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Original Article

Effects of chronic treprostinil treatment on experimental right heart hypertrophy and failure

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Abstract *Background:* Right heart function is an important predictor of morbidity and mortality in pulmonary arterial hypertension and many CHD. We investigated whether treatment with the prostacyclin analogue treprostinil could prevent pressure overload-induced right ventricular hypertrophy and failure. *Methods:* Male Wistar rats were randomised to severe pulmonary trunk banding with a 0.5-mm banding clip (n = 41), moderate pulmonary trunk banding with a 0.6-mm banding clip (n = 36), or sham procedure (n = 10). The banded rats were randomised to 6 weeks of treatment with a moderate dose of treprostinil (300 ng/kg/minute), a high dose of treprostinil (900 ng/kg/minute), or vehicle. *Results:* Pulmonary trunk banding effectively induced hypertrophy, dilatation, and decreased right ventricular function. The severely banded animals presented with decompensated heart failure with extracardial manifestations. Treatment with treprostinil neither reduced right ventricular hypertrophy nor improved right ventricular function. *Conclusions:* In the pulmonary trunk banding model of pressure overload-induced right ventricular hypertrophy and failure, moderate- and high-dose treatment with treprostinil did not improve right ventricular function neither in compensated nor in decompensated right heart failure.

Keywords: Right ventricular dysfunction; pulmonary hypertension; prostacyclin

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RIGHT HEART FUNCTION IS THE MOST IMPORTANT predictor of morbidity and mortality in pulmonary arterial hypertension and many types of CHD.[1–3] Sustained pressure overload is a major cause for right ventricular hypertrophy and failure in pulmonary hypertension and CHD. Initially, right ventricular hypertrophy is an appropriate and beneficial response of the right ventricle to maintain a sufficient cardiac output; however, with prolonged pressure overload, cardiac contractile force decreases, leading to right ventricular dilatation and a further mismatch between oxygen supply and demand that impairs right ventricular function leading to terminal right heart failure.[4]

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The present treatment for pulmonary arterial hypertension focusses on dilatation of the pulmonary vessels and secondary unloading of the right ventricle through decreased afterload. Despite optimal treatment, pulmonary arterial hypertension is associated with an estimated 3-year survival of 55%.[5] At present, there are no therapies directly targeting the dysfunctional and failing right ventricle, and conventional treatment for left ventricular failure has not been proven to be effective in treating right ventricular failure.[6]

Patients suffering from pulmonary arterial hypertension have endothelial dysfunction in the pulmonary arterial circulation causing impaired production of vasodilatatory and anti-proliferative endogenous substances such as prostacyclin.[7] Prostacyclin analogues are well-established first-line therapy for patients with pulmonary arterial hypertension in functional class III and IV because of

their vasodilatatory and anti-remodelling properties in the pulmonary vessels.[8] It is not clarified whether the beneficial effect of prostacyclin on the right ventricle solely relies on decreased afterload or also on a direct positive inotropic effect. Previous in vivo studies have suggested inotropic properties of the prostacyclin analogue iloprost in both acute and chronic settings.[9,10]

Treprostinil is a prostacyclin analogue currently used for the treatment of pulmonary arterial hypertension. The benefits of treprostinil compared with other prostanoids are a longer half-life in blood and the possibility of oral administration.[8] The direct effect of treprostinil treatment on right ventricular hypertrophy and failure has not been previously investigated. In this study, we investigated the chronic effects of the prostacyclin analogue treprostinil in an experimental model of pressure overload-induced right heart hypertrophy and failure.

Materials and methods

Study design

Rats were randomised to severe pulmonary trunk banding (PTB) with a clip compressed to an inner diameter of 0.5 mm (0.5PTB; n = 41), moderate PTB with a clip compressed to an inner diameter of 0.6 mm (0.6PTB; n = 36), or sham procedure (n = 10); 8 days after surgery, each group was randomised to 6 weeks of treatment with a moderate dose (300 ng/kg/minute) of treprostinil (0.6PTB-300Tre; n = 11, 0.5PTB-300Tre; n = 12), a high dose (900 ng/kg/minute) of treprostinil (0.6PTB-900Tre; n = 10, 0.5PTB-900Tre; n = 13), or vehicle (0.6PTB-veh; n = 14, 0.5PTB-veh; n = 15). Sham-operated rats received vehicle. Treprostinil and vehicle were administered by continuous subcutaneous infusion by means of an osmotic minipump inserted under the skin (ALZET Durect 2ML4 and 2006; Durect Corporation, Cupertino, California, United States of America). Dosing was selected based on results of pharmacokinetic pilot studies.

After 6 weeks of treatment, cardiac function was evaluated by echocardiography, MRI, and invasive pressure–volume measurements. Blood samples were drawn, and clinical signs of heart failure were evaluated before euthanisation of the animals. The heart was excised, weighed, and quick frozen for molecular analysis.

Dose-finding study

A dose-finding study was performed before initiation of the main study. Healthy male Wistar rats (250 g, n = 12) and rats operated by PTB (150 g, n = 15) were randomised to 14 days of treatment with treprostinil in low (100 ng/kg/minute), moderate (300 ng/kg/minute), or high (900 ng/kg/minute) dose administered using ALZET osmotic minipumps. Plasma concentration of treprostinil was analysed in blood samples drawn from the tail vein at day 1, 4, 7, 10, 13, and 15, and at the end of the study rats were phenotyped by echocardiography, MRI, and invasive pressure–volume analysis (detailed protocol and results are given in the Supplementary material).

