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Inhaled Nitric Oxide Therapy for Pulmonary Disorders of the Term and Preterm Infant

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Abstract

The 21st century began with the FDA approval of inhaled nitric oxide therapy for the treatment of neonatal hypoxic respiratory failure associated with pulmonary hypertension in recognition of the two randomized clinical trials demostrating a significant reduction in the need for extracorporeal support in the term and near-term infant. Inhaled nitric oxide is one of only a few therapeutic agents approved for use through clinical investigations primarily in the neonate. This article provides an overview of the pertinent biology and chemistry of nitric oxide, discusses potential toxicities, and reviews the results of pertinent clinical investigations and large randomized clinical trials including neurodevelopmental follow-up in term and preterm neonates. The clinical investigations conducted by the Eunice Kennedy Shriver NICHD Neonatal Research Network will be discussed and placed in context with other pertinent clinical investigations exploring the efficacy of inhaled nitric oxide therapy in neonatal hypoxic respiratory failure.

Index Words

newborn; hypoxic respiratory failure; inhaled nitric oxide; pulmonary hypertension; surfactant

Approximately twenty-five years ago, medical researchers began investigating nitric oxide (NO) as a potential therapeutic inhalational agent. The interest in this gas began in 1980 when Furchgott and Zadawaski, investigating acetylcholine's relaxing effect on arterial smooth muscle noted the requirement of vascular endothelium for acetycholine to produce a smooth muscle relaxing effect.[1] They proposed acetylcholine stimulated the formation of a substance in the endothelial cell that subsequently caused smooth muscle relaxation. This

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observation initiated a near decade long search for the "endothelial-derived relaxing factor" (EDRF). Three investigators independently identified the simple molecule NO as EDRF, and were rewarded with the Nobel Prize in medicine and physiology.[2–4] Shortly thereafter, animal and clinical investigations revealed that inhaled nitric oxide (iNO) was a pulmonary-specific vasodilator prompting excitement about its potential use for pulmonary diseases.[5,6]

Prior to these observations, NO was known primarily as an atmospheric contaminant generated during combustive processes.[7] In the atmosphere, in the presence of oxygen it reacts to form nitrogen dioxide (NO_2). Both NO and NO_2 are air pollutants and contribute to the formation of smog. Within biologic systems, the enzyme nitric oxide synthase (NOS) facilitates the production of NO. To produce NO, NOS requires a number of cofactors in the presence of oxygen, and utilizes arginine as a nitrogen donor to yield the products of citruline and NO. Three isoforms of NOS exist in mammals: endothelial, neuronal, and inducible.[8] Endothelial NOS is important for regulating vascular smooth muscle tone. The neuronal form produces NO to act as a diffusible neurotransmitter. The inducible form generates larger cytotoxic amounts of NO which depending upon the circumstances can be protective or pathologic. Thus the NO molecule can play many roles in the organism: protective, regulatory, or toxic.

Figure 1 illustrates the role NO and NOS play in regulating vascular tone. When NOS in the endothelial cell is stimulated it will generate a molecule of NO. As NO is a small lipophilic molecule it can easily transverse cellular membranes and enter the adjacent vascular smooth muscle cell. There NO can bind and activate another enzyme – soluble guanylate cyclase to produce cyclic guanosine monophosphate (cGMP). After being phosphorylated it initiates the process of smooth muscle relaxation, thereby reducing vasculature resistance.

iNO a pulmonary-specific vasodilator

NO's chemical characteristic of existing as a gas facilitated its ability to elude investigators searching for the EDRF, however this characterist captivated the attention of clinicians treating pulmonary hypertension. Being a gas, it provides the ideal way to deliver a therapeutic vasodilator to the target organ (the blood vessels in the lung) thereby avoiding systemic hypotension. Another characteristic of NO that enhances iNO's pulmonary-specific vasodilatory effect is NO's high affinity for heme proteins.[9] Because of NO's high affinity for heme proteins, any excess iNO delivered into the lung and reaching the blood stream rapidly binds to hemoglobin, eliminating the potential for systemic vasodilitation, and enabling iNO to be a pulmonary-specific vasodilator. However, binding to heme proteins is not without cost as methemoglobin is formed, a potentially toxic by-product of iNO use.

Potential toxicites of iNO therapy

Methemoglobin formation As described above, the high affinity of NO for hemoglobin facilitates pulmonary-specific vasodilator ability when provided by inhalation. Unfortunately it also contributes to a potential toxicity – the formation of methemoglobin.[10–12] Fortunately, in individuals with adequate methemoglobin reductase activity, exposure to concentrations of iNO at 20 ppm or less are well tolerated. However when iNO is used in high

concentrations or in rare individuals with deficiencies in methemoglobin reductase, methemoglobin may accumulate resulting in methemoglobinemia and the potential for decreased oxygen delivery to tissues. Nitrogen dioxide exposure As mentioned earlier, whenever NO is in the presence of oxygen, NO_2 will be formed. NO_2 is a more toxic gas than NO and can be a pulmonary irritant provoking asthma exacerbations at concentrations in the 1 part per million (ppm) range.[12–14] According to the termolecular reaction of NO with oxygen, the rate of NO_2 formation is dependent upon the concentration of oxygen, the square of the concentration of NO, and the duration of time the 2 gases are exposed to each other.[15,16] Therefore to minimize the amount of patient NO_2 exposure, iNO should be delivered into the ventilatory circuit as close as possible to the patient allowing for adequate mixing of the gas while limiting the amount of dwell time for NO_2 formation. Likewise, using the lowest necessary concentration of iNO and oxygen will minimize the amount of NO_2 formed.

Decreased platelet aggregation / Prolongation of bleeding time Investigators observed decreased platelet aggregation in adults with ARDS receiving iNO therapy, however they did not experience a prolongation of their bleeding time.[17] However, healthy adult volunteers breathing 30 ppm of iNO for 15 minutes experienced a prolongation of their bleeding time which returned to baseline in a short time after the iNO exposure stopped.[18] Physiologically the vascular endothelium likely contributes in preventing platelet aggregation by releasing small amounts of NO. Despite this observed prolongation of bleeding time in adult volunteers, randomized clinical trials in neonates have not revealed a difference in bleeding complications. However caution is prudent in patients with bleeding diatheses.

Peroxynitrite formation The NO molecule is a free-radical and therefore highly reactive. In the presence of superoxide (another free-radical) it will readily react to form peroxynitrite (also a free-radical) with potential of oxidant injury. Whether NO plays the role of an oxidant or antioxidant likely depends on the local mileu and the concentrations of other potential interacting substances present.[8,12]

In summary, whatever the potential iNO toxicity, the lower the concentration of iNO used, the less likely the concern for adverse events.

Rebound pulmonary hypertension/hypoxemia During the early clinical investigations with iNO, life-threatening hypoxia was reported when abruptly discontinuing iNO from concentrations   10 ppm.[19,20] This rapid decrease in oxygenation correlated with a rebound of pulmonary hypertension which could be avoided by weaning iNO to lower concentrations prior to discontinuation.[21] As treating infants with iNO improves oxygenation, it seems intuitive that reducing the amount of iNO will result in some reduction of oxygenation. The Neonatal Research Network anticipated this phenomenon and carried out a prospective observational study accessing the change in oxygenation that occurs when weaning iNO therapy. In general, weaning the concentration of iNO is well tolerated with a modest reduction in oxygenation (19 torr). However greater reductions occur when discontinuing the drug (42 torr). Therefore iNO should be gradually reduced to 1 ppm or less before discontinuation.[22]

Efficacy of iNO Therapy for Neonatal Hypoxic Respiratory Failure

To establish the safety and efficacy of iNO therapy, the NICHD Neonatal Research Network in collaboration with the Canadian Inhaled Nitric Oxide Study Group initiated the first large randomized clinical trial (RCT) of iNO therapy, the Neonatal Inhaled Nitric Oxide Study (NINOS).[23] This trial tested the primary hypothesis that administration of iNO to infants 34 weeks of gestation with hypoxic respiratory failure (HRF) would reduce the risk of death (by day 120) or the initiation of ECMO from 50% in control infants to 30% in infants treated with iNO (a relative reduction of 40%). An oxygenation index (OI) = [(mean airway pressure × FiO$_2$ × 100)/PaO$_2$]   25 was believed to predict that level of risk at that time. The trial randomized 235 patients with HRF due to meconium aspiration, persistent pulmonary hypertension of the newborn (PPHN), pneumonia or sepsis, respiratory distress syndrome (RDS), or suspected pulmonary hypoplasia associated with oligohydramnios and premature rupture of membranes. Echocardiographic evidence of pulmonary hypertension was not required. Center specific patient management guidelines were established to optimize conventional therapy prior to study entry. Administration of surfactant or use of high frequency ventilation was allowed if initiated prior to randomization.

