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Abstract

The nitric oxide pathway plays a pivotal, yet diverse, role in human physiology, including modulation of vascular tone, neural transmission and inflammation. Inhaled nitric oxide is a selective pulmonary vasodilator that has emerged rapidly as an important therapeutic agent. It finds its best applications in paediatrics; the use of iNO in term neonates with hypoxaemic respiratory failure, in the assessment of pulmonary vascular reactivity and in the treatment of postoperative pulmonary hypertension in congenital heart disease is well recognised and accepted. This review details the delivery and monitoring aspects of inhaled nitric oxide, its potential toxic and side effects and its applications in several cardiopulmonary disorders in paediatrics.

MeSH: Heart defects, congenital, Hypertension, pulmonary, Infant, premature, Newborn, Nitric oxide, Paediatrics

Introduction

Inhaled nitric oxide (iNO) therapy is nowadays recognised as an important tool for the treatment and diagnosis of pulmonary vascular and airspace disease. Nitric oxide (NO) is a naturally occurring gas with multiple biological actions. Endogenous NO plays a role in the modulation of vascular tone,[1,2] the regulation of platelet function, neuronal transmission and the inflammatory response.[3] NO is formed, in the endothelial cell, from L-arginine and molecular oxygen in a reaction catalysed by the enzyme nitric oxide synthase (NOS).[4] Three different isoforms of NOS exist: NOS 1, a constitutive form first identified in neurons named nNOS; NOS2, an inducible form, first found in activated leukocytes called iNOS; and NOS 3, identified in endothelial cell also called eNOS. Even if very similar in structure, they show some differences. NOS 1 and 3 are constitutively expressed and are calcium calmodulin dependent. NOS 2, in contrast, is seen after induction by inflammatory stimuli, and is calcium independent. Nitric oxide

synthase 2 can produce large amount of NO measured in nanomoles, instead of the picomoles produced by the nitric oxide synthase 1 and 3. NO thus formed is a small reactive molecule which diffuses readily to adjacent vascular smooth muscle. In the vascular smooth muscle NO activates guanylate cyclase, which transforms GTP into cyclic guanylate monophosphate (cGMP), which in turn induces vasodilatation.[5] As NO exists as a gas, it can be delivered by inhalation, hence NO can vasodilate constricted blood vessels in close proximity to the ventilated lung. The rapid inactivation of NO by haemoglobin, when NO reaches the intravascular space, limits its effects on the pulmonary circulation.[6]

In this review, we will first describe the delivery and monitoring of inhaled NO (iNO) as well as its potential adverse effects and toxicity. These aspects are essential to the safe use of iNO. We shall then discuss its different applications in paediatric practice.

Rationale for the use of inhaled nitric oxide

For many years, physicians involved with the care of patients with pulmonary hypertension were in search of the ideal pulmonary vasodilator, which should be easy to administer, have a short duration of action and above all, be selective for the pulmonary circulation. Approaches to manipulate the pulmonary vascular tone were limited to oxygen supplementation[7,8] and respiratory or metabolic alkalosis.[9,10] Drug therapy included several intravenous vasodilators such as prostaglandins,[11,12] tolazoline[13] and magnesium sulphate.[14,15] All were characterised by their lack of selectivity, leading to a fall in systemic arterial pressure and an increase in intrapulmonary shunt. The introduction of inhaled NO and other recent advances in vascular biology have drastically changed the therapeutic approach of pulmonary hypertension.

As nitric oxide exists as a gas, it can be easily administered by inhalation. The anatomical proximity of the airspaces to the muscular arterioles allows NO to diffuse. Nitric oxide is lipophilic and thus crosses the membranes easily. By inducing vasodilation of aerated airspaces, NO can redirect blood flow from poorly ventilated areas, atelectatic or diseased lung regions, to better aerated air spaces and improves oxygenation and ventilation perfusion mismatch. This effect is the so-called microselective effect of iNO. As for endogenous NO, this vasodilator effect is induced through the cyclic cGMP pathway, as demonstrated by an increase in plasma cGMP during iNO therapy.[16,17] When it reaches the vascular lumen, NO avidly binds to haemoglobin and is thereby inactivated with a half-life of 2-6 seconds.[18] This rapid inactivation limits its effects on the pulmonary circulation and accounts for its selective action.[6,19] This is the macroselective effect of iNO. Indeed, red blood cells act as scavengers of iNO as demonstrated by Deem et al.[20] Between 75 and 90% of iNO is absorbed during inhalation. The metabolic fate of iNO is indeed similar to endogenous NO with the formation of nitrites and nitrates eliminated in the urine,[21] and more than 70% of the inhaled gas will appear in the urine as nitrates within 48 hours of inhalation.[22,23]

In summary, iNO fulfils most of the properties required to be the ideal pulmonary vasodilator; thus its use has become of major clinical interest.