Animals

Male Wistar GALAS rats (n = 87; 94–115 g; M&B Taconic, Ry, Denmark) were housed two per cage in a room with a 12-hour light–dark cycle and a temperature of 23°C with free access to tap water and standard rat chow (Altromin no. 1324; Altromin, Lage, Germany).

PTB

Right heart hypertrophy and failure was induced by PTB as previously described.[11,12] In brief, rats were sedated, intubated, and mechanically ventilated with sevoflurane (Abbott Scandinavia, AB, Solona, Sweden) (induction: 7.0% in 2:1 O_2/N_2O mix; maintenance: 3.5% in 2:1 O_2/N_2O mix) at 75 breaths/minute and a tidal volume of 10 ml/kg (7%).

A lateral thoracotomy was performed, the pulmonary trunk was exposed, and a titanium clip with an inner diameter of 0.5 or 0.6 mm was applied. Buprenorphine was injected subcutaneously (0.12 mg/kg) peroperatively and administered in the drinking water (7.4 µg/ml) for a total of 3 days postoperatively to relieve pain. Sham animals underwent the same procedure without applying the clip.

Echocardiography

Transthoracic echocardiography was performed on superficially anaesthetised rats (sevoflurane and oxygen: induction: 7.0%; maintenance: 2.6%) using a MS250 line array transducer on a Vevo 2100 Imaging System (VisualSonics Inc., Toronto, Ontario, Canada) scanning at a frequency of 14–21 MHz.

The right ventricle outflow tract and the pulmonary trunk were visualised in the parasternal long-axis view. Pulsed wave Doppler measurements were obtained, and stroke volume was calculated (stroke volume = pulmonary $\text{trunk}_{\text{diameter}}/2)^2 \times 3.14 \times$ velocity time integral). A parasternal short-axis view was used for visualisation of diastolic bulging of the septum into the left ventricle. Tissue Doppler measurements, the presence of tricuspid regurgitation, and tricuspid annular plane systolic excursion were

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obtained from an apical four-chamber view. All images were analysed offline (Vevo® 2100; Fujifilm VisualSonics Inc., Amsterdam, The Netherlands). The observer was blinded to the clinical source of the sample.

MRI

MRI was performed using a 3-Tesla whole-body MR scanner (Skyra; Siemens, Erlangen, Germany). The animals were anaesthetised (sevoflurane and oxygen: induction: 7.0%; maintenance: 2.9%), placed in a 16-element wrist coil, and maintained with spontaneous respiration during the scan. Electrocardiography and respiration were recorded to trigger scanning between expiration and inspiration (SA11; Stony Brook, New York, United States of America). The position and orientation of the left ventricle long axis were obtained by performing a number of cine – multiple-heart phase – acquisitions. In total, six slices encompassing the heart from the base to the apex in the cardiac short-axis orientation were acquired. The data were evaluated using Segment (http://segment.heiberg.se, Medviso) cardiac analysis software. The right ventricular endocardium was traced manually, and the ejection fraction was calculated from end-systolic and end-diastolic volumes. Stroke volume was measured in the pulmonary artery and in the aorta using a phase contrast sequence. Flow values were obtained using specific analysis software (Siswin). All data evaluation was performed with the observer blinded to the sample source.

Pressure–volume relations

Pressure–volume relations were recorded using Millar MikroTip conductance catheters (SPR-869NR; Millar Instruments Inc., Houston, Texas, United States of America) under general anaesthesia (sevoflurane and oxygen: induction: 7.0%; maintenance: 3.5%) with the animals intubated and ventilated. Catheters were pre-soaked for ~20 minutes in sterile saline, connected to a signal-conditioning box (PCU-2000; Millar Instruments Inc.), and calibrated before insertion. The left carotid artery was exposed and the catheter was inserted to obtain systemic blood pressure measurements. Subsequently, an open-chest approach was performed and the catheter was inserted into the right ventricle.

End-systolic volume and end-diastolic volume derived from the conductance signal were calibrated, using the volumes derived from MRI, and the analysis was performed using software from Notocord (Notocord Systems SAS, Midland, North Carolina, United States of America) and PVAN (Millar Instruments Inc.).[11]

Anatomy and histology

Rats were euthanised under general anaesthesia by excision of the heart. The heart was dissected into right ventricle and left ventricle + septum and weighed separately. The lungs, liver, kidneys, and spleen were removed and weighed. The right tibia was dissected, and the length of the tibia was measured using a vernier caliper. The presence of hydrothorax and ascites was assessed by visual inspection. The liver was examined for nutmeg signs or black discolouration as evidence of varying degree of liver damage. The right ventricle was separated into three different pieces. Pieces of the right ventricle were immersion fixated in 4% formaldehyde buffer (pH 7). The tissue was dehydrated in graded ethanol. After tissue embedding in paraffin, 2-μM sections were cut and stained to estimate the degree of fibrosis (Sirius Red), cardiomyocyte diameter and quantity per area (haematoxylin–eosin), and right ventricular capillary density (CD31 immune histochemistry).

Data analysis and statistics

Unless otherwise stated, normally distributed quantitative data are expressed as mean ± SEM, and non-normally distributed data are expressed as means with 95% confidence intervals. All statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, San Diego, United States of America). Data were tested for normal distribution using the Shapiro–Wilk normality test and non-parametric tests were used if not normally distributed. Ordinary one-way analysis of variance test or the non-parametric Kruskal–Wallis test was used to evaluate the model (PTB versus sham) and the effects of treatments (PTB-veh versus PTB-300Tre, PTB-veh versus PTB-900Tre); $p < 0.05$ was considered to be statistically significant.

Results

Effects of PTB

The effects of the PTB procedure were evaluated by comparison between sham animals and PTB animals treated with vehicle.

