Continuous Infusion of Epoprostenol Improves the Net Balance Between Pulmonary Endothelin-1 Clearance and Release in Primary Pulmonary Hypertension

David Langleben, MD; Robyn J. Barst, MD; David Badesch, MD; Bertron M. Groves, MD; Victor F. Tapson, MD; Srinivas Murali, MD; Robert C. Bourge, MD; Neil Ettinger, MD; Eileen Shalit, RN; Linda M. Clayton, PharmD; Maria M. Jöbsis, BA; Shelmer D. Blackburn, BA; James W. Crow, PhD; Duncan J. Stewart, MD; Walker Long, MD

Background—Primary pulmonary hypertension results from progressive narrowing of the precapillary pulmonary vasculature. A variety of endothelial abnormalities have been identified, including a net reduction in pulmonary clearance of the vasoconstrictor and smooth muscle mitogen endothelin-1. In many patients, net pulmonary release of endothelin-1 is observed. Chronic infusions of epoprostenol (prostacyclin) improve functional capacity, survival, and hemodynamics in patients with advanced primary pulmonary hypertension. We hypothesized that the epoprostenol infusions, as compared with conventional therapy, might alter the abnormal pulmonary endothelin-1 homeostasis.

Methods and Results—Using a subset of patients from a larger randomized study comparing epoprostenol plus conventional therapy (n=11 in the present study) with conventional therapy alone (n=7 in the present study), we determined the ratio of plasma endothelin-1 levels in systemic arterial blood leaving the lung to levels in mixed venous blood entering the lung both before randomization and after 88 days of continuous therapy. There were no differences between the 2 groups before therapy, but by day 88, the epoprostenol-treated group had a greater proportion of patients (82%) with an arterial/venous ratio <1 than did the conventional therapy group, in which only 29% of patients had a ratio <1 (P<0.05).

Conclusions—These results suggest that continuous epoprostenol therapy may have a beneficial effect on the balance between endothelin-1 clearance and release in many patients with primary pulmonary hypertension and may provide one explanation for the salutary effect of epoprostenol in this disease. (Circulation. 1999;99:3266-3271.)

Key Words: hypertension, pulmonary ■ endothelin ■ epoprostenol ■ prostaglandins

Primary pulmonary hypertension (PPH) causes progressive elevation of pulmonary vascular resistance, leading to right heart failure and death. Although vasoconstriction may play a role, microvascular narrowing and obliteration by cellular proliferation are important to the pathogenesis of PPH. The cause of PPH is as-yet unidentified, but abnormalities of vascular and endothelial homeostasis, including reduced epoprostenol (formerly called prostacyclin) production, increased thromboxane production, possibly decreased nitric oxide synthase levels, and altered pulmonary handling of endothelin-1, have been identified in patients with established PPH. It is not known whether any 1 of these abnormalities is the initiating cause of PPH, but all favor vasoconstriction and cellular proliferation and could contribute to the progression of the disease.

Endothelin-1 (ET-1) is a vasoconstrictor peptide and smooth muscle mitogen that has been implicated in the pathogenesis of several models of pulmonary hypertension. The human lung normally acts as a clearance organ for ET-1, removing 60% to 70% of circulating ET-1 from the blood on each passage through the lungs. However, as assessed by the ratio of ET-1 levels in systemic arterial
plasma leaving the lung to that of mixed venous plasma entering it, many PPH patients have ratios close to or greater than unity, suggesting reduced net clearance and, in some patients, net release. Histologic studies of lung tissue from patients with PPH demonstrate excess ET-1 production and increased expression of prepro-ET-1. Given its actions on the microvasculature, ET-1 could contribute to the progressive rise in pulmonary vascular resistance seen in PPH.