Delivery and monitoring

The potential toxicity of iNO calls for reliable modes of delivery and availability of monitoring systems. Large and extensive reviews of the diverse modes of iNO delivery have been published.[24–32] The exact method of delivery and monitoring of NO may vary with the clinical indication and duration of treatment as well as the type of ventilator used. NO source tanks should be medical grade quality gas

manufactured by a process accepted by the medical administration and is available in different concentrations of NO in N_2, from 100 to 10000 ppm. By using continuous flow ventilators, stable NO concentrations can be easily delivered by titrating NO directly from the tank into the inspiratory limb of the ventilator. The theoretical concentration can be calculated using the following formula: $NO_{conc} = NO_{tank} x (Flow_{NOtank}/Flow_{ventilator})$. However, as demonstrated by Betit et al.[32] NO concentrations can be underestimated and direct measurement of NO is mandatory during therapy.

NO can also be administered with demand valve systems using a similar technique but a stable concentration of NO may be difficult to obtain. It has been proposed to deliver NO with a synchronised inspiratory injection technique to avoid the inconvenience of delivery during expiration.[33] Ventilators equipped with in-built NO delivery and monitoring systems are currently available. They simplify delivery and improve safety. The recent development of new ventilatory techniques such as high frequency oscillation ventilation (HFO) in newborns or infants with respiratory failure open new challenges for iNO deliveries, but because HFO is also delivered at constant flow, it seems possible to obtain stable NO concentrations.[34,35] Fujino et al reported that mixing NO during HFO was acceptable at all injection sites with a preference for the prehumidifier injection, which offers less fluctuation of NO concentration.[36] However, with this latter ventilatory technique, measurements may not be accurate, as a prolonged residency time of NO in the airways is possible; also the concentrations measured at the inspiratory limb of the ventilator do not accurately reflect the effective NO concentrations in the alveoli.

In spontaneously breathing patients NO can be administered by mask or hand ventilation with a bag.[37–39] Administration through nasal prong is also possible, opening the possibility of long-term treatment with iNO.[40]

The appropriate dose of iNO to assess pulmonary vascular resistance or treat pulmonary hypertension is not completely defined. Dose response studies have been performed in persistent pulmonary hypertension of the newborn (PPHN) and ARDS[41–46] and in congenital heart disease.[47,48] Inhaled NO doses required to treat pulmonary hypertension are higher than those required for improvement of ventilation perfusion mismatch and oxygenation.[41] The recommended dose by the FDA for the treatment of neonatal hypoxic respiratory failure is 20 ppm. Recently, Tworetzky et al suggest an initial dose of 20 ppm for the treatment of PPHN, as it produced an improvement in the pulmonary to systemic arterial pressure ratio, even though 5 ppm iNO was enough to produce peak improvement in oxygenation.[49] The exact dose required may indeed vary not only with the pathology but also with duration of therapy. Nelin et al. showed that the effective dose (the smallest dose effective to obtain a beneficial response) decreases as therapy continues.[50]

It has been shown that there is little haemodynamic benefit obtained above 20 ppm. The dose used to assess pulmonary vascular reactivity varies between 10 and 40 ppm, rarely 80 ppm, but higher doses had no further effect. The same may be applied for postoperative cardiac patients with a starting dose between 10 and 20 ppm. If there is no response, a trial at 40 and 80 ppm may be attempted but questions about the diagnosis must be raised. Higher doses give little or no benefit but may be associated with an increased risk of toxicity, in particular NO_2 and methaemoglobin formation. When a response is obtained, the dose must be decreased progressively to the lowest effective dose to avoid potential toxic effects. Slightly higher doses may be required for PPHN but Finer et al. showed that maximal response was obtained with 5 ppm.[44] Adequate

ventilation and lung recruitment is necessary in these patients to deliver NO in the alveoli and obtain a result. ARDS patients sometimes respond to very low doses of some hundreds ppb.
Guidelines on the use of NO with an emphasis on safety have emerged from a NHLBI workshop.[51] An ideal delivery system uses medical grade NO, limits to the maximum the residency time of NO in the ventilatory circuit, allows for precise concentrations of NO with a uniform mixing, has alarms notifying when excessive concentrations of NO are administered or if inadvertent discontinuation of NO occurs. Most important of all, it allows on line monitoring of NO and NO_2.
The formation of NO_2 is in part correlated to the amount of NO administered and a correct measurement of NO concentrations is of utmost importance. Two systems are currently available for monitoring NO and NO_2 concentrations. Chemiluminescence technique remains the gold standard with an accuracy of some ppb.[52] Manufacturers have adapted industry materials to the requirement of medicine with rapid response analysers requiring small amount of air sampling and therefore not interfering with ventilation. However, these devices are expensive and one may prefer the electrochemical devices that are somewhat inaccurate to measure very low amount of NO (such as for measurement of exhaled NO) but accurate enough by far for treatment monitoring.[53]

