University of Groningen

Treprostinil in advanced experimental pulmonary hypertension

van Albada, Mirjam E.; van Veghel, Richard; Cromme-Dijkhuis, Adri H.; Schoemaker, Regien G.; Berger, Ro F. M. E.

Published in:
Journal of Cardiovascular Pharmacology

DOI:
[10.1097/01.fjc.0000248229.87510.9b](https://doi.org/10.1097/01.fjc.0000248229.87510.9b)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

van Albada, M. E., van Veghel, R., Cromme-Dijkhuis, A. H., Schoemaker, R. G., & Berger, R. F. M. E. (2006). Treprostinil in advanced experimental pulmonary hypertension: Beneficial outcome without reversed pulmonary vascular remodeling. *Journal of Cardiovascular Pharmacology*, 48(5), 249-254. <https://doi.org/10.1097/01.fjc.0000248229.87510.9b>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Treprostinil in Advanced Experimental Pulmonary Hypertension: Beneficial Outcome Without Reversed Pulmonary Vascular Remodeling

Mirjam E. van Albada, MD,* Richard van Veghel,† Adri H. Cromme-Dijkhuis, MD, PhD,§
Regien G. Schoemaker, PhD,† and Rolf M. F. Berger, MD, PhD*

Introduction: Beneficial effects of treprostinil, a stable prostacyclin analogue, were demonstrated in patients with pulmonary arterial hypertension (PAH). Although regression of pulmonary vascular remodeling has been suggested as therapeutic mechanism, its mode of action remains unknown.

Methods: Flow-associated PAH was created in rats by injection of monocrotaline (60 mg/kg) combined with an abdominal aortocaval shunt. Subsequently, rats were treated with subcutaneous treprostinil (50 ng/kg/min, treated; $n = 8$) or saline (untreated; $n = 9$). A control group underwent sham-surgery ($n = 8$). Animals were sacrificed at symptoms of cardiac failure, together with their matched controls.

Results: Dyspnea and weight loss determined the moment of sacrifice in 8/9 untreated animals (89%) versus in one of eight treated animals (13%; log-rank test survival curves; $P = 0.02$). Mean pulmonary arterial pressure increased in the model (42 ± 2 mm Hg in untreated vs. 18 ± 1 in controls; $P < 0.01$) and decreased by 8 mm Hg after therapy (34 ± 3 mm Hg, $P = 0.04$ vs. untreated). No effects of treatment on right ventricular hypertrophy could be demonstrated. Quantitative morphometry of pre- and intra-acinar pulmonary arteries revealed no effects of treatment on vessel histopathology.

Conclusions: Treprostinil treatment improved clinical course and ameliorated symptoms of heart failure in a model of advanced PAH. However, beneficial effects were not associated with reversed structural remodelling of the pulmonary vasculature.

Key Words: pulmonary hypertension, pulmonary vascular histopathology, prostacyclin, monocrotaline, surgical arteriovenous shunt

(*J Cardiovasc Pharmacol*™ 2006;48:249–254)

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive and often fatal disease that is characterized by proliferation of intimal and vascular smooth muscle cells in the pulmonary arteries. This pulmonary vascular remodeling leads to obliteration of small pulmonary arteries, increased pulmonary vascular resistance, and eventually to right ventricular failure and death.^{1–5}

Continuous intravenous epoprostenol therapy has demonstrated clinically beneficial effects in patients with PAH, such as improvements in pulmonary hemodynamics, exercise capacity, quality of life, and survival.^{6–9}

Epoprostenol has a very short half life of 1 to 2 minutes and thus requires continuous intravenous administration. Therefore, more stable prostacyclin analogues have been developed allowing for other modes of delivery. Treprostinil is more stable than epoprostenol, also activates prostacyclin receptors and has comparable clinical effects. The advantage of treprostinil is that it allows for continuous, subcutaneous administration.^{10–12}