The introduction of epoprostenol as a therapy for advanced PPH has resulted in improved functional capacity, hemodynamics, and survival. Although epoprostenol is a potent vasodilator and platelet antiaggregant, it also inhibits smooth muscle cell growth in vitro. In many patients who have only a minimal acute vasodilator response to epoprostenol, long-term intravenous therapy results in a gradual reduction of pulmonary vascular resistance. It has, therefore, been postulated that some of the beneficial effects of epoprostenol may relate not to its vasodilator action, but to other, as yet unidentified effects on the growth and synthetic function of vascular cells. It is not known whether epoprostenol can beneficially alter the homeostasis of other, potentially detrimental vascular mediators, such as ET-1. In the present study, by measuring the ratio of ET-1 levels in plasma leaving and entering the lung, we show that chronic epoprostenol infusions help restore the balance toward normal in many PPH patients.

Methods

Patient Population

Our patients were a subset of the participants in the North American Primary Pulmonary Hypertension Group study. All had NYHA functional class III or IV PPH, as determined using established criteria. All patients underwent a baseline cardiac catheterization (day 0) and then were randomized to receive either conventional therapy (oral calcium blockers, diuretics, digoxin, oxygen, and warfarin) or conventional therapy plus chronic intravenous infusions of epoprostenol (Flolan, Glaxo Wellcome) for a period of 3 months. The epoprostenol was given through an indwelling subclavian venous catheter tunneled through the pectoral tissues to the skin surface. At the end of 3 months (day 88), a repeat cardiac catheterization was performed.

Seven patients in the conventional-therapy group and 11 in the epoprostenol-therapy group provided blood samples at the time of both cardiac catheterizations. Written informed consent was obtained in a protocol approved by the Research Ethics Committee of the Jewish General Hospital and the committees of the other contributing centers. The number of patients participating was limited by the willingness of referring centers to contribute samples, patient consent and willingness to complete the study, availability of paired blood samples at the start and end of the study, and proper handling of the samples.

Blood Sampling Protocol

At each cardiac catheterization, 6 mL of mixed venous blood (VEN) was withdrawn from the proximal port of a thermomodulation catheter that had been inserted through the internal jugular, subclavian, or femoral vein and passed into the pulmonary artery for hemodynamic measurements. Simultaneously, 6 mL of arterial blood (ART) was withdrawn from the proximal port of a thermodilution catheter that had been inserted through the internal jugular, subclavian, or femoral vein and passed into the pulmonary artery for hemodynamic measurements. After centrifugation (1800g at 4°C) for 20 minutes, the plasma was transferred to polypropylene tubes and frozen at −70°C until analysis. All samples were handled in a similar fashion.

ET-1 Measurement

All samples were processed in the laboratory in Montreal, and immunoreactive ET-1 levels in plasma were measured using a standardized immunoassay, as previously described. The cross-reactivity to big ET was 10%, and it was 5% to ET-3. Final plasma levels were corrected for a 25% loss during extraction.

Calculation of the Arterial/Venous Ratio

The arterial/venous plasma ET-1 ratio (ART/VEN ratio) was calculated by mathematical division. Normally, the lung demonstrates net clearance of ET-1, resulting in a ratio <1. With decreased clearance, pulmonary ET-1 synthesis, or a combination of the two, the ratio rises toward 1 and may be >1 if synthesis exceeds clearance.

Statistics

All data are presented as group mean±SEM. To examine differences between means of the 2 groups, ANOVA was performed, followed (where appropriate) by Tukey’s test. To compare means between the 2 time points within the same treatment group, the paired t test was performed. To compare the proportion of patients with a given ART/VEN ratio ≥1 versus the proportion with a ratio <1, a χ² analysis was performed. Because of the small number of patients in the study, a more conservative test of proportions, the Fisher Exact Test, was also used. Correlations were examined using least-square linear regression. Two-tailed probability values <0.05 were considered significant.

