vevo 2100 (Visual Sonics, Toronto, ON, Canada) equipped with a 21-MHz solid state transducer (MS250S). Body temperature and heart rate were monitored throughout the procedure.

Hearts were initially imaged in the modified parasternal long-axis view of the RV outflow tract. M-mode window was positioned to go through the widest portion of the RV chamber using the aorta as a landmark. The following parameters were obtained from the M-mode once in this position: right ventricle internal diameter during diastole (RVIDd), right ventricle internal diameter during systole (RVIDs) and right ventricle free wall thickness (RVFWT, measured during end-diastole) were obtained. The probe angle was then adjusted until the pulmonary valve (PV) was clearly visualized, and the PV diameter was obtained. The pulmonary blood outflow was obtained using the pulsed-wave (PW) Doppler mode to measure pulmonary acceleration time (PAT), pulmonary ejection time (PET), pulmonary peak systolic velocity (PSV), heart rate (HR) and cardiac cycle length (CL). The pulmonary velocity time integral (PV VTI) was also obtained and the cardiac output (PVCO) and stroke volume (PVSV) determined. The PAT/PET and PAT/CL ratios were calculated.

The apical four-chamber view of the right side of the heart was obtained to simultaneously view the RV and right atrium (RA). The RA area was obtained from the b-mode image at end-systole and end-diastole. The end-diastolic area (RVEDA) and end-systolic area (RVESA) were obtained from the apical four-chamber view of the right ventricle, and the fractional area change calculated [(RVEDA-RVESA)/RVEDA]. Tricuspid valve flow patterns were assessed in the apical four-chamber view by PW Doppler, and the peak velocities of flow in the early phase of diastole (E), tricuspid closure open time (TCO) and ejection time (ET) obtained. The RV index of myocardial performance (RVMPI) was calculated using the formula: (TCO-ET)/ET. Additionally, the tricuspid annular velocity at early diastole (E') and the tricuspid annular velocity systole (S') were obtained with the sample volume at the free wall side of the tricuspid annulus in the four-chamber view. The E/E' ratio was calculated. The M-mode Doppler beam was placed through the lateral annulus of the tricuspid valve plane to obtain the tricuspid annular plane systolic excursion (TAPSE).

Echocardiographic images were analyzed off-line using VevoLab Software (version 5.7.1). Measurements and calculations were averaged from 3 consecutive cycles and performed according to the American Society of Echocardiography guidelines.

Morphometric Analysis: On Study Day 24, the rats were euthanized by exsanguination and heart-lung block was removed and gently infused via the vasculature with ice cold saline until the perfusate ran clear. The heart and lungs were separated, aorta removed, excess saline drained, and each organ weighed separately. The atria were removed and discarded. The left ventricle with septum (LV+S) was separated from the RV. The ventricles were weighed separately. The left tibia was removed and separated from the soft tissue. A longitudinal measurement was obtained with a digital caliper.

RESULTS

Morbidity, Mortality and Clinical Observations

- All vehicle-treated animals (n = 8) survived to the terminal procedures on Study Day 24.
- Three MCT-treated animals died prior to the terminal procedure: 1 animal died during echocardiography on Study Day 22 while 2 animals were found dead prior to Study Day 24 (Study Day 20 and Study Day 24; n = 1 each day).
- Clinical and necropsy observations were noted only in the MCT-treated animals.
  - Clinical observations were limited to dried crusts around the nares (n = 2) and thin appearance (n =2).
  - Necropsy observations included fluid in the thoracic cavity (n = 3), discolored lungs (n = 2) and enlarged heart and atria (n = 2).

MCT treatment resulted in right ventricle hypertrophy

Morphometric Analysis of MCT-induced PAH in Sprague Dawley Rats (Study Day 24)

Morphometric Parameters	Experimental Groups: Vehicle n = 8 mean ± SD	MCT[a] n = 7 mean ± SD
RV/(LV+S)	0.2814 ± 0.0259	0.6629 ± 0.1564[b]
RV/Tibia (mg/mm)	5.76 ± 0.53	10.65 ± 2.39[b]
(LV+S)/Tibia (mg/mm)	20.55 ± 1.81	16.11 ± 1.00[b]
Heart Wt/Tibia (mg/mm)	29.41 ± 2.40	30.81 ± 3.22
Lung Wt/Tibia (mg/mm)	40.29 ± 2.03	55.77 ± 6.75[b]
Tibia (mm)	39.32 ± 1.03	38.73 ± 1.74

[a] MCT, monocrotaline (60 mg/kg) as a single dose administered on Study Day 0.