Toxicity

There has been proper concern about the potential toxicity of NO therapy. NO is a common pollutant. The US Occupational Safety and Health Administration has set a limit of NO exposure time to 25 ppm when breathed for 8h/day in the workplace.[54]
No severe side effects of iNO have been described, using concentrations up to 40 to 80 ppm (the maximal doses used in clinical practice). Studies in rats have shown that inhalation of NO up to 1500 ppm for 15 min caused no demonstrable injury.[55] Moreover cigarette smoke contains NO concentrations up to 1000 ppm[56] and no acute toxic effects have been reported. However, NO may be one of the substances involved in long-term lung toxicity of cigarette smoke. If extreme doses are used it seems to lead rapidly to methaemoglobinemia and a toxic pulmonary oedema, which may be more related to the transformation of NO to NO_2than to a direct effect of NO.[57] Therefore, particular attention has been focused on the possible acute toxic effects such as the production of NO_2 (a pulmonary toxin), methaemoglobinemia (which should remain < 5%), and cellular toxicity.

NO_2

One of the main concerns of iNO therapy is the chemical reaction of NO to NO_2 in the presence of oxygen within the ventilatory system or the airways. NO_2 is thought to be responsible for the pulmonary injury caused by exposure to NO. NO_2 is clearly cytotoxic[55] and in aqueous solutions is transformed to nitric and nitrous acids. It causes pulmonary epithelial cell damage, interstitial atrophy and fibrosis.[55] It is generally accepted that concentrations of NO_2 over 5 ppm are toxic.[54,58] Production of NO_2 is function of the NO concentration squared, the fraction of inspired oxygen and the time of residency in the ventilatory system.
Based on these findings guidelines should be followed to minimise the risk of toxicity related to the formation of NO_2:

1. Administer the lowest effective dose of iNO with a maximal dose of 40 to 80 ppm

2. Administer the lowest possible concentration of O_2
3. Monitor O_2 , NO and NO_2 concentrations
4. Minimise the transit time in the ventilator by using high gas flow rates to flush out alveolar gases
5. Minimise the exposure time of NO to oxygen before it reaches the patient

Methaemoglobin

Inhaled NO is quickly absorbed into the blood stream, avidly bound to haemoglobin and thereby, inactivated with the formation of methaemoglobin. This oxidised form of haemoglobin has impaired oxygen transport function. Methaemoglobin is restored to its oxygen carrying capacity by methaemoglobin reductase. Increased levels of methaemoglobin in patients receiving iNO therapy are unusual, and remain for the great majority in a safe range (<5%). High levels of methaemoglobin have rarely been reported.[59,60] Neonates have an immature methaemoglobin reductase system and may be more susceptible to increased levels.[61] Of note, methaemoglobin reductase deficiency is common in Native Americans.[62]

Methaemoglobin will indeed remain in a safe range with doses of iNO of 40 ppm and less. If necessary, excess methaemoglobin may be treated by reducing the iNO concentrations, or administrating vitamin C or methylene blue.[24,63–65] Particular caution must be taken when intravenous nitrovasodilators are associated with iNO, i.e. in the postoperative cardiac patient, where in our experience higher levels of methaemoglobinemia might be encountered.

Cellular effects

NO reacts with O_2 and superoxide anion (O_2-) to form peroxinitrite. Peroxinitrite is a potent oxidant that may damage a wide range of biological molecules.[66] In particular, it can induce lipid peroxidation of the cell membrane. Peroxinitrite also cause cell apoptosis by DNA strand breakage and by inhibition of mitochondrial respiratory enzymes.[67] However, NO is also considered as a free radical scavenger which can counteract and stabilise peroxinitrite. The significance of peroxinitrite-induced toxicity during inhaled NO therapy remains unknown. The antioxidant versus oxidant balance of these reactions may depend of the relative concentrations of these molecules and potential toxicity may only appear with high doses of NO not routinely used in clinical practice.

NO can react with thiol groups and form S-nitrosothiols.[68] These forms may act as NO carriers and transport NO far from its delivery site in the lung. Indeed S-nitrosothiols have similar platelet and vasorelaxant activities as NO and may prolong its effects or displace its site of action.[69]

One of the other major potential toxic effects at the cellular level is the effect on DNA. NO can inhibit DNA synthesis[70] and it has been shown that NO can alter DNA by the direct modification and strand breakage of DNA and also by inhibition of enzymes that are necessary to repair DNA lesions.[71,72]

Long term follow-up

Long-term studies of iNO in paediatrics are still scarce. In 12 newborns who received iNO for up to 4 days, no signs of lipid peroxidation product, impaired surfactant activity or changed cytokine profile were observed.[73,74] On the other hand, nitrotyrosine was detected after 10 days of life in two infants requiring prolonged ventilation, suggesting potential toxicity of NO.[73] Follow-up studies of adult patients treated with iNO for ARDS showed no difference in pulmonary

function compared to ARDS patients not treated with iNO.[75] Dobyns et al. came to the same conclusions in infants treated with iNO for PPHN and studied 4 to 12 months after discharge from the hospital.[76] In hypoxaemic term neonates, iNO is not associated with an increase in neurodevelopmental, behavioral or medical abnormalities at 2 years of age.[77] A recent follow up review of patients with PPHN randomised to a treatment with iNO demonstrated no adverse health or neurodevelopmental outcomes have been observed among infants treated with NO vs controls.[78]
In summary, so far no long-term toxic effects have been reported but caution must be the rule with this molecule with its wide variety of biological activities and potential toxicity.

