

# Inhaled Nitric Oxide Versus Aerosolized Iloprost in Secondary Pulmonary Hypertension in Children With Congenital Heart Disease

## Vasodilator Capacity and Cellular Mechanisms

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**Background**—Inhaled nitric oxide (iNO) has been used to assess the vasodilator capacity of the pulmonary vascular bed in children with congenital heart disease and elevated pulmonary vascular resistance. Inhaled iloprost is a pulmonary vasodilator for the long-term treatment of pulmonary hypertension (PHT). Because these 2 vasodilators act through different pathways (release of cGMP or cAMP, respectively), we compared the pulmonary vasodilator capacity of each.

**Methods and Results**—A total of 15 children with congenital heart disease and PHT who had elevated pulmonary vascular resistance (preoperative,  $n=10$ ; immediately postoperative,  $n=5$ ) were first given 20 ppm of iNO for 10 minutes; then, after baseline values were reached again, they were given aerosolized iloprost at  $25 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for another 10 minutes. Finally, iNO and iloprost were given simultaneously for 10 minutes. With iNO, the pulmonary vascular resistance and systemic vascular resistance ratio decreased from  $0.48 \pm 0.38$  to  $0.27 \pm 0.16$  ( $P < 0.001$ ). Similarly, iloprost decreased the ratio from  $0.49 \pm 0.38$  to  $0.26 \pm 0.11$  ( $P < 0.05$ ). The combination had no additional effect on the resistance ratio. Plasma cGMP increased from  $17.6 \pm 11.9$  to  $34.7 \pm 21.4$  nmol/L during iNO ( $P < 0.01$ ), and plasma cAMP increased from  $55.7 \pm 22.9$  to  $65.1 \pm 21.2$  nmol/L during iloprost inhalation ( $P < 0.05$ ).

**Conclusions**—In children with PHT and congenital heart disease, both iNO and aerosolized iloprost are equally effective in selectively lowering pulmonary vascular resistance through an increase in cGMP or cAMP, respectively. However, the combination of both vasodilators failed to prove more potent than either substance alone. Aerosolized iloprost might be an alternative to iNO for early testing of vascular reactivity and for the postoperative treatment of acute PHT. (*Circulation*. 2001;103:544-548.)

**Key Words:** hypertension, pulmonary ■ heart defects, congenital ■ vasodilatation ■ nitric oxide ■ prostaglandins

The assessment of pulmonary vascular reactivity plays an important role in the management of patients with pulmonary hypertension (PHT) and congenital heart disease (CHD). We and others have shown that the response to early testing with inhaled nitric oxide (iNO) seems to depend on the degree of established vascular disease and, thus, may be helpful in selecting patients for operation.<sup>1-4</sup> iNO mediates vasodilatation through the stimulation of soluble guanylate cyclase to produce cGMP. Prostacyclin, an unstable arachidonic acid metabolite, is another endothelial-derived vasodilator that increases the concentration of cAMP in vascular smooth muscle cells.<sup>5,6</sup> Recently, inhaled prostacyclin ( $\text{PGI}_2$ ) was proven to act as a selective pulmonary vasodilator, and it has been proposed as an alternative to iNO for the treatment of adult respiratory distress syndrome<sup>7</sup> and after repair of CHD.<sup>8</sup> It has been demonstrated that iloprost, a stable

prostacyclin analog, is as potent in its selective vasodilator effect as  $\text{PGI}_2$ .<sup>9,10</sup>

We compared the hemodynamic effects and changes in plasma cGMP and cAMP levels induced by iNO and iloprost alone or in combination in patients with PHT and CHD preoperatively or postoperatively.

