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Reversibility of the monocrotaline pulmonary hypertension rat model

To the Editor:

Pulmonary hypertension (PH) is a disease characterised by progressive remodelling of the pulmonary vasculature eventually leading to right heart failure. Various animal models have been used to mimic the disease, involving pigs, dogs, rats and mice [1]. The most commonly used model is the monocrotaline (MCT) rat model. In this model MCT is injected subcutaneously and becomes metabolically activated, as a pyrrolizidine alkaloid, by hepatic cytochrome P450 3A [2, 3]. The active MCT pyrrole is pneumotoxic and damages the pulmonary artery endothelial cells (PAECs), which leads to a disturbed barrier function [4]. Other features of MCT-induced pulmonary vascular remodelling are arterial medial hyperplasia of axial arteries, interstitial oedema, adventitial inflammation, haemorrhage and, eventually, fibrosis [1, 2, 5, 6]. As a result, pulmonary vascular resistance (PVR) increases and the right ventricle compensates by hypertrophy and eventually fails [7, 8].

Besides the MCT PH rat model, chronic hypoxia with or without Sugen 5416 and pulmonary artery banding are used to study experimental PH [1]. The PH animal model of choice is mostly dependent on the research question that needs to be answered. Ideally, an animal model would recapitulate the progressive and irreversible pulmonary vascular remodelling, which is the hallmark of human PH [1, 4, 9]. However, none of the animal models fulfil this criterion. Concerns have been raised about the MCT rat model since many therapies were successful in MCT rats but not in humans with PH [4].

We, therefore, investigated the long-term progression and reversibility of MCT-induced PH in rats over 12 weeks, using a dose of 40 $mg \cdot kg^{-1}$ in a randomised placebo-controlled study design. Since it is known that a high dose of MCT (60 or 80 $mg \cdot kg^{-1}$) is fatal within 3–6 weeks, the only possible way to study the long-term effects of MCT was to use a lower dose [7, 8, 10, 11]. Alterations in the lungs and heart were measured 4, 8, and 12 weeks after administration of MCT or saline.

We randomly assigned 22 male Wistar rats (Harlan Laboratories, Horst, The Netherlands) to a subcutaneous injection of either 40 $mg \cdot kg^{-1}$ MCT or saline when their body weight was 175–200 g. Rats were sacrificed at either 4 weeks (n=3 control, n=5 MCT), 8 weeks (n=3 control, n=4 MCT), or 12 weeks (n=3 control, n=4 MCT). Echocardiography was performed 3 weeks after MCT injection, to determine baseline haemodynamics, and on the day of sacrifice. Before sacrifice, all animals underwent a right heart catheterisation to construct right ventricle pressure–volume loops. Internal organs were harvested for histology. All methods have been described in detail previously [12]. Data are presented as mean±SD. The study was approved by the local animal ethics committee.

At baseline echocardiography, 3 weeks after MCT injection, all MCT rats had a higher PVR index compared with controls (mean of the three MCT groups: 4.33 ± 2.58 $mmHg \cdot mL^{-1} \cdot min^{-1} \cdot mg^{-1}$ *versus* controls: 0.50 ± 0.13 $mmHg \cdot mL^{-1} \cdot min^{-1} \cdot mg^{-1}$; $p<0.001$) with decreased cardiac index (0.14 ± 0.06 *versus* 0.29 ± 0.04 $mL \cdot min^{-1} \cdot g^{-1}$; $p<0.001$) and lower tricuspid annular plane systolic excursion (TAPSE) (2.3 ± 0.5 *versus* 3.6 ± 0.3 mm; $p<0.001$). Body weight was significantly lower in the MCT rats sacrificed at 4 weeks (311 ± 21 g) compared with controls (354 ± 6 g; $p<0.05$). After 8 and 12 weeks body mass of the MCT rats (405 ± 27 and 455 ± 27 g, respectively) was similar to age-matched controls (408 ± 36 and 496 ± 46 g, respectively).

We observed a significantly higher PVR index in the MCT rats sacrificed at 4 weeks compared with controls (fig. 1a) with an increased right ventricle systolic pressure (57.8 ± 10.3 *versus* 15.6 ± 2.3 mmHg; $p<0.05$). Although stroke volume and heart rate were decreased, cardiac index was relatively preserved (fig. 1b–d). However, cardiac index was negatively correlated to estimated right ventricle systolic pressure in all MCT rats ($r= -0.65$; $p=0.02$, fig. 1e) but not in control rats ($r=0.30$; $p=0.46$, data not shown). Diminished right ventricle function compared with controls was seen by means of lower TAPSE (2.0 ± 0.6 *versus* 3.7 ± 0.3 mm; $p<0.05$), a trend to increased right ventricle wall thickness (1.4 ± 0.2 *versus* 1.0 ± 0.1 mm; $p=0.07$) and increased right ventricle end-diastolic diameter (6.2 ± 1.3 *versus* 3.5 ± 0.5 mm; $p<0.05$). In line with these findings, invasive haemodynamic measurements demonstrated high right ventricle afterload (E_a), increased right ventricle diastolic stiffness (E_{ed}) and increased right ventricle systolic elastance (E_{es}, a measure of contractility) in MCT rats sacrificed at 4 weeks compared with age-matched controls (fig. 1f–h). Because E_a and E_{es} are increased to the same extent, ventriculo-arterial coupling, represented by E_{es}/E_a ratio, is preserved in all animals (fig. 1i). Interestingly, all aforementioned parameters are normalised at 8 and 12 weeks after MCT injection (fig. 1a–i).