Adverse effects

Some of the unwanted effect of nitric oxide may not be categorised as toxic effects but as adverse effects.

Rebound pulmonary hypertension

When iNO is administered over a prolonged period, such early as after cardiac surgery or in newborns with hypoxaemic respiratory failure, a considerable rise in pulmonary artery pressure may appear at time of abrupt withdrawal of iNO.[79–83] This may be a self resolving problem in a few minutes but can also progress to right ventricular failure and low cardiac output which may be life threatening. Different mechanisms can explain this reaction, ie: a possible down regulation of the endogenous production of NO by the administration of exogenous iNO, a slow recovery from cell dysfunction taking several days or a combination of both. Recently, an increase in endogenous endothelin has also been suggested as a potential mechanism.[84]
A negative feedback mechanism is supported by several studies both in vitro and in vivo[85–89] and is probably the main effect responsible of this phenomenon.
Exhaled NO is reduced after cardiopulmonary bypass due to either a decreased production or increased breakdown.[90] Endothelial cell dysfunction after cardiopulmonary bypass, as demonstrated by Wessel et al., may therefore take some days to recover and resume production of endogenous NO.[16] Withdrawal of iNO during this period leads to rebound pulmonary hypertension. This was also supported by Combes et al. showing that NO inhalation for 48 hours increases the reactivity of the pulmonary vascular bed to vasoconstricting agents.[89]
In order to avoid this rebound phenomenon, a stepwise slow reduction of iNO is mandatory and this even at very low levels of iNO such as 1 ppm. Regular attempts to wean should be performed to avoid self-maintaining treatment with iNO leading to a prolonged postoperative course.[91] Several other methods have been proposed: a transient increase in the fraction of inspired oxygen by Aly et al.,[92] the use of dipyridamole, a phosphodiesterase inhibitor allowing to maintain high levels of cGMP in the smooth muscle cell, thus prolonging the effect of iNO.[93,94] The use of sildenafil has also been reported recently.[95]

Platelet function

Endogenous NO has multiple biological functions among which the regulation of platelet function.[96] Hoggman et al. first reported that iNO inhibits platelet function and prolongs bleeding time in rabbits.[97] In patients with ARDS, NO decreased platelet aggregation but had no effect on bleeding time.[98] Intracranial haemorrhage is one of the most serious concerns in neonates, particularly in preterm neonates. Therefore the application of iNO in this group of patients

raises some concerns. Studies in neonates are conflicting: George et al.[99] showed that the bleeding time was increased after 30 min of 40 ppm iNO, whereas several other clinical studies demonstrated no change in the frequency of bleeding events in NO treated compared to placebo treated neonates.[35,59,100] Furthermore, recent studies in near terms and premature neonates did not demonstrate an increased risk of intracranial haemorrhage.[100,101]
We have shown that, in healthy volunteers, 10 min iNO at 30 ppm induced an inhibition of platelet aggregation accompanied by an increase in plasma and platelet cGMP.[102] This effect is of short duration and platelet aggregation studies should be performed as soon as the blood sampling is performed.
Even though, they are conflicting these studies open news questions; if iNO is selective for the pulmonary circulation in regard to its vasodilatory activity, this may not be true for others actions such as changes in platelet function; here the effect may be mediated by stable compounds such as nitrosothiols formed in the pulmonary circulation during inhalation and act systemically.

Left ventricular dysfunction

There are several reports of the negative effects of inhaled NO in patients with left ventricular dysfunction and elevated pulmonary vascular resistance.[103–108] Inhaled NO produces selective pulmonary vasodilatation. However, in patients with elevated left atrial pressure due to left ventricular dysfunction, a decrease in pulmonary vascular resistance (induced by iNO) will lead to an increase in pulmonary venous return and hence to an increase in left atrial and left ventricular filling pressures; this may not be tolerated by a failing left ventricle working on the flat portion of the Frank-Starling curve.[108] This effect may lead to rapid left heart failure and pulmonary oedema, most marked if the right ventricular pressure is suprasystemic and the left cavity small.[103] Such a phenomenon has been confirmed by Rosales et al. in a patient with a small non compliant left atrium and ventricle after repair of total anomalous pulmonary venous return.[109] Indeed several years ago, Wood already suggested that an elevation in pulmonary vascular resistance may protect the lungs from pulmonary oedema in severe mitral stenosis.[110] A similar postulate may be applicable in patients with severe left ventricular dysfunction or poor left atrial and ventricular compliance. This pathophysiologic explanation has been confirmed, and a direct negative inotropic effect excluded by recent studies[111,112] in pigs and in patients with normal left ventricular function.[113] Loh et al.,[114] in a canine model of cardiomyopathy, showed that iNO decreased pulmonary vascular resistance and increased left ventricular filling pressures, which was related to an increase in pulmonary venous return, without any effect on the contractile or relaxation properties of the left ventricular. This effect was further confirmed by Hayward et al. in patients with dilated cardiomyopathy,[115]
Even though iNO does not seem to have direct negative inotropic effects, these factors highlight the need for careful observation and intensive monitoring during NO inhalation in patients with left ventricular failure, if left ventricular afterload is not lowered concomitantly.