[b] p < .001 vs vehicle

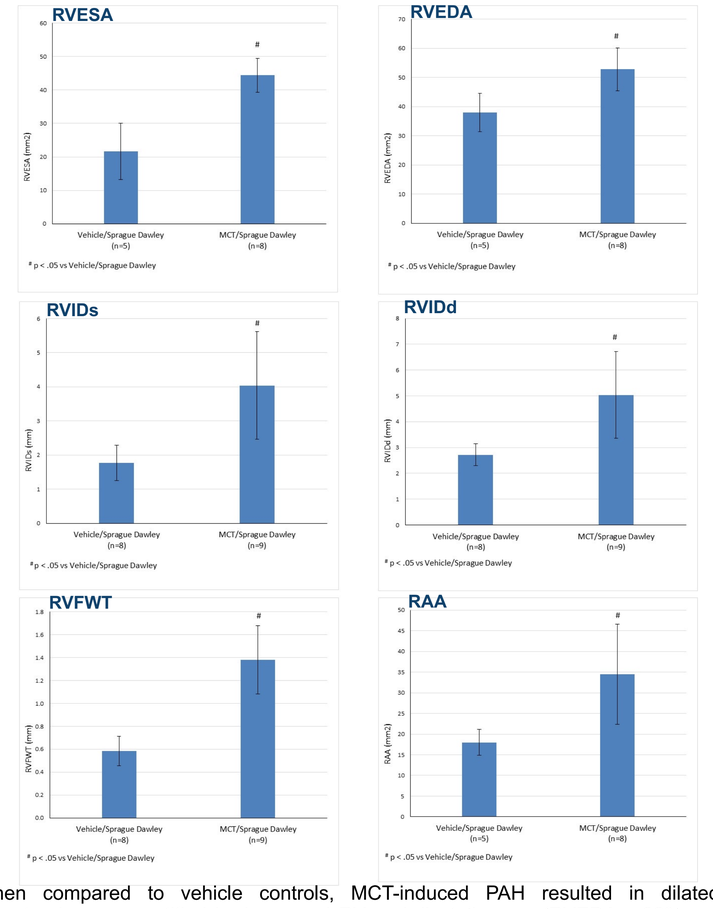
LV + S, left ventricle + septum; RV, right ventricle.

- MCT treatment resulted in RV hypertrophy as shown by an increase in both the Fulton index (RV weight normalized to LV + septum weight) and RV weight to tibia length ratio (+136% and +85% compared to vehicle control, respectively; p < .001).
- The left ventricle + septum weight to tibia length ratio was decreased in MCT-treated rats as (-22% compared to vehicle control; p < .001).
- Pulmonary congestion was observed with MCT treatment as shown by an increase in the lung weight to tibia length ratio (+38% when compared to vehicle control; p < .001).

Echocardiography showed dilated hypertrophy and impairment of systolic and diastolic function in MCT-treated (PAH) rats as compared to vehicle-treated rats

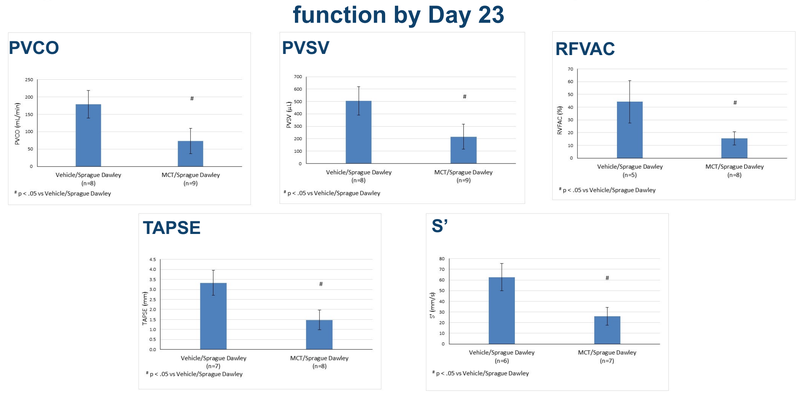
- Body weights on Study Day 23 were 32% lower in PAH rats vs vehicle control rats (0.264 ± 0.022 kg vs 0.388 ± 0.028 kg).
- Of note, heart rate was comparable between PAH rats and vehicle control rats on Study Day 23 (325 ± 42 bpm vs 359 ± 43 bpm; NS) during echocardiography.

