Abstract

Inhaled nitric oxide (NO) plays an important role in treating persistent pulmonary hypertension of the newborn (PPHN), which is marked by a pathologic elevation of pulmonary vascular resistance. There is good evidence that the use of inhaled NO reduces the need for extracorporeal membrane oxygenation for term babies with severe PPHN of any cause, except in those infants with congenital diaphragmatic hernia, for which a benefit has not been shown. Although reducing the need for extracorporeal membrane oxygenation is beneficial in terms of cost and morbidity, inhaled NO has not been shown to decrease mortality in any neonatal population. Inhaled NO has also been shown to improve oxygenation in premature infants, although longer-term benefits have not been consistently demonstrated. This article will review the physiology of NO, its mechanisms of action in PPHN, and examine the evidence that supports its use in term and preterm infants with pulmonary hypertension.

Inhaled Nitric Oxide

By Kelly R. Wendel, RN, MSN, NNP, and Amy T. Nathan, MD

In 1980, while studying how acetylcholine relaxes vascular smooth muscle, Robert Furchgott discovered another substance that had an effect on vascular smooth muscle and named this substance endothelium-derived relaxing factor.1 After further research, in 1986 Furchgott and colleagues identified the substance as nitric oxide (NO),2 a discovery for which they later won the Nobel Prize in Medicine. Before this revelation, NO was primarily known as an environmental pollutant. The recognition of its key role in numerous physiologic systems fueled interest in using exogenous NO as medical therapy. Initially studied in newborn lambs,3-8 inhaled NO was found to reverse pulmonary vasoconstriction and was soon shown in adults to decrease pulmonary vascular resistance (PVR). The first publications of its use in neonates came out in 1992, with two separate reports demonstrating that inhaled NO produced a significant improvement in oxygen saturation when given to infants with persistent pulmonary hypertension of the newborn (PPHN).9,10 Subsequent small studies supported these findings and were the impetus for larger, randomized controlled clinical trials to be performed.

Nitric Oxide Physiology

Nitric oxide is a colorless, odorless gas that is produced by the endothelial cells lining blood vessels. It plays a key role in regulating vascular muscle tone. It is generated from the amino acid L-arginine by one of the forms of the enzyme NO synthase (NOS). Normally, the activity of NOS is increased by factors such as birth, shear stress, and oxygen exposure. Endogenous NO is crucial for the normal transition of the pulmonary circulation that occurs at delivery, and failure of this pulmonary vascular relaxation results in persistent pulmonary hypertension.11,12 Inhaled NO is the most effective way to selectively dilate the pulmonary vasculature because it acts directly on those areas to which it is delivered, improving ventilation-perfusion matching.

Inhaled NO diffuses quickly through the alveolar-capillary membrane into the smooth muscle layer of adjacent pulmonary vessels.13 Here it causes relaxation of the vessel wall through an increase in cyclic guanylic acid, a cyclic nucleotide that acts as a second messenger to transmit a signal from the cell surface to the cytosol or nucleus. This leads to a decreased concentration of free calcium in smooth muscle cells. The half-life of inhaled NO is incredibly short (on the order of seconds14) because it is quickly bound by red blood cells once it enters the circulation. The avid binding of NO to hemoglobin is also responsible for one of the toxicities seen with NO therapy, namely, methemoglobinemia.15 Methemoglobin is not directly toxic, but hemoglobin that has bound NO cannot carry oxygen. Levels of methemoglobin higher than 10% result in methemoglobinemia, which is characterized by cyanosis, respiratory distress, and
tachycardia. Ideally, patients receiving inhaled NO should have levels of methemoglobin less than 2%, and this should be monitored every 24 hours while on NO therapy. The other byproduct of NO metabolism is nitrate. It is estimated that almost 70% of inhaled NO is excreted within 48 hours as nitrate in the urine. Nitrate itself is not particularly toxic to the body. However, excess quantities can promote the conversion of nitrate (NO₃⁻) to nitrite (NO₂⁻), which in turn can result in increased production of both methemoglobin and ammonia. If ammonia cannot be excreted in the urine, excess quantities can lead to hepatic and central nervous system toxicity.