Inhaled nitric oxide applications

Neonates with hypoxaemic respiratory failure

Inhaled NO was targeted early to the newborn with persistent pulmonary hypertension of the newborn (PPHN). The first application of iNO in the newborn

was described almost 10 years ago by Roberts et al. and Kinsella et al.[116,117] They used iNO in term neonates with hypoxaemic respiratory failure and found a rapid improvement in oxygenation without any lowering of the systemic pressure. Several studies have then confirmed its efficacy in this group of patients.[59,118–120]
PPHN is a syndrome associated with diverse neonatal cardiopulmonary disorders, which are characterised by a high pulmonary vascular resistance with right to left shunt of deoxygenated blood across the ductus arteriosus and/or the foramen ovale. The role of echocardiography to confirm the diagnosis and conduct therapy is therefore essential. Echocardiography also excludes structural congenital heart disease, which would contraindicate the use of iNO. In neonates with hypoxaemic respiratory failure the success of iNO was mainly measured by the number of neonates in whom ECMO could be avoided.[46,121] A multicentre, randomised study with 248 neonates > 34 weeks who required mechanical ventilation and had an OI of 25 or above, showed that fewer neonates required ECMO in the group treated with iNO. However, the mortality was similar in both groups.[121] The most significant effect occurred in the newborns with PPHN. Patients with isolated PPHN generally show an immediate and dramatic response to iNO, whereas patients with parenchymal lung disease have less impressive responses. The latter requires specific and adapted mechanical ventilation to obtain adequate lung inflation and recruitment. This allows iNO to reach alveoli and diffuse to the smooth muscle cells. In the presence of atelectasis and mucous plugs, iNO can not reach the alveolocapillary membrane and thus may appear ineffective. This explains why iNO response is mainly disease related in this group of patients.
Wessel and al speculated that the use of specific lung expansion with the use of NO lead to a reduced use of ECMO in neonates with PPHN. Kinsella et al. reported that high frequency oscillation with iNO was better than high frequency oscillation alone, or iNO with conventional ventilation.[118] Again, among infants ventilated by high frequency oscillation, those receiving inhaled NO had a reduced need for ECMO, but no change in mortality in a prospective randomised study.[122] In conclusion, pure hypoxia associated with PPHN but without additional parenchymal lung disease has a high rate of clinical response to iNO.
Extensive supportive data demonstrate the overall safety and efficacy of iNO in hypoxaemic respiratory failure in term neonates, as evidenced by its recent approval by the United States Food and Drug administration.
Neonates diagnosed with congenital diaphragmatic hernia represent a particular group of patients within the neonates with hypoxaemic respiratory failure. Although iNO may be effective in some patients, as a group, neonates with congenital diaphragmatic hernia, are poor responders.[123] This may be due to several factors including pulmonary hypoplasia, left ventricular size and function as well as structural abnormalities of the pulmonary vascular bed.

NO application in premature neonates

In preterm neonates, hypoxaemic respiratory failure is almost invariably associated with hyaline membrane disease and respiratory distress syndrome (RDS). Preliminary studies in premature neonates with severe hypoxaemic respiratory failure support the efficacy of iNO to improve arterial oxygenation. Peliowski et al. reported their experience with iNO therapy in 8 premature neonates between 24 and 31 weeks of gestation.[124] Significant reduction of oxygen index and mean airway pressures were described during iNO inhalation. Skimming et al compared the effect of 5 ppm versus 20 ppm in 23 preterm

neonates with severe RDS.[125] Both concentrations of iNO induced an improvement in oxygenation.