Signs of right ventricle adaptation to high afterload after 4 weeks of MCT were also found at autopsy, since heart weight was increased compared with controls (1.54 ± 0.02 *versus* 1.27 ± 0.08 g; $p<0.05$). This was mainly due to an increase in right ventricle weight indicated by the high right ventricle/left ventricle+septal weight ratio (0.63 ± 0.11 *versus* 0.28 ± 0.06 in controls; $p<0.05$). Lung weight was greater in MCT rats sacrificed at 4 weeks (1.73 ± 0.06 *versus* 1.36 ± 0.19 g; $p<0.05$), but liver weight was reduced compared with controls (11.8 ± 0.4 *versus* 14.8 ± 1.1 g; $p<0.05$). In MCT rats sacrificed at 8 and 12 weeks, the right ventricle/left ventricle+septal weight ratio was decreased compared with MCT rats sacrificed at 4 weeks, but not completely normalised (8 weeks: 0.37 ± 0.03 and 12 weeks: 0.40 ± 0.07, both $p<0.01$ *versus* 4 weeks MCT). In addition, lung wet weight reduced to 1.74 ± 0.17 g in MCT rats sacrificed at 8 weeks and 1.59 ± 0.10 g in MCT rats sacrificed at 12 weeks. Liver weight increased to control values in MCT rats sacrificed at 8 and 12 weeks (14.0 ± 0.67 and 15.2 ± 1.4 g, respectively).

Lung histology showed increased media muscularisation in the smallest arterioles (<40 μm) after 4 weeks of MCT ($32 \pm 4\%$; $p<0.01$ *versus* controls) which returned to control values ($24 \pm 1\%$) after 8 weeks ($25 \pm 6\%$) and 12 weeks after MCT administration ($21 \pm 7\%$; $p<0.05$ *versus* 4 weeks MCT) (fig. 1j–l). Right ventricle cardiomyocyte cross-sectional area was considerably increased in the MCT rats sacrificed at 4 weeks (388 ± 91 μm^2) compared with controls (258 ± 44 μm^2; $p<0.001$) and remained at that level at 8