Results

Characteristics of the Groups on Day 0

At the initial catheterization, there were no differences between the conventional therapy group and the epoprostenol-treated group in mean pulmonary artery pressure (60.5±2.9 versus 59.9±4.0 mm Hg), cardiac output (2.85±0.26 versus 2.84±0.15 L/min), or pulmonary vascular resistance (19.2±2.5 versus 18.8±2.0 Wood units). Immunoreactive ET-1 levels in VEN plasma were also similar between the 2 groups (1.41±0.15 versus 1.62±0.35 pg/mL, respectively).

Characteristics of the Groups on Day 88

The patients in this study were a subgroup of a larger study that established that chronic epoprostenol therapy improved exercise capacity, survival, dyspnea/fatigue rating, quality of life, and hemodynamics as compared with conventional therapy alone.

The present study had a smaller number of patients. When comparing the conventional therapy and epoprostenol-treated groups, respectively, on day 88, a trend toward lower mean pulmonary artery pressure (62.6±2.9 versus 59.9±2.5 Wood units, P=0.07) and higher cardiac output (3.3±0.2 versus 3.0±0.1 L/min, P=0.01) were noted. The differences between groups were not significant. However, compared with day 0, the mean pulmonary vascular resistance was significantly reduced (P=0.01) on day 88 in the epoprostenol-treated group but not in the conventional therapy group, and there were trends toward lower pulmonary artery pressure (P=0.07) and higher cardiac output (P=0.08) in the epoprostenol-treated group.
epoprostenol-treated group. Immunoreactive ET-1 levels in VEN plasma were similar between the 2 groups (2.24\(\pm\)0.59 versus 1.84\(\pm\)0.41 pg/mL, respectively), as were the levels in ART plasma (1.53\(\pm\)0.31 versus 2.08\(\pm\)0.64 pg/mL, respectively), and these levels did not differ significantly from day 0 levels.

**ART/VEN Ratios of ET-1**

On day 0, there were no significant differences between the treatment group means for ART/VEN ratios (0.87\(\pm\)0.18 for conventional versus 1.21\(\pm\)0.15 for epoprostenol), but the range of individual ratios was large (Figure 1). When the effect of therapy on the proportion of subjects with a given ART/VEN ratio was examined (Figure 2) on day 0, there was no difference in the proportion of subjects with a ratio <1 versus those with a ratio \(\geq\)1 between the treatment groups. If anything, there was a trend to more patients with a ratio <1 in the conventional therapy group. However, on day 88, there was a significantly greater proportion (82\%) of epoprostenol-treated patients with ratios <1 compared with the conventional-therapy group, in which only 29\% of patients had ratios <1 and the majority (71\%) now had ratios \(\geq\)1 (\(P=0.02\) by \(\chi^2\) and \(P=0.01\) by Fisher Exact Test).

The relationship of percent change in baseline ART/VEN ratio to absolute or percent change in ART plasma ET-1 levels was examined (Figure 3). There was a statistically significant relationship in both instances, with a better correlation for percent change in ART ET-1 level.

For conventionally treated patients, there was a strong relationship between the percent change in pulmonary vascular resistance over the 88-day study and the day-88 ART/VEN ratio (Figure 4). This relationship was not apparent for the epoprostenol-treated patients, despite the fact that most of these patients experienced a decrease in pulmonary vascular resistance during the study and had ratios <1 at the end of the study.

**Discussion**

This is the first study to demonstrate that the administration of "vasodilator" therapy with epoprostenol has salutary effects on the pulmonary homeostasis of a second vasoactive and mitogenic mediator, endothelin-1, in many patients with NYHA functional class III and IV PPH. The North American Primary Pulmonary Hypertension study\(^\text{17}\) offered a unique and unrepeatable opportunity for this study; it might now be considered unethical to have a conventional therapy group in future studies of advanced NYHA class III and IV PPH given the now-proven efficacy of epoprostenol.