We observed a 2.5-fold increase in right ventricular weight normalised to tibia length in PTB animals as evidence of right ventricular hypertrophy. No difference in right ventricular weight was observed between 0.5PTB animals and 0.6PTB animals (Fig 1a).

Histology revealed an increased amount of fibrosis in 0.5PTB animals compared with sham animals, and a non-significant increased amount of fibrosis was seen in the 0.6PTB animals (Fig 1b). PTB animals

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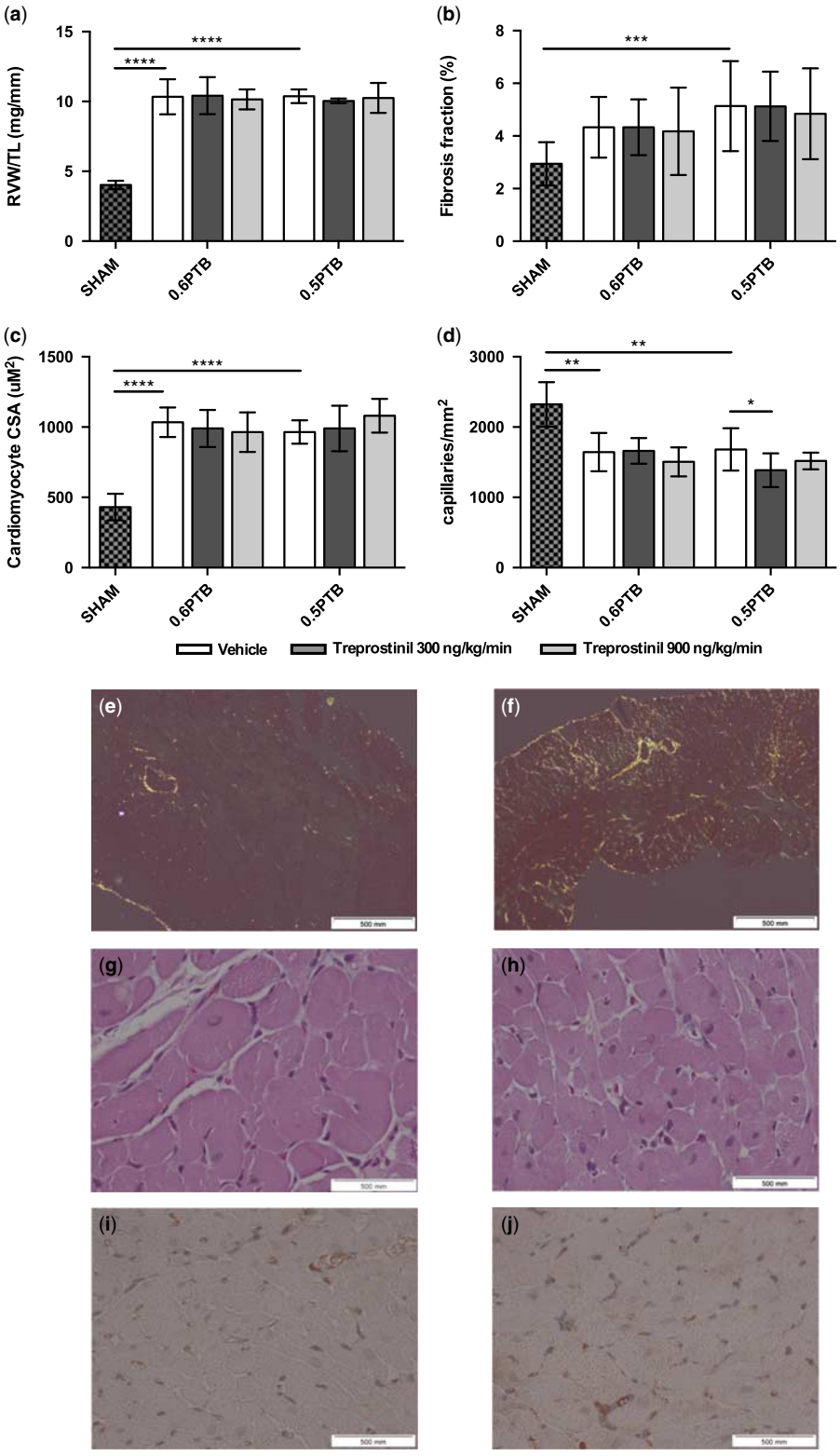

Figure 1.

*Anatomical and histological effects. (**a**) Right ventricular weight (RVW)/tibia length (TL). (**b**) Right ventricular (RV) fibrosis fraction. (**c**) Cardiomyocyte size. (**d**) Capillary density. Histological sections stained with Sirius Red from (**e**) sham and (**f**) 0.5PTB rats. Haematoxylin and eosin from (**g**) sham and (**h**) 0.5PTB rats. Immunohistochemical staining for the endothelium marker CD31 from (**i**) sham and (**j**) 0.5PTB rats. Results are expressed as mean ± SEM (7–14 animals/group). 0.5PTB = pulmonary trunk banding with a 0.5-mm banding clip; 0.6PTB = pulmonary trunk banding with a 0.6-mm banding clip; sham = sham-operated animals treated with vehicle (saline); vehicle = pulmonary trunk banding animals treated with saline; CSA = cross-sectional area. *$p \leqslant 0.05$, **$p \leqslant 0.005$, ***$p \leqslant 0.0005$, ****$p \leqslant 0.00005$.*