Subhedar et al. included 42 preterm neonates < 32 weeks, who were recruited at 4 days of age if they were at high risk of developing bronchopulmonary dysplasia.[126] Neonates were randomised to receive either iNO or dexamethasone, or both or none of the treatments. There was no difference in survival, chronic lung disease, or intracranial haemorrhage between iNO treated infants and control group. One of the major restrictions of these studies is the small number of neonates randomised. Two recent double blind randomised studies have been published. Kinsella et al assessed the effects of low dose iNO (5ppm) in 80 premature neonates with severe hypoxaemic respiratory failure with survival to discharge as a primary endpoint.[101] iNO induced a significant improvement in oxygenation after 60 minutes. There was, however, no difference in survival. The low dose of iNO did not increase the risk of bleeding complications. They further hypothesised that low dose iNO may be effective as a lung anti-inflammatory therapy because this treatment may affect neutrophil adhesion in the microcirculation. The Franco Belgium Collaborative NO trial group randomly assigned 204 preterm (<33 weeks) and near term neonates (≥ 33 weeks) with oxygenation indices from 12.5 to 50 and 15 to 40, respectively, to 10 ppm iNO (n=105) or control ventilation therapy without iNO (n=99).[100] Neonates with a gestational age ≥ 33 weeks had a significant reduction in OI, duration of mechanical ventilation and duration of NICU stay. In the group < 33 weeks there were no significant differences.

The use of iNO remains somewhat controversial in premature neonates. Low doses of iNO can be of benefit for premature neonates with severe hypoxaemic respiratory failure allowing improvement of oxygenation and decreasing the need for mechanical ventilation but so far no effect on mortality has been demonstrated. The possibility that iNO may have a preventive effect on chronic lung disease is a hypothesis that has not yet been studied. However, there are no data to support the use of iNO routinely in premature neonates and it should be used in the context of prospective trials.

Hypoxaemic respiratory failure in infants and children

Acute respiratory distress syndrome (ARDS) is an acute form of severe alveolar-capillary injury that evolves after a direct or indirect lung insult. It begins as noncardiogenic pulmonary edema and develops into a neutrophilic alveolitis, and, later, pulmonary fibrosis.[127]

Patients with adult respiratory distress syndrome and hypoxaemic respiratory failure may also benefit from iNO.[42,80,128,129] Pulmonary hypertension and hypoxaemia secondary to a ventilation/perfusion mismatch, or a right to left shunt through a foramen ovale or both are prominent features of the disease. However, despite improved oxygenation iNO did not change mortality in large groups of patients.[130,131]

Dobyns et al included 108 childrens with a median age of 2.5 years in a prospective, multicenter study.[132] The children were randomised between a placebo group (n=55) and a control group (n=53) with 10 ppm of iNO for 3 to 7 days. A statistically significant improvement in both OI and PaO2/FiO2 was found in the iNO group at both 4 and 12 hours of treatment, but as in adults, no change in mortality was obtained.

From the data provided available so far, iNO had no effect on mortality and only transiently improved oxygenation in ARDS in children and/or adults.[132–135]

In conclusion iNO, may be useful as a rescue treatment for a short period of time in patients with ARDS. The transient improvement in oxygenation following administration of iNO may be explained by a reduced ventilation/perfusion mismatching. In the long run however, pulmonary damage secondary to volume and barotraumatism, oxygen toxicity, and cytokines, may over-ride any potential benefit of iNO.

Inhaled nitric oxide and cardiac disease

Assessment of pulmonary vascular reactivity

The assessment of pulmonary vascular reactivity plays an important role in the management of chronic pulmonary hypertension. In children with heart disease and significantly increased pulmonary blood flow or pulmonary venous hypertension, progressive pulmonary vessel wall damage can lead to medial and intimal changes, smooth muscle cell hypertrophy and hyperplasia and finally fibrosis and obliteration.[136,137] The increase in muscularity induces some degree of vasoconstriction. Therefore, elevated pulmonary vascular resistance, encountered at all stages of increased muscularity, may in part be due to this reversible vasoconstriction. Different diagnostic techniques are offered to evaluate the degree and progression of pulmonary vascular disease.

Morphologic criteria based on lung biopsies have been first described by Heath and Edwards.[138] They described six grades of progressive structural changes in the small pulmonary arteries of patients with congenital heart disease. Rabinovitch, then described a morphometric method which evaluated the extension of muscle into usually non muscularised peripheral arteries.[139] Despite reports showing a good correlation of biopsy with postoperative haemodynamics,[140] the predictive value of lung biopsies remains controversial as lesions may be unequally distributed within the lungs; thus the degree of lesions may be misinterpreted. Moreover, this static description does not take into account the dynamics of the pulmonary vascular bed. In addition, open lung biopsy is required, as transbronchial route does not offer adequate material, and this it is not without risk.[141,142]

Standard catheterization and assessment of pulmonary vascular reactivity remains however, the test performed in most cardiac centers to assess operability, even though there are some limitations, such as difficulties in measurement of cardiac output and failure to take into consideration the pulsatile nature of the pulmonary circulation.

