Alternatives to nitric oxide

Stuart M. Lowson

Department of Anesthesiology, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA

Inhaled nitric oxide (INO) is a selective pulmonary vasodilator that has the ability to produce vasodilation in the pulmonary vascular bed without causing it in the systemic circulation. This property of INO has made it a useful therapy in the management of both adult and paediatric patients with a variety of conditions associated with pulmonary hypertension (PH), with or without hypoxia. Toxicity, cost and negative-outcome studies have prompted a search for alternative agents. These include inhaled prostacyclin and alternative prostaglandin preparations such as inhaled iloprost, treprostinol and beraprost. The phosphodiesterase inhibitors show real potential in the management of both acute and chronic forms of PH, and antagonists of endogenous pulmonary vasoconstrictors, such as endothelin and thromboxane, are being evaluated for the long-term treatment of conditions such as primary pulmonary hypertension.

Introduction

Nitric oxide (NO) is a non-polar gaseous molecule that is involved in many biological processes.1 From the clinical perspective, its most important property is the ability to produce relaxation of vascular smooth muscle via activation of guanylate cyclase and the conversion of guanosine-5-triphosphate to cyclic guanosine monophosphate (cGMP). When inhaled, INO produces pulmonary vasodilation in those lung areas receiving ventilation and thus can divert blood flow away from atelectatic/non-ventilated areas and decrease intrapulmonary shunting. Any NO that is absorbed from the lungs into the circulation is rapidly inactivated by combination with haemoglobin. Therefore the vasodilator effects of INO are confined to the pulmonary circulation, producing a decrease in pulmonary arterial pressure and pulmonary vascular resistance without affecting systemic arterial pressure. This is known as ‘selective pulmonary vasodilation’. Prior to the first major clinical trial of INO,2 many systemic vasodilators had been unsuccessfully evaluated in the hope of finding a selective pulmonary vasodilator. The ability of inhaled NO to produce such an effect was a major advance in the treatment of pulmonary arterial hypertension (PH) and prompted many investigations of its
effects in pathophysiological conditions associated with increased pulmonary arterial pressure.

Inhaled NO has shown clinical efficacy in decreasing the need for extracorporeal membrane oxygenation in the treatment of persistent PH of the newborn and in decreasing mortality and the occurrence of chronic lung disease in neonatal respiratory failure. However, sustained improvements in outcome have not been demonstrated in adult patients. Although INO therapy results in a temporary increase in oxygenation in patients with acute respiratory distress syndrome (ARDS), it has not been shown to improve survival. This factor, combined with its potential toxicity, difficulties in administration and cost (a major consideration in the USA), has prompted the search for alternative selective pulmonary vasodilators.

The argument for selective pulmonary vasodilation

Although the World Health Organization classification defines five types of PH, clinically it can be broadly divided into acute and chronic forms, albeit with considerable overlap. The classic acute form of PH is acute massive pulmonary embolism. While complete obstruction of pulmonary blood flow is invariably fatal, lesser degrees of obstruction produce an acute increase in right ventricular afterload, right ventricular failure, decreased cardiac output and systemic hypotension. Right ventricular coronary perfusion pressure depends upon both the upstream systemic arterial pressure and the downstream right ventricular end-diastolic pressure. Coronary blood flow to the right ventricle is decreased because of the combination of systemic hypotension and increased right ventricular end-diastolic pressure (secondary to the increased right ventricular afterload and failure). Systemic hypotension decreases right ventricular coronary perfusion pressure and oxygen delivery, and can exacerbate the right ventricular failure. This in turn produces a further decrease in cardiac output and systemic arterial pressure, and establishes a vicious circle of continued haemodynamic deterioration (Fig. 1). To break this cycle, therapy must be targeted towards decreasing the elevated pulmonary arterial pressure without exacerbating the systemic hypotension. Decreased pulmonary arterial pressure and right ventricular afterload permit an increased right ventricular ejection fraction and cardiac output. Avoiding systemic hypotension maintains perfusion to both the right ventricle and the rest of the organism.

