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Inhaled Nitric Oxide and Neuroprotection in Preterm Infants

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Abstract

Infants born very prematurely (<32 weeks gestation) are well documented to be at risk for neurodevelopmental impairments, including blindness, deafness, cerebral palsy, and global cognitive delay, as well as more subtle cognitive deficits, such as language delay, learning disabilities, and attention and executive function abnormalities. Longitudinal studies of several large cohorts of extremely premature (<28 weeks gestation) infants including the Victorian Infant Collaborative Study Group (VICS) in Australia¹ (<28 weeks gestation), the EPICure study group in the United Kingdom^{2,3} (<26 weeks gestation), and the National Institute for Child Health and Development (NICHD) in the United States, have provided a rough consensus regarding the incidence of abnormal neurobehavioral outcomes at 18 to 30 months for these smallest infants born during the 1990s (for recent reviews, see Anderson and Doyle⁴ and Aylward⁵). Reported rates of cerebral palsy vary between 8% for infants less than 28 weeks² to as high as 21% for infants less than 25 weeks,⁶ whereas rates of severe cognitive delay (scores on the Bayley Scales of Infant Development⁷ [BSD-II, Bayley] < 70) are reported to vary between 18% and 30%.³ Blindness and deafness are relatively uncommon, with an incidence for each around 2%. The percentages of children of these gestational ages reported as having no neurodevelopmental impairment is disturbingly small: fewer than half the children in the VICS 1997 cohort and 21% for children less than 25 weeks gestation in the NICHD cohort.

Keywords

Chronic lung disease; Periventricular leukomalacia; Intraventricular hemorrhage; Neurodevelopment; Bayley

Risk Factors for Abnormal Neurodevelopmental Outcomes in Preterm Infants

The risk of abnormal neurodevelopmental outcome is well documented to increase at progressively earlier gestational ages; neurologic morbidity becomes a significant concern with infants with birth weights less than 1000 g. Those infants born at less than 27 weeks gestation are at high risk, and this risk progressively increases with each 1-week decrease in gestational age to 24 weeks. Biological risks factors contributing to an abnormal neurodevelopmental outcome can be partitioned into (a) those occurring before birth; (b) direct brain injury sustained at or after birth; (c) co-morbidities sustained during maturation to term; (d) ongoing challenges to brain development encountered following discharge from the intensive care nursery.

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Chronic placental insufficiency leading to ongoing hypoxia, acute placental insufficiency and asphyxia, as well as chorioamnionitis constitute important risks before birth.^{8,9} Intraventricular hemorrhage and periventricular leukomalacia are the two primary signs of acute brain injury in the premature infant contributing significantly to abnormal neurodevelopmental outcome. Infants who have suffered major complications of prematurity, including hypotension, sepsis, and necrotizing enterocolitis have a greater overall illness severity and are at greater risk of abnormal neurodevelopmental outcomes.¹⁰ The presence of chronic lung disease at discharge is an independent risk factor for abnormal neurodevelopmental outcome,^{11–13} as is exposure to prolonged postnatal courses of corticosteroids.^{14,15} Infants discharged home on oxygen or with apnea are at risk for continuing episodes of hypoxia and subsequent brain injury.¹⁶ Discharge to families challenged by low socioeconomic class, with the attendant lack of appropriate, supportive home environments, educational supports, and decreased access to health care constitutes socioeconomic risk for abnormal neurodevelopmental outcome.¹⁷

Despite increasing understanding of the risk factors leading to abnormal neurodevelopmental outcomes, and despite intensive investigation,^{18,19} until recently little progress had been made in the development of interventions during the initial hospitalization to improve neurodevelopmental outcomes. Recently, however, evidence has been accruing that inhaled nitric oxide (iNO) may provide an unanticipated therapy to improve neurodevelopment in preterm infants.

Inhaled Nitric Oxide in Preterm Infants: A Treatment to Improve Cardiopulmonary Outcomes

Inhaled NO is currently approved for use for the treatment of hypoxic respiratory failure and persistent pulmonary hypertension in the full- and near-term newborn. Inhaled NO promotes pulmonary vasodilatation, decreases pulmonary artery pressure, decreases right-to-left extra- and intra-cardiac shunting and intrapulmonary shunting, and improves arterial oxygenation.^{20–22} In preterm infants, trials of iNO therapy were undertaken initially to test the overall hypothesis that iNO, by improving ventilation–perfusion matching in the immature lung, would allow infants to be supported with lower levels of mechanical ventilation than would be necessary in the absence of iNO. This potentially decreased support was hypothesized to reduce the incidence of chronic lung disease and death. Thus, trials of iNO therapy in preterm infants were initiated to improve cardiopulmonary outcomes.