The NINOS trial accumulated two study groups with similar characteristics at the time of randomization. Seventy-eight percent of infants had evidence of pulmonary hypertension on echocardiography. Over 70% of the infants received surfactant therapy, 50% within the 6 hours prior to randomization. The qualifying blood gases in both groups revealed OI's in the mid-40s, placing most infants near ECMO criteria at enrollment. The results of the trial revealed the control group experienced the primary outcome of ECMO/death 64% of the time vs. 46% for the iNO group; RR = 0.72; 95% CI 0.57 – 0.91. There was no difference in death between the groups, the improved primary outcome arose from a reduced need for ECMO in the patients treated with iNO; 39% vs. 55% (p = 0.014). With the initial administration of iNO, oxygenation improved by 58 torr vs. 10 torr in the control group (p<0.001).

When the NINOS trial was taking place, the maximum dose of iNO being studied in neonatal clinical trials was 80 ppm. NINOS patients randomized into the iNO group were initiated at 20 ppm, but for those who did not receive an adequate response, an option to increase to 80 ppm was allowed. The majority of patients responded to 20 ppm of iNO with only a few experiencing added benefit at 80 ppm. Eleven of the patients treated with iNO at 80 ppm required a reduction of their iNO concentration because of elevated methemoglobin levels. However, no infant required discontinuation because of toxic effects.

Simultaneously with the NINOS trial, the Neonatal Research Network conducted a separate parallel trial investigating iNO therapy in infants with congenital diaphragmatic hernia (CDH).[24] Using a similar study design and primary hypothesis as the NINOS trial, 53 patients with CDH were randomized to iNO or control. The baseline characteristics of the 2 study groups were similar at the time of enrollment. Seventy-six percent of the infants displayed evidence of pulmonary hypertension on echocardiography. Over 80% of the infants received surfactant therapy prior to enrollment. Similar to the NINOS cohort, the mean OI in the CDH trial at baseline was in the mid-40s. In this population, there was no

early improvement in oxygenation and no difference in the primary outcome between the iNO and control group (96% vs. 82%). However, more infants in the iNO group received ECMO: 80% vs. 54%, (p = 0.043). The only observed benefits of iNO therapy in this patient population were a short-term stabilization of infants (for transport or ECMO cannulation) and an improvement in oxygenation in several patients following ECMO therapy when iNO was added.

The second large RCT demonstrating the efficacy of iNO therapy was the "Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn study" conducted by the Clinical Inhaled Nitric Oxide Research Group investigators (CINRGI).[25] They studied a similar group of neonates with HRF requiring an OI   25 (or requiring a pH higher than 7.55 to maintain arterial oxygen of   60 torr) with the additional requirement of clinical or echocardiographic evidence of pulmonary hypertension. In this trial, infants were randomized by the diagnostic categories of meconium aspiration, pneumonia, RDS, lung hypoplasia syndromes including CDH, or PPHN; to receive iNO at 20 ppm or nitrogen (control). The study emphasized low-dose and a minimal duration of iNO exposure, attempting to reduce the iNO to 5 ppm every 4 hours if the infant was stable during the first day of treatment, then requiring a reduction at 24 hours of treatment, and discontinuation after 96 hours of therapy.

Two hundred forty-eight neonates were enrolled in the study. The baseline characteristics between the 2 groups were similar except for increased prenatal care before the third trimester in the iNO group and increased stable air-leak in the control group. Thirty-eight percent of infants received surfactant prior to enrollment. The primary outcome of the study (receiving ECMO) occurred in 64% of the control group and 38% of the iNO group (p = 0.001). The median duration of successful iNO treatment was 44 hours, with all but 2 patients weaned off by 96 hours. In addition to the reduced need for ECMO in the iNO treated infants, they also experienced less chronic lung disease (defined as oxygen requirement at 30 days) 7% vs. 20%, (p = 0.02). The stratification by respiratory diagnosis enabled the CINRGI study to show that iNO therapy reduced the need for ECMO in most diagnostic catagories except for CDH. Together, these 2 large multi-center RCTs of iNO therapy in the term and late-preterm infant validated the safety and efficacy of iNO therapy (Figure 2).

The Case for Early Intervention in Neonatal Hypoxic Respiratory Failure

The clinical course of HRF can be highly variable with some infants presenting in extremis shortly after birth while others progress in severity over time. Many infants presenting with respiratory failure are treated with gradual escalation of support while assessing their response to treatment. Conventional management of these infants includes administration of supplemental oxygen for hypoxia and positive pressure ventilation for hypercarbia. These therapies are often maximized prior to initiation of lung-specific therapies such as surfactant and pulmonary vasodilation with iNO. This is based on ready access to supplemental oxygen and positive pressure ventilation, and the perception of iNO initiation as a significant escalation of therapy. Recent studies suggest that both supplemental oxygen and mechanical ventilation can induce structural and biochemical injury to the lung.[26] Use of iNO therapy,

which allowed weaning of FiO_2 led to preservation of mitochondrial function in the lungs during ventilation for PPHN. There are potential benefits to earlier intervention with two specific therapies: surfactant use for alveolar recruitment and iNO for pulmonary vasodilation. These therapies are more effective when introduced before alveolar atelectasis is widespread. Alveolar distribution and response to iNO in HRF is more effective when lung recruitment is optimal.[27,28] Earlier use of therapies to optimize lung recruitment and oxygenation can avoid the adverse effects of oxygen toxicity and barotrauma. Although surfactant and iNO use are associated with increased cost, recent evidence suggests that benefits of minimizing lung injury and decreasing hospital stay may outweigh the cost of using these therapies.[29] These potential benefits are summarized below. Early use of inhaled nitric oxide The initial RCTs of iNO enrolled neonates that were in severe respiratory failure with OIs near 40 at the time of study gas administration.[23,25,30] Although the majority of infants who received iNO showed a consistent improvement in oxygenation with a decreased risk for ECMO/mortality, their oxygenation response rate was approximately 50%, and their need for ECMO or risk of mortality was close to 40%. The need to further improve outcomes led to the investigation of iNO therapy earlier in the course of HRF in 3 RCTs. The Franco-Belgian collaborative trial investigated the effects of early iNO or control ventilation without iNO on outcomes in neonates with HRF.[31] The study stratified infants by gestational age, based on preterm (<33 weeks) or near term (33 weeks) gestational age at birth. The term and late preterm cohort in this study consisted of 107 neonates who were eligible if their OI was between 15 and 40 on 2 consecutive measurements at least 1 hour apart. The median OI at the time of randomization was 25.9 for the iNO group and 21.7 for the control group, both lower than the initial large RCTs of iNO therapy. Control group infants received iNO therapy if their OI increased to >40. Overall, 51% of iNO treated infants compared to 33% of control group of infants had at least a 33% decrease in OI by 2 hours, the primary study endpoint. Infants assigned to early iNO had a trend for fewer days on the ventilator (6±3 for iNO and 7±3 for control, p=0.05) and fewer days in the NICU (9±6 vs. 12±9, p=0.02), although the total length of hospitalization was not different between the 2 groups. There were several methodological limitations with the study, including an early time point to assess the primary outcome, a higher OI at enrollment in the iNO compared to the control group, lack of masking, and lack of information on the availability or use of ECMO.

The second trial of early iNO was reported from Chile by Gonzalez in a setting where ECMO therapy was not available.[32] This study enrolled 56 newborns with moderate HRF defined as an OI of 10–30 on 2 consecutive post-ductal blood gas measurements at 48 hours of postnatal age. Infants were randomly assigned to early iNO or conventional mechanical ventilation with oxygen after a screening echocardiogram ruled out congenital heart disease and noted pulmonary hypertension. Infants in the control group who reached an OI >40 were treated with iNO. The severity of respiratory failure at baseline was similar in both groups. In the early iNO group the OI decreased from 22 at baseline to 19 at 4 hours ($p < 0.05$) and remained lower at all subsequent time points. The control group infants experienced an increase in OI that remained significantly higher than the early iNO group ($p < 0.01$) during the first 48 hours of the study. Fewer infants receiving early iNO developed an OI >40 (25% vs. 61%, $P<0.05$), the primary endpoint of the study. There were no

differences in mortality, days of mechanical ventilation, or rates of chronic lung disease between the 2 groups. In addition to the improved oxygenation, newborns receiving early iNO had a reduction in duration of oxygen exposure vs. controls (p < 0.03). These results suggest that early use of iNO therapy can decrease the progression of HRF and potentially decrease lung injury. However, the impact of early iNO on the outcome of ECMO could not be assessed due to unavailability of this therapy.