Prior to the discovery of selective pulmonary vasodilators for the treatment of PH, clinicians were faced with the difficult task of finding the precise dose of a given systemic vasodilator that produced the greatest decrease in pulmonary arterial pressure for the smallest decrease in systemic arterial pressure. The number of systemic vasodilators that have
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The classic form of chronic PH is primary pulmonary hypertension, an idiopathic condition that is invariably fatal. Secondary PH is usually associated with cardiopulmonary disease (left ventricular failure, mitral valve disease, chronic obstructive pulmonary disease) and to a lesser extent with connective tissue diseases or thromboembolism. Both primary and secondary PH are associated with extensive ‘remodelling’ of the structure of the pulmonary vessels such that there is both an obstructive (fixed) and a dynamic component to the PH. The dynamic component may respond to vasodilators. Traditionally, these have been ‘non-selective’, such as calcium-channel blockers or intravenous prostacyclin (PGI₂).
While such therapies have produced improvements in both functional ability and survival, they are associated with several problems. Novel therapies have been explored in the hope of finding simpler routes of drug administration, more selective pulmonary vasodilators that may be associated with fewer systemic side effects and improved outcome. In addition, new approaches are being targeted towards reversing the cellular abnormalities/remodelling in the pulmonary circulation rather than simply treating the PH *per se*.

**Prostaglandins**

Prostacyclin is a member of the prostaglandin family derived from arachadonic acid. Similar to NO, it is produced by the vascular endothelium and is involved in the regulation of vascular tone and in localized thrombotic and inflammatory processes. PGI₂ stimulates the endothelial release of NO, while NO, in turn, increases the synthesis of endogenous PGI₂. *In vivo*, PGI₂ is spontaneously hydrolyzed to its inactive metabolite 6-keto-prostaglandin-F₁α with a half-life of 3–6 min.⁶

Intravenous PGI₂ is an established treatment for primary PH, and has also been studied in more acute conditions such as ARDS. Disadvantages of i.v. PGI₂ include the need for long-term intravenous access, the lack of pulmonary selectivity, resulting in systemic side effects, and the potential for causing increased intrapulmonary shunting (in patients with lung injury). In contrast, inhaled PGI₂ produces selective pulmonary vasodilation, can potentially improve oxygenation and does not cause the systemic side effects associated with the intravenous route.

Inhaled PGI₂ and INO have been compared in animal models of PH and in several clinical studies. Inhaled PGI₂ produces comparable effects to INO, with a trend (in both animal and clinical studies) for inhaled PGI₂ to produce greater decreases in pulmonary vascular resistance while INO produces greater improvements in oxygenation.⁶ In patients with ARDS, inhaled PGI₂ was shown to produce selective pulmonary vasodilation and increases in PaO₂ comparable to those obtained with INO.⁷,⁸ Approximately 53% of patients with ARDS respond to inhaled PGI₂ with an increase of >10% in the PaO₂/FiO₂ ratio, which is again comparable to that produced by INO therapy.

The ability to produce selective pulmonary vasodilation has been of particular value in both adult⁹ and paediatric patients with PH and right ventricular failure after cardiac surgery. INO therapy has been a major advance in the management of this condition based on its ability to selectively decrease pulmonary arterial pressure without exacerbating the systemic hypotension commonly associated with this clinical state. Inhaled PGI₂ has also been shown to be an effective therapy for acute
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PH during and after cardiac surgery. Della Rocca et al. have shown that inhaled PGI, produced a selective decrease in pulmonary arterial pressure and a 25% decrease in intrapulmonary shunting in patients undergoing lung transplantation.

There have been only a few reports of the use of inhaled PGI2 in children or neonates. Most of these consist of case reports or small case series and demonstrate that inhaled PGI2 produces selective pulmonary vasodilation in neonates with hypoxic respiratory failure associated with PH. Recently, Dahlem et al. performed a randomized placebo-controlled trial of inhaled PGI2 in 14 infants (mean age 54 months) with acute lung injury. Inhaled PGI2 at a dose of 30 ng/kg/min produced a 26% improvement in the oxygenation index compared with placebo. Fifty-seven percent of the children responded with >20% improvement in oxygenation.

Prostacyclin and its metabolites are remarkably non-toxic compared with NO. The side effects of inhaled PGI2 include inhibition of platelet aggregation. Impaired in vitro platelet aggregation was noted after 2 h of inhaled PGI2 in patients undergoing cardiac surgery, but was not associated with an increase in chest tube drainage or transfusion requirements even when therapy was continued for 6 h. Similar effects on platelet aggregation have been recorded in response to INO, again with no clinical evidence of increased bleeding noted. There is conflicting evidence concerning the effects of PGI2 on bronchial tone, with some studies reporting bronchodilation and others bronchoconstriction. While bronchospasm has not been reported in any of the clinical studies published to date, at least one potential case of bronchospasm in response to inhaled PGI2 has been noted in a paediatric patient at our institution. Systemic hypotension is a potential side effect of inhaled PGI2, but this has only been reported in one study and only in female patients at a dose 10 times that normally administered. Increased blood levels of the inactive metabolite of PGI2 have been found in some studies of inhaled PGI2 but not in others, suggesting that there is minimal absorption of inhaled PGI2 from the lungs into the systemic circulation. Abrupt withdrawal of inhaled PGI2 may cause rebound increases in PH, as also reported for INO.