The therapeutic mechanism, leading to the beneficial effects of prostacyclin and its analogues in patients with PAH, is largely unknown. Patients who do not show any acute pulmonary vasodilatory response to epoprostenol, nitric oxide, or other pulmonary vasodilators, do show improvement in outcome after several months of prostanoid treatment. These observations suggest an effect on structural remodeling of the diseased pulmonary arteries, rather than effects on pulmonary vascular tone.^{7,9,13} Indeed, in vitro treprostinil is shown to have antimitogenic properties on vascular smooth muscle cells.¹³ Therefore, prostanoid therapy is assumed to beneficially affect structural remodeling of the pulmonary arteries present in PAH. However, such an effect of treprostinil therapy has never been demonstrated in vivo.

In the current study, we investigated the effects of subcutaneous treprostinil therapy on outcome, hemodynamics, and pulmonary vascular histopathology in an animal model for flow-associated pulmonary arterial hypertension that is associated with advanced pulmonary vascular disease, including neointimal lesions.^{14,15}

METHODS

Animals

Twenty-six male Wistar rats, weighing 320 to 360 g, were fed ad libitum. Animal care and experiments were

From the *Department of Pediatrics, Division of Pediatric Cardiology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; †Department of Experimental Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; ‡Department of Pharmacology, Erasmus Medical Center, Rotterdam, the Netherlands; and §Department of Pediatrics, Division of Pediatric Cardiology, Erasmus Medical Center, Rotterdam, the Netherlands.

Reprints: M. E. van Albada, Antonius Deusinglaan 1, 9713 AV Groningen, the Netherlands (e-mail: m.e.van.albada@med.umcg.nl).

Copyright © 2006 by Lippincott Williams & Wilkins

conducted according to guidelines from the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The Erasmus University Animal Care and Use Committee approved the experimental protocol.

Study Design

Rats were randomly assigned to one of three experimental groups: 1) a control group (control; $n = 8$) that underwent sham surgery; 2) an experimental group (untreated; $n = 9$), in which a model for flow-associated pulmonary hypertension was created as previously described, using a combination of a monocrotaline injection (60 mg/kg sc, Sigma Chemical Co, St. Louis, MO) followed by the creation of an abdominal aortocaval shunt 1 week later in order to create flow-associated pulmonary hypertension and to induce advanced vascular neointimal lesions^{15,16}; or 3) an experimental group (treated; $n = 9$) that received continuous subcutaneous treatment with the prostacyclin analogue treprostinil (50 ng/kg/min; United Therapeutics Corporation, Research Triangle Park, NC) via osmotic minipumps (model 2004, Alzet, Palo Alto, CA). Implantation of the osmotic minipumps occurred on the same day as the creation of the shunt. At that time point, pulmonary vascular remodeling due to the monocrotaline has already started, whereas right ventricular hypertrophy is not yet present.¹⁷ Treatment was continued until sacrifice, usually during 4 to 5 weeks. Animals were sacrificed when a significant deterioration in clinical condition was noted, simultaneously with the two matched animals from the other groups.

Echocardiography

In a subgroup of rats from each group (control, $n = 4$; untreated, $n = 4$; treated, $n = 5$), echocardiography was performed under pentobarbital anesthesia 4 weeks after the administration of monocrotaline using a 12-MHz phased array transducer (Sonos 5500, Hewlett-Packard Inc, Andover, MA). Ventricular dimensions and flow profiles over the aortic valve were assessed in standard views.¹⁸ Stroke volume was calculated using the following equation: stroke volume = aortic valve-area $\times$ aortic velocity-time integral.

Hemodynamics

At the time of sacrifice, animals were anesthetized with pentobarbital (60 mg/kg intraperitoneally). Pulmonary arterial pressures were measured with the technique of Rabinovitch, as described previously.^{14,19} By introducing a catheter via the left carotid artery into the aorta, systemic arterial pressures as well as heart rate were measured.