The Neonatal Research Network also conducted a study using early iNO. This investigation arose from an observation from the NINOS post-hoc subgroup analysis showing infants enrolled in the lowest OI subgroup (25–29.9) experienced the greatest reduction of ECMO/ mortality (61% control vs. 28% iNO), a relative reduction of 46%, suggesting that intervention at a lower acuity of illness may further reduce the use of ECMO/mortality in term/late preterm infants with HRF.[23] This hypothesis generating observation led to the development of a second multi-center RCT of iNO conducted by the NINOS centers. The "Early iNO study" enrolled 299 infants of a projected target sample of 400 infants, over a 3 year period.[33] Trial recruitment was stopped early due to slowing enrollment over the final 12-month period. Infants were eligible for the study if they were delivered at 34 weeks gestation and were <14 days postnatal age with an OI 15 and <25 on two blood gases done in a 12 hour period. Surfactant therapy was encouraged prior to enrollment in the trial. All infants in the trial received standard iNO therapy if their OI reached 25. Results of the study showed that 70% of infants in the early iNO group experienced an improvement in oxygenation, a greater portion of infants than observed in the NINOS trial. Also, infants in the early iNO group were less likely to progress to an OI>40 compared to the control group. The primary outcome of ECMO/mortality was not different between the early iNO (17%) and control (20%) group; however, the mean OI at the time of enrollment (19.7 early iNO and 19.2 control) was very close to the qualifying OI for the administration of standard iNO in the control group contributing to limited separation between the 2 study groups.

A reanalysis of the study data from the Early iNO study stratified infants in each study group by an OI cut point of 20, based on receiver operated characteristic curve analysis.[28] Univariate and multivariate logistic regression analysis revealed that infants treated with iNO at an OI of 15<20 were less likely to receive ECMO or die than infants who received iNO at an OI of 20 25 (OR 0.26; 95% CI 0.08–0.67, p=0.02 for early treatment). Additionally, this post-hoc analysis demonstrated that neonates in the original cohort treated with early iNO at an OI of 15 25 were less likely to progress to an OI>30 and were also less likely to meet the composite end point of progression to OI >30 and/or to receive ECMO (early iNO 25% vs. control 38%, p=0.02). Use of iNO at an OI of 15<20 was associated with a significant decrease in the length of hospital stay, compared to control group infants enrolled at the same OI who received standard iNO at an OI>25 (Figure 3). Thus 50% of early iNO infants were discharged home by 18 days compared to 27 days for the control infants enrolled at the same OI (Figure 3). This post-hoc analysis suggested favorable outcomes with early administration of iNO in moderate HRF compared to later administration at OI 25.

Figure 4 summarizes the short term outcomes of ECMO/mortality risk for infants enrolled into RCTs who received iNO treatment compared to their OI at enrollment.[34] It demonstrates the strong correlation of ECMO/mortality outcome with OI at enrollment.

Infants in the initial RCTs who received iNO therapy at a mean OI of >40 had an ECMO/
death rate >40%, while the recent post-hoc analysis of the Early iNO trial showed that
infants who receive iNO at an OI of 15<20 had an ECMO/death rate of 10.2%.

Earlier use of iNO therapy can potentially increase the cost of providing care to these infants
since more infants with HRF will be exposed to iNO therapy. To address this question,
pharmaco-economic modeling was done to weigh the relative costs of earlier use of iNO for
HRF compared to the overall cost of care for these infants.[29] Decision tree analysis was used
to compare the costs of providing iNO to all infants with HRF at an OI of 15 to <20
compared to the potential cost savings achieved by earlier hospital discharge for these
infants. The model utilized a hypothetical case population of 1000 patients, based on the
Early iNO study data, and a probabilistic sensitivity analysis was completed where clinical
inputs were varied. The deterministic sensitivity analysis included both hospital cost inputs
and the cost of iNO. A Monte Carlo simulation was completed with 1000 simulations. Both
deterministic analyses showed that early iNO use was cost saving with a per-infant lowering
of costs by $2,000–$14,000. This analysis shows that despite the significant cost of iNO
therapy, earlier use in neonatal HRF can provide medical benefits without increasing the
overall cost of caring for these infants.

Early surfactant for the treatment of HRF in term and late preterm neonates The therapeutic
benefit of surfactant therapy for premature infants with RDS is well established. However,
there are limited investigations into the potential benefits of surfactant therapy for term and
late preterm infants with HRF on oxygenation, need for ECMO, and mortality.

Among the first studies, Findley and colleagues conducted a pilot RCT investigating
improvement in oxygenation in infants with meconium aspiration syndrome (MAS).[35] Forty
infants were enrolled and the infants in the surfactant (beractant) group were eligible to
receive up to 4 doses every 6 hours. The mean arterial-to-alveolar PO_2 ratio values increased
modestly after the first dose of surfactant and oxygenation improved significantly and
cumulatively after the second and third doses of surfactant. In addition, the secondary
outcomes of need for ECMO and duration of oxygen therapy and hospitalization were
improved with surfactant therapy (p<0.05 for each).

Additional evidence for the early use of surfactant in HRF for parenchymal lung diseases
came from 2 subsequent larger multi-center trials in HRF. The first study by Lotze
randomized term infants with gestational age   36 weeks with HRF to surfactant (beractant,
n=167) or placebo (n=161) prior to ECMO treatment.[36] Neonates were stratified by
diagnosis (PPHN, sepsis, or MAS) and by OI at the time of enrollment (15 – 22, 23 – 30, 31
– 39) as a surrogate for the severity of lung disease. The surfactant treated group experienced
approximately a 10% absolute reduction in ECMO rate compared to the control group
(p=0.038). The benefit was observed primarily in infants with MAS and sepsis, not for
infants with PPHN; an observation consistent with the presence of parenchymal lung disease
as the cause of HRF. In addition, infants enrolled at an OI 15 to 22 (less severe or early
disease) experienced a three-fold reduction in the ECMO rate with surfactant vs. controls
(p=0.013). In contrast, neonates enrolled late in the course of HRF (OI 31 to 39) experienced

no benefit, and those with a moderate degree of HRF (OI 23 to 30) had a modest reduction in ECMO rate.

A second multi-center trial demonstrating a beneficial effect from early surfactant use is the post-hoc analysis of the Early iNO study.[28] This study enrolled 299 infants with HRF secondary to a variety of lung diseases including primary PPHN and parenchymal lung disease secondary to MAS, RDS, and pneumonia/sepsis. Overall 64% of the study cohort received surfactant therapy for the management of HRF prior to reaching the target OI of 15–25 to be eligible for the study. The severity of HRF in this cohort is therefore similar to the low OI group in the Lotze trial. Although surfactant use was not randomized in this trial, univariate analysis of the data revealed that infants who received surfactant prior to OI of 15–25 had a significant decrease in the incidence of ECMO/mortality (surfactant treatment 13.5%, no treatment 26%, p = 0.008). Surfactant therapy did not affect the ECMO/mortality risk for infants with primary pulmonary hypertension. However, for infants with parenchymal lung disease (MAS, RDS, or pneumonia), the use of surfactant was associated with a highly significant reduction in ECMO/mortality (9.3% surfactant treatment, 30% no treatment, p<0.001). In addition, surfactant treated infants had shorter duration of ventilator therapy and decreased length of hospitalization. The relative reduction in ECMO/death rate for the infants in these 2 trials is very similar among the infants enrolled with an OI <25 showing consistency of therapeutic response. Together, the results of these 2 trials reinforce the concept that the early use of surfactant for infants with HRF secondary to parenchymal lung disease is beneficial and improves oxygenation and discharge outcomes.

Neurodevelopmental Follow-up of Term and Near-Term Infants with Hypoxic Respiratory Failure from NINOS and the Early iNO Trial

Previously conducted follow-up studies revealed the vulnerability of survivors of neonatal HRF to neurodevelopmental impairments and hearing loss. These studies reported associations between the use of hyperventilation, systemic alkalosis, duration of ventilator support, and certain pharmacologic agents with an increased risk of hearing loss.[37,38] The neurodevelopmental follow-up studies of the Neonatal Research Network's iNO RCTs used the Bayley Scales of Infant Development to assess the mental development index (MDI) and the psychomotor development index (PDI) along with a structured neurologic exam to detect the presence and severity of cerebral palsy. The follow-up assessments included hearing loss, characterized as unilateral or bilateral, conductive or sensorineural and an assessment for uncorrectable vision (20/200 in the better eye) or blindness. Neuro-developmental impairment was defined as the presence of one of the following factors: MDI or PDI score <70, presence of moderate or severe CP, or bilateral hearing or vision loss.