The exact dose of inhaled PGI2 has yet to be established. Van Heerden et al. studied the differential effects of a 10–50 ng/kg/min dose range of inhaled PGI2 in patients with ARDS. There was a significant increase in oxygenation in response to the 10 ng/kg/min dose and a trend (non-significant) for oxygenation to improve as the dose increased to 50 ng/kg/min. This dose range had no effect on pulmonary arterial pressures; however, none of the patients had PH. Similar to INO, inhaled PGI2 appears not to produce pulmonary vasodilation in patients with normal pulmonary arterial pressures. Mikhail et al. studied the pulmonary arterial pressure response to inhaled PGI2 over a 15–50 ng/kg/min dose range in patients with PH. There was a significant decrease in pulmonary arterial
pressure in response to the lowest dose with no further response at the higher doses, suggesting a plateau dose–response relationship over this range. At our institution we start patients with a dose of 50 ng/kg/min and titrate downwards according to the response. We have observed a further decrease in pulmonary arterial pressure in response to a 100 ng/kg/min dose in the rare patient, but usually we do not exceed the 50 ng/kg/min dose. The PGI₂ solution (at the required dilution) is prepared in the hospital pharmacy and provided in 50-ml syringes. The solution is delivered to the nebulizer by a syringe pump at a rate of 8 ml/h to replace the volume nebulized. More sophisticated delivery systems have been described by other authors.

The response to inhaled PGI₂ may be increased by certain adjuncts. INO and PGI₂ act through different cellular messengers: cGMP and cyclic adenosine phosphate (cAMP), respectively (Fig. 2). An additive effect of combined therapy with INO and inhaled PGI₂ has been reported in both animal models and clinical studies. cAMP is degraded by phosphodiesterase enzymes (PDEs), predominantly of type 3. Milrinone, a type 3 PDE inhibitor, has been shown to augment the effects of inhaled PGI₂ in both animal models and patients after cardiac surgery.

One of the major advantages of selecting inhaled PGI₂ over INO is cost. In the USA, the cost of administering INO is $3000 per day. In contrast, the cost of inhaled PGI₂, delivered at a dose of 50 ng/kg/min to a 70 kg adult, is approximately $200 per day.

Prostacyclin is supplied as a powder that is dissolved in a glycine buffer (supplied by the manufacturers) prior to use. After reconstitution, PGI₂ is stable for 12 h at room temperature and for 48 h if refrigerated. The solution must be protected from light to avoid photodegradation. The solution pH is 10.5, but apart from a report of mild tracheitis in piglets at nine times the normal dose of diluent that would be administered to a patient, there have been no reports of the solution causing airway injury in humans. A separate study was unable to detect any evidence of pulmonary toxicity in healthy lambs after inhaling PGI₂ for 8 h. We have administered inhaled PGI₂ for as long as 3 weeks to one patient with no evidence of airway toxicity on bronchoscopy. The solution is administered via a standard continuous nebulizer apparatus positioned adjacent to the patient’s endotracheal tube.

In summary, inhaled PGI₂ is a useful alternative to INO that is easier to administer, less toxic and considerably less expensive.

**Prostaglandin preparations**

Prostaglandin E₁ (PGE₁) is readily available in most hospitals caring for neonates and is used to keep the ductus arteriosus open in cases of
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insufficient pulmonary blood flow. Similar to PGI₂, PGE₁ is produced within the lungs and undergoes rapid metabolism. In a study of 10 adults with ARDS, inhaled PGE₁ (at a dose of 6–15 ng/kg/min) produced a decrease in pulmonary arterial pressure and increase in PaO₂ comparable to that produced by 2–10 p.p.m. INO.²⁰