## Methods

### Study Population

Approval by the institutional ethics committee and written informed consent were obtained before recruiting 15 children (mean age,  $5.4 \pm 4.3$  years) with PHT and CHD to participate in the study. Among the 15 children, 10 were studied in the catheterization laboratory preoperatively (group 1) and 5 were studied in the intensive care unit immediately after surgical repair (group 2). PHT was defined as a mean pulmonary pressure  $>30$  mm Hg and an

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**TABLE 1. Baseline Hemodynamics of All Patients**

Patient	Diagnosis	Age, y-mo	Weight, kg	Mean SAP	Mean PAP	Qp/Qs	PVR, U · m <sup>2</sup>	Rp/Rs
Preoperative								
1	VSD	5–7	17	78	69	2.9	5.45	0.31
2	AVSD	1–9	8	63	58	1.6	9.7	0.56
3	TGV corrected, VSD*	8–6	16	64	45	1.8	5.9	0.35
4	TGV corrected, VSD*	4–0	12	64	40	1.8	2.6	0.27
5	VSD	5–2	15	87	83	2.4	7.4	0.36
6	VSD, PDA	2–11	8	65	45	1.7	2.9	0.27
7	PDA, coarctation	6–5	10	85	61	2.9	3	0.21
8	VSD, PDA	11–10	17	70	40	2.6	4.14	0.38
9	ASD, PDA	14	27	85	71	2.5	6.35	0.4
10	ASD, VSD	1–1	8	47	46	0.54	18.9	1.8
Postoperative								
11	VSD closure	4–0	11	62	56	...	5.4	0.47
12	VSD closure	1–8	6	53	34	...	5.7	0.47
13	AVSD repair	1	5	51	29	...	2.9	0.45
14	VSD, PDA closure	1–11	7	71	34	...	9.7	0.52
15	VSD closure	11–11	17	86	31	...	6.3	0.3

VSD indicates ventricular septal defect; ASD, atrial septal defect; AVSD, atrioventricular septal defect; TGV, transposition of the great vessels; PDA, patent ductus arteriosus; SAP, systemic arterial pressure; and PAP, pulmonary arterial pressure.

\*Post-Senning procedure with VSD left open.

indexed pulmonary vascular resistance  $>2.5$  Wood U · m<sup>2</sup>. Baseline demographic and hemodynamic data are given in Table 1.

### Study Design

The following protocol was used in all patients: (1) baseline measurements, (2) administration of 20 ppm iNO for 10 minutes, (3) return to baseline for 10 minutes, (4) administration of 25 ng · kg<sup>-1</sup> · min<sup>-1</sup> iloprost for 10 minutes, and (5) administration of iNO and iloprost for 10 minutes. Normoventilation (pH 7.38 to 7.42) and inspired oxygen concentration (21% in preoperative patients and 40% in postoperative patients) were kept constant throughout the protocol to avoid confounding factors. At the end of each step, hemodynamic changes were recorded, arterial blood gases were measured to ensure stable pH conditions, and blood samples were drawn from the left atrium or systemic artery for measurements of cGMP and cAMP.

Children in group 1 were premedicated with midazolam (Dormicum; 0.5 mg/kg PO; maximum dose 15 mg). For heart catheterization, the children were intubated and sedated using the following protocol: 10 μg/kg atropine as a single dose; 10 μg/kg alfentanil (Rapifen) as a single dose; 0.5 mg/kg atracurium as a single dose to facilitate intubation; and 2.5 to 3.5 mg/kg propofol (Disoprivan) as a bolus, followed by 10 to 15 mg · kg<sup>-1</sup> · h<sup>-1</sup> propofol as a continuous infusion.

Children in group 2, who were already intubated and ventilated, were sedated by a continuous infusion of morphine (40 to 80 μg · kg<sup>-1</sup> · h<sup>-1</sup>) and midazolam (0.2 mg · kg<sup>-1</sup> · h<sup>-1</sup>), as it is standard practice in our unit in the early postoperative period. Normoventilation was assured during the test protocol for both groups.

### Drug Administration

Medical-grade quality NO at 300 ppm in nitrogen was administered through a separate flowmeter into the inspiratory limb of the ventilator circuit, where it was volumetrically diluted to 20 ppm and

continuously measured with an electrochemical device during NO administration (PrinterNOx, Micro Medical Ltd).