Initial Trials of Inhaled Nitric Oxide Therapy in Preterm Infants: Lack of Increased Central Nervous System Complications

Trials of iNO have been undertaken in the preterm infant amid theoretic concerns regarding its safety in this population, particularly with respect to bleeding, methemoglobinemia, and oxidative stress. Inhaled NO increases bleeding times in adults²³ and full-term infants,²⁴ and an early, uncontrolled study of iNO in premature infants showed a concerning incidence of intraventricular hemorrhage (IVH).²⁵ Methemoglobinemia has been reported in full-term infants treated with high concentrations of inhaled nitric oxide (80 ppm) but not in infants receiving less than 20 ppm,^{20,22,26} the maximum concentration used in the best-run studies discussed here.^{27–29} A recent study of infants weighing less than 1250 g who were randomly assigned to receive iNO (20 ppm, weaned to 2 ppm) or placebo for 24 days found no significant differences between control and treated infants for concentrations of 3-nitrotyrosines or protein carbonylation, markers of oxidative stress.³⁰ As will be reviewed below, studies have ranged in sophistication from retrospective, uncontrolled studies to prospective, double-blind, randomized controlled trials. We focus here on the impact of iNO therapy on markers of

neurologic injury during hospitalization and, where possible, the effect of iNO therapy on neurodevelopmental outcomes during early childhood.

An early study in the United Kingdom enrolled premature infants less than 32 weeks gestation into a four-armed open trial of iNO therapy and early dexamethasone.³¹ Mechanically ventilated infants treated with surfactant were eligible if they were calculated to have a high risk for the development of chronic lung disease, using a previously published metric validated by logistic regression.³² Infants were randomly assigned to receive either iNO (20 ppm weaned to 5 ppm, if possible, for 72 hours), dexamethasone (0.5-1 mg/kg/day × 6 days), both treatments, or neither. Mean birth weights across the groups varied from 750 g (iNO) to 870 g (dexamethasone). Neither iNO nor dexamethasone treatment decreased the primary outcome of chronic lung disease or death before hospital discharge. In this small study of 42 babies, only 60% of infants survived to discharge, suggesting that these infants were very ill. This conclusion is supported by the entry criterion of having a high risk for chronic lung disease. There are few data provided regarding acute neurologic outcomes in these patients; although rates of IVH did not differ between groups, the group sizes were small. Only grades I and II IVH were reported; periventricular leukomalacia (PVL) was not reported.

At 30 months corrected age, surviving infants (N = 25) underwent formal, blinded developmental assessments.³³ Children were classified as having mild, moderate, or severe neurodevelopmental delay based on mental or physical developmental indices (MDI or PDI) of the Bayley⁷ (mild: 71–85 [1–2 standard deviations below the mean], moderate: 50–75 [>2 standard deviations below the mean]; severe < 50). Children with “significant” abnormalities of tone or movement received a diagnosis of cerebral palsy. There was a nonsignificant trend toward less neurodevelopmental delay in the iNO group, and no children in the iNO group received a diagnosis of cerebral palsy compared with two of 14 children in the control group. The very small numbers of this study, the selection bias toward very ill children, and the open nature of the study conspire to render the results unhelpful in determining the safety of iNO in preterm infants, let alone any effects on neurodevelopmental outcome.

The first double-blind, randomized controlled trial of iNO therapy in preterm infants²⁶ enrolled 80 infants less than 34 weeks gestation from 12 centers in the United States. Infants had a mean birth weight of about 1000 g and a mean gestational age of about 27 weeks. The primary entry criterion was severe hypoxic respiratory failure unresponsive to standard therapies (alveolar to arterial ratio <0.1 on two sequential blood gas measurements). Infants were randomly selected to receive iNO (5 ppm) or placebo for 7 days. The primary outcome was survival to discharge. The trial was stopped after a planned interim analysis showed no significant difference in the primary outcome between the groups, and that with the enrollment rate at the time, detection of a difference was unlikely in a reasonable time frame. Infants in the two groups did not vary in the rates of grades II to IV IVH at study entry. Relevant to this review is the observation that, after detailed analyses, there were no differences in the rates of IVH, mild or severe, in survivors and nonsurvivors, between the iNO and placebo groups at study end. Using a worst-case scenario analysis, in which infants without cranial ultrasound or pathologic data regarding IVH were assigned grade IV IVH in the iNO group and no IVH in the control group, the authors determined that a clinical trial designed to detect a difference in grade IV IVH in this population of infants with this high level of illness severity would require enrollment of a minimum of 15,000 infants.