Infants seen in follow-up from the NINOS study (n = 172) were part of a cohort with a mean entry OI >40 with 37% of infants requiring ECMO, while infants in the Early iNO study (n = 234) enrolled with a mean OI ~19 with 11% of infants requiring ECMO.[39,40] For the infants that were seen in follow-up, the incidence of those who presented with one or more neurodevelopmental disabilities (either cerebral palsy, MDI < 70, PDI < 70, blind or hearing impaired) was 32% for NINOS compared to 26% for the Early iNO study. For both RCTs

there were similar degrees of neurodevelopmental disability between the iNO and control groups and the rate of disability was not affected by the requirement for ECMO. Although the Early iNO study observed a significantly lower PDI score for the early iNO exposed infants, the variability for the PDI scores was large, resulting in a large standard deviation. Fewer infants in the iNO group of the NINOS trial reported having seizures (4.7% vs. 14.9%, p=0.046), however, the incidence of seizures among infants in the Early iNO study for each treatment group was 3%. Survivors of the CDH trial experienced a higher rate of sensorineural hearing loss, 59% of controls and 43% in the iNO group.[39]

Use of iNO in the Preterm Infant

Large RCTs and meta-analyses Interest in the use of iNO for the premature infant with respiratory distress increased following the positive results seen in RCTs in term and near term infants with HRF associated with PPHN.[23,25] The effectiveness of iNO in term and near term infants is primarily due to its role as a selective pulmonary vasodilator; however, basic and animal model investigations have demonstrated that deficient endogenous NO disrupts pulmonary parenchymal and vascular development and that exogenous NO may benefit the developing lung by its effects on vascular remodeling, inflammation and pulmonary edema, lung mechanics, lung growth, angiogenesis, and airway smooth muscle.[41–44] Unfortunately, randomized clinical trials assessing the efficacy of iNO for prevention of bronchopulmonary dysplasia (BPD) in preterm infants have yielded mixed results and a specific preterm population that clearly benefits from the use of nitric oxide has not been identified.[45–53] The trials in preterm infants have been broadly categorized based on the entry criteria; entry in the first three days based on high oxygenation index (rescue), routine use in intubated preterm babies (prophylactic), and later enrollment for increased risk of BPD (BPD prevention).[54] This categorization is useful when comparing trial results, but other variables including gestational age, birth weight, chronologic age at initiation, dose, duration of use, and mode of ventilation are other potentially important parameters.

The first large randomized trial of iNO in preterm infants was performed by Schreiber et al.[45] It was a single center study with a high proportion of black infants and concluded that iNO at a dose of 10 ppm administered prophylactically to preterm infants less than 34 weeks and less than 2 kilograms resulted in a lower rate of death or BPD when compared to placebo (49% versus 64%, p=0.03). In addition, they noted a lower rate of severe intracranial hemorrhage (ICH) or periventricular leukomalacia (PVL) in the iNO group (12% versus 24%, p=0.04). Surprisingly, subgroup analysis found that the benefit of iNO was seen only in less ill infants with an OI below 6.9. Infants were also randomized to either conventional mechanical ventilation or high frequency oscillation. Subgroup analysis favored iNO use on conventional mechanical ventilation; however, the interaction was not significant (p=0.11).

The next trial, published by the NICHD Neonatal Research Network focused on iNO use as a rescue for babies with severe respiratory failure and high OI after surfactant treatment.[47] This multi-center trial performed in 16 academic centers found no difference in the rate of death or BPD (80% with iNO versus 82% in control, p=0.52). The trial was stopped early after a planned interim analysis with 420 of planned 440 infants enrolled due to a higher incidence of severe ICH or PVL in the iNO group. When all the trial data were available for

analysis the difference in severe ICH or PVL was no longer statistically significant (39% versus 32%, p=0.11). Subgroup analysis found a significant interaction of treatment and birth weight on outcome (p=0.02). Infants 1000 g (n=316) had increased rates of BPD and death when treated with iNO, as well as increased rates of severe ICH or PVL while infants >1000 g (n=104) had a statistically significant reduction in BPD or death when treated with iNO (50% versus 69%, RR 0.72, 95% CI 0.54–0.96, p=0.03). The results were in contrast with the benefit seen in the Schreiber trial, but were likely related to the differences in the patient populations. The NICHD trial was a larger, multicenter trial enrolling less mature, lower birth weight infants with significantly higher mean OIs (see Table 1). The proportion of black infants in the NICDH trial was lower. The higher rate of ICH in the infants <1000 g is worrisome and plausible given the effect of iNO on bleeding time and platelet aggregation. Higher rates of ICH had also been seen in case studies of iNO use in preterm infants.[55,56]

A pilot study of iNO use in larger preterm infants with birth weight >1500 grams and gestational age <34 weeks was performed concurrently with the NICHD trial.[48] Pilot data collection done in NICHD Neonatal Network centers had established that there would be low numbers of infants in this weight range. Concurrent collection of randomized data for this weight group was seen as desirable despite the likelihood that there would be insufficient power. In the twenty-nine infants enrolled, iNO did not decrease the rate of death or BPD after adjustment for differences in surfactant use, mode of ventilation, and OI entry strata.

A secondary analysis of NICHD trial data using stepwise logistic regression models and Classification and Regression Tree (CART) models was undertaken to identify variables predicting death or BPD.[57] The major factors associated with death or BPD were lower birth weight, male gender, increased severity of illness as measured by OI, and outborn status. The magnitude of improvement in PaO_2 in response to iNO was not found to be associated with death or BPD, indicating that the initial response to iNO in premature infants with severe respiratory failure may not be a good indication of whether iNO should be continued.

A small multi-center randomized controlled trial was performed in the UK to determine if iNO use in preterm infants was both clinically effective and cost effective.[49] Infants were eligible if the clinician was uncertain about whether an infant might benefit from iNO. The primary outcome of the trial was death prior to discharge or disability at 1 year corrected age. Recruitment into the trial was hampered by open-label use and unwillingness to randomize critically ill infants and the trial was stopped short of the desired sample size of 200 with 145 enrolled. There was no evidence of an effect of iNO on the primary outcome (RR 0.99; 95% CI 0.76–1.29; p=0.94). Mean total costs per infant at 1 year corrected age were significantly higher in the iNO group partly due to the cost of iNO, but mainly due to a longer length of ventilation in the iNO group. The conclusion was that there was evidence of prolongation of intensive care with associated increased costs without any evidence of benefit and for these reasons iNO could not be recommended for preterm infants with severe respiratory failure.

Hascoet et al. reported on the use of iNO in preterm infants <32 weeks randomized to either 5 ppm iNO or placebo if they met criteria for hypoxic respiratory failure (FiO2 >40% and arterio-alveolar ratio <0.22).[50] This trial was performed at 10 centers in France and Belgium and infants developing refractory respiratory failure were given open-label iNO per French Drug Agency recommendations. The primary outcome was intact survival at 28 days of age defined as no respiratory support or oxygen use, no ICH greater than grade I, and no refractory hypoxemia. There were 145 infants who developed HRF. There was no improvement in the primary outcome; however, the risk of BPD was lower in the iNO group when compared to control group. No other differences in secondary or safety outcomes were seen.

One of the largest multi-center trials performed was the prophylactic use of low dose iNO in all intubated preterm infants <34 weeks gestation by Kinsella et al.[51] No difference in the rate of BPD or death was seen; however, infants receiving iNO had a lower rate of brain injury defined as either severe ICH, ventriculomegaly or PVL (p=0.03). Effects varied significantly by birth weight. Brain injury was significantly lower in the iNO-treated group with birth weights 750 to 999 grams (p=0.006), while BPD was lower in the iNO-treated group with birth weights 1000–1250 g (p=0.001). The potential mechanism by which iNO might provide neuroprotection is not known.