New techniques using intravascular ultrasound and assessing the pulsatility and distensibility of the pulmonary arteries are being studied, and may offer new insights in the assessment of pulmonary vascular disease in the future.[143–145]

Since the early 60's, when high percentage inspired oxygen was recognised as a pulmonary vasodilator in congenital heart disease,[7,8] 100% oxygen (O_2) has been used to assess pulmonary vascular reactivity routinely. Conclusions based on this unique test however were not considered as sufficient.[146] Other agents such as tolazoline,[147] prostacyclin,[11] or calcium channel blockers,[148] have been used but they all share the same problem.[149] As they require intravenous administration, their results may be confounded by changes in systemic hemodynamics and intrapulmonary shunts. The use of a selective pulmonary vasodilator such as iNO offers the clinician important advantages in the investigation of pulmonary vascular reactivity. Several studies have been performed to assess the effect of iNO on pulmonary vascular resistance in congenital heart disease. First, Roberts et al studied the effect of 20 and 80 ppm

inhaled nitric oxide in 10 patients with congenital heart disease.[39] The maximum reduction in pulmonary vascular resistance was obtained with the highest dose. There was an additive effect when a high dose of inspired oxygen was delivered simultaneously. Winberg et al showed, in addition, that children with a normal pulmonary vascular tone did not respond to nitric oxide.[150] Day et al failed to demonstrate an increased response to 60 ppm as compared to 12 ppm.[151] Berner et al studied the effect of 35 ppm nitric oxide in children with long-standing pulmonary hypertension and congenital heart disease.[37] They showed that the decline of the selective response to nitric oxide seems to parallel the progression of established vascular disease and thus may be helpful for the selection of patients for corrective surgery. Atz et al studied the effects of nitric oxide and high levels of inspired oxygen. Inhalation of 80 ppm nitric oxide and 100% oxygen produced a similar degree of pulmonary vasodilation.[152] In addition the combination of both vasodilators provides additional pulmonary vasodilation and may identify patients who would not respond to each substance alone. Rimensberger et al compared the effects of 20 ppm nitric oxide and aerosolised iloprost (25ng/Kg/min).[153] They concluded that in children with pulmonary hypertension and congenital heart disease both inhaled nitric oxide and aerosolised iloprost are equally effective in selectively lowering pulmonary vascular resistance. The combination, however, failed to prove more potent than either substance alone.

End-stage cardiac failure and cardiopulmonary transplantation

Severe pulmonary vascular changes have been found in patients with right ventricular failure after heart transplant.[154,155] Therefore, the preoperative assessment of the degree of vasoconstriction versus fixed obliterative disease is crucial in the decision to transplant the heart alone or to proceed with heart and lung transplantation.[156] There are several reports of the effects of iNO in patients with elevated pulmonary vascular resistance before transplantation.[103,104] NO produces selective pulmonary vasodilation without systemic hypotension, which is a common limiting feature of intravenous prostacyclin and sodium nitroprusside.[156,157] Inhaled NO provides an effective assessment of pulmonary vascular resistance before cardiac transplantation.

Post-operative differentiation between anatomic obstruction and vasospasm

Pulmonary hypertension after surgery for congenital heart disease may pose diagnostic and therapeutic management dilemmas and complicate considerably the early post-operative course.[47,82,147,158] Pulmonary hypertension may be episodic or sustained in the immediate postoperative period with resolution once recovery from cardiopulmonary bypass is complete. Certain patients, particularly neonates with obstructed anomalous pulmonary venous drainage, truncus arteriosus and hypoplastic left heart syndrome, have marked pulmonary vascular lability postoperatively. Even with invasive monitoring it may be difficult to differentiate pulmonary vascular lability from residual obstruction at the surgical anastomosis. Indeed, the measurement of elevated pulmonary artery pressures from a proximally inserted catheter or the noninvasive recognition of an elevated right ventricular pressure by Doppler interrogation may represent either distal pulmonary artery obstruction (amenable to treatment by surgical or interventional catheterisation techniques) or pulmonary vasospasm. It is especially important to resolve vasospasm from fixed obstruction to pulmonary blood flow when the

temporary support of the children of the circulation with ECMO is considered.[159,160]

Two studies showed that a trial of inhaled nitric oxide may differentiate anatomic obstruction from pulmonary vasoconstriction.[161,162] Both demonstrated that failure to respond to inhaled nitric oxide in neonates after cardiac surgery should prompt investigations looking for lesions amenable to surgical correction. A trial of inhaled nitric oxide after cardiac surgery may be useful to differentiate reversible pulmonary vasoconstriction from fixed anatomic obstruction and may provide information if temporary support with extracorporeal membrane oxygenation is considered.

Management of pulmonary hypertension after cardiac surgery

Sudden pulmonary hypertensive crises may punctuate the post-operative course despite accurate surgery and are associated with significant mortality and morbidity.[158] For many years strategies aimed at minimising increases in pulmonary vascular resistance following repair of congenital heart disease have included hyperventilation to induce a respiratory alkalosis[9,163] and the use of sedation and paralysis.[164] Also, various vasodilator drugs such as tolazoline, prostacyclin, phenoxybenzamine and nitrodilators[12,13,147,165,166] have been used to reduce pulmonary arterial pressure. However, their lack of selectivity renders their use particularly risky in congenital heart disease patients. There is particular appeal in the therapeutic opportunities afforded by iNO in this setting.

