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# Macitentan treatment retards the progression of established pulmonary arterial hypertension in an animal model☆

I.P. Temple [a,*,1], O. Monfredi [a,1], G. Quigley [a,1], H. Schneider [a,1], M. Zi [a,1], E.J. Cartwright [a,1], M.R. Boyett [a,1], V.S. Mahadevan [b,2], G. Hart [a,1,2]

[a] Institute of Cardiovascular Sciences, University of Manchester, UK
[b] Central Manchester University Hospitals NHS Trust, UK

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ABSTRACT

*Background:* Macitentan is a new endothelin receptor antagonist that is used to treat pulmonary arterial hypertension in humans. Treatment of established pulmonary hypertension with macitentan was studied using the monocrotaline model of pulmonary hypertension.
*Methods:* Three groups of rats were created (n = 12): control (CON: macitentan only), monocrotaline (MCT: monocrotaline only) and macitentan (MACI: macitentan and monocrotaline). Monocrotaline (60 mg/kg) was injected in the MCT and MACI groups on day 0; volume matched saline was injected in the CON groups. Macitentan therapy (30 mg/kg/day) was commenced on day 11 in the CON and MACI groups. Serial echocardiography and ECGs were performed. The rats were sacrificed if they showed clinical deterioration.
*Results:* The MCT and MACI rats showed signs of pulmonary hypertension by day 7 (maximum pulmonary velocity, CON 1.15 ± 0.15 m/s vs MCT 1.04 ± 0.10 m/s vs MACI 0.99 ± 0.18 m/s; $p < 0.05$). Both the MCT and MACI groups developed pulmonary hypertension, but this was less severe in the MACI group (day 21 pulmonary artery acceleration time, MCT 17.55 ± 1.56 ms vs MACI 22.55 ± 1.00 ms; pulmonary artery deceleration, MCT 34.72 ± 3.72 m/s$^2$ vs MACI 17.30 ± 1.89 m/s$^2$; $p < 0.05$). Right ventricular hypertrophy and QT interval increases were more pronounced in MCT than MACI (right ventricle wall thickness, MCT 0.13 ± 0.1 cm vs MACI 0.10 ± 0.1 cm; QT interval, MCT 85 ± 13 ms vs MACI 71 ± 14 ms; $p < 0.05$). Survival benefit was not seen in the MACI group ($p = 0.50$).
*Conclusions:* Macitentan treatment improves haemodynamic parameters in established pulmonary hypertension. Further research is required to see if earlier introduction of macitentan has greater effects.


## 1. Introduction

Pulmonary arterial hypertension (PAH) is a disease characterised by raised pulmonary vascular resistance. It has a poor prognosis typically resulting in progressive right ventricular failure and death. Treatment in PAH has advanced rapidly over the past decade with the use of $Ca^{2+}$ channel blockers, prostanoids, endothelin receptor antagonists (ERAs) and phosphodiesterase-5 inhibitors [1]. PAH often has an insidious onset, which means that diagnosis and treatment are usually not begun until the disease is advanced. Recent studies have looked at patients with less severe disease (World Health Organisation (WHO) class II), and have shown that early initiation of therapy can delay the progression of the disease [2].

Endothelin is a 21-amino acid peptide which is produced mainly by the vascular system and acts in a paracrine manner to regulate vasoconstriction, cell proliferation, cell migration and fibrosis [3]. Activation of the endothelin system plays a central role in the pathogenesis of PAH [3]. ERAs are widely used in clinical practice for patients with WHO class II to IV symptoms, either as monotherapy or in combination with other agents [1,4,5]. They have beneficial effects on haemodynamic parameters, objective measurements of exercise capacity and subjective symptom scores [1,4,5]. Data regarding ERAs and mortality are limited, although registry data suggests a survival benefit with the ERA bosentan [1,4,5]. Macitentan is a new ERA which has been shown in animal studies to have improved tissue penetration, longer receptor binding and affinity for both the endothelin A and B receptors compared with the older ERA bosentan [6]. The use of macitentan to treat PAH has been investigated in a phase III clinical trial enrolling WHO class II to IV patients showing a significant improvement in exercise capacity

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* Corresponding author at: Institute of Cardiovascular Sciences, University of Manchester, Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, UK.
*E-mail address:* ian.temple@manchester.ac.uk (I.P. Temple).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.
[2] VSM and GH are joint senior authors.