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**Fig. 2** The physiological effect of NO and prostacyclin production by the endothelial cell (EC) on the vascular smooth muscle cell (SMC). cGMP mainly activates protein kinase G (PKG) and to a lesser extent protein kinase A (PKA), and is metabolized predominantly by PDE type 5. cAMP mainly activates PKA and to a lesser extent PKG, and is metabolized predominantly by PDE type 3. Both PKA and PKG act via the sarcolemmal potassium (K⁺) and calcium (Ca²⁺) pumps to decrease cytosolic calcium and thereby induce smooth muscle relaxation. R, receptor; NOS, nitric oxide synthase; PCS, prostacyclin synthase; GC, guanylate cyclase; AC, adenylate cyclase; GTP, guanosine triphosphate.
Iloprost is a more stable carbacyclin derivative of PGI₂ with a half-life of 20–30 min (compared with only 3 min for PGI₁). The pulmonary vasodilator effect of inhaled iloprost is ~20–60 min. Clinical studies have demonstrated that inhaled iloprost has comparable pulmonary haemodynamic effects to INO and PGI₂. It has been shown to produce selective pulmonary vasodilation in patients with primary PH, producing a 30% decrease in pulmonary vascular resistance which was greater than that produced by 40 p.p.m. INO. Repeated daily nebulizations of inhaled iloprost have also been used for the long-term management of PH and were shown to improve pulmonary arterial pressures and increase exercise capacity. In an additional study of 10 patients with primary PH, inhaled iloprost (15–20 µg) was significantly more effective at lowering pulmonary vascular resistance than INO (40 p.p.m.) or oral sildenafil (50–100 mg). One of the potential advantages of the inhaled over the intravenous route for the management of primary PH is the lack of a need for a permanent intravenous access. However, not all patients with primary PH respond to inhaled iloprost and some require transfer back to an intravenous formulation to achieve therapeutic benefit.

Inhaled iloprost has also been shown to produce selective pulmonary vasodilation in both adult and paediatric patients with PH undergoing cardiac surgery.

Two other long-acting analogues of PGI₂ are treprostinol and beraprost. Treprostinol can be administered by either the intravenous or the subcutaneous route. A continuous subcutaneous infusion of treprostinol was shown to improve exercise capacity at 12 weeks in patients with PH. Treprostinol is associated with many of the side effects seen with intravenous PGI₂ therapy, such as diarrhoea, systemic vasodilation-related flushing, headaches, jaw pain and hypotension. Beraprost is the only commercially available oral PGI₂ analogue. Peak concentrations are achieved after 30 min and it has an elimination half-life of 40 min. In the clinical studies performed to date in patients with PH, beraprost was shown to improve functional ability at 12 weeks and 6 months, but not at 9 or 12 months.

Phosphodiesterase inhibitors

Both cGMP and cAMP are metabolized by PDEs (Fig. 2). While cGMP is metabolized predominantly by type 5 PDEs, cAMP is metabolized mainly by type 3 PDEs. Type 5 PDEs are expressed in relatively high amounts in the pulmonary vasculature and animal models suggest that type 5 PDE inhibitors are more effective at decreasing pulmonary arterial pressures than type 3 PDE inhibitors. Type 5 PDE inhibitors such as sildenafil (Viagra) enhance the response of exogenously administered...
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Pulmonary vasodilators such as INO and can prevent the rebound PH associated with INO withdrawal. Atz et al demonstrated that oral sildenafil (0.3 mg/kg) augmented the pulmonary vasodilator effects of INO in an infant with postoperative PH. Interestingly, sildenafil has also been shown to enhance the pulmonary vasodilation produced by inhaled iloprost, which is most likely explained by the fact that the various PDE subtypes are not completely specific for a particular ligand. Sildenafil is administered orally and the effective dose in patients with PH appears to be in the range of 25–100 mg. Mickelakis et al have demonstrated that oral sildenafil produces selective pulmonary vasodilation in patients with primary PH and is as effective as INO. The finding that pulmonary selectivity can be produced by a systemically administered agent (in particular, an orally administered agent) is very promising for long-term therapy. However, not all studies have confirmed this selectivity. While i.v. sildenafil 0.35mg/kg significantly decreased pulmonary vascular resistance and enhanced the effects of INO in paediatric patients after cardiac surgery, it also produced a decrease in systemic arterial pressure and oxygenation (from 138 to 108 mmHg). Lack of selectivity, with decreases in both systemic and pulmonary arterial pressures, has also been reported in both animal models of PH and patients with chronic PH given sildenafil. Systemically administered type 5 PDE inhibitors have also shown conflicting effects on oxygenation. Theoretically, a systemically administered vasodilator should inhibit hypoxic pulmonary vasoconstriction in poorly ventilated lung regions and potentially worsen intrapulmonary shunting. While this has been found in animal models of acute lung injury, it has not been reported in clinical studies to date.

In summary, type 5 PDE inhibitors show considerable promise in the management of both acute and chronic forms of PH. Both pulmonary selectivity and the effect on intrapulmonary shunting may depend on the dose and the age and clinical condition of the patient.