Pulmonary Vascular Remodeling

After completion of hemodynamic measurements, the presence of pleural fluid was noted. The left lung was fixed in 3.6% formalin. Pulmonary sections were stained with hematoxylin-eosin and resorcin-fuchsin elastin stain for qualitative and quantitative morphometric analysis of vascular dimensions. All transversally cut arteries with a diameter > 50 μm (preacinar arteries) and 40 randomly chosen vessels (10 in each left lung quadrant) with a diameter < 50 μm (intra-acinar vessels) were assessed using an image analysis system (CZ KS400, Imaging Associates, Bicester, UK) by measuring three

different vascular areas: outer vessel area, inner vessel area, and luminal area. Outer vessel area was defined as the area within the lamina elastica externa. The area within the lamina elastica interna was denoted as inner vessel area. Wall area was calculated by subtracting luminal area from outer vessel area. Medial area was defined as outer vessel area minus inner vessel area. Intimal area was calculated by subtracting luminal area from the inner vessel area and expressed as a percentage of inner vessel area.

Vessels < 50 μm usually do not have a clearly discernible internal elastic lamina. Therefore, a vascular occlusion score was calculated in these vessels as opposed to the calculation of a medial wall thickness to lumen ratio in the larger pulmonary arteries. Occlusion was calculated in the intra-acinar pulmonary vessels according to the following formula: (outer vessel area – luminal area)/(outer vessel area).

Pulmonary arteries were excluded from measurement if they had a longest/shortest diameter of more than 2, an incomplete circular shape or a collapse of more than one quarter of the vessel wall. Muscularization of the intra-acinar arteries was scored separately.¹⁴

Statistical Analysis

Power analysis prior to the study revealed that in order to be able to demonstrate a 25% reduction in mean pulmonary pressure, right ventricular hypertrophy or pulmonary vascular occlusion score, with a power of 0.8 and an alpha of 0.05, a sample size of 7 to 8 rats per group was needed. Data were analyzed using SPSS version 12.0.2. Mortality data were analyzed with a log-rank test. Morbidity data (the presence of pleural fluid) were analyzed using Fisher's exact test. Other differences between healthy control animals and untreated animals and between the untreated and treated animals were analyzed using one-way analysis of variance testing with Fisher's protected least squared difference post-hoc testing. Nonparametric testing was performed when data were not normally distributed. Alpha was chosen to be 0.05.

RESULTS

Abdominal aortocaval shunt surgery was performed successfully in all but one rat, in the treated group, that died from postoperative complications.

Weight loss and dyspnea were observed in the PAH animals (both treated and untreated) in the 5th and 6th week after monocrotaline administration. This led to the sacrifice of most rats within this period (median survival after monocrotaline injection: 35 days). Symptoms appeared earliest and most severe in the untreated rats, leading to preliminary death in one out of nine of these rats. Consequently, hemodynamic data could not be obtained in this animal. Once was the moment of sacrifice determined by a rat from the treated group, versus eight times by rats from the untreated group (Table 1, log-rank test on survival curves, $P = 0.02$). Thoracic fluid was observed in eight of the nine untreated rats and in five of the eight treated rats (Table 1).

Echocardiography

Increased pulmonary blood flow due to the presence of the aortocaval shunt was confirmed echocardiographically by

TABLE 1. Animal Characteristics and Heart Weights

	Animals With Excessive Thorax Fluid (>12 mL)	Animals That Determined the Moment of Sacrifice	Body Weight at Sacrifice	Heart Rate (bpm)	Cardiac Pathology		
					RV (g)	RA (g)	RV/LV+IVS
Control	0/8 (0%)	0/8 (0%)	428 ± 14	371 ± 11	0.240 ± 0.013	0.030 ± 0.004	0.284 ± 0.013
Untreated	8/9 (89%)	8/9 (89%)*	373 ± 20	273 ± 22*	0.497 ± 0.010*	0.112 ± 0.014*	0.629 ± 0.036*
Treated	5/8 (63%)	1/8 (13%)†	401 ± 24	281 ± 14*	0.459 ± 0.039*	0.083 ± 0.011*	0.591 ± 0.061*

Body weight is shown as mean ± SEM.

