Influence of Inhaled Iloprost on Transpulmonary Gradient of Big Endothelin in Patients With Pulmonary Hypertension

H. Wilkens, MD; M. Bauer, MD; N. Forestier; J. König, MD; A. Eichler, MD; S. Schneider; H.J. Schäfers, MD; G.W. Sybrecht, MD

Background—The pulmonary circulation is an important site for the production and clearance of endothelin (ET)-1, a potent vasoactive and mitogenic peptide. In healthy individuals, 40% to 50% of circulating ET-1 is removed on each passage through the lungs resulting in an arteriovenous ratio of <1, whereas many patients with pulmonary arterial hypertension (PAH) have ratios >1, indicating reduced clearance or increased release of endothelin. The influence of inhaled prostanooids on endothelin clearance is unknown.

Methods and Results—In a prospective investigation, plasma concentrations of big endothelin-1 (big ET-1, Elisa) were measured in 15 patients with pulmonary hypertension undergoing right heart catheterization with iloprost inhalation (4 m, 11 f, aged 35 to 75 years, mean pulmonary arterial pressure (PAPm) 54±2.3 mm Hg, pulmonary vascular resistance (PVR) 1061±141 dyn × sec × cm⁻5). There was a significant transpulmonary gradient for big ET-1 with 31% ±11% higher concentrations in the radial artery than in the pulmonary artery (P<0.001). After inhalation of iloprost a significant decrease in the AV-ratio from 1.31±0.11 to 0.92±0.06 (P<0.007) was observed. The pulmonary net release of 3.10±0.65 pmol/min big ET-1 at baseline decreased to -1.24±1.32 pmol/min (P=0.013) within 15 minutes indicating a restored balance. Patients under long-term treatment with iloprost (n=7) tended to have a lower net release and AV-ratio for big ET-1 than patients without pretreatment.

Conclusion—An increase in pulmonary clearance of big-ET could be a mechanism contributing to the beneficial effects of inhaled prostanooids in the treatment of PAH. (Circulation. 2003;107:1509-1513.)

Key Words: hypertension, pulmonary • endothelin • prostaglandins

Pulmonary arterial hypertension (PAH) is a progressive disease with poor prognosis leading to fatal right heart failure.¹ In the last few years, significant improvements have been made about insights in pathophysiology and treatment options. Although vasoconstriction may play a role, microvascular narrowing and obliteration by cellular proliferation contribute to the progression of the disease. Treatment with prostanooids has been shown to improve prognosis, exercise capacity, and hemodynamic variables.²³ The mechanisms of action, however, are not fully understood so far, in addition to vasoconstriction antiproliferative effects have been hypothesized.² Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor and smooth-muscle mitogen that is overexpressed in the plasma and lung tissue of patients with PAH.⁵⁶ The ET-1 gene is translated to a 203 amino acid precursor, which is then cleaved to form big ET-1. Big ET-1 is subsequently cleaved by the ET-converting enzyme into functional ET-1. There is increasing evidence that ET-1 has a pathogenic role in PAH and that blockade of endothelin receptors may be beneficial.⁷ The human lung normally acts as a clearance organ for ET-1; in healthy individuals, 40% to 50% of circulating ET-1 is removed on each passage through the lungs, resulting in an arteriovenous ET-1 gradient of <1. Many patients with PAH have ratios close to or greater than unity, suggesting reduced clearance or increased release of endothelin.⁸ The interactions among vasoactive factors in the vasculature include those of the prostanooids and endothelins. Several studies have shown an endothelin-induced release of prostacyclin.⁹¹⁰ Presently, however, there is little information about clinical significant effects of prostanooids on the endothelin system. One study in humans suggested that continuous intravenous epoprostenol therapy may have a beneficial effect on the balance between ET-1 release and clearance in patients with primary pulmonary hypertension.¹¹ The vasodilator action of the prostanooids may thus result partially from their ability to inhibit the production of endothelin. The objective of the present study was to assess the response of plasma big ET-1 levels to nebulized iloprost in patients suffering from PAH.

Methods
Fifteen patients with PAH were included and gave written, informed consent. All patients fulfilled the diagnostic criteria for PAH of the
Patient Characteristics

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Mean ± SEM 57±3 ... 4±2.4 ... 66.3±2.4 165±2.1 54.5 2.16±0.16 1061±141 10±1.2 61±1.7 ...