The first study within the "BPD prevention" category was the Nitric Oxide (to Prevent) Chronic Lung Disease (NO CLD) study. Ballard et al.[52] found that iNO therapy improved survival without BPD at 36 weeks' gestation (43.9% with iNO versus 36.8% in control, p=0.042). This trial had a vastly different strategy focusing on BPD prevention using a starting dose of 20 ppm, delaying the start of treatment until 7 to 21 days after birth, and continuing treatment for a minimum of 24 days. By treating only those infants requiring mechanical ventilation at least 7 days after birth, infants with developing lung disease who were at very high risk for BPD were targeted.[58] Post hoc analyses indicated that iNO had the greatest benefit in infants when starting treatment 7 to 14 days after birth, with no appreciable benefit for those infants starting later than postnatal day 14. Furthermore, the effect of iNO appeared to differ according to race (p=0.05 for the interaction).

The EUNO trial was a large, multi-center RCT performed in 36 NICUs in 9 countries in the European Union designed to test whether prophylactic, low dose iNO administered for a minimum of 7 days and up to 21 days improved survival without BPD in infants requiring either surfactant or CPAP with a FiO2 of at least 30%.[53] The target population for this trial differed from earlier trials as infants with severe lung disease with FiO2 at entry greater than 0.50 were specifically excluded. This design was chosen based on the post hoc analysis of the Schreiber trial, noting a reduction in death or BPD in the cohort with OI<6.94. The trial enrolled 800 newborns and found no significant differences in survival without BPD between iNO treated and controls (65% versus 66%, p=0.73). The authors concluded that low dose iNO started within 24 hours of birth and continued for a median of 3 weeks does not improve survival without BPD.

An industry-sponsored multi-center trial, the NEWNO trial, was designed and conducted with the goal to replicate or improve upon the results of the Ballard trial.[46] Study entry was

changed from 5 to 14 days instead of 7–21 days given the post-hoc analysis of the Ballard trial. Like the Ballard trial, the initial dose of iNO was 20 ppm for 72 to 96 hours and iNO was weaned to a dose of 5 ppm for a total of 24 days. The trial enrolled 451 preterm newborns and concluded that iNO did not improve survival without BPD compared with placebo (35% versus 32%, p=0.43). There were no significant differences in any of the secondary outcome measures or adverse events. The reasons for the inconsistency in the outcome results between the Ballard and NEWNO trials are unclear. The results of the trial have not yet been published or included in meta-analysis.

Several meta-analyses of iNO trials have been published, all with similar conclusions. The most recent Cochrane review by Finer and Barrington in 2010 was based on review of 14 RCTs, grouped them into 3 categories based on entry criteria used as previously described.[54] No important clinical benefit was seen in any of the subgroups (Figure 5). Rescue use for critically ill preterm infants was not effective and was associated with a non-significant 20% increase in severe ICH. Early routine use did not improve brain injury or improve survival without BPD. Later use of INO did not demonstrate a significant benefit in this analysis based on summary data, but the review did suggest that further study was needed. The Ballard trial enrolled a high proportion of multiple births and randomized multiples as clusters. The statistical technique, multiple outputation, used to deal with possible correlation of outcomes between siblings from multiple births, could not be reproduced using the data available and therefore the meta-analysis differs from the original publication.

To further determine if there were any subgroups that benefit from iNO therapy an individual-patient data (IPD) meta-analysis was performed.[59] This analysis involved the central collection of data from each patient enrolled in a trial. The advantage is that uniformity in defining patient characteristics and outcomes are achieved. The specific goal was to determine if the effects of iNO differed according to patient or intervention-related factors such as gestational age, birth weight, race, antenatal steroid use, age at randomization, severity of illness, ventilation mode, iNO dose and duration. The IPD analyzed data from 3298 infants enrolled in 12 of the 14 trials performed. The conclusion was that there was no statistically significant effect of iNO on death or BPD or severe neurologic events on neuroimaging. In addition, there were no differences in iNO effect according to patient level characteristics; however, in trials using a starting iNO dose 5 ppm there was evidence of benefit (interaction p=0.02). This latter finding was based on benefit seen in Ballard trial, which also had other trial design differences. The results of the IPD meta-analysis of international trial data did not support a recommendation for routine use of iNO for preterm infants with respiratory failure.

iNO use in infants with PPROM, oligohydramnios and pulmonary hypoplasia Neonates with a history of preterm premature rupture of membranes (PPROM) and subsequent oligohydramnios are at significant risk for lethal pulmonary hypoplasia. In an older study, neonates with PPROM prior to 25 weeks gestation with severe oligohydramnios for more than 14 days had a predicted mortality rate of greater than 90%.[60] The pulmonary hypoplasia is often accompanied by significant pulmonary hypertension and several case reports have suggested improved oxygenation with iNO.[61,62] Infants with PPROM, oligohydramnios and suspected pulmonary hypoplasia enrolled in the NICHD Preemie iNO

study were identified and the effect of iNO on this subset of infants was analyzed.[63] Twelve of 449 infants had suspected pulmonary hypoplasia associated with PPROM and oligohydramnios. Six were exposed to iNO and 6 were control. The iNO treated group had a mean increase in PaO_2 of 39 ± 50 mm Hg versus a mean decrease of 11 ± 15 mm Hg in the control group. Mortality was 33% versus 67%, BPD 40% versus 100%, and severe ICH or PVL 20% versus 50% in the iNO and control groups, respectively. None of these changes reached statistical significant due to the small sample size. However, review of the limited number of cases from this large multicenter trial suggests that iNO use in this specific patient population may decrease the rate of BPD and death without increasing severe ICH and PVL. Pulmonary hypertension seen in preterm newborns with PPROM, oligohydramnios, and pulmonary hypoplasia has a pathophysiology similar to that seen in term infants. At least two other recent case studies in this patient population report an improved survival with iNO treatment.[64,65] Unfortunately, a randomized controlled trial is not felt to be feasible due to the low frequency of this condition and the number of centers required to perform such a trial. The second reason, and maybe most important, is lack of equipoise.[66]

Long-term Medical, Respiratory, and Neurodevelopmental outcomes There is a significant body of literature describing the long-term medical and neurodevelopmental outcomes of newborns enrolled in the 8 RCTs described previously.[67–75] Only the French/Belgian trial and the recent NEWNO trial do not have publications reporting outcomes after hospital discharge. The only trial to report improved neurodevelopmental outcomes in the iNO-treated group was the single center trial.[67] Mestan et al. found abnormal neurodevelopmental outcome in 24% of iNO-treated infants at two years corrected age compared to 46% of control infants (p=0.01). This effect persisted after adjustment for birth weight, sex, chronic lung disease, and severe ICH or PVL. At 5.7 years of age iNO-treated infants continued to have better outcomes with fewer chronic morbidities or technology dependence (p=0.05) and less functional disability (p=0.05).[68] Only one study reported improved respiratory outcomes in iNO-treated survivors. In the NO-CLD trial infants treated with iNO received significantly less bronchodilators, inhaled steroids, systemic steroids, diuretics, and supplemental oxygen after discharge.[74] However, the iNO-treated cohort had no reduction in hospitalizations, and the rate of neurodevelopmental impairment at 2 years of age was not lower when compared to the placebo group.[73] The remainder of the publications describing medical, respiratory, and neurodevelopmental outcomes in iNO-treated survivors attribute neither adverse or beneficial impact.

NIH Consensus Development Conference and Committee on Fetus and Newborn With the goal of providing health care professionals, families, and the public with an assessment of the accumulated data on the benefits and risks of iNO in premature infants, the *Eunice Kennedy Shriver* NICHD, National Heart, Lung, and Blood Institute (NHLBI) and the Office of Medical Applications of Research (OMAR) of the National Institutes of Health convened a consensus-development conference held over a 2-day period in 2010. The independent panel authored a report after presentations from investigators, examination of a new systematic literature review, and questions and statements from conference attendees.[76] The panel stated that although there is biologic plausibility and the results in term infants were positive, combined evidence from the 14 randomized controlled trials in premature

infants 34 weeks gestation showed equivocal effects on pulmonary outcomes, survival, and neurodevelopmental outcomes. They concluded that the available evidence did not support early-routine, early-rescue, or later rescue regimens, however, they acknowledged that there were "rare clinical situations, including pulmonary hypertension or hypoplasia, that have been inadequately studied in which iNO may have benefit". They recommended that future research should attempt to understand the gap between basic research and clinical trials. In addition, they warned that subgroup and post hoc analyses showing potential benefits in subgroups serve to generate hypotheses for future research but are prone to false positive results.