* $P < 0.05$ vs. control animals, † $P < 0.05$ vs. untreated.

RV, right ventricular weight in grams; RA, right atrial weight in grams; RV/LV+IVS, right ventricular to left ventricular plus interventricular septal weight ratio.

an increased stroke volume at 4 weeks in the PAH animals compared to the nonshunted control rats (0.48 ± 0.04 mL in PAH animals vs. 0.29 ± 0.02 mL in nonshunted control rats, $P = 0.03$).

Right ventricular wall thickness, as indication for increased right ventricular load, increased nonsignificantly in the untreated animals compared to control animals (1.09 ± 0.08 vs. 0.79 ± 0.09 mm in control, $P = 0.086$). In this sample, therapy did not alter right ventricular wall thickness (1.06 ± 0.13 mm in treated animals, NS vs. untreated). The internal diastolic diameter of the right ventricle was increased in PAH animals (6.43 ± 0.68 mm in untreated vs. 3.87 ± 0.04 in control, $P = 0.04$). In this small number of animals, treprostinil treatment did not alter right ventricular diameter significantly (5.31 ± 0.57 mm in treated, NS vs. untreated).

Hemodynamics

Hemodynamic data are shown in Figure 1. Mean pulmonary arterial pressure (mPAP) and the ratio of mPAP to mSAP were significantly increased in the untreated animals compared to control animals, whereas mean systemic arterial pressure (mSAP) and heart rate (273 ± 22 bpm in untreated rats vs. 371 ± 11 bpm in control animals, $P = 0.001$) were

significantly reduced in these animals compared to controls. In the animals treated with treprostinil, mPAP decreased compared to the nontreated animals (treatment effect -8 mm Hg, $P = 0.04$). No differences could be demonstrated for the other hemodynamic parameters between treated and untreated animals (Fig. 1; heart rate 281 ± 14 bpm in treated animals, NS vs. untreated animals).

Pathology

Right ventricular and atrial weights are shown in Table 1. Right ventricular hypertrophy, expressed as right ventricular to left ventricular plus septal weight ratio, did not differ between untreated and treated animals (Table 1).

Pulmonary Vascular Remodeling

Preacinar Pulmonary Arteries (>50 μ m)

Compared to control rats, the total number of preacinar pulmonary arteries was augmented in the PAH animals (Fig. 2B). Both the inner and outer diameter of the larger pulmonary arteries increased significantly (inner vessel diameter: 121.4 ± 7.0 μ m in untreated vs. 82.7 ± 4.3 μ m in control animals; outer vessel diameter: 148.1 ± 7.0 μ m in untreated vs. 101.1 ± 4.3 μ m in control animals; both $P < 0.05$). Furthermore, medial wall thickness was increased (Fig. 2A). Neointimal formation was not found in these preacinar arteries (data not shown).

Treprostinil treatment did not change any of the described morphologic parameters of vascular remodelling (inner vessel diameter: 113.2 ± 3.8 μ m; outer vessel diameter: 140.4 ± 4.6 μ m, both NS vs. untreated animals; Fig. 2A, B).

Intra-acinar Pulmonary Vessels (<50 μ m)

In contrast to control rats, the intra-acinar vessels of PAH rats showed increased muscularization and luminal occlusion (Fig. 2C–E). In these rats, the outer diameter of the intra-acinar vessels did not change (outer vessel diameter 32.2 ± 0.8 μ m in untreated vs. 33.3 ± 0.5 μ m in control animals). However, the luminal diameter decreased significantly (luminal diameter 26.3 ± 0.7 μ m in untreated vs. 32.4 ± 0.6 μ m in control animals; $P < 0.05$ vs. control). Thus, wall thickness was increased in the animal model (2.98 ± 0.20 μ m in untreated vs. 0.42 ± 0.13 μ m in controls, $P < 0.001$).

Additionally, the percentage of intra-acinar pulmonary vessels that was completely muscularized was increased ($20.6 \pm 2.4\%$ in untreated vs. $6.9 \pm 2.4\%$ in controls, $P = 0.002$, Fig. 2D).