PPH indicates primary pulmonary hypertension; CTEPH indicates chronic thrombembolic PH; and SPH indicates secondary pulmonary hypertension (6 idiopathic pulmonary fibrosis, 13 Langhans cell granulomatosis). WHO classification and suffered from severe pulmonary hypertension with NYHA stage III or IV. Patient characteristics are listed in Table 1. No patients were taking high-dose calcium channel blockers. Seven patients were treated with aerosolized iloprost for 2 to 4 years.

Measurements were performed after overnight rest before intake of regular medication. The patients were admitted to the intensive care unit, and a fiberoptic pulmonary artery catheter (Baxter) was placed via the jugular or cubital vein and a catheter was inserted into the radial artery. The patients were recumbent during the measurements. After a 60-minute equilibration period (to perform oxygen response testing), baseline hemodynamic variables were recorded. Subsequently, the acute hemodynamic response to aerosolized iloprost was measured. Iloprost 50 μg (Ilomedin, Schering) was diluted in 4.5 mL of isotonic saline, aerosolized in a jet nebulizer (Ilo-Neb, Nebu-Tec) and administered over a period of 12 to 15 minutes, which resulted in a cumulative dose of nebulized iloprost between 8.4 and 10.5 μg.

Immediately after inhalation and every 15 minutes thereafter for up to 2 hours, the hemodynamic variables were measured. Samples for big ET-1 were collected before and 15 minutes after inhalation of iloprost.

Pulmonary net release of big ET-1 was derived with individual pulmonary concentration gradients (PCG) as described previously by Stangl et al: PCG = arterial - pulmonary arterial concentration (pmol/L). Individual pulmonary plasma flow (PFPF) was equal to the CO (L/min) and corrected for individual hematocrit (HC): PFPF = CO × (1-HC). Pulmonary net release (pmol/min) was calculated as follows: ET release = PCG × PFPF = PCG × CO × (1-HC).

Hemodynamic Monitoring

Heart rate, pulmonary and systemic arterial pressure, right atrial pressure (CVP), as well as transcutaneous and pulmonary arterial oxygen saturation were monitored continuously. The pulmonary capillary wedge pressure (PCWP) was determined at the end of each evaluation period. Cardiac output was measured by the thermodilution method with the mean of triplicate measurements. A radial artery catheter was used to measure systemic arterial pressure (SAP) and systemic arterial oxygen saturation (SaO₂). The PVR and the systemic vascular resistance (SVR) were calculated by standard hemodynamic formulas.

Measurement of Plasma Big ET-1

For big-endothelin measurements, 10 mL of mixed venous blood was withdrawn after a 60-minute equilibration period from the pulmonary artery through the pulmonary artery catheter. Simultaneously, 10 mL of arterial blood was obtained from the radial artery catheter. Blood samples were collected in prechilled tubes containing EDTA (Sarstedt) and immediately placed on ice until centrifugation. Particular attention was paid to avoid hemolysis. After centrifugation (1800g at 4°C) for 20 minutes, the plasma was transferred to polypropylene tubes and frozen at −70°C until analysis.

Plasma levels of big ET-1 were measured by means of an immunoassay with polyclonal capture and monoclonal detection antibodies highly specific for human big ET-1, with a detection limit of 0.1 pg/mL (big ET-1 enzymatic immunoassay, Biomedica) as described previously.

Statistical Analysis

All values are presented as mean ± standard error. The arterial/central venous plasma big-ET-ratio was calculated. Treatment effects were compared by two-sided sign test or paired two-sided t-test where appropriate. Constancy over time in hemodynamics (PAPm), cardiac output, PVR, blood pressure, heart rate, CVP, and mixed venous oxygen saturation after iloprost were tested with repeated measure ANOVA.

Correlations were tested by Pearson’s correlation coefficient with the corresponding test. A probability value of < 0.05 was considered statistically significant.