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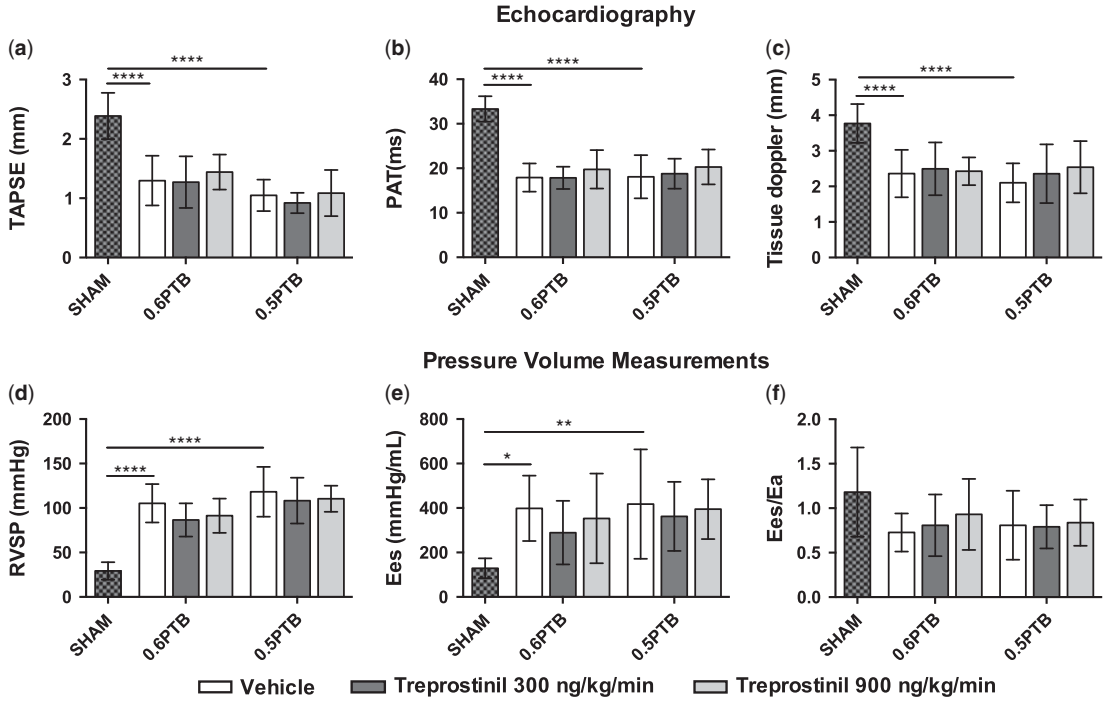

Figure 2.

*Echocardiography and pressure–volume measurements. (**a**) Tricuspid annular plane systolic excursion (TAPSE) measured by echocardiography, (**b**) peak acceleration time measured by echocardiography, (**c**) tissue Doppler measured by echocardiography, (**d**) right ventricular systolic pressure (RVSP) measured by pressure–volume measurements, (**e**) end-systolic elastance measured by pressure–volume measurements. (**f**) End-systolic elastance divided by arterial elastance measured by pressure–volume measurements. Results are expressed as mean ± SEM. *$p \leqslant 0.05$, **$p \leqslant 0.005$, ****$p \leqslant 0.00005$. 0.5PTB = pulmonary trunk banding with a 0.5-mm banding clip; 0.6PTB = pulmonary trunk banding with a 0.6-mm banding clip; sham = sham-operated animals treated with vehicle (saline); vehicle = pulmonary trunk banding animals treated with saline; Ees = end systolic elastance; Ea = arterial elastance.*

presented a twofold increase in cardiomyocyte size in both groups compared with sham animals (Fig 1c). Capillary density was decreased almost equally in the 0.6 and 0.5 groups compared with sham animals (Fig 1d).

Haemodynamic signs of right ventricular failure were increasingly present with increased banding severity as measured by tricuspid annular plane systolic excursion, stroke volume, cardiac output, right ventricular volumes, ejection fraction, and tissue Doppler (Figs 2a–c, 3a–e).

Tricuspid regurgitation was present in all 0.5PTB animals and in most 0.6PTB animals. Tricuspid regurgitation was not observed in sham animals (Table 1). Extracardiac manifestations of right ventricular failure as seen by the presence of nutmeg liver and/or ascites and hydrothorax were observed in a few 0.6PTB animals and in most of the 0.5PTB animals. None of the sham animals showed signs of extracardiac manifestations of right ventricular failure (Table 1).

Invasive pressure–volume measurements showed increased right ventricular systolic pressure in PTB animals (Fig 2d). We observed increased inotropic properties in PTB animals as measured by end-systolic elastance (Fig 2e) but a trend towards a less-favourable ventriculo-arterial coupling (Fig 2f).

Analysis of mRNA expression levels of genes related to right ventricular hypertrophy and failure showed significantly increased levels of atrial natriuretic peptide and brain natriuretic peptide in PTB animals compared with sham animals. Furthermore, we observed a non-significant increase in the 0.5 group compared with the 0.6 group ($p = 0.16$ for atrial natiuretic peptide 0.6PTB-veh versus 0.5PTB-veh; $p = 0.11$ for brain natriuretic peptide 0.6PTB-veh versus 0.5PTB-veh) (Fig 4a and b). The banding also caused increased levels of the β-isotype of myosin heavy chain, also with non-significant higher levels in the 0.5 group compared with the 0.6 group ($p = 0.12$ for 0.6PTB-veh versus 0.5PTB-veh) (Fig 4c). The analysis of collagen expression revealed significant higher levels of both collagen 1 and collagen 3 in PTB animals compared with sham animals (Fig 4d and e).