FIGURE 1. Mean pulmonary arterial pressure, mean systemic arterial pressure, and the ratio between these parameters in the three animal groups. * $P < 0.05$ vs. control. † $P < 0.05$ vs. untreated. mPAP, mean pulmonary arterial pressure; mSAP, mean systemic arterial pressure; mPAP/mSAP, ratio between mean pulmonary arterial pressure and mean systemic arterial pressure.

FIGURE 2. Pulmonary vascular remodelling in the different animal groups. A, Medial wall thickness of the pre-acinar pulmonary arteries. B, Number of preacinar arteries. C, Luminal occlusion score of the intra-acinar pulmonary vessels. D, Muscularization of the intra-acinar pulmonary vessels. E, Examples of intra-acinar pulmonary vessel remodelling. To the left, an unobstructed intra-acinar vessel in healthy control animal, hematoxylin-eosin stain; in the middle, the vessel of an untreated animal with intra-acinar vessel obstruction; to the right, a treated animal showing an unchanged pattern of pulmonary vascular remodelling. * $P < 0.05$ vs. control.

No alterations in this morphologic pattern of vascular remodelling could be demonstrated after treatment with treprostinil (outer vessel diameter $30.9 \pm 0.9 \mu\text{m}$, luminal diameter $26.2 \pm 0.9 \mu\text{m}$, wall thickness $2.35 \pm 0.39 \mu\text{m}$ and percentage of vessels that was totally muscularized $18.2 \pm 3.5\%$, all NS vs. untreated animals; Fig. 2C–E).

DISCUSSION

Our findings demonstrate that continuous subcutaneous treatment with the prostacyclin analogue treprostinil has a beneficial effect on morbidity, mortality, and hemodynamics in a rat model of PAH associated with advanced pulmonary vascular lesions. Mean pulmonary artery pressure decreased after treprostinil treatment by 8 mm Hg. In this model of advanced pulmonary vascular disease, no beneficial effects of treprostinil therapy on the structural remodelling of the diseased pulmonary arteries could be demonstrated. To our

knowledge, this is the first study that investigates the effects of continuous, subcutaneous administration of treprostinil on structural pulmonary vascular remodelling in an in vivo situation. In our study, we started treatment 1 week after the administration of monocrotaline, when severe pulmonary vascular lesions are already present.²⁰ Thus, this study was designed as a regression study in a model with advanced pulmonary vascular disease, including occlusion of small pulmonary vessels by neointima formation, because this mimics the stage of disease progression in which patients with PAH do present in clinical practice.

The data on hemodynamics and outcome from this study closely resemble the effects of prostacyclin treatment in patients with pulmonary arterial hypertension. Intravenous epoprostenol has been shown to improve quality of life, symptoms, exercise tolerance, hemodynamics, and survival in patients with pulmonary arterial hypertension in a randomized clinical trial.¹²

Treprostinil is a tricyclic benzidine prostacyclin analogue with a half life of 3 to 4 hours, which is suitable for subcutaneous administration.^{10,11,21} Its major advantage is therefore that it eliminates the need for continuous intravenous access and its complications as the occurrence of infections, thrombosis, and acute discontinuation of the drug. In a recent trial, the clinical effects of treprostinil in patients with PAH were reported by Simonneau and co-investigators.¹² The primary endpoint in this 12-week, double-blind, placebo-controlled study consisted of the 6-minute walk test, reinforced by indices of dyspnea and fatigue. These improved significantly in patients treated with treprostinil compared to placebo-treated patients. Hemodynamic parameters, as secondary endpoints, also improved with treprostinil treatment compared to placebo. However, this improvement of hemodynamics was rather modest, as was illustrated by a treatment effect on mean pulmonary arterial pressure of 8 mm Hg. This is consistent with findings in trials with other prostacyclin analogues, in which improved functional outcome parameters, including survival, were also associated with relatively modest effects on pulmonary hemodynamics.⁷ With respect to the improved clinical outcome as well as to the modest improvement in hemodynamic parameters, our findings in this rat model are congruent to the findings in these human studies.