Results

Hemodynamics After Iloprost Inhalation

Within the first minutes of iloprost inhalation, PAPm started to decrease in all patients. At the end of the inhalation period, a reduction of PAPm of 7.21 ± 1.07 mm Hg (-15.3 ± 2.6% of baseline, P = 0.01) was observed (Figure 1). The maximal effect was seen after 15 to 30 minutes (-15.8 ± 23.5%, P < 0.02) with a return to baseline levels after 120 minutes.
Cardiac output increased from 3.8±0.37 to 4.9±0.34 l/min (53.8±8.3%, P<0.007).

Correspondingly, PVR decreased markedly after iloprost inhalation by 46±4.2% (P=0.002, Figure 1)

**Plasma Big-ET-Concentrations**

Before inhalation of iloprost, plasma big-ET-concentrations were significantly higher in the radial artery than in the pulmonary artery (Figure 2, 2.46±0.29 versus 2.72±0.27 pg/mL, P<0.0001) resulting in an AV-ratio of 131.7±11.5% (Figure 2).

Immediately after inhalation, this AV-ratio decreased to 91.8±5.9% (P<0.007) with a tendency of increasing plasma big-ET-concentrations in the pulmonary artery (2.74±0.28 pg/mL) and decreasing plasma big-ET-concentrations in the radial artery (2.53±0.32, Figures 2 and 3). Values of the individual patients are shown in Figure 4.

Patients under pretreatment with inhaled iloprost tended to have lower AV-ratios before inhalation than patients inhaling iloprost for the first time (113.8±5.1% versus 149.0±21.1%, P=0.23).

The pulmonary net-release of big endothelin was positive at baseline, whereas a significant decline was observed within 15 minutes after inhalation with a restored balance (from 3.10±0.65 pmol/min to -1.24±1.32 pmol/min, P=0.013). Patients under long-term iloprost therapy tended to have a lower net-release before (2.24±0.9 pmol/min) and after inhalation (-2.65±2.2 pmol/min) than patients without pretreatment (3.95±0.9 pmol/min and 0.18±1.1 pmol/min, Figure 5).

There was a weak correlation between plasma big-ET-concentrations in the radial artery and PAPm (r=0.46, P=0.085) and RAP (r=0.650; P=0.012). A very weak correlation was found between the mixed venous big-ET level and PAPm (r=0.30, P<0.05), PVR (r=0.34, P<0.5), RAP (r=0.32, P<0.01) with a negative correlation between the mixed venous big-ET level and cardiac output (r= -0.36, P<0.1).

**Figure 1.** Time course of PAPm, PVR, cardiac output, and heart rate in 15 patients with PAH after iloprost inhalation. The time scale shows the baseline (0) and minutes between the measurements. Iloprost produced a reduction of PAPm and PVR with significant increases in cardiac output and a return to baseline levels after 120 minutes. There were no significant changes in heart rate.

**Figure 2.** Plasma big ET-1 concentrations in the radial artery and the pulmonary artery before and after iloprost inhalation. Before inhalation of iloprost plasma, big-ET concentrations were significantly higher in the radial artery than in the pulmonary artery (2.46±0.29 versus 2.72±0.27 pg/mL, P<0.0001) resulting in an AV-ratio of 131.7±11.5% (Figure 2).

**Figure 3.** Arteriovenous ratio of plasma big ET-1 concentrations before and after iloprost inhalation. At baseline there was an AV-ratio of 131.7±11.5%. Immediately after inhalation this AV-ratio decreased to 91.8±5.9% (P<0.007).
No correlation was observed between the changes in hemodynamics and the decrease of big-ET net release or AV-ratio.

Discussion
In the present study, we show that treatment with an inhaled prostanoid has significant effects on big ET-1 in patients with PAH. A central role in the pathogenesis of PAH has been proposed for ET-1 because it is overexpressed in patients with PAH and has been implicated in vascular remodeling.5,6 High concentrations of endothelin in plasma or lung tissue5 with an increased arterial/central venous ratio of plasma endothelin5,15 have been documented in various forms of pulmonary hypertension. In our patient population, the level of the precursor big ET-1 was significantly higher than normal values and values of patients undergoing coronary bypass grafting.14 So far, therapy with prostanooids is the most important treatment option to improve prognosis, exercise capacity and quality of life in patients with PAH. However, little is known about the influence of prostanoid therapy on endothelin and the endothelin system. Our results show that the administration of iloprost by inhalation leads to a transient normalization of this ratio concomitant with an improvement in hemodynamics. This effect has a rapid onset and can be measured within 15 minutes from start of inhalation.