Statements from the Canadian Pediatric Society Fetus and Newborn Committee and the American Academy of Pediatrics Committee on Fetus and Newborn were published in 2012 and 2014, respectively.[62,77] The Canadian statement mirrored the NIH Consensus report stating that iNO did not appear to be effective as a rescue or routine treatment; however, it may be beneficial for a small number of critically ill neonates such as those with respiratory failure associated with oligohydramnios.[62] Surprisingly, the AAP statement went farther and did not acknowledge any indications for iNO use in preterm infants.[77]

Usage of iNO in the recent era Despite less than encouraging recommendations from the Consensus statement and the Committee on Fetus and Newborn, iNO use for premature infants has become more prevalent in many practice settings. An analysis of off-label use of iNO in premature infants 23–29 weeks gestation utilizing the Pediatrix Medical Group Clinical Data Warehouse for the period 2009–2013 showed a relative increase of 23%, from 5.03% to 6.19% (p=.003).[78] iNO utilization rates were inversely proportional to gestational age; iNO was used in 13.9% of infants at 23–24 weeks compared to 0.6% of infants born at 33 weeks. The reason for use was not collected. The authors estimated that this use at Pediatrix hospitals cost payers $19.6 million dollars in 2013. To answer a related question regarding the use of iNO for prevention of BPD following the Consensus Development Conference, Truog et al. investigated iNO use when initiated at 7 days of age within 13 NRN centers comparing 2011 use to 2008–2010. Overall use decreased from 4.6% to 1.6% (p<0.001).[79] Twelve of 13 sites demonstrated a significant reduction, often eliminating iNO use in this population, and there was also an overall reduction in inter-site variability. This finding is in contrast to most other reports where use is on the rise. Using propensity analysis, the use of iNO at 7 days of age in infants <29 weeks was not associated with an improvement in death or severe BPD consistent with the findings of meta-analyses. Another analysis of iNO use was performed using the California Perinatal Quality Care Collaborative.[80] The authors examined iNO use in preterm infants 22 to <34 weeks gestation during the period 2005–2013. Overall, 2.6% were exposed to iNO; however, exposure was the highest in the lowest gestational age cohort and in regional centers compared to community NICUs. In the cohort at 22 to 24+6/7 week gestation cared for at regional centers, the median exposure rate was 10.63% with a hospital interquartile range of 3.8% to 22.6%. The reason for the prevalent use of off-label iNO despite any clear evidence of improved survival is unclear. Several authors have speculated that the increase in oxygen saturation following iNO exposure leads neonatologists to attribute their survival to this physiologic response.[66,78] Finer and Evans suggest that evidence-based guidelines for the use of iNO in preterm infants should be developed by units.[66] Guidelines should leave room

for individual judgment; however, they should also recognize the accumulated data which has shown the highest risk and lowest efficacy in premature infants <1000 grams. A recent commentary by Kinsella et al. focuses on the treatment of severe pulmonary hypertension in premature newborns.[81] They emphasize that the role of iNO in the acute management of severe HRF with associated pulmonary hypertension has not been fully addressed and also acknowledge that a RCT is not feasible for the reasons previously discussed. They propose a prospective registry of newborns with severe pulmonary hypertension who are treated with iNO, other vasodilators, as well as those not treated to further define the role of iNO in this subpopulation.

NICHD Neonatal Research Network Contributions

The NICHD Neonatal Research Network has made substantial contributions to the investigation of iNO therapy in both term and preterm newborns. The NINOS trial was the first RCT demonstrating that iNO therapy reduced the need for ECMO in term and near-term neonates with HRF without significant toxicity. Planned post hoc analyses provided additional insights into clinical iNO therapy demonstrating that regardless of evidence of pulmonary hypertension, iNO therapy improved oxygenation in neonates with severe hypoxic respiratory failure. Patients with the primary diagnosis of PPHN experienced the greatest reduction in primary outcome, however the beneficial effect of iNO was not limited to this subgroup as the entire cohort (all primary diagnoses, except CDH) experienced improved oxygenation and a reduction in the need for ECMO or death. The multiple dosing strategy suggested that a dose of 80 ppm provided little benefit over 20 ppm but increased the potential for toxicity. The observation that patients in the lowest severity of illness subgroup experienced a better oxygenation response and reduction in primary outcome with iNO therapy generated the hypothesis for the Early iNO study. The NINOS trial results including the neurodevelopmental follow-up component were essential for the FDA's approval of iNO therapy for clinical use.

The Early iNO study showed that more infants treated with iNO at a lower acuity of illness experienced improved oxygenation and were less likely to progress to severe HRF. The post hoc reanalysis suggested infants treated with iNO at an OI 15 20 were less likely to receive ECMO or die and had a decreased hospital stay. Early surfactant treatment was associated with a decreased incidence of ECMO/death in infants with parenchymal lung disease and a reduction in days of ventilation and hospitalization. The neurodevelopmental follow-up of both term/near-term infants enrolled in the iNO RCTs showed that survivors of neonatal HRF remain vulnerable to neurodevelopmental disabilities despite current rescue therapies.

Despite biologic plausibility and positive results in animal models, randomized clinical trials assessing the efficacy of iNO for prevention of BPD in preterm infants have yielded mixed results. Meta-analysis of trials and individual patient met-analysis have found no important clinical benefit on reducing the risk of BPD, neurologic injury or mortality for any of these categories of use. The Network Preemie iNO trial focused on the rescue use of iNO in critically ill preterm neonates and clearly showed the lack of efficacy in this population. The NIH Consensus Development Conference, as well as statements from the Fetus and Newborn Committees of the Canadian Pediatric Society and the American Academy of

Pediatrics have all emphasized the lack of benefit of iNO for preterm newborns. The Consensus Development Conference did acknowledge that it may be beneficial for critically ill newborns with PPROM, oligohydramnios and suspected pulmonary hypoplasia. Despite evidence for lack of benefit, many NICUs continue to use rescue iNO therapy in premature infants with hypoxic respiratory failure. Whether iNO therapy can benefit a select group of neonates with pulmonary hypoplasia and pulmonary hypertension requires further study.

Acknowledgments

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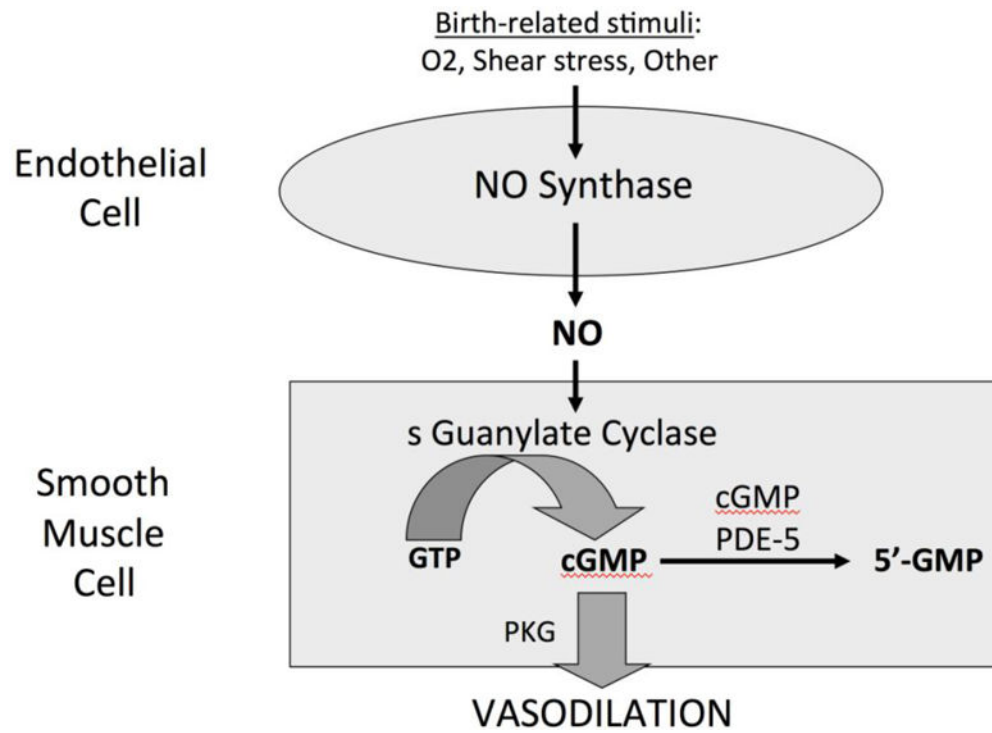
Figure 1.
The NO-cGMP pathway for initiation of pulmonary vasodilation. Nitric oxide (NO) synthase in the endothelial cell is activated by birth-related stimuli to produce NO, which then diffuses to the nearby smooth muscle cell and activates the enzyme soluble guanylate cyclase to produce cyclic GMP (cGMP). Activation of cGMP-dependent protein kinase (PKG) then initiates relaxation of vascular smooth muscle cell and vasodilation.