Effects of treprostinil treatment

To evaluate the effects of treprostinil, we compared animals treated with treprostinil in a moderate or

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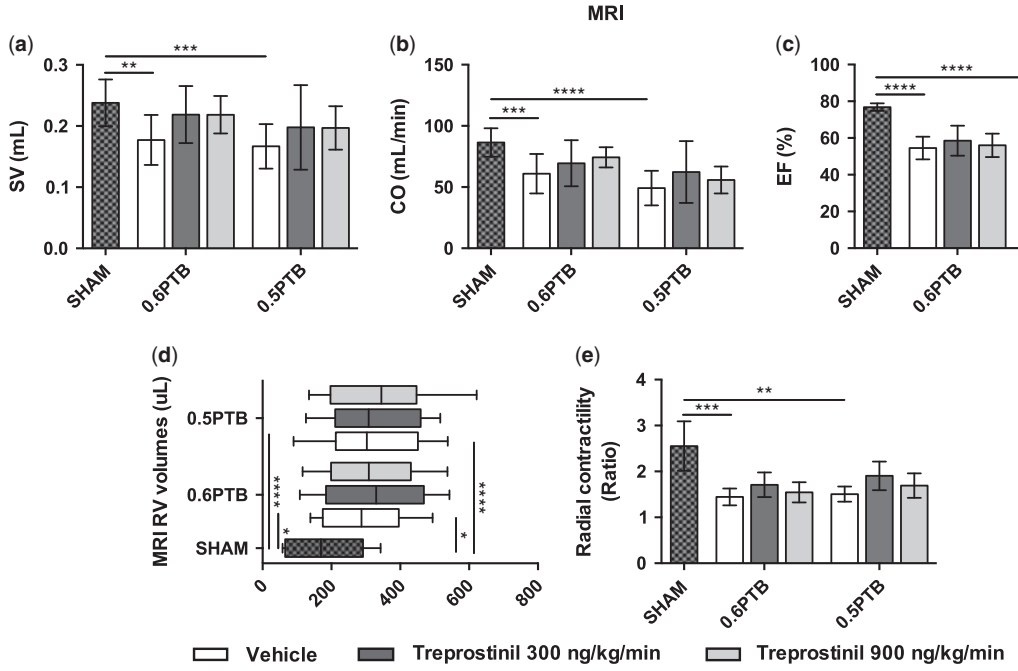

Figure 3.

*MRI. (**a**) Stroke volume (SV), (**b**) cardiac output (CO), (**c**) ejection fraction, (**d**) right ventricular (RV) volumes, (**e**) radial contractility. Results are expressed as mean ± SEM (6–14 animals/group). *$p \leqslant 0.05$, **$p \leqslant 0.005$, ***$p \leqslant 0.0005$, ****$p \leqslant 0.00005$. 0.5PTB = pulmonary trunk banding with a 0.5-mm banding clip; 0.6PTB = pulmonary trunk banding with a 0.6-mm banding clip; sham = sham-operated animals treated with vehicle (saline); vehicle = pulmonary trunk banding animals treated with saline; EF = ejection fraction.*

high dose (300 and 900 ng/kg/minute) with vehicle-treated animals.

Treprostinil did not reverse or reduce right ventricular hypertrophy neither in moderate nor in high doses (Fig 1a).

With regard to haemodynamics, no statistically significant differences were observed between vehicle and 0.5PTB or 0.6PTB groups treated with treprostinil (Figs 2 and 3). On MRI, we observed a non-significantly increased stroke volume and cardiac output (Fig 3a and b). The non-significant increase was also observed on ejection fraction (Fig 3c).

There was no improvement in tricuspid annular plane systolic excursion in either treatment groups (Fig 2a). A non-significant increased radial contractility in animals treated with treprostinil was observed ($p = 0.08$ for 0.6PTB-veh versus 0.6PTB-300Tre) (Fig 3e).

Treatment with treprostinil did not influence the right ventricular pressure–volume relations significantly. Animals treated with treprostinil showed a non-significant decreased right ventricular systolic pressure (Fig 2d) and a non-significant decreased inotropy as measured by end-systolic elastance (Fig 2e).

There was no significant difference in the fraction of animals with tricuspid regurgitation (Table 1). No difference in fibrosis fraction or cardiomyocyte size was observed between groups (Fig 1b and c). The capillary density was reduced in the 0.5PTB group treated with moderate-dose treprostinil compared with vehicle (Fig 1d).

Treatment with treprostinil did not cause significant increased or decreased levels of atrial natriuretic peptide or β-myosin heavy chain in any of the treatment groups (Fig 4a and c). On the other hand, 0.6PTB animals treated with high-dose treprostinil showed significant higher levels of brain natriuretic peptide compared with vehicle. This finding was not reproduced in any other treatment group (Fig 4b). The high dose of treprostinil reduced the expression of collagen 1 in the 0.5PTB group. No effect of treprostinil treatment on collagen 1 or 3 expression was observed in the other treatment groups (Fig 4d and e).

Discussion

In this study, rats with pressure overload-induced right heart hypertrophy and failure were treated with a moderate or high dose of treprostinil for 6 weeks. The PTB procedure resulted in hypertrophy, dilatation, and haemodynamic deterioration in all animals subjected to the procedure. Treatment with treprostinil did not show any direct anti-hypertrophic

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Table 1. Anatomical and haemodynamic data at the end of the study.