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and haemodynamic parameters at 6 months and a significant reduction in morbidity over a followup period of up to 36 months [7].

Monocrotaline is a pyrrolizidine alkaloid, extracted from the plant *Crotalaria spectabilis*. A single injection has been shown to generate severe pulmonary hypertension in several species and has been widely used as an animal model of pulmonary hypertension in the rat [8,9]. The effects of monocrotaline on pulmonary arterial pressures, pulmonary vascular resistance and right ventricular hypertrophy have been studied using invasive methods with direct pressure methods and non-invasive methods including echocardiography (echo) and magnetic resonance imaging (MRI) [10,11]. These studies have demonstrated a characteristic change in the pulmonary velocity profile from the typical rounded shape to a 'spike and dome' morphology [10,11]. The echo parameter 'pulmonary artery deceleration' (PAD) is correlated to pulmonary arterial pressure measured invasively and the echo parameter 'pulmonary artery acceleration time' (PAAT) is inversely correlated to both pulmonary pressure and pulmonary vascular resistance measured invasively [10,11].

Experiments using the monocrotaline model have given positive results from drug therapy including ERAs, sildenafil, statins and beta blockers [6,12–15]. The experimental design of these studies has varied such that some studies have started therapy on the same day as the monocrotaline injection, i.e. a 'prevention' strategy, whereas others have waited until there is evidence of the animals displaying pulmonary hypertension before commencing therapy, i.e. a 'treatment' strategy. In cases where 'prevention' and 'treatment' have been compared there has been a greater effect with 'prevention' than with 'treatment' [13,14,16]. These findings raise questions about the extent to which 'prevention' studies are applicable to clinical practice, particularly given that the dramatic successes seen in 'prevention' studies have not been borne out in clinical practice.

Animal studies with macitentan administration, given as a 'prevention' strategy, have shown a significant mortality benefit. In order to more closely to reflect current clinical practice, we have investigated the safety and efficacy of macitentan administration *after* the development of pulmonary hypertension in the monocrotaline model.

## 2. Methods

All procedures were carried out in accordance with the UK Animals Scientific Procedures Act (1986). Invasive pulmonary pressure monitoring in monocrotaline injected rats has demonstrated that pulmonary pressures are significantly raised by day 10 and increase progressively, leading to RV failure and death [11,17]. In the light of such previous studies we elected to initiate therapy at day 11, in order to mirror the clinical situation with respect to initiation of treatment. Male Wistar Harlan rats (n = 36; weight 200 g; Charles River, UK) were arbitrarily assigned to three equal groups (n = 12). All animals received pulverised chow only from day 0 to day 11. The control group (CON) received saline injection (3 ml/kg) by intraperitoneal injection on day 0 and macitentan (Actelion Pharmaceuticals Ltd, Allschwil, Switzerland) 30 mg/kg/day admix to pulverised chow from day 11 to the day of termination. The monocrotaline only group (MCT) received monocrotaline 60 mg/kg by intraperitoneal injection on day 0 and pulverised chow only from day 11 to the day of termination. The macitentan treated group (MACI) received monocrotaline injection 60 mg/kg by intraperitoneal injection on day 0 and macitentan 30 mg/kg/day admix to pulverised chow day 11 to the day of termination [6]. Monocrotaline (Sigma-Aldrich Ltd, UK) was dissolved in 1 M hydrochloric acid, then made up to a concentration of 20 mg/ml with 0.9% saline, the pH corrected to 7.4 using 4 M NaOH.

ECG and echo recording was carried out under general anaesthesia with 2% isoflurane. Electrodes were inserted subcutaneously with the negative electrode in the right forepaw, the positive electrode in the left forepaw and the ground electrode in the right hindpaw. The electrodes were connected to a Bioamp and Powerlab analogue to digital converter (AD instruments, New Zealand). Signals were recorded using Labchart (AD Instruments, New Zealand) and analysed offline. All intervals were measured from the average of 100 beats using Chart software. ECG was recorded on day 0 immediately prior to injection, and on day 7, day 14 and day 21. QTc was calculated using Bazett's formula.