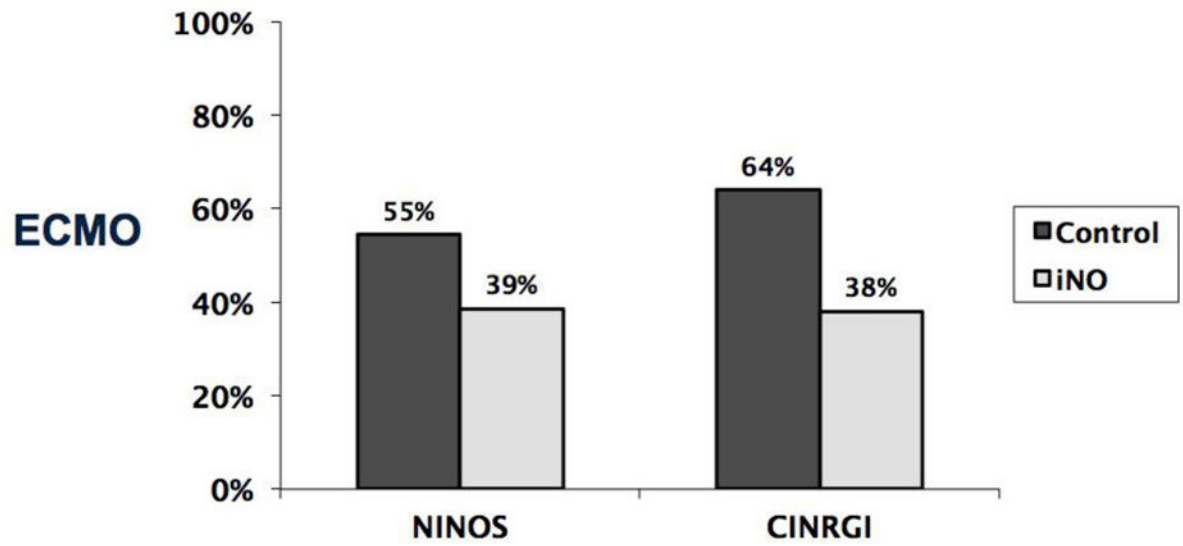**Figure 2.**
The reduced need for ECMO with inhaled nitric oxide (iNO) therapy as demonstrated in the neonatal inhaled nitric oxide study (NINOS) and the clinical inhaled nitric oxice research group investigators (CINRGI) multi-center RCTs.[23,25]
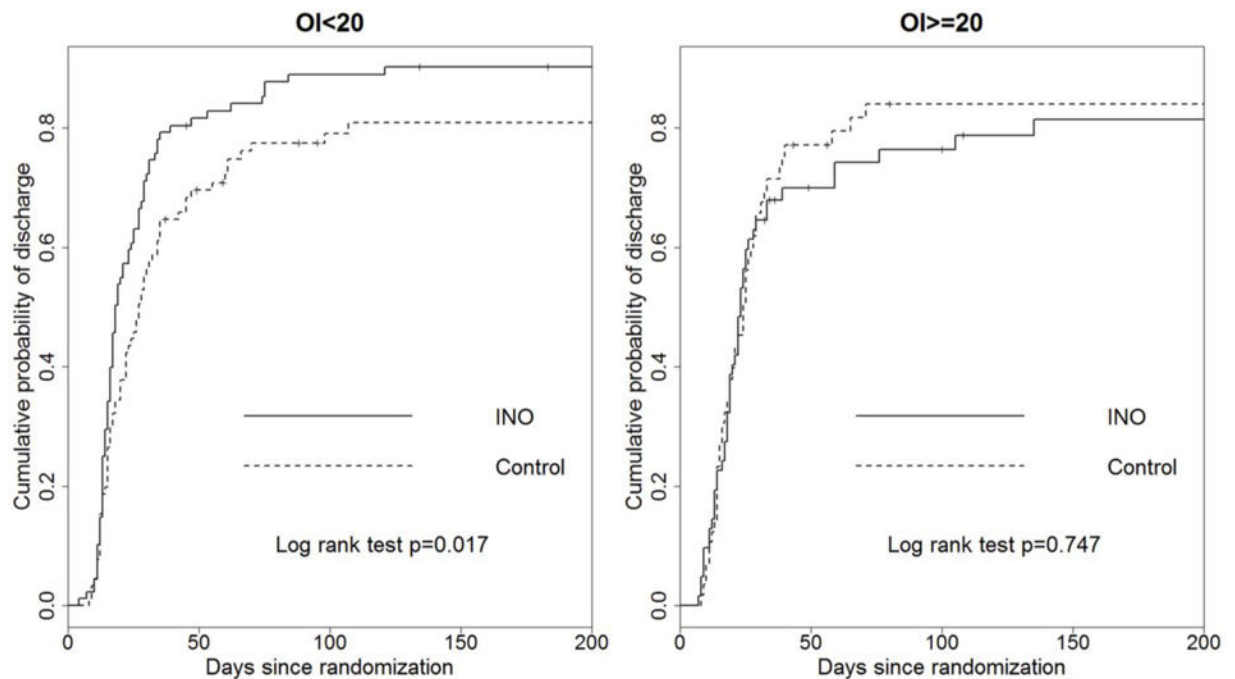
Figure 3.
The influence of early initiation of inhaled nitric oxide (iNO) compared to placebo at an oxygenation index (OI) of 15–20 on the time to discharge from NICU, plotted as a Kaplan Meir survival curve. Infants in the placebo group who progressed to an OI of 25 received standard iNO therapy. Early initiation of iNO was associated with earlier discharge home, with 50% of infants in the early iNO group going home by 18 days compared to 27 days for the placebo group (p=0.017 by log rank test). No difference was seen between the 2 groups when iNO was initiated at an OI 20. Reproduced with permission from Konduri, GG et al., J Perinatol, 2013; 33: 944–949.[28]
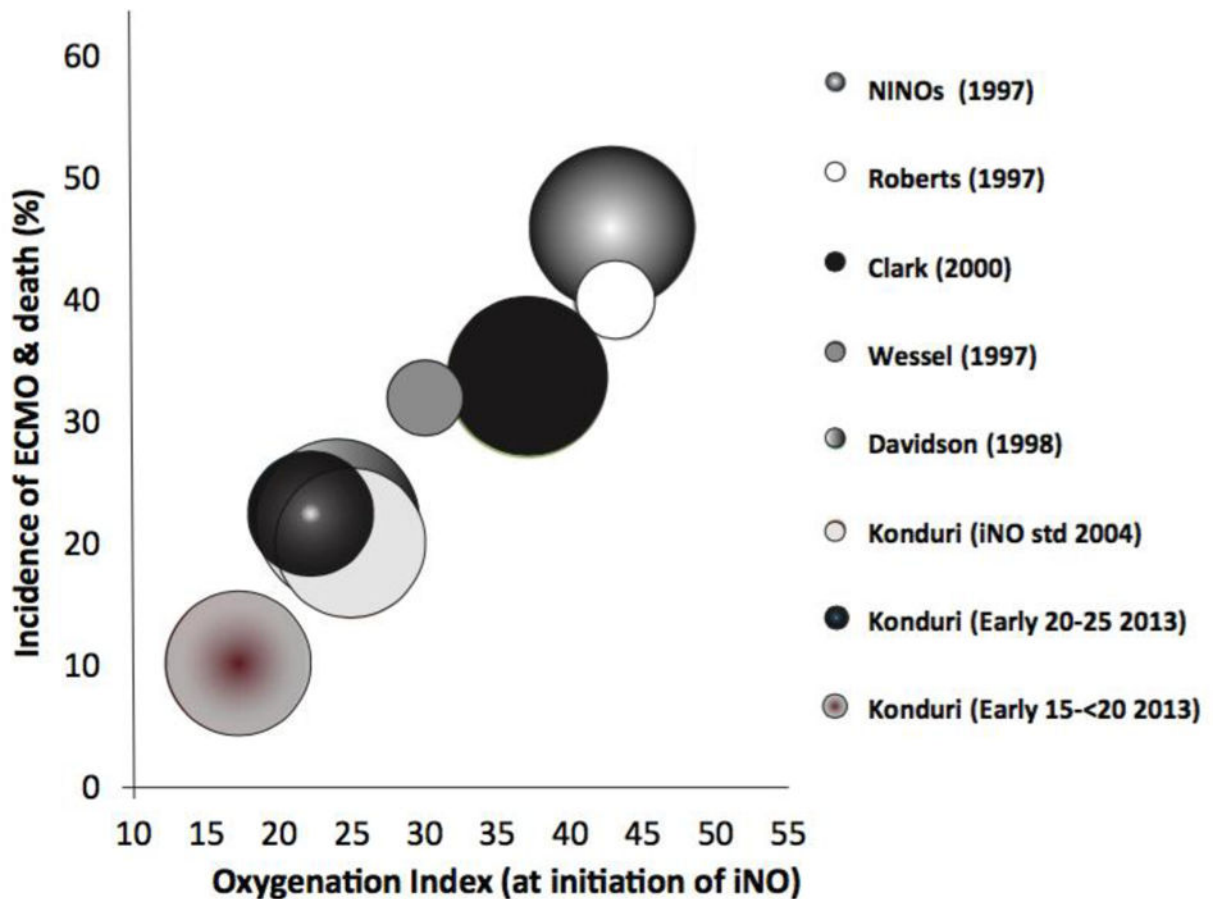
Figure 4.
Correlation of ECMO/mortality risk with oxygenation index (OI) at the initiation of iNO in different randomized clinical trials of inhaled nitric oxide (iNO) therapy. The ECMO/mortality rate reported as primary outcome for the infants randomized to iNO in different trials was shown as bubbles whose size was shown proportional to the sample size in the trial. The ECMO/mortality rate was close to 40% for trials that enrolled infants at an average OI of 40 or above, while the rate was 10.2% when iNO was initiated at an OI of 15–20 based on post-hoc analysis of data from the trial reported by Konduri GG, et al.[28] (Copyright for the figure: Drs. Satyan Lakshminrusimha and Girija G. Konduri. Modified from Lakshminrusimha S. The pulmonary circulation in neonatal respiratory failure. Clin Perinatol. 2012; 39(3):655–83.)[34]