	Sham Vehicle (n = 10)	0.6PTB Vehicle (n = 10)	0.6PTB 300Tre (n = 11)	0.6PTB 900Tre (n = 9)	0.5PTB Vehicle (n = 14)	0.5PTB 300Tre (n = 7)	0.5PTB 900Tre (n = 7)
Anatomical data							
Body weight (g)	368 ± 5	318 ± 12***	377 ± 7	319 ± 7	317 ± 8***	332 ± 7	324 ± 5
RV (g)	0.16 ± 0.00	0.37 ± 0.02****	0.41 ± 0.01	0.37 ± 0.01	0.38 ± 0.01****	0.38 ± 0.01	0.38 ± 0.01
LV + s (g)	0.61 ± 0.02	0.71 ± 0.03*	0.73 ± 0.03	0.68 ± 0.02	0.70 ± 0.02*	0.66 ± 0.03	0.70 ± 0.03
Tibia length (mm)	40 ± 0.16	37 ± 0.43****	39 ± 1.09	37 ± 0.57	37 ± 0.45****	38 ± 0.52	37 ± 0.46
Haemodynamic measures echocardiography							
HR (beats per minute)	365 ± 9	344 ± 12	326 ± 7	349 ± 6	303 ± 7****	312 ± 12	313 ± 10
Tricuspid regurgitation (%)	0	80	82	78	100	100	100
Septal bulging (%)	0	40	45	55	100	100	86
SV (μl)	289 ± 18	254 ± 22	274 ± 11	267 ± 18	237 ± 15	208 ± 9	244 ± 26
CO (ml/minute)	105.87 ± 5.2	88.15 ± 9.76	89.70 ± 4.0	93.70 ± 6.4	76.61 ± 5.44**	82.87 ± 13.4	77.79 ± 6.9
RVOT VTI (mm^2)	40.62 ± 1.8	44.13 ± 3.3	44.36 ± 1.9	43.93 ± 2.5	40.54 ± 3.2	37.92 ± 2.3	44.39 ± 3.63
Haemodynamic measures MRI							
CI (ml/minute)	234 ± 8	202 ± 23	201 ± 17	233 ± 10	158 ± 16**	188 ± 32	173 ± 13
Mean Vel PUL (cm/s)	14.06 ± 0.7	11.19 ± 1.0*	12.55 ± 0.6	12.82 ± 1.2	8.85 ± 0.7***	10.89 ± 1.1	11.19 ± 0.3
Act Pul (ms)	28.9 ± 1.5	52.04 ± 7.5**	55.73 ± 3.5	59.77 ± 6.8	65.44 ± 3.8****	64.86 ± 7.0	61.30 ± 8.5
Haemodynamic measures pressure–volume relations							
MAP (mmHg)	114 ± 3	99 ± 4	89 ± 7	89 ± 5	94 ± 4*	93 ± 6	83 ± 5
PRSW	20.6 ± 2.0	54.2 ± 8.4*	48.6 ± 5.0	56.4 ± 9.6	73.3 ± 12.3***	73.7 ± 10.4	74.0 ± 17.9
EDP-BDP	2.10 ± 0.28	6.84 ± 0.40*	7.19 ± 1.20	7.60 ± 1.17	7.11 ± 0.92**	6.05 ± 0.90	9.81 ± 0.63
k value	6.33 ± 0.63	9.03 ± 1.29	6.55 ± 0.94	7.70 ± 0.78	10.93 ± 2.35	7.59 ± 0.93	7.47 ± 0.58

0.5PTB = pulmonary trunk banding with a 0.5-mm banding clip; 0.6PTB = pulmonary trunk banding with a 0.6-mm banding clip; 300Tre = pulmonary trunk banding animals treated with treprostinil (300 ng/kg/minute); 900Tre = pulmonary trunk banding animals treated with treprostinil (900 ng/kg/minute); Act Pul = acceleration time in pulmonary artery; BDP = begin diastolic pressure; CI = cardiac index; CO = cardiac output; EDP = end-diastolic pressure; HR = heart rate; LV + s = left ventricle + septum; MAP = mean arterial pressure; Mean Vel Pul = mean velocity in pulmonary artery; PRSW = preload recruitable stroke work; RV = right ventricle; RVOT VTI = right ventricular outflow tract velocity time integral; Sham = sham-operated animals treated with vehicle (saline); SV = stroke volume; vehicle = animals treated with saline

Results are expressed as mean ± SEM (7–14 animals/group)

*p ⩽ 0.05 versus sham, **p ⩽ 0.005 versus sham, ***p ⩽ 0.0005 versus sham, ****p ⩽ 0.00005 versus sham

properties in the right ventricle and did not significantly improve right ventricular function.

Effects of PTB

The PTB procedure effectively induced hypertrophy, pressure overload, and functional impairment of the right ventricle. Different settings of the clip applier made it possible to investigate both compensated and decompensated right heart failure, as most of the animals banded with a 0.5-mm clip showed extra-cardiac manifestations of right ventricular failure and also had increased deterioration of right ventricular function compared with animals banded with a 0.6-mm clip in spite of no difference in right ventricular hypertrophy. Thus, previous findings of PTB being a precise model able to discriminate between compensated and decompensated right heart failure were reproduced in this study.[11] Right ventricular weight divided by the length of the tibia almost tripled in PTB animals compared with sham animals, consistent with previous findings in PTB studies.[11,13] The increase was consistent among animals as evidence of a very reproducible disease model (Table 1).