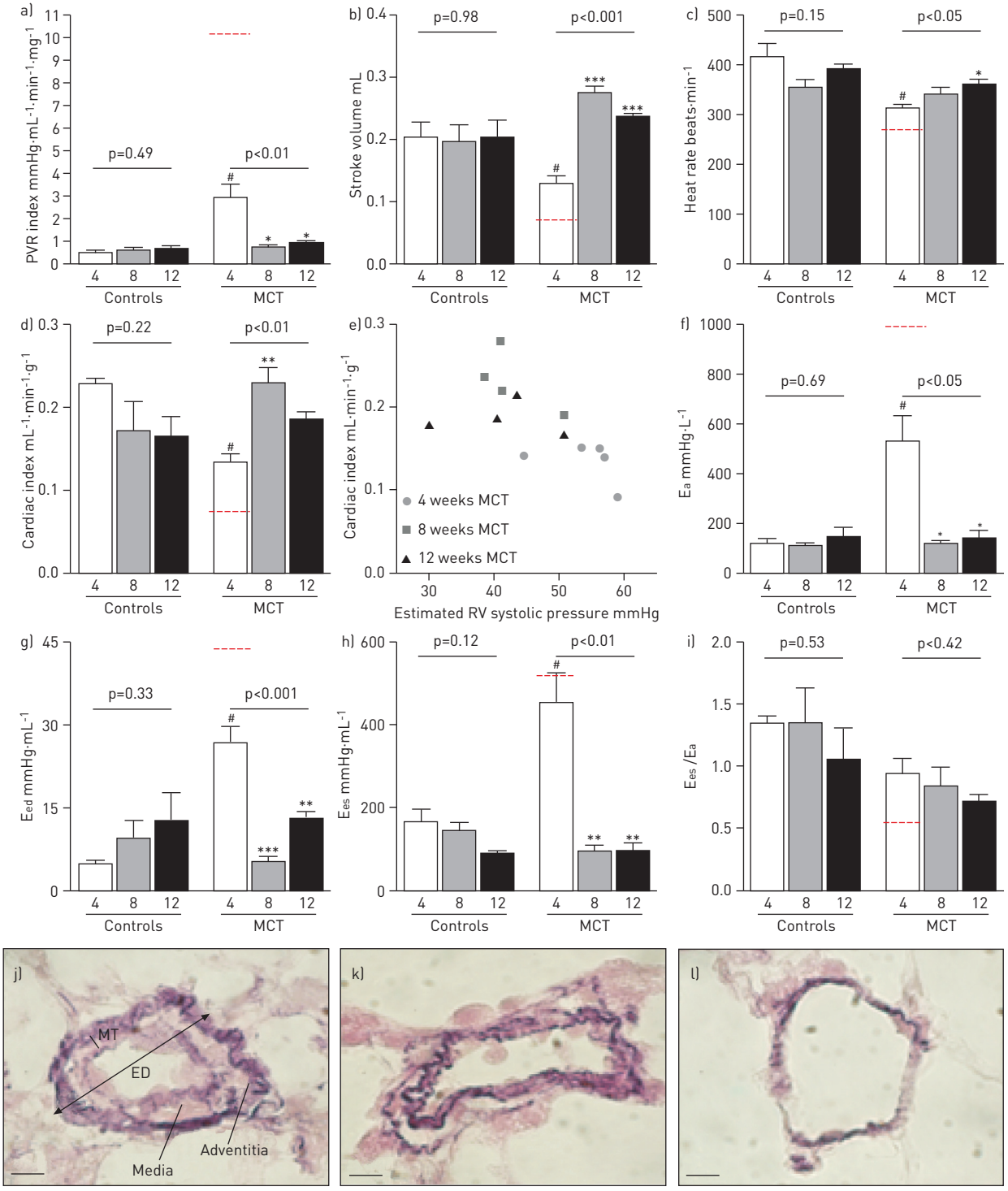

FIGURE 1 Echocardiographic, invasive haemodynamic and histology measurements. Data are presented as mean±SEM. a) Pulmonary vascular resistance (PVR), b) stroke volume, c) heart rate, d) cardiac index, e) cardiac index *versus* estimated right ventricular systolic pressure (eRVSP), f) arterial elastance (Ea) (right ventricle afterload), g) end diastolic elastance (Eed) (diastolic right ventricle stiffness), h) end systolic elastance (Ees) (right ventricle contractility), i) ventriculo-arterial coupling of the right ventricle (Ees/Ea ratio). In a–i) the red dotted lines represent values at 4 weeks after injection with 60 mg·kg^{-1} monocrotaline (MCT), data from De Man *et al.* [12]. The three control and MCT-groups are compared with each other using one-way ANOVA with Bonferroni *post hoc* testing and p-values are given on the graphs. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ compared with MCT rats sacrificed at 4 weeks. #: $p<0.05$ for comparison of MCT rats with age-matched control values performed by Mann–Whitney testing. Histology of pulmonary arterioles from rats after j) 4, k) 8, and l) 12 weeks of MCT. Pulmonary arterioles were stained with the Elastica von Gieson method to visualise the tunica media, which is hypertrophied in animals sacrificed at 4 weeks. In addition, the adventitial layer is thickened compared with controls and MCT rats sacrificed at 8 and 12 weeks. Media thickness was measured as: (2×media thickness(MT)/external diameter(ED))×100%.

and 12 weeks (344±76 μm^2 and 358±57 μm^2, respectively). Although right ventricle cardiomyocyte size was greater in MCT rats, the number of capillaries was similar in all MCT groups (mean 1.2±0.5 capillaries per cardiomyocyte). Right ventricle fibrosis was similar at all time points in MCT animals and controls (0.6±0.3 *versus* 0.6±0.4% collagen; p=0.88). In addition, right ventricle inflammation, expressed as the number of CD45 positive leukocyte cells per unit area was similar in all MCT animals (mean 25.8±15.4 $CD45^+$ nuclei·mm^{-2}) and similar to controls (mean 19.5±15.8 $CD45^+$ nuclei·mm^{-2}; p=0.17), indicative of a compensated rather than a failing right ventricle.

The MCT model is considered, by some, to be a toxic model and it has been suggested that MCT rats die from hepatic veno-occlusive disease with liver failure instead of right ventricle failure [13]. In the current study, liver weight was lower in MCT rats sacrificed at 4 weeks compared with controls. However, right ventricle systolic pressure and PVR are largely increased in these rats and increased pulmonary arteriolar muscularisation is found, which cannot be explained by liver damage alone.

To conclude, we demonstrated that 40 mg·kg^{-1} MCT induces acute muscularisation of the smallest pulmonary arterioles, together with high PVR and right ventricle hypertrophy at 4 weeks after MCT administration. However, at 8 and 12 weeks after MCT administration, the pulmonary arteriolar abnormalities were restored accompanied by normalisation of right ventricular function, although cardiomyocyte hypertrophy was maintained. This shows that MCT induced PH is reversible after 4 weeks and does not resemble the progressive nature of human PH. Therefore, this model is not suitable for therapeutic studies after 4 weeks of a low dose of MCT.

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Pulmonary vascular remodelling and right ventricular adaptation in rats treated with monocrotaline 40 mg·kg^{-1} is completely restored after 8 and 12 weeks http://ow.ly/luztH

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