Iloprost (Ilomedin, Schering AG, Schlieren, Switzerland) was prepared from a vial of 50 μg/0.5 mL and diluted to obtain a theoretical alveolar deposition of 25 ng · kg<sup>-1</sup> · min<sup>-1</sup>. Dilution was calculated according to the following formula: aerosol concentration/mL = (dose × body weight [kg])/nebulization rate (mL/min) of the nebulizer. For nebulization, we used the Respirgard II (Marquest Medical Products) nebulizer connected to the inspiratory limb of the ventilator or to a modified Jackson-Rees system for bag ventilation, as described previously.<sup>11</sup> This jet nebulizer shows the following characteristics: mass median aerodynamic diameter, 2.1 μm; mass distribution, 80% of droplets were  $<6$  μm, allowing a high intrapulmonary deposition in small children; and nebulization rate,  $\approx 0.15$  mL/min at a flow rate of 6 L/min.<sup>12</sup> Iloprost was continuously aerosolized at a flow rate of 6 L/min.

### Hemodynamic Assessment

Catheters were inserted femorally and placed in the superior vena cava, the pulmonary artery, the left atrium, and the aorta (or a peripheral systemic artery) to allow simultaneous measurements of pressure and blood gases and to avoid time-consuming and confounding measurements due to catheter manipulations. For patients with an atrial septal defect or a patent foramen ovale, a catheter was placed in a pulmonary vein instead of the left atrium. In the absence of an interatrial communication, pulmonary arterial wedge pressure was measured instead of left atrial pressure.

Intravascular pressures were measured with fluid-filled transducers, and oxygen content was calculated from hemoglobin concentrations and oxygen saturations. Blood gases were measured from systemic arterial blood at each level. Cardiac index and indexed pulmonary and systemic blood flow (Qp and Qs, respectively) calculations, based on the Fick principle, were obtained from assumed oxygen consumption. Systemic and pulmonary vascular resistances were calculated with standard formulas and indexed to body surface area. The pulmonary-to-systemic vascular resistance

drug administration, because the calculation of Rp/Rs avoids the influence of oxygen consumption changes. Hemodynamic parameters were measured at baseline and at the end of each period of drug exposure.

### cGMP and cAMP Assays

Blood samples were collected in an EDTA vacutainer (Becton Dickinson Vacutainer systems) and immediately placed on ice. Plasma was obtained by centrifugation at 1600g for 10 minutes at 4°C and then stored at -70°C until analysis. cGMP and cAMP levels were determined in batches using a commercial radioimmunoassay kit (Immunotech catalog #1118 and 1117). The cGMP assay had a measurement range of 0.1 to 100 nmol/L and a sensitivity of 10 pmol/L. The cAMP assay had a measurement range of 5 to 50 nmol/L, and the sensitivity of the assay was 0.2 nmol/L. cGMP and cAMP levels are expressed in nmol/L.

### Statistical Analysis

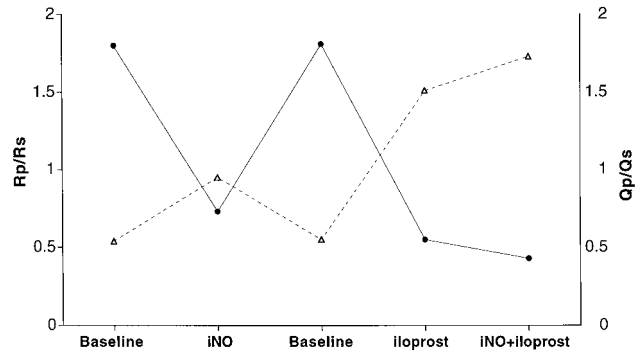
The data were computerized and analyzed using the GraphPad Prism Software package. Mean, median, SD, and lower and upper 95% confidence intervals were calculated for all parameters. Results are expressed as mean±SD. Analyses were performed for all patients and separately for groups 1 and 2. For each parameter, repeated measures ANOVA for normally distributed data or the Friedman's test for non-normally distributed data were used, with post hoc corrections as appropriate, for all pairwise multiple comparisons (Tukey's or Dunn multiple comparison test, respectively).  $P<0.05$  was considered significant.