The haemodynamic data supported right ventricular failure in the PTB animals. We observed a significant decrease in tricuspid annular plane systolic excursion in both PTB groups as seen in previous studies of decompensated right heart failure in rats[14] and in patients with pulmonary arterial hypertension with impaired right ventricular function.[15,16] Haemodynamic deterioration was observed as decreased cardiac output and ejection fraction in PTB animals. Tricuspid regurgitation was seen in all 0.5PTB animals and in most 0.6PTB animals, coinciding with our knowledge that right ventricular dilatation develops with long-term pressure overload.[17] The PTB procedure caused a fourfold increase in right ventricular systolic pressure, which coincides with previous findings.[12] We observed an increase in right ventricular systolic function in PTB rats measured by end-systolic elastance. This is expected, as right ventricular adaptation to increased afterload implies hypertrophy, leading to increased cardiac muscle

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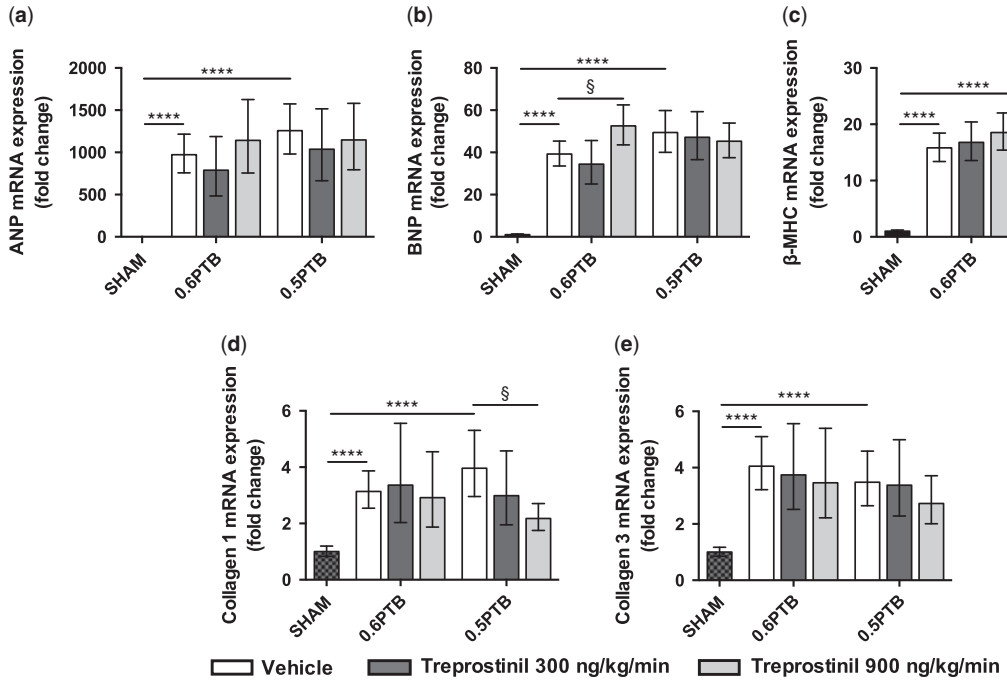

Figure 4.

*Effects of pulmonary trunk banding and treprostinil treatment on gene expression levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), and collagen 1 and 3. (**a**) mRNA expression of ANP, (**b**) mRNA expression of BNP, (**c**) mRNA expression of β-MHC, (**d**) mRNA expression of collagen 1, and (**e**) mRNA expression of collagen 3. Results are expressed as mean ± SEM (6–14 animals/group). $^{\S}p \leqslant 0.05$; ****$p \leqslant 0.00005$. 0.5PTB = pulmonary trunk banding with a 0.5-mm banding clip; 0.6PTB = pulmonary trunk banding with a 0.6-mm banding clip; sham = sham-operated animals treated with vehicle (saline); vehicle = pulmonary trunk banding animals treated with saline.*

mass and increased contractile force. The increase in end-systolic elastance does, however, not fully compensate for the increase in afterload applied by the banding clip, evident by decreased ventriculo-arterial coupling. In a model with a fixed afterload, ventriculo-arterial coupling is a complex variable to interpret with regard to treatment effect. We do, however, believe that it is an important part of the characterisation of the disease model.

The haemodynamic variables were supported by the histological findings of increased cardiomyocyte diameter, fibrosis fraction, and increased collagen 1 and 3 mRNA expressions in PTB animals, suggesting a relative increased amount of extracellular matrix and a decreased amount of contractile filaments, coinciding with findings in right ventricular biopsies from patients with pulmonary arterial hypertension.[18] The decrease in capillary density indicates that the increased oxygen consumption in the enlarged myocytes is not met by a sufficient increase in oxygen supply. This capillary rarefaction has been hypothesised to play an important role in the development of heart failure.[19]

Gene expression analysis confirmed the presence of hypertrophy and right heart failure at the molecular level with increased levels of atrial natriuretic peptide, brain natriuretic peptide, and β-myosin heavy chain in the right ventricle of PTB animals. Atrial natriuretic peptide is considered to be a phenotypic marker of hypertrophy,[18] whereas brain natriuretic peptide levels have demonstrated to correlate well with the degree of right ventricular dysfunction in patients with pulmonary arterial hypertension.[20] Increased levels of β-myosin heavy chain are caused by an isotope switch from α- to β-myosin heavy chain. In heart failure, this alteration in gene expression of myosin heavy chain isoforms is related to decreased myosin ATPase velocity and slow speed of contraction, which is also seen in humans with right heart failure caused by pulmonary arterial hypertension.[18,19]

Using the well-established PTB model, we successfully induced compensated and decompensated right ventricular hypertrophy and failure corresponding to right heart failure seen in patients suffering from pulmonary arterial hypertension with regard to anatomical, haemodynamic, and molecular characteristics.