Considering the observed effects of prostanoid treatment, theories about the mechanism of action of these drugs have emerged. The major activities of prostacyclin analogues are mediated by cell surface prostanoid receptors, the IP receptors, that increase cell cAMP,²² leading to pulmonary vascular dilatation.²³ Further experimental evidence exists that prostacyclin analogues actually decrease vascular smooth muscle cell proliferation.^{13,24,25} This was supported by the findings of Schermuly and coworkers, who found that inhaled iloprost induced regression of medial hypertrophy in the monocrotaline rat model. It should be noted, however, that these animals did not have advanced pulmonary vascular lesions.²⁶ The mechanisms by which cell proliferation is inhibited still have to be determined.

In vitro data have demonstrated that treprostinil induces a larger and more sustained increase in cAMP compared to other analogues.¹³ Moreover, treprostinil appeared to be a particular potent inhibitor of smooth muscle cell proliferation.¹³ Additionally, treprostinil was shown to mediate anti-proliferative effects on lung fibroblasts.²⁷

Taken together, these observations suggest that treprostinil could exert its effects in patients with pulmonary arterial hypertension by reversing remodeling of the pulmonary vascular lesions. This hypothesis has not been tested in an in vivo situation before. Moreover, it has not been tested in pulmonary hypertension associated with advanced vascular lesions, such as neointimal lesions, as is the case in patients with pulmonary arterial hypertension. In this study, using a rat model for advanced pulmonary vascular disease, however, we found no support for this hypothesis: the extensive morphological analysis of the pulmonary vascular bed performed in this study could not demonstrate any changes in pulmonary vascular histopathology induced by treprostinil treatment.

We studied the animals in an advanced stage of pulmonary vascular disease. In the used design, the time of

sacrifice of the trios of rats was determined by a significant clinical deterioration of one of the three animals. It is important to realize that the two matched animals from the other study groups that were sacrificed simultaneously had not clinically deteriorated yet. In other words, our study suggests that the observed better clinical condition of the treated animals at this timepoint apparently was not due to a delayed or reversed pulmonary vascular remodelling process.

Several factors may have contributed to the differences found between the in vitro and the in vivo effects of prostanoids. Due to the design of this study, cell proliferation is already present in the studied animal model before the start of treprostinil treatment. This is similar to the situation in patients with advanced pulmonary vascular disease. Therefore, a limitation in cellular proliferation rate by treprostinil might not be sufficient to reduce the vascular lesions that are already present. Furthermore, in the pathobiology of vascular remodeling, multiple mechanisms and cell types are involved. It is a process that is not restricted to vascular smooth muscle cell proliferation alone.

Taking together the results of our study and the observations in patients treated with prostacyclin analogues, the mechanism for the improved clinical outcome after prostanoid therapy is unlikely to be fully explained by the modest reduction in pulmonary artery pressure or by regression of structural pulmonary vascular remodeling.¹² Speculating on a mechanism, one could think of prostacyclin analogues having positive inotropic properties^{28,29} or increasing vascular capacitance. Indeed, Castelain et al demonstrated that prostacyclin altered the relation between cardiac index and pulmonary pressures during exercise.³⁰ The current study does not provide data to address this hypothesis. More detailed hemodynamic evaluation, including exercise testing, would be required to study this issue.

Limitations of the Study

The dose of treprostinil administered in this study was 50 ng/kg/min. This dose was chosen to create a plasma concentration in rats that matches plasma concentrations that are effective in humans.¹² Furthermore, we tried to avoid a dose that would have significant effects on systemic hemodynamics. Because patient data suggest a strong dose-dependency of the effects of treprostinil,¹² it can not be excluded that a higher dosage could lead to effects on pulmonary vascular remodelling.

As stated previously, this study was powered to detect a 25% reduction in pulmonary vascular occlusion score, mean pulmonary arterial pressure, or right ventricular hypertrophy. We can therefore not rule out that smaller changes in the pulmonary vascular remodelling process may have occurred that may have contributed to the observed beneficial clinical effect in this experiment.