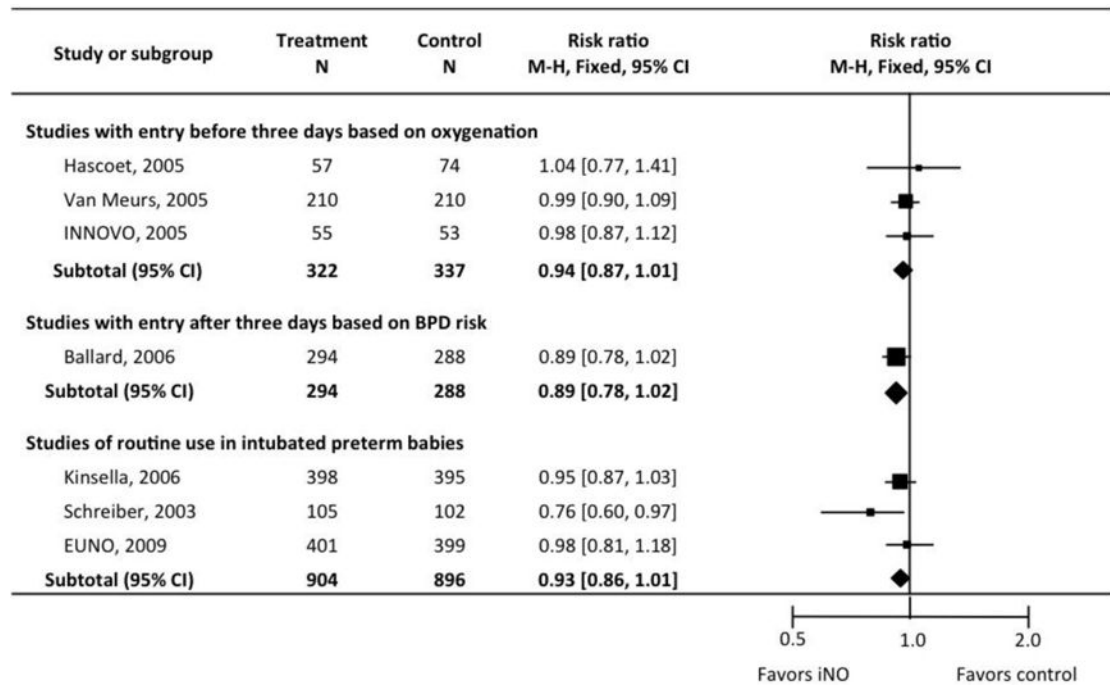| Study or subgroup	Treatment N	Control N	Risk ratio M-H, Fixed, 95% CI	Risk ratio M-H, Fixed, 95% CI
Studies with entry before three days based on oxygenation				
Hascoet, 2005	57	74	1.04 [0.77, 1.41]	
Van Meurs, 2005	210	210	0.99 [0.90, 1.09]	
INNOVO, 2005	55	53	0.98 [0.87, 1.12]	
Subtotal (95% CI)	**322**	**337**	**0.94 [0.87, 1.01]**	
Studies with entry after three days based on BPD risk				
Ballard, 2006	294	288	0.89 [0.78, 1.02]	
Subtotal (95% CI)	**294**	**288**	**0.89 [0.78, 1.02]**	
Studies of routine use in intubated preterm babies				
Kinsella, 2006	398	395	0.95 [0.87, 1.03]	
Schreiber, 2003	105	102	0.76 [0.60, 0.97]	
EUNO, 2009	401	399	0.98 [0.81, 1.18]	
Subtotal (95% CI)	**904**	**896**	**0.93 [0.86, 1.01]**	

0.5   1.0   2.0

Favors iNO   Favors control

Figure 5.
Meta-analysis of the effect of iNO on death or BPD at 36 weeks post-menstrual age (Adapted from Inhaled nitric oxide for respiratory failure in preterm infants. Cochrane Review Update, Neonatology 2012;102:251–3.)[54]

Table 1

Large randomized clinical trials of inhaled nitric oxide in premature infants

Trial author and group (date of publication)	N	Entry Criteria	Age at study entry	Mean BW (grams)	Mean GA (weeks)	Initial iNO dose (ppm)	Duration iNO use	Primary outcome and subgroup analysis
Schreiber (2003)[45]	207	< 34 weeks < 2 kg Need for ventilation	< 72 hours	975	27.2	10	7 days	Decreased death/BPD (p=0.03) Lower severe ICH/PVL (p=0.04) Benefit in subgroup with OI< 6.94 (p=0.02)
Van Meurs/NICHD (2005)[47]	420	< 34 weeks 401–1500 g Need for ventilation OI criteria ^	< 120 hours	838	26.0	5 or 10	Response dependent	No difference death/BPD (p=0.52) Lower BPD/death in >1000 g (p=0.03) Higher ICH/PVL 1000 g (p=0.03)
Field/INNOVO (2005)[49]	126	MD uncertain of iNO benefit	< 28 days	978	27.0	5 or 10	Response dependent	No difference death or disability at 1 year (p=0.94) Longer length of ventilation iNO group (p=0.06) Costs higher in NO group
Hascoet (2005)[50]	145	< 32 weeks FiO2>40% and aAO2 < 0.22+	< 48 hours	Not available	25.7	5	Response dependent	No difference intact survival (p=0.94)
Kinsella (2006)[51]	793	< 34 weeks Need for ventilation	< 48 hours	790	25.6	5	21 days *	No difference death/BPD (p=0.24) Less brain injury (0.03) Lower BPD in 1000–1250 g (p=0.004)
Ballard (2006)[52]	582	< 1250 g Need for vent or CPAP++	7–21 days	760	26.0	20#	24 days	Increased survival without BPD (P=0.042) Less supplemental oxygen (0.006) Discharged earlier (p=0.04)
Mercier/EUNO (2010)[53]	800	24 to < 29 weeks >500 g Need for vent or CPAP>	< 24 hours	857	26.5	5	7–21 days	No difference survival without BPD (p=0.73)
Yoder/NEWNO[46]	451	< 1250 g < 30 weeks Vent or CPAP#	5–14 days	737	25.6	20#	24 days	No difference death/BPD (p=0.43)

^ OI criteria were >10 on 2 gases 30 minutes to 12 hours apart (Strata 1) then revised to >5 followed by >7.5 30 minutes to 24 hours apart (Strata 2)

[+]Excluded for refractory hypoxemia defined as PaO2 < 50 on FiO2 1.0

[*]iNO used for 21 days or until extubation

[++]eligible if on CPAP and < 800 g

[#]iNO started at 20 ppm for 48–96 hours then weaned at weekly intervals

[>]Infants were excluded if on FiO2 greater than 0.5 to maintain saturation above 85%