### Results

Patient demographics and hemodynamic data are given in Table 1. Inhaled NO caused a selective pulmonary vasodilatation in 14 of 15 patients and an overall decrease in Rp/Rs from  $0.48\pm 0.38$  (baseline 1) to  $0.27\pm 0.16$  ( $P<0.001$ ). Similarly, aerosolized iloprost resulted in a selective pulmonary vasodilatation in the same 14 patients, and Rp/Rs decreased from  $0.49\pm 0.38$  (baseline 2) to  $0.26\pm 0.11$  ( $P<0.05$ ). The combination of both drugs did not result in an additional decrease of Rp/Rs ( $0.26\pm 0.11$  to  $0.24\pm 0.09$ ).

One patient (patient 3 in Table 1), who had a ventricular septal defect after a previous palliative atrial switch procedure (Senning) for transposition of the great arteries, did not respond at all. One patient (patient 10 in Table 1), who had a ventricular septal defect and an atrial septal defect, presented with suprasystemic pulmonary arterial pressures and a right-to-left shunt (Qp/Qs, 0.54; Rp/Rs, 1.8) before testing. The intracardiac shunt diminished under iNO (Qp/Qs, 0.95) and reversed under aerosolized iloprost (Qp/Qs, 1.51) due to a decrease of Rp/Rs to 0.73 and 0.55, respectively. Rp/Rs decreased further to 0.43 when both substances were combined (Figure 1). When this patient was excluded from statistical analysis because of his very high initial pulmonary resistance, results still remained significant. The Rp/Rs decreased from  $0.39\pm 0.11$  (baseline 1) to  $0.24\pm 0.10$  ( $P<0.001$ ) under iNO and from  $0.39\pm 0.11$  (baseline 2) to  $0.24\pm 0.08$  ( $P<0.001$ ) under aerosolized iloprost. The combination of both drugs did not further decrease Rp/Rs ( $0.24\pm 0.08$  to  $0.22\pm 0.08$ ; Figure 2).

Plasma cGMP levels (Table 2) increased by 97%, from  $17.6\pm 11.9$  nmol/L (baseline 1) to  $34.7\pm 21.4$  nmol/L, during

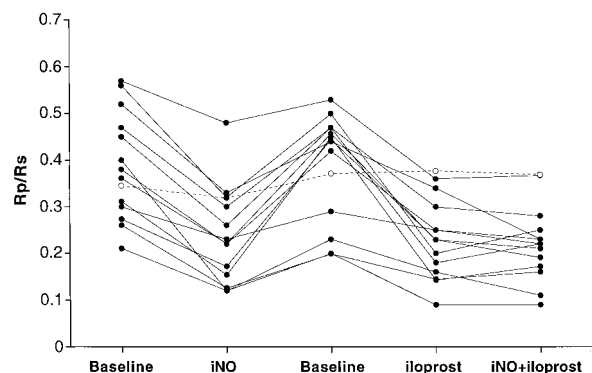


**Figure 1.** Changes in Rp/Rs (●, solid line) and intracardiac shunt (Qp/Qs; △, dotted line) in patient 10, showing inversion of shunt with iNO and under iloprost aerosolization.

ation, cGMP increased from  $17.4\pm 14.1$  nmol/L (on iloprost alone) to  $36.1\pm 15.2$  nmol/L ( $P<0.001$ ). Plasma cAMP levels (Table 2) remained stable under iNO (baseline,  $55.7\pm 22.9$  nmol/L; during iNO,  $54.0\pm 21.8$  nmol/L). During the 10-minute administration of iloprost, cAMP increased by 20%, from  $54.0\pm 21.8$  to  $65.1\pm 21.2$  nmol/L ( $P<0.05$ ). It remained elevated, at  $66.0\pm 19.85$  nmol/L, during the combined administration of iNO and iloprost ( $P<0.05$  versus baseline and iNO). Subgroup analyses of preoperative or postoperative patients showed no differences in response when compared with all patients.