Echo images were acquired on an ACUSON Sequoia™ (Acuson Universal Diagnostics Solution, USA) with a 15 MHz 15L8 transducer. All images were stored on optical media disks for subsequent offline analysis. M-mode recordings were taken in the parasternal short axis view allowing recording of left ventricle (LV) anterior and posterior wall thickness and the internal diameter of the LV in both systole and diastole. Right ventricle (RV) wall thickness was measured from M-mode recordings in the parasternal long axis view. Continuous wave Doppler recordings through the pulmonary artery were used to assess the pulmonary velocity profile. The maximum pulmonary velocity (PVmax), time from the onset of pulmonary outflow to maximal flow (pulmonary artery acceleration time, PAAT) and the rate of deceleration of pulmonary flow (pulmonary artery deceleration time, PAD) were measured (Fig. 1). Echo was recorded on day 0 immediately prior to injection, on day 7, day 14, and day 21.

### 2.1. Symptomatic endpoints

The animals were weighed and their clinical condition was assessed twice weekly in the first 18 days, and daily thereafter. Animals were sacrificed on the day that the following pre-specified endpoints were met, namely evidence of clinical deterioration with reduced movement, increased respiratory rate, piloerection and weight loss of >10 g over 2 days. Animals that did not meet these criteria were electively sacrificed on day 28. The animals were sacrificed by stunning and cervical dislocation; the heart and lungs were excised and weighed.

### 2.2. Statistical methods

The distribution of the data was analysed using the Shapiro–Wilk test. The data were found to be normally distributed and therefore analysis of the differences between body weight, heart weight and lung weight was performed using Student's *t*-test. Comparisons of the echo and ECG parameters at day 0, day 7, day 14 and day 21 were made using a two-way repeated measures ANOVA with the two factors being time and treatment group; time was the repeated measure. Comparisons were made between the three treatment groups at each timepoint using the Tukey test to correct for multiple comparisons. Survival analysis was performed using the log-rank (Mantel–Cox) test.

## 3. Results

Table 1 shows that both the MCT group and the MACI group had increased heart weight and decreased body weight compared with the CON group. The differences between the MCT group and MACI group were not significant. There was no difference in lung weight between the MACI treated group and the CON group. Although the MCT group did show an increase in lung weight compared with the CON group, there was no significant difference when the MCT group and MACI group were compared directly.

Fig. 2 shows that both the MCT and MACI group developed echo evidence of pulmonary hypertension with a change from a 'rounded' pulmonary velocity profile to a 'spike and dome' morphology. The timings of these changes are summarised in Fig. 3. The earliest changes in echo parameters were seen at day 7 with a reduction in PVmax of 9% in the MCT group and 13% in the MACI group compared with the CON group (Fig. 3A). No other parameters were significantly altered by day 7. The reduction of PVmax in both MCT and MACI groups when compared with the CON group suggests that pulmonary hypertension had begun to develop by day 7.

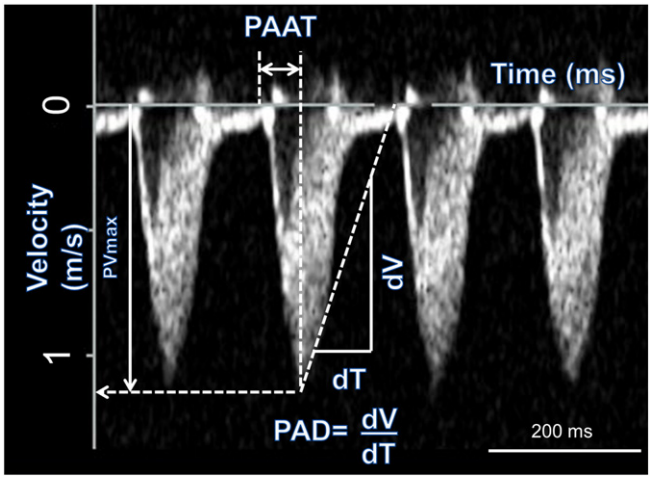


**Fig. 1.** Pulsed wave Doppler recording through the pulmonary artery and measurement of PVmax, PAAT and PAD. The x axis measures time and the y axis measures velocity. PAAT is the time from the beginning of flow to the peak velocity, measured from the x axis. PVmax is the maximum velocity measured from the y-axis. PAD is the gradient of the initial deceleration of the pulmonary velocity profile.