Other possible toxicities of NO relate to its ability to act as a free radical (it has an unpaired electron). Nitric oxide reacts with oxygen to form nitrogen dioxide, which is a more reactive and toxic free radical than NO itself. As inhaled NO is usually given to infants who are also receiving high concentrations of oxygen, this is a relevant concern. At high concentrations, NO has pro-inflammatory and prooxidant effects. However, lower levels of NO have been shown to have anti-inflammatory properties, and improve surfactant function. Concentrations of more than 20 parts per million (ppm) provide little additional hemodynamic benefit in most patients; therefore, 20 ppm is generally the recommended starting dose, and even lower doses are often sufficient. Prolonged treatment (longer than 10 days) has been associated with pulmonary toxicity. Finally, inhalation of NO has been shown by some investigators to inhibit platelet function and lengthen bleeding time, although most of the controlled neonatal trials have not shown a significant increase in bleeding complications in infants receiving NO.

Nitric Oxide in Term and Near-Term Infants

Persistent pulmonary hypertension of the newborn is a cardiopulmonary disorder characterized by systemic arterial hypoxemia secondary to elevated PVR with resultant shunting of blood from the pulmonary to the systemic circulation. The elevated PVR diverts the blood through the patent ductus arteriosus and the patent foramen ovale (Fig 1). Persistent pulmonary hypertension of the newborn is marked by failure of the PVR to decrease after birth, despite improved alveolar oxygenation and lung expansion. After birth the umbilical cord is cut, which removes the baby from the placental circulation and increases systemic vascular resistance. If the PVR remains high, the ductus arteriosus and foramen ovale remain open as blood continues to flow through them, bypassing the lungs. The inability to increase pulmonary blood flow leads to worsening hypoxemia, which may lead to death in severe cases. The high PVR puts a great strain on the heart, and with continued hypoxemia the performance of the myocardial muscle becomes compromised. If the pulmonary hypertension is not corrected it can lead to right heart dilation, tricuspid insufficiency, and right ventricular failure.

Early recognition of PPHN is essential to treat and possibly reverse the disease process. Diagnosis is not always easy and, in many cases, is made by exclusion or by response to treatment. Echocardiography is a useful diagnostic tool, but may not be readily available. The clinical examination can be misleading, as PPHN can present with a murmur that mimics complex cardiac defects, and radiologic evidence may or may not show
lung disease. Pre- and postductal oxygen saturations can help in making the diagnosis but have limitations, as postductal saturations will be lower in infants with ductal-dependent heart lesions, even with normal pulmonary pressures. In cases where an echocardiogram is not easily obtained, the clinical course and response to therapy may be helpful in making the diagnosis.

A decade ago, many infants with severe PPHN required extracorporeal membrane oxygenation (ECMO) because specific pulmonary vasodilators were not available. Most vasodilatory drugs are not specific for the pulmonary bed but act systemically, which can lead to acute hypotension. The mainstays of therapy for infants with PPHN were aggressive ventilator management with induced respiratory alkalosis or infusion of alkali, as well as sedation and/or paralysis. The discovery of NO and its availability as a gas introduced for the first time the opportunity to use a vasodilator localized to the pulmonary circulation. Inhaled NO was seen as the “silver bullet” that would finally be able to reverse pulmonary hypertension in critically ill newborns.

The Neonatal Inhaled Nitric Oxide Study was a multicenter trial that enrolled infants who were at least 34 weeks’ gestation, were younger than 14 days, and had poor oxygenation but no structural heart disease. All babies were ventilated, and the treatment group received inhaled NO at 20 ppm in a blinded fashion (the placebo gas was 100% oxygen). The study was stopped early, after enrollment of 121 infants in the control group and 114 infants in the NO group, as the results were showing a significant reduction in the combined outcome of death or need for ECMO. However, further analysis revealed no difference in mortality rates, only that babies treated with NO were less likely to require ECMO. A few years later, another placebo-controlled, randomized, and blinded multicenter trial studied a similar patient population, but treated them with 20 ppm of inhaled NO for less than 24 hours, then dropped the dose to 5 ppm for no more than 96 hours. A total of 248 babies were randomized, and results again showed a reduction in the need for ECMO, although no fewer deaths in those infants treated with NO. There was, however, less chronic lung disease in the babies who had received inhaled NO. This study included neonates who had pulmonary hypertension for a variety of reasons, including congenital diaphragmatic hernia. Upon subgroup analysis, only infants with congenital diaphragmatic hernia did not seem to benefit from NO. Other studies have shown that with adequate lung recruitment, even those infants with respiratory distress syndrome (RDS) or meconium aspiration syndrome were less likely to need ECMO when treated with inhaled NO. Meta-analysis of several of these studies has reinforced the finding that inhaled NO reduces the need for ECMO in infants with hypoxic respiratory failure regardless of the cause, although it does not appear to decrease mortality or the length of hospital stay. Compared with ECMO, inhaled NO therapy is cheaper and has less associated morbidity, making the risk/benefit ratio more favorable.