Effects of treprostinil treatment

Treatment with treprostinil in a moderate or high dose did not reverse or attenuate right ventricular

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hypertrophy. This is in concordance with previous findings, as van Albada et al[21] found no reduction in right ventricular weight following treprostinil treatment in the monocrotaline model of pulmonary arterial hypertension. On the other hand, right ventricular size seems to be susceptible to other prostacyclin analogues. Schermuly et al[9] found decreased right ventricular weight after long-term treatment with iloprost in the monocrotaline model. This effect of iloprost is not reproduced in models of surgically induced right ventricular pressure overload with fixed afterload, suggesting that the effect found by Schermuly et al is secondary to pulmonary vasodilatation.[22] The diverging effects of different prostanoids on right ventricular hypertrophy imply that the effects of one specific prostacyclin analogue cannot be extrapolated to others. In vitro studies have shown that different analogues act as agonists for different receptors and activate different adenylyl cyclase isoforms.[23,24] Moreover, clinical research has suggested differences between prostacyclin and its analogues, which suggests that the effects are not fully clarified and that further research in differential mechanism of actions between prostacyclin analogues is needed.[25] The apparently diverse effects of different prostanoids on the right ventricle should be taken into consideration when considering them as specific therapies for the right ventricle.

The differences among prostanoids are retrieved in our histological findings, as they suggest no difference in fibrosis fraction or capillary density between groups. Gomez-Arroyo et al[22] demonstrated a reversal of established fibrosis after iloprost treatment in rats with SU5416/hypoxia-induced right ventricular dysfunction, and van Albada et al[26] demonstrated increased capillary density in iloprost-treated rats with an aortocaval shunt and monocrotaline-induced pulmonary hypertension. To our knowledge, similar effects have never been demonstrated for treprostinil. Our histology analysis did not reveal reduced deposition of fibrosis in treprostinil-treated rats. We did, however, find decreased collagen 1 mRNA expression in treprostinil-treated animals with severe PTB. This finding suggests that treprostinil has some degree of anti-fibrotic effects, and would be a relevant topic for future research.

We did observe a trend towards a better haemodynamic profile in animals treated with treprostinil in several independent parameters. Thus, stroke volume and cardiac output was insignificantly increased in treated animals measured by echocardiography and MRI. Moreover, there was a trend towards increased ejection fraction in treated animals on MRI. Comparison of treprostinil-treated animals with sham animals reveals loss of significance on some MRI flow parameters, suggesting that right heart function of treated animals approaches the one of sham animals, supporting the theory of an actual effect of treprostinil despite lack of significance. Nevertheless, this finding was not reproduced on echocardiographic parameters.

Gomez-Arroyo et al[22] recently demonstrated increased tricuspid annular plane systolic excursion after iloprost treatment in rats with SU5416/hypoxia-induced pulmonary hypertension and right ventricular dysfunction. We did not see this effect with treprostinil in PTB rats; however, we observed an insignificant increase in radial contractility in treprostinil-treated animals. It can be speculated that changes in the geometrical shape of the pressure-overloaded right ventricle might favour radial more than longitudinal contractility.[27]

Decreased right ventricular systolic pressure after prostacyclin treatment has been demonstrated for iloprost in rats with monocrotaline-induced pulmonary hypertension in an acute setting,[22] but has never been investigated for treprostinil.

To our knowledge, cardiac function has until now never been evaluated after chronic treprostinil treatment in any in vivo model of experimental right heart hypertrophy and failure. In an in vitro set-up, treprostinil has shown to slightly increase contractile dynamics of cardiomyocytes at clinically relevant concentrations and to significantly potentiate the positive inotropic effects of catecholamines.[28] The effects of iloprost have been studied more extensively in previous studies. Positive inotropic properties and improvement in haemodynamics have been demonstrated in vivo in both acute and chronic settings with fixed and non-fixed afterload[9,10] and abilities of fibrosis reduction have been proven.[22]

Treprostinil, however, has a favourable pharmacokinetic profile compared with iloprost and other prostacyclin analogues manifested by a longer half-life in blood – 3 hours compared with 0.5 hours for iloprost – facilitating less-complex administration requirements.

A few limitations of the study have to be noted. First, we used outbred Wistar rats for the experiments. The difference between rats and humans should be taken into consideration, and experiments in other animal models using different strains should be performed before clinical translation. Second, rats were anaesthetised for all haemodynamic measures. This may possibly blunt differences between groups. To minimise the effects of anaesthesia on the results, we followed a well-tested protocol of anaesthesia and we used the smallest possible amount of anaesthesia when performing non-invasive methods such as echocardiography and MRI. Third, previous studies have observed reduced cardiac output already 1 week

after the inflicted pressure overload,[11] suggesting a more abrupt introduction of pressure overload than seen in human with pulmonary hypertension, leading to a possible different response to therapy. This should be taken into consideration when interpreting our results. Finally, the comparison of studies using different methods for induction of right ventricular failure is associated with some degree of uncertainty, as the PTB model with a fixed mechanical resistance might behave differently from other standard models of right ventricular failure, such as the monocrotaline and SU5416/hypoxia model.

In this study, no adverse effects of chronic treatment with treprostinil in moderate or high doses were observed in any of the measured haemodynamic parameters. Although treprostinil showed no significant direct stimulatory effect on the right ventricle, this drug did not display any cardiodepressant effect on the hypertrophic and failing right ventricle.

In conclusion, we find that in a well-established rat model of fixed pressure overload-induced right ventricular hypertrophy and failure, moderate- and high-dose treatment by treprostinil did not improve right ventricular function neither in compensated nor in decompensated right heart failure. We did observe a trend towards a better haemodynamic profile in animals treated with treprostinil in several independent parameters; however, an actual positive effect of treprostinil on right ventricular failure cannot be concluded.

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Conflicts of Interest

This study was supported by United Therapeutics.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the Danish national guidelines and has been approved by the National Ethics Review Board and conducted in accordance with the Danish law for animal research (Authorisation number 2012-15-2934-00384 Danish Ministry of Justice).

Supplementary materials

For supplementary material referred to in this article, please visit http://dx.doi.org/10.1017/S1047951116000160

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