At the time this trial was designed, concerns regarding the safety of iNO in preterm infants had not been addressed. Consequently, patient selection for this randomized, controlled trial was necessarily restricted to extremely ill infants whose respiratory disease was refractory to conventional methods. The observation, therefore, that IVH rates were not significantly higher in iNO-treated infants compared with control infants, although far from definitive proof that

iNO did not increase the risk of IVH, nonetheless provided an indication that it might be safe in this population. A lack of increase in IVH after iNO treatment in preterm infants was also found in several other studies.^{34–36} Finally, a small (N = 34) randomized, placebo-controlled trial of iNO treatment³⁷ was performed to ascertain safety of iNO in preterm infants. In this study, infants with birth weights between 500 and 2000 g were enrolled within 72 hours of birth. Infants (mean birth weight approximately 900 g and mean gestational age 27 weeks) were randomly assigned to receive iNO (20 ppm, weaned to 5 ppm over 24–48 hours) or placebo. In this study, mean oxygen indices were about 12. No significant difference in the rates of severe IVH between iNO and placebo-treated groups was observed. Similarly, the incidences of other acute complications of prematurity, as well as early neonatal death, were comparable between the iNO and placebo-treated groups. This pilot study provided reassurance that iNO did not increase severe IVH in at-risk preterm infants and allowed further study of the efficacy of iNO in this population.

Inhaled Nitric Oxide Therapy Decreases Central Nervous System Complications of Prematurity: Randomized Trials

The first study to assess the role of iNO in less sick preterm infants was performed by Schreiber and colleagues.²⁹ In this single-center, double-blinded, randomized, controlled trial performed at the University of Chicago, 207 preterm infants less than 34 weeks gestation who were receiving mechanical ventilation for respiratory distress syndrome and who had received surfactant were, within 3 days of birth, randomly assigned to receive iNO (10 ppm on day 1, followed by 5 ppm) or placebo for the shorter of 7 days or until extubation. The primary hypothesis was that iNO would decrease the incidence of death and chronic lung disease among surviving infants. Mean birth weight of this cohort was approximately 1000 g, and mean gestational age was 27 weeks. Inhaled NO treatment conferred a significant 0.76 relative risk of death or chronic lung disease compared with treatment with placebo gas.

A key difference between this study and previously performed trials was the illness severity of the population. In contrast to previous studies, in which patient enrollment was restricted to infants with severe respiratory disease,^{26,32} the median initial oxygenation index (OI) of the enrolled population was about 7. In fact, iNO therapy decreased significantly the risk of the primary outcome (by 47%) for infants having an initial OI below the median but not for the remainder of the infants. While suggesting that iNO reduced chronic lung disease and death only in a less-ill subset of infants, this observation was derived from a post hoc analysis and should be interpreted cautiously.

Among the secondary outcomes evaluated for this study, the overall incidence of IVH and PVL did not differ between iNO- and placebo-treated infants. Unexpected, however, was a marked, significant reduction in severe (grades III or IV) IVH and PVL in iNO-treated infants compared with placebo-treated infants (risk reduction 47%).

Previously, only antenatal corticosteroids have been shown to reduce IVH in preterm infants.^{38–40} Indeed, phenobarbital,^{41,42} indomethacin,^{43,44} and vitamin E⁴⁵ have all, at one time, appeared to be promising therapies. Therefore, although this unanticipated finding from a post hoc analysis was intriguing, these data could hardly be taken as clear evidence of iNO being a neuroprotective agent, especially before information regarding long-term neurodevelopmental outcomes of this cohort of infants became available.