No ideal animal model for pulmonary arterial hypertension exists. Although our rat model mimics the human disease with respect to clinical symptoms, hemodynamics and pulmonary vascular lesions, the presence of interspecies differences, or the use of monocrotaline may induce response patterns to treatment that differ from those in humans with PAH.

Finally, one should be cautious to extrapolate the results of this study to other prostacyclin analogues. In vitro work

showed that different analogues might act as agonists for different receptors and activate different adenylyl cyclase isoforms.^{13,22,27} Moreover, clinical research suggests differences between prostacyclin and its analogues: When the transition from treatment with epoprostenol to treatment with intravenous treprostinil was studied, pulmonary hemodynamics appeared to alter in adverse direction, showing an increase in pulmonary arterial pressure and a decrease in cardiac index.³¹ Whether these differences between prostacyclin and its analogues are of clinical importance requires further research.

CONCLUSION

Our findings demonstrate that continuous subcutaneous treatment with the prostacyclin analogue treprostinil reduces morbidity and mortality in a rat model for advanced pulmonary vascular disease while hemodynamics improved modestly. These observations closely resemble the beneficial effects of subcutaneous treprostinil treatment in patients with PAH. The mechanism of action of treprostinil has been assumed to include reversal of pulmonary vascular lesions. However, in this model of advanced pulmonary vascular disease, we could not demonstrate treprostinil to improve structural pulmonary vascular remodelling.