The starting dosage of inhaled NO is typically 20 ppm, which is then decreased, using oxygenation as a measure of the response to therapy. Some studies have used 80 to 100 ppm, but higher doses have not been shown to result in more sustained improvement in oxygenation. When an infant fails to respond to NO, ECMO may be needed. Therefore, inhaled NO treatment failure plans need to be predetermined for units that cannot provide ECMO. In addition, these units need to have the capability for transporting infants on inhaled NO because some patients, although they have not responded, will deteriorate with any attempts to wean.

The risks of using inhaled NO include increased bleeding time, which may be especially worrisome for preterm babies who are at risk for intraventricular hemorrhage. As mentioned earlier, there is a risk of methemoglobinemia, which can reduce the oxygen-carrying capacity of red blood cells. There is also a risk of oxidative lung injury, especially when inhaled NO is paired with high inspired concentrations of oxygen. Finally, inhaled NO is an extraordinarily expensive therapy, and care must be used in only offering it to those infants who will truly benefit.

When evaluating a potential therapy for infants, long-term outcomes are particularly important. Although many studies show initial improvement in pulmonary outcome and a decrease in the requirement for ECMO, this improvement has not translated into a reduced need for outpatient treatment of lung disease, reduced hospitalization rates, or even improved survival. The follow-up studies are as short as 1 year, and much longer follow-up is needed.

Nitric Oxide in Preterm Infants

In contrast to term infants who often have persistent pulmonary hypertension and little parenchymal lung disease, preterm infants typically manifest pulmonary hypertension as a result of their underlying lung disease, most often RDS. Although premature neonates with respiratory failure have an increase in pulmonary artery pressure, this elevation is rarely sufficient to cause reverse flow through the ductus arteriosus (as is often seen in term babies). There is also a subset of neonates who have pulmonary hypertension related to pulmonary hypoplasia, with an overall smaller surface area of vessels in the lung for the blood to pass through, which increases the pressure within these blood vessels. As inhaled NO was being shown to provide dramatic improvements in oxygenation for term infants, there was much interest in treating
premature neonates with inhaled NO as well.\textsuperscript{1} It was recognized that inhaled NO could provide benefit by selectively dilating the pulmonary vasculature, which improves ventilation-perfusion matching,\textsuperscript{25} as well as acting as an anti-inflammatory and antioxidant\textsuperscript{26} in the lung. However, the availability of inhaled NO coincided with a surge in the use of antenatal steroids as well as surfactant. As a result, there are now fewer preterm babies with severe acute respiratory failure, those who are ventilated (and therefore eligible for trials) have tended to be sicker than in the past, and trials in premature infants have lagged behind those for full-term neonates.\textsuperscript{27}

The most recent Cochrane review on the use of inhaled NO for respiratory failure in preterm infants includes three trials for a total number of 207 babies studied.\textsuperscript{28-30} Each of the individual trials reported improvement in short-term outcomes such as oxygenation, but none was able to show any intermediate- or long-term benefits from inhaled NO therapy. The conclusion of the Cochrane reviewers was that there is currently no published evidence to support the use of inhaled NO in preterm infants.\textsuperscript{31}

Since the Cochrane review was published, there have been additional studies looking at inhaled NO use in preterm babies. A double-blind, randomized, placebo-controlled, single-center trial showed that inhaled NO during the first week of life significantly decreased the incidence of chronic lung disease and death by 24\% in premature infants with RDS.\textsuperscript{32} A total of 207 infants with less than 34 weeks’ gestation and weighing less than 2 kg were enrolled, all requiring mechanical ventilation at 72 hours of life. The treatment group received inhaled NO at 10 ppm on the first day, followed by 5 ppm for 6 days or until extubation. The neonates were also randomized to intermittent mandatory ventilation or high-frequency oscillatory ventilation. Further analysis of the data revealed that the most benefit was seen in the least sick infants (those who had an oxygenation index of <6.94). The same study demonstrated a 47\% decrease in severe intraventricular hemorrhage and periventricular leukomalacia in babies treated with inhaled NO. Follow-up of these infants at 2 years of age showed a significantly lower risk of an abnormal neurodevelopmental outcome in those patients treated with inhaled NO.\textsuperscript{33} Although the original analysis showed reduction in the combined outcomes of chronic lung disease and death, the improvement in neurodevelopmental outcome was seen even after controlling for these intermediate variables. Their hypothesis was that by decreasing right ventricular afterload, NO may attenuate venous stasis and prevent infarction of the germinal matrix, and/or it may limit venous thrombosis by reducing platelet aggregation. Unusual features of this study included a low rate of antenatal steroid use (around 55\%) and a high morbidity rate in the placebo group (24\% had severe intraventricular hemorrhage or periventricular leukomalacia).\textsuperscript{34}