Right ventricular function may be compromised following congenital heart disease repair because of cardiopulmonary bypass and direct injury by the surgical procedure itself. Increased pulmonary vascular resistance further compromises right ventricular function, and dilate the right ventricle inducing an intrapericardial tamponnade of the left ventricle.[167] This secondary diastolic dysfunction further reduces cardiac output leading to aortic hypotension and coronary hypoperfusion of the right ventricle leading to a vicious circle.[168] Inhaled NO improves right ventricular systolic function by decreasing afterload and reduces the left ventricular tamponnade thus restoring aortic pressure and coronary perfusion.[169–171] It has been applied in several different congenital heart disease, other than left to right shunts, where an increased PVR may complicate post operative course such as mitral valve stenosis,[172] bicavopulmonary anastomosis,[173,174] the Fontan circulation[175,176] or post cardiac and/or lung transplant.[63,177–180] Several reports confirm that iNO corrects right ventricular dysfunction after left ventricular assist device implantation[181] perhaps through an increase in pulmonary venous return and left atrial pressure, and facilitating pump flow.[182]

Wessel et al. showed that pulmonary endothelial dysfunction was present after cardiopulmonary bypass: thus the response to acetylcholine was attenuated but the response to inhaled nitric oxide was maintained.[16] They hypothesized that a dysfunctional endothelium with reduced endogenous nitric oxide release may contribute to post operative pulmonary hypertension. Journois et al demonstrated that inhaled nitric oxide was a useful therapy for pulmonary hypertensive crisis refractory to conventional treatment.[183] Miller et al. demonstrated that low doses of nitric oxide (2 ppm) appear to be effective in similar patients.[47] Beghetti et al. demonstrated that the effect of low dose nitric oxide was maintained over several days at concentrations carrying little risks of toxicity.[82] Nitric oxide has been used with success in several different congenital heart defects where increased pulmonary vascular resistance may complicate post operative course such as mitral valve stenosis,[172] total anomalous pulmonary venous return,[79] bidirectional

Glenn anastomosis,[173] the Fontan circulation[176] or post heart and/or lung transplant.[63] A beneficial effect in patients with cavopulmonary anastomosis is not consistently reported and this despite of an increase in cGMP levels which is proof of effective deliver.[184]
Two recent studies reported the prophylactic use of iNO after cardiac surgery. They showed controversial results. Miller et al showed that in infants at high risk of pulmonary hypertension, routine use of inhaled nitric oxide after congenital heart surgery can lessen the risk of pulmonary hypertensive crises and shorten the postoperative course, with no toxic effects,[185] whereas Day et al showed no benefit.[186]

Other potential applications

After lung transplantation, acute but transient graft dysfunction (manifested by pulmonary hypertension with hypoxaemia) challenges and escalates the post-operative management.[63,180] Inhaled NO is effective in reducing pulmonary vascular resistance and intrapulmonary shunt after lung transplantation. It was indeed more effective than acetylcholine, suggesting that ischemic preservation of the donor lung may result in endothelial dysfunction and contribute to pulmonary hypertension.[63]
Some reports demonstrated the efficacy of iNO to increase PaO_2 and decrease pulmonary vascular resistance in patients with chronic obstructive airway disease.[187–189] The bronchodilatory effect of iNO in obstructive airway disease remains a matter of debate. Human observations[190] do not suggest a marked bronchodilator role for NO compared to guinea pigs studies.[191] Inhaled NO may also be useful in severe asthma.[192] Some studies have shown that endogenous NO production appears to be increased in asthmatics secondary to the stimulation of inducible NO synthase by mediators of inflammation.[193–196]
The number of pathologies in which iNO has been tested with success is continuously growing (primary pulmonary hypertension,[157,197–201] pulmonary embolism,[202] and sickle cell disease.[203–205]

Conclusion

Inhalation of nitric oxide has made the journey from the bench to the bedside more rapidly than any other drug. Inhaled NO is a selective vasodilator for the constricted pulmonary vascular bed and improves ventilation perfusion matching in respiratory failure. In paediatrics, the use of iNO in term neonates with hypoxaemic respiratory failure, in the assessment of pulmonary vascular reactivity and in the treatment of postoperative pulmonary hypertension in congenital heart disease is well recognised and accepted. Several other applications have been described but caution must be the rule with a drug that shows potential adverse and toxic effects. Still, randomised double blind trials are required to define its effects in premature neonates and even after cardiac surgery, to define its effects on morbidity and mortality. Several questions are still unanswered regarding its safety in regards to long-term administration. Meanwhile, this unique molecule continues to provide us with insight into the mechanisms of pulmonary vascular disease. In addition, new roles in inflammation may include the modification of the platelet and /or leukocytes function and its effects in the development of chronic lung disease.

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