REFERENCES

- Wagenvoort CA. Lung-Biopsy Specimens in the Evaluation of Pulmonary Vascular-Disease. *Chest*. 1980;77:614–625.
- Wagenvoort CA, Mooi WJ. Plexogenic arteriopathy. In: Munro Neville A, Walker F, Gottlieb LS, eds. *Biopsy Pathology of the Pulmonary Vasculature*. London: Chapman and Hall Medical; 1989:56–113.
- Pietra GG, Capron F, Stewart S, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol*. 2004; 43:25S–32S.
- Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med*. 2004;351:1425–1436.
- Geiger R, Berger RM, Hess J, et al. Enhanced expression of vascular endothelial growth factor in pulmonary plexogenic arteriopathy due to congenital heart disease. *J Pathol*. 2000;191:202–207.
- Barst RJ, Rubin LJ, McGoon MD, et al. Survival in primary pulmonary hypertension with long-term continuous intravenous prostacyclin. *Ann Intern Med*. 1994;121:409–415.
- Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N Engl J Med*. 1996;334:296–302.
- Rubin LJ, Mendoza J, Hood M, et al. Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin (epoprostenol). Results of a randomized trial. *Ann Intern Med*. 1990;112:485–491.
- Higenbottam T, Wheeldon D, Wells F, et al. Long-term treatment of primary pulmonary hypertension with continuous intravenous epoprostenol (prostacyclin). *Lancet*. 1984;1:1046–1047.
- Wade M, Baker FJ, Roscigno R, et al. Pharmacokinetics of treprostinil sodium administered by 28-day chronic continuous subcutaneous infusion. *J Clin Pharmacol*. 2004;44:503–509.
- Laliberte K, Arneson C, Jeffs R, et al. Pharmacokinetics and steady-state bioequivalence of treprostinil sodium (Remodulin) administered by the intravenous and subcutaneous route to normal volunteers. *J Cardiovasc Pharmacol*. 2004;44:209–214.
- Simonneau G, Barst RJ, Galie N, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med*. 2002;165:800–804.
- Clapp LH, Finney P, Turcato S, et al. Differential effects of stable prostacyclin analogs on smooth muscle proliferation and cyclic AMP generation in human pulmonary artery. *Am J Respir Cell Mol Biol*. 2002; 26:194–201.
- van Albada ME, Schoemaker RG, Kemna M, et al. The role of increased pulmonary blood flow in pulmonary arterial hypertension. *Eur Respir J*. 2005;26:487–493.
- Tanaka Y, Schuster DP, Davis EC, et al. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. *J Clin Invest*. 1996; 98:434–442.
- Okada K, Tanaka Y, Bernstein M, et al. Pulmonary hemodynamics modify the rat pulmonary artery response to injury. A neointimal model of pulmonary hypertension. *Am J Pathol*. 1997;151:1019–1025.
- Jones JE, Mendes L, Rudd MA, et al. Serial noninvasive assessment of progressive pulmonary hypertension in a rat model. *Am J Physiol Heart Circ Physiol*. 2002;283:H364–H371.
- Kato Y, Iwase M, Kanazawa H, et al. Progressive development of pulmonary hypertension leading to right ventricular hypertrophy assessed by echocardiography in rats. *Exp Anim*. 2003;52:285–294.
- Rabinovitch M, Gamble W, Nadas AS, et al. Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. *Am J Physiol*. 1979;236:H818–H827.
- Wilson DW, Segall HJ, Pan LC, et al. Mechanisms and pathology of monocrotaline pulmonary toxicity. *Crit Rev Toxicol*. 1992;22:307–325.
- Steffen RP, de la Mata M. The effects of 15AU8I, a chemically stable prostacyclin analog, on the cardiovascular and renin-angiotensin systems of anesthetized dogs [corrected]. *Prostaglandins Leukot Essent Fatty Acids*. 1991;43:277–286.
- Turcato S, Clapp LH. Effects of the adenylyl cyclase inhibitor SQ22536 on iloprost-induced vasorelaxation and cyclic AMP elevation in isolated guinea-pig aorta. *Br J Pharmacol*. 1999;126:845–847.
- McLaughlin VV, Gentner DE, Panella MM, et al. Reduction in pulmonary vascular resistance with long-term epoprostenol (prostacyclin) therapy in primary pulmonary hypertension. *N Engl J Med*. 1998;338: 273–277.
- Tuder RM, Zaiman AL. Prostacyclin analogs as the brakes for pulmonary artery smooth muscle cell proliferation: is it sufficient to treat severe pulmonary hypertension? *Am J Respir Cell Mol Biol*. 2002;26:171–174.
- Li RC, Cindrova-Davies T, Skepper JN, et al. Prostacyclin induces apoptosis of vascular smooth muscle cells by a cAMP-mediated inhibition of extracellular signal-regulated kinase activity and can counteract the mitogenic activity of endothelin-1 or basic fibroblast growth factor. *Circ Res*. 2004;94:759–767.
- Schermlay RT, Yilmaz H, Ghofrani HA, et al. Inhaled iloprost reverses vascular remodeling in chronic experimental pulmonary hypertension. *Am J Respir Crit Care Med*. 2005;172:358–363.
- Ali FY, Egan K, Fitzgerald GA, et al. Role of Prostacyclin Receptor Versus PPAR{beta} with Treprostinil Sodium on Lung Fibroblast Proliferation. *Am J Respir Cell Mol Biol*. 2006;34:242–246.
- Sebbag I, Rudski LG, Therrien J, et al. Effect of chronic infusion of epoprostenol on echocardiographic right ventricular myocardial performance index and its relation to clinical outcome in patients with primary pulmonary hypertension. *Am J Cardiol*. 2001;88:1060–1063.
- Rich S, McLaughlin VV. The effects of chronic prostacyclin therapy on cardiac output and symptoms in primary pulmonary hypertension. *J Am Coll Cardiol*. 1999;34:1184–1187.
- Castelain V, Chemla D, Humbert M, et al. Pulmonary artery pressure-flow relations after prostacyclin in primary pulmonary hypertension. *Am J Respir Crit Care Med*. 2002;165:338–340.
- Gomberg-Maitland M, Tapson VF, Benza RL, et al. Transition from Intravenous Epoprostenol to Intravenous Treprostinil in Pulmonary Hypertension. *Am J Respir Crit Care Med*. 2005;172:1586–1589.