The neonatal ventilation with inhaled nitric oxide versus ventilatory support without inhaled nitric oxide for preterm infants with severe respiratory failure trial was a multicenter trial based in the United Kingdom.\textsuperscript{27} Their recruitment goal of 200 infants was not met as neonatologists seemed unwilling to enroll any but the sickest infants (mortality rate in the study was around 60\%). Neonates younger than 34 weeks were eligible if they had severe respiratory failure requiring ventilation at less than 28 days. The study was not blinded, and treatment began with 5 ppm of inhaled NO, with stepwise doubling of the dose up to a maximum of 40 ppm. Of those babies who received inhaled NO, there appeared to be short-term improvements in oxygenation, but no effect on mortality or disability was seen at 1-year follow-up. The trial also showed significantly increased costs in the group that received inhaled NO. In addition to the actual expense of the gas, the increased cost was related to a trend of longer periods of ventilation and time in the hospital for those babies who were treated with NO, which predominantly reflected the group that eventually died.

The most recent published study is a multicenter, randomized trial in the United States that enrolled a total of 415 preterm infants with less than 34 weeks’ gestation, with birth weights of 401 to 1500 grams who had respiratory failure 4 hours after treatment with surfactant.\textsuperscript{35} Neonates received either inhaled NO (5–10 ppm) or placebo (simulated flow) in a blinded fashion. The trial was stopped just short of its completion because of concerns for increased rates of severe intraventricular hemorrhage in the treatment group, which were not substantiated upon review of the head ultrasounds, and subsequent analysis of all enrolled infants.\textsuperscript{34} No reduction of the combined incidence of death or chronic lung disease was demonstrated overall for those babies who received inhaled NO. A post hoc analysis of the smallest infants (birth weight <1000 grams) demonstrated a higher incidence of severe hemorrhagic or ischemic brain injury in the group treated with NO and an apparently higher mortality rate. In contrast, post hoc analysis of the subgroup of babies with birth weight greater than 1000 grams did show a significant reduction in the combined outcome of death or chronic lung disease with inhaled NO.

The conclusion at this time is that inhaled NO should not be used routinely in premature infants with respiratory failure.\textsuperscript{36} as there are not convincing long-term benefits\textsuperscript{37} (especially for the sickest and smallest neonates), and there may be increased risks of brain injury. There is a suggestion of potential benefit for babies with mild lung disease, and new directions for research include using...
inhaled NO in infants who are on nasal continuous positive airway pressure. A pilot study that added 10 ppm of NO to nasal continuous positive airway pressure showed improvement in oxygenation.38

It is known from animal studies that a deficiency of NO disrupts development of lung tissue as well as pulmonary vasculature.39 There is much interest in the effects of inhaled NO on lung development, including modulation of blood vessel formation as well as maturation of lung parenchyma and airway smooth muscle, especially when given at low doses for an extended period. Animal data seem to indicate positive effects of NO exposure in terms of normalizing vascular growth.40,41 Further studies will likely focus on developmental effects of exogenous NO, as well as its use in infants with less severe lung disease.

Conclusion

As one of the newer therapies for pulmonary hypertension, inhaled NO provides another avenue of treatment for severe PPHN. Many babies have rapid, dramatic improvement in oxygenation, and numerous studies have verified its beneficial effects in reducing the need for ECMO in term or near-term infants with hypoxic respiratory failure. Inhaled NO is now considered standard of care when more conventional treatment has failed. Many neonatal units are now offering this therapy. Despite these advances, there has been no improvement in overall mortality from severe pulmonary hypertension. In contrast to term newborns, premature neonates (<34 weeks) have been shown only to benefit from inhaled NO in the most short-term outcomes. They do have initial improvement in oxygenation, but overall mortality is not decreased, and rates of chronic lung disease are similar between infants treated with NO or placebo. In addition, the suggestion that inhaled NO may increase central nervous system morbidity or even mortality in infants of less than 1000 grams compels us to restrict our use of NO in this population to randomized, controlled trials.

References

1. Ramanathan R. Nitric oxide: what is this stuff? Symposium on Critical Care and Transport; Center for Advanced Medical Education; Children’s Hospital of Los Angeles, Oct. 4, 2001.
27. Field D, Elbourne D, Truesdale A. Neonatal ventilation with inhaled nitric oxide versus ventilatory support without inhaled nitric