Endothelin and thromboxane antagonists

Pathological PH is associated with increased expression of vasoconstrictors such as endothelin and thromboxane, and decreased expression of vasodilators such as PGI₂ and NO. Antagonists to these endogenous vasoconstrictors have been developed and evaluated in clinical trials. Endothelin-1 exerts its biological effect at two receptors, ET-A and ET-B, with vasoconstriction being predominantly mediated via the ET-A receptor and vasodilation via the ET-B receptor. Endothelin antagonists are described as either non-specific (blocks both receptors) or specific to one receptor subtype. Bosentan (a non-specific antagonist) has been
investigated in patients with chronic PH (primary and secondary to connective tissue disease) and was shown to improve exercise capacity significantly at 16 weeks compared with placebo.\(^{39}\) Furthermore, echocardiographic studies have demonstrated that bosentan improves right ventricular systolic function, and leads to decrease right ventricular dilation in patients with chronic PH.\(^{40}\) Preliminary data also suggest a possible long-term survival benefit of oral bosentan therapy. Studies to date suggest that the use of endothelin antagonists that are selective for the ET-A receptor do not demonstrate increased clinical efficacy compared with the non-selective antagonists like bosentan.\(^{41}\)

Terbogrel, a combined thromboxane synthetase inhibitor and receptor antagonist, has been evaluated in patients with primary PH. No improvement in exercise capacity was found and treatment was associated with an unacceptable incidence of leg pain which prompted early termination of the one study performed to date.\(^{42}\)

### Adrenomedullin

Adrenomedullin is a long-lasting vasodilator peptide that was originally isolated from human pheochromocytoma. There are multiple binding sites for adrenomedullin within the lung and plasma levels increase in proportion to the severity of PH. The vasodilatory effects of adrenomedullin are mediated by both cAMP- and NO-dependent mechanisms. In animal models of PH, repeated inhalation of adrenomedullin decreased pulmonary arterial pressures and significantly improved survival.\(^{43}\) The same authors demonstrated that inhaled adrenomedullin (10 µg/kg) significantly decreased pulmonary arterial pressures (from 54 ± 3 to 47 ± 3 mmHg), and caused a 22% decrease in pulmonary vascular resistance without affecting systemic arterial pressures.\(^{44}\) These effects persisted for 45 min after the end of the adrenomedullin treatment and corresponded to the time course of measured plasma levels of adrenomedullin. Therefore adrenomedullin produces selective pulmonary vasodilation and is a potential alternative to INO that requires further evaluation.

### NO donors

Both sodium nitroprusside and nitroglycerin exert their vasodilating effects via enzymatic release of NO. When given intravenously, these agents decrease both pulmonary and systemic pressures and potentially increase intrapulmonary shunting. However, selective pulmonary
vasodilation has been observed when these agents were administered by the inhaled route.

In animal models, the pulmonary selectivity of inhaled sodium nitroprusside is dose dependent, with loss of selectivity and decreased systemic arterial pressures being observed at higher doses. In a newborn piglet model of acute lung injury, inhaled sodium nitroprusside produced a significant decrease in pulmonary arterial pressures and an increase in \( P_{aO_2} \).\(^6\) In the only clinical study published to date, inhaled sodium nitroprusside produced a mean increase in \( PaO_2 \) from 32 to 94 mmHg in 10 cyanotic neonates\(^6\).

Inhaled nitroglycerin produced selective pulmonary vasodilation in animal models of PH and in patients with PH after cardiac surgery for mitral valve replacement. Administration of a dose of 2.5 \( \mu g/kg/min \) of inhaled nitroglycerin produced significant decreases in pulmonary arterial pressure, pulmonary vascular resistance and intrapulmonary shunting without affecting systemic arterial pressures.\(^4\)\(^5\)

NO has been chemically attached to a number of other delivery molecules, which are known as nitric oxide–nucleophile adducts. These compounds release NO spontaneously in physiological solutions, are stable in the solid form and have the potential to function as aqueous slow-release forms of NO.\(^6\) Animal studies have demonstrated that the response to inhalation of these compounds in terms of their pulmonary selectivity and duration of effect is dependent upon their specific chemical structure. While the development of novel NO donors is an active area of current research, the compounds that are already available, like nitroglycerin, should not be forgotten and perhaps should be evaluated further for their ability to produce reliable selective pulmonary vasodilation.

**Conclusion**

INO has been a major advance in the treatment of a number of clinical conditions in both paediatric and adult medicine. However, the outcome studies to date suggest that INO therapy does not produce an improvement in survival, at least in adult patients and under the clinical conditions in which it has been tested. Whether the potential alternatives to INO described in this article are able to improve survival must await further study. Unfortunately, worthwhile outcome studies require multi-centre studies and major capital funding, which frequently is provided by the pharmaceutical industry. Whether industry is willing to invest in such studies is open to question, particularly in the case of 'old' agents such as PGI\(_2\). Therefore we may never really know whether any of these agents are more than just an 'alternative' to INO.
References