The improved AV-ratio may be due to decreased production or increased clearance of big ET-1. Because there was a tendency of increasing big ET-1 levels in the pulmonary artery and decreasing levels in the radial artery simultaneously with increasing cardiac output, an increased pulmonary clearance of big ET-1 has to be assumed.

Calculation of the net release showed a significant pulmonary net release of big ET-1 at baseline with a significant decline within 15 minutes after inhalation indicating a restored balance. However, plasma-levels of big ET-1 must be interpreted with caution, because ET-1 is a paracrine mediator mainly diffusing into the vascular media, with some spillover into the blood. That spillover, modified by active clearance mechanisms, appears in the arterial blood. The increase in big endothelin levels in the central venous blood (pulmonary artery) could be explained by increased cardiac output in response to a decrease in PVR and/or by increased spillover due to peripheral vasodilatation induced by small systemic effects of iloprost inhalation, because, for instance, the skin is an important source of ET-1-production.17

There seems to be an important interaction between the prostaglandins and endothelin. In animal models the vasoconstrictor peptide endothelin-1 stimulates the production of both vasodilatory and vasoconstrictor prostanoids,18–20 whereas the vasodilators prostacyclin (PGI2) and PGE2 inhibit ET-1 production.21 In cultured endothelial cells, PGE2 and PG12 cause an ~40% inhibition of basal ET-1 secretion and a 50% inhibition of serum-stimulated ET-1 secretion in a dose-related and time course fashion by possibly stimulating particulate guanylate cyclase.22 There is one study showing an effect of prostacyclin on endothelin in patients with PPH so far. In this study, chronic intravenous prostacyclin-therapy for 88 days leads to a greater proportion of patients with an AV-ratio <1 compared with patients receiving conventional treatment.11 Similar to our data there is no significant change of absolute values of ET-1 during treatment, therefore the AV-ratio may be a more sensitive measure of net pulmonary clearance and release than the absolute values of endothelin. In our patients, the acute improvement of the AV-ratio was maintained even after long-term treatment with inhaled iloprost, with a tendency of lower baseline AV-ratios compared with patients without pretreatment. The ET-1 gene is subject to transcriptional control and consistent with the immediate effect observed in the present study an effect of inhaled iloprost on the clearance seems likely. However, the trend of lower AV-ratios found in patients on long-term prostanoid therapy would also be consistent with a direct effect of prostanoids on big ET-1 production.

The intravenous administration of vasodilative agents, such as epoprostenol, lacks pulmonary selectivity. The application of aerosol techniques for alveolar deposition of vasodilatory drugs induces a preferential vasorelaxation in the pulmonary circulation, perhaps reaching areas with severe vasoconstriction better than IV administration. Therefore, inhaled iloprost might have more profound acute effects than IV administration on the ET-system. However, to differentiate between these two routes of administration, studies are required to address AV-ratios after inhalation as compared with IV administration.

The mechanisms of reduced pulmonary endothelin-clearance in PAH are unknown. ET-1 clearance is mediated by the ETB receptor, however no conclusive data exist on the effects of prostanoids on this activity. Future studies will be
required to directly address the effects of prostanoids on ET-1 clearance.

There are no data about an additive or synergistic effect by combining an endothelin receptor antagonist with a prostanoid. Although the present data suggest that the therapy with inhaled iloprost normalizes the net-uptake of endothelin in the lungs, a combined therapy with iloprost and bosentan further improves right heart hemodynamics in our hands (own unpublished observation, 2002). A controlled study investigating the effect of combination therapy versus single therapy is necessary to answer this question.

In summary, our data demonstrate that therapy with inhaled iloprost leads to increased pulmonary clearance of big ET-1 with improvement of the AV-ratio. These data may have an impact on clinical trials on the interactions between vasoactive and pro- or antimitogenic mediators in PAH for gaining insights into the mechanisms of action of therapies for this devastating disease.

References
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_Circulation._ 2003;107:1509-1513; originally published online February 24, 2003;
doi: 10.1161/01.CIR.0000056104.49686.4B
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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