Our patients treated with conventional therapy demonstrated the natural history of PPH, with no improvement in hemodynamics during the 3-month period. The proportion of patients who progressed to a worse balance in pulmonary ET-1 clearance/release, as measured by an ART/VEN ratio \(\geq\)1, increased. In contrast, the epoprostenol-treated group had improved hemodynamics, and the proportion of patients with...
a more normal pattern of net pulmonary ET-1 clearance/release increased. This finding identifies a nonvasodilator action of epoprostenol in vivo and offers 1 explanation for its beneficial effects in patients who do not have an initial acute vasodilator response.

ET-1 may be produced in altered hemodynamic states such as shock or heart failure. However, in PPH, pulmonary ET-1 production, possibly combined with decreased clearance, may result in high local levels that could contribute to the pathogenesis of PPH. Although plasma levels of ET-1 did not differ between the 2 groups in our study, the ART/VEN ratio may be a more representative and sensitive measure of net pulmonary clearance and release. The greater proportion of patients with improved ART/VEN ET-1 ratios seen with epoprostenol therapy could be due to a direct effect of epoprostenol on ET-1 clearance and/or release or, less likely, a consequence of improved hemodynamics in these patients. In support of the former, epoprostenol has been shown to inhibit ET-1 production by endothelial cells in vitro, possibly by stimulating particulate guanylate cyclase. ET-1 clearance is mediated by the ETa receptor, and the effects of epoprostenol on this activity are unknown. Future studies using radiolabeled compounds will be required to directly address the effects of epoprostenol on ET-1 clearance.

The percent change in ART/VEN ratio over the period of the study correlated with the absolute and percent change in ART ET-1 during the same time period. Immunohistochemical studies of arteries from patients with PPH show significant ET-1 production locally in the microvasculature. Much of this ET-1 must diffuse into the vascular media, but some spills out into the blood that is passing by. That spillover, moderated by any clearance mechanisms that are active, appears in the ART blood. Previous studies have shown, at best, variable correlations between VEN ET-1 levels and pulmonary hemodynamics in PPH. Local levels and net balance between clearance and release may be of greater relevance to the disease process. Moreover, we show that progression of the disease (as measured by the percent change in pulmonary vascular resistance) over the study period
correlated well with the ART/VEN ratio at the end of the study in conventionally treated patients. This may represent the “natural history” of the disease. A similar relationship was not found for the epoprostenol-treated patients. The hemodynamic response to epoprostenol may be complex and have multiple determining factors. It is certainly not just dependent on ET-1 homeostasis. Another possible explanation for the lack of a correlation in the epoprostenol-treated group is that while epoprostenol reduced ET-1 release, it may not have significantly increased ET-1 clearance. Thus, epoprostenol treatment would reduce the ART/VEN ratio to <1 but could not reduce it much more because further reduction would be dependent mainly on increased clearance.

The lung circulation may have greater recuperative capacity than was previously imagined. Improvements have been demonstrated in ET-1 levels or ART/VEN ratios after the repair of congenital heart disease or with recovery from Adult Respiratory Distress Syndrome. Previous studies have also identified other nonvasodilator actions of epoprostenol in PPH, including improvement in coagulation and platelet function. Future studies drawing on the results of these and the present study will shed light on the interactions between vasoactive and pro- or antimitogenic mediators in PPH, advancing our understanding of the mechanisms of action, other than vasodilatory, of therapies for the disease.

Acknowledgments
This work was supported in part by the Bank of Montreal Center for Heart Disease in Women. Dr Langleben is a Chercheur-Boursier (Research Scientist) of the Fonds de la Recherche en Sante du Quebec. We thank Farida Mohammed for expert technical assistance and Sylvain Rochelleau, Linda Houle, and Dr Francois Lebel of Burroughs Wellcome Canada for assistance with planning the transportation of the samples.

References


Continuous Infusion of Epoprostenol Improves the Net Balance Between Pulmonary Endothelin-1 Clearance and Release in Primary Pulmonary Hypertension


Circulation. 1999;99:3266-3271
doi: 10.1161/01.CIR.99.25.3266

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/25/3266

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/