Figure 1. MCT-treatment led to RV structural changes reflective of hypertrophy in Sprague Dawley rats by Day 23



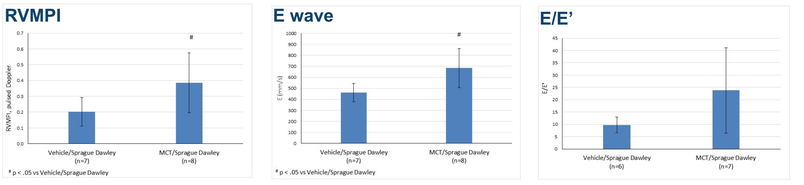
- When compared to vehicle controls, MCT-induced PAH resulted in dilated RV hypertrophy, as quantified by increased RV end-diastolic area (RVEDA: +39%; p=.004) and RV end-systolic area (RVESA: +105%; p<.001). RV dilation was confirmed by an increased RV internal diameter during diastole (RVIDd: +85%; p=.002) and systole (RVIDs: +128%; p=.002).
- MCT treatment also led to right atrial dilation (right atria area [RAA] increase: +92%; p=.01) and an increase in the RV free wall thickness (RVFWT: +136%; p<.001) as compared to vehicle controls.

Figure 2. Rats with MCT-induced PAH showed impaired RV systolic function by Day 23



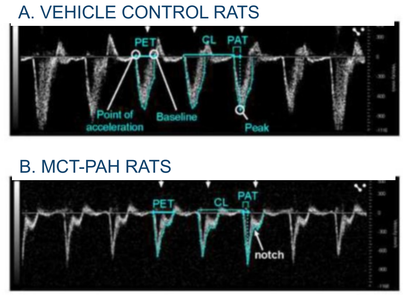
- Decreases in pulmonary valve cardiac output (PV CO: -59%; p < .001) and PV stroke volume (PV SV: -57%; p < .001) were seen in rats with MCT-induced PAH when compared to vehicle control rats.
- MCT-treated rats had a decrease in RV fractional area (-65%; p = .001)
- PAH rats also had decreases in tricuspid annular plane systolic excursion (TAPSE: -56%; p < .001) and Doppler-derived peak systolic velocity (S': -59%; p < .001) as compared to vehicle control.

Figure 3. Rats with MCT-induced PAH showed impaired RV diastolic function by Day 23

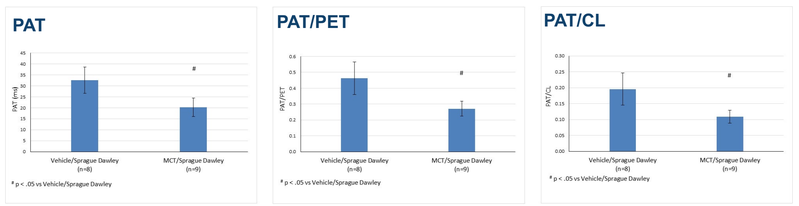


- Echocardiography showed impaired diastolic function as evidenced by a significantly higher RV myocardial performance index (+90%, p = .04) and E wave (+49%, p = .01) and a tendency towards an increase in the E/E' ratio (+143%, p = .08) for MCT-PAH rats vs vehicle control rats.

Figure 4. PAH rats exhibited slower pulmonary artery flow on Day 23



- Pulmonary artery flow in vehicle control rats was as expected, with a symmetrical V shape and peak velocity in mid systole. PAH rats had a slower peak velocity that occurred earlier in systole as compared to vehicle control rats (-606 ± 170 mm/s vs -1032 ± 101 mm/s, respectively).



- The slower peak velocity in PAH rats resulted in a shortened pulmonary acceleration time (PAT: -38% vs vehicle control; p < .001) as well as decreases in the PAT:pulmonary ejection time (PET) ratio and the PAT:cycle length (CL) ratio (-42% and -44% when compared with vehicle controls, respectively; p < .001).

CONCLUSION

Use of transthoracic echocardiography showed dilated RV hypertrophy and impairment of RV systolic and diastolic function in PAH rats compared to vehicle control rats, suggesting that this technique supports the understanding of RV remodeling in drug development PAH studies.