### Discussion

In our study population, inhaled NO and aerosolized iloprost, at the concentration or doses used, were proven to be equipotent and equally selective in lowering pulmonary vascular resistance through an increase in either cGMP or cAMP, respectively. We observed no changes in systemic vascular resistance or in systemic arterial pressures during iloprost inhalation such as other investigators have reported,<sup>13</sup> although we delivered slightly higher doses of  $25 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over a longer time (ie, over a total of 20 minutes when combining both substances) than those authors did. This may be explained by the use of a nebulizer, different characteristics of the aerosol spray, and the different intrapulmonary deposition characteristics between children and adults and between intubated and nonintubated patients.



**Figure 2.** Individual responses from all patients in Rp/Rs to iNO

**TABLE 2. Levels of cGMP and cAMP in Plasma Samples**

	Baseline	iNO	Iloprost	iNO+Iloprost
cGMP, nmol/L	18±12	35±21*	17±14	36±15*
cAMP, nmol/L	56±23	54±22	65±21†	66±19†

Values are mean ± SD.

\* $P < 0.01$  vs baseline; † $P < 0.05$  vs baseline or iNO (repeated measures ANOVA).

However, the positive hemodynamic response and the significant increase in cAMP levels with aerosolized iloprost exclude an underdosing of this substance in our study.

Although a synergistic effect might be theoretically expected when combining both substances, because the vasodilatation is induced by different second messengers, we failed to demonstrate such a synergistic effect except in one patient. The combination of 2 different vasodilators directly applied to the airways has, to our knowledge, never been tested clinically, although results from animal studies would suggest to do so.<sup>14,15</sup> In these animal studies, the combination of PGI<sub>2</sub> and iNO produced a more enhanced vasodilator effect compared with their separate effects, and this was dose-dependent. It was suggested that the combination of both drugs might be useful in the management of PHT. Also, a synergistic effect on pulmonary vasodilatation has been shown in clinical studies in children with PHT, in which oral beraprost, a PGI<sub>2</sub> analog, was combined with iNO.<sup>16–18</sup> All these studies showed major differences compared with the present study. The results may differ for the following reasons. (1) The doses of prostacyclin used by Ikeda et al<sup>15</sup> in animals were ≈100-fold greater than those commonly used clinically (and those tested to be safe and without the risk of overdosing); this must result in a spillover into the systemic circulation, with a subsequent decrease of arterial pressure.<sup>19</sup> (2) An oral preparation of a PGI<sub>2</sub> analog was used in 3 studies<sup>16–18</sup>; therefore, drug dosing can hardly be compared with an application of iloprost by aerosol. Indeed, there are no studies in children with CHD and PHT thus far in whom prostacyclin or iloprost was given by aerosolization into the airways.

All but 1 of our 15 patients responded to iNO and to iloprost. The only nonresponder to either substance even failed to respond to the combination, despite a net increase in cGMP and cAMP levels (Table 3). This observation suggests that mechanisms other than the vascular smooth muscle cell concentration and release of the 2 second messengers may play an important role in vasodilatation. A defect further down the pathway, fixed rigid vessels caused by increased

**TABLE 3. Hemodynamics and cGMP and cAMP Levels of Patient 3 (Nonresponder)**

	Baseline	iNO	Iloprost	iNO+Iloprost
Mean SAP, mm Hg	64	65	70	67
Mean PAP, mm Hg	45	47	49	46
Rp/Rs	0.35	0.32	0.37	0.37
cGMP, nmol/L	13.9	19.6	14.1	24.6
cAMP, nmol/L	24	25	56	58

extracellular matrix, or plexiform obstructive vascular lesions may be responsible for the absent reaction. Although we did not see a synergistic effect of the combination of both vasodilators when statistical analysis were performed, there were a few patients who showed a further decrease in Rp/Rs when both substances were combined. One patient with both ventricular and atrial septal defects and a right-to-left shunt (Qp/Qs, 0.54) showed an impressive response to both iNO and iloprost alone, with inversion of the intracardiac shunt on aerosolized iloprost, and further improvement of Rp/Rs when both substances were combined (Figure 1). This observation suggests that there might be a subgroup of patients who will respond to a combined treatment better than to a single treatment.<sup>18</sup> Whether this is of clinical or prognostic importance remains to be evaluated.

iNO increased cGMP and iloprost increased cAMP (Table 2). Both responses were highly selective, indicating different independent mediator pathways for the 2 vasodilators. Although initially different messenger pathways will be activated, the final pathway leading to smooth muscle cell relaxation is probably the same for both substances (ie, a decrease in the concentration of free intracellular cytosolic Ca<sup>2+</sup>).<sup>20,21</sup> This might explain the lack of a synergistic effect when combining both pulmonary vasodilators. We found no evidence for an iloprost-induced endothelial cell release of NO, which would again stimulate soluble guanylate cyclase to produce cGMP, as has been postulated to be the case for PGI<sub>2</sub>.<sup>14,22</sup>

PGI<sub>2</sub> has been proposed as an alternative to iNO after the repair of CHD.<sup>8</sup> The nebulization of PGI<sub>2</sub> or iloprost might have some advantages over the inhalation of NO, such as its lack of toxic reactions<sup>23,24</sup> (which require monitoring NO<sub>2</sub> and methemoglobin formation during NO inhalation) and its easy administration by conventional nebulizers compared with the more complicated delivery systems required for NO. Furthermore, possibly life-threatening rebound phenomena have been described with iNO<sup>25,26</sup> but not with aerosolized PGI<sub>2</sub> or iloprost withdrawal. These advantages might favor the use of iloprost for the treatment of PHT; however, because of the lack of well-documented studies with prostacyclin or prostacyclin analogs, it might be too early to recommend the use of iloprost instead of iNO for the postoperative management of patients with PHT and CHD. The use of iNO has been shown to be helpful for the assessment of pulmonary vascular reactivity in selecting patients for surgery.<sup>1–4</sup> In this setting, the aerosolization of prostacyclin might offer a real alternative to iNO. However, as with any nebulizer device, some uncertainty will exist regarding the amount of prostacyclin effectively delivered into the alveolar space. This leads to a potential risk to falsely classify some patients as nonresponders. The problem may be more present in children, in whom aerosol deposition shows more variability and is less well studied than in adults.<sup>27,28</sup>

Our study has several limitations. First, iloprost was always given after iNO because of its longer half-life; iloprost has a documented hemodynamic effect of ≈1 to 2 hours,<sup>9,13</sup> which would have excluded a 2-group study protocol with a



acceptable for ethical reasons. Furthermore, it would have been more difficult to insure stable conditions and a return to baseline over such a long period. Second, although we made sure of a return back to baseline hemodynamics after iNO inhalation before administering iloprost, a potential interaction between iNO-induced effects and the iloprost-induced vasodilator capacities on the endothelial cell cannot be excluded. However, the return to baseline of cGMP levels after iNO inhalation under iloprost makes this unlikely. Finally, dose-response studies were not performed. Therefore, we cannot exclude that higher doses of iloprost might have resulted in a dose-dependent additional decrease in pulmonary arterial resistance. However, this is unlikely on the basis of other reports.<sup>10,29,30</sup>

In children with PHT and CHD, both iNO and aerosolized iloprost are equally effective in selectively lowering pulmonary vascular resistance through a selective increase in cGMP or cAMP, respectively. However, the combination of both vasodilators failed to prove more potent than either substance alone. Although aerosolized iloprost may be an alternative to iNO for early testing of vascular reactivity and for the postoperative treatment of acute PHT, it may prove to be more useful for the prolonged treatment of PHT.

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