Similar reductions in central nervous system (CNS) complications by iNO therapy, however, have been recently reported by Kinsella and colleagues,²⁷ at the University of Colorado, in a large (N = 793), 16-center, randomized, double blind, placebo-controlled trial performed in the United States. Infants less than 34 weeks gestation were randomly assigned within 48 hours

of birth to receive iNO (5 ppm) or placebo for 21 days. Similar to the Chicago study, the primary outcome was the incidence of death or chronic lung disease in survivors. Mean birth weight of this population was about 800 g, and mean gestational age 25.6 weeks gestation, about 2 weeks more premature than those in the Chicago study. More than 70% of infants had mothers who received antenatal steroids (higher than the 50% in the Chicago study). There were no differences between iNO- and placebo-treated groups in baseline ventilator requirements. Overall, there was no difference between the two groups in the incidence of the primary outcome. However, prespecified subgroup analyses by birth weight strata showed that iNO-treated infants weighing 1000 to 1250 g had a significantly lower incidence of the primary outcome as well as a lower incidence of chronic lung disease alone, compared with placebo-treated infants. Most importantly, iNO-treated infants, across all weight strata, had a significantly decreased incidence of PVL, the primary ischemic lesion of the preterm brain. The combined outcome of severe IVH, ventriculomegaly, and PVL was also significantly and markedly reduced among infants weighing 750 to 999 g. Bolstering the findings from the Chicago study, these observations from the Colorado study strongly suggest that low-dose iNO therapy can safely improve short-term neurologic outcomes in preterm infants. The OI in this cohort of less than six, combined with a mean birth weight that was approximately 200 g smaller than that in the Chicago cohort, makes direct comparison of this population's risk for complications of prematurity with that of the Chicago study more difficult. Nonetheless, these two, well-designed studies, encompassing 1000 infants in total, have shown that low-dose iNO therapy, begun within the first 72 hours of birth, decreases the incidence of CNS complications, particularly severe IVH and PVL, by 40% to 50%.

Infants with severe IVH are at risk for abnormal neurodevelopmental outcome,^{46–48} as are infants with PVL.^{49–52} Similarly, the presence of chronic lung disease adversely affects long-term neurodevelopmental outcome.¹¹ Reductions in severe IVH and chronic lung disease in preterm infants by low-dose iNO therapy, therefore, would be predicted to result in improved neurodevelopmental outcomes. This prediction has been borne out in the only follow-up study to date of a double-blind, randomized, placebo-controlled trial of iNO treatment in which infants received iNO for more than several days.

Children enrolled in the Chicago trial (10 ppm day 1, 5 ppm days 2–7 vs. placebo)²⁹ were studied in a prospective, longitudinal follow-up study at 2 years corrected age.⁵³ The primary outcome variable—abnormal neuro developmental outcome—was defined as either disability (cerebral palsy, blindness, or hearing loss) or delay (a Bayley score more than two standard deviations below the mean, ie, below 70). Eighty-two percent of surviving infants enrolled in the initial study were captured for assessment at two years of age by means of close contact with their families after discharge from the intensive care nursery. Children were examined by a pediatrician and a neurologist, and infant development was assessed by a single, certified, neonatal occupational therapist. Importantly, all assessors (and families) were unaware of the children's assignments to iNO or placebo. In the follow-up cohort, the percentage of iNO- to placebo-treated children was similar to that in the initial report (53% versus 51%, respectively).

Inhaled NO-treated survivors had approximately half the risk of abnormal neurodevelopmental outcome than did placebo-treated survivors. After adjustment of the primary outcome individually for each of the factors potentially associated with abnormal neurodevelopmental outcomes, including birth weight, sex, socioeconomic status, type of ventilation (high frequency or conventional), and postnatal exposure to corticosteroids, the decreased risk of an abnormal neurodevelopmental outcome remained unchanged. Furthermore, simultaneous adjustment for the two most powerful epidemiologic confounders, ie, birth weight and sex, did not alter the magnitude of the risk reduction by iNO. These statistical analyses demonstrate that iNO treatment cut the incidence of abnormal neurodevelopmental outcome by about half, independent of the effects of confounding variables. Therefore, in this cohort, iNO treatment

provided powerful neuroprotection from the multifactorial causes of abnormal neurodevelopmental outcome. Comparative follow-up data from the Colorado study are not yet available.

What aspects of abnormal neurodevelopmental outcome did iNO treatment impact in the Chicago follow-up study? The incidence of disability (cerebral palsy, blindness, hearing loss) was not significantly different between iNO- and placebo-treated infants. Children with these conditions constituted less than 10% of the cohort. Although these numbers are certainly too low to detect a significant difference, no trend toward decrease on disability is apparent. It is clear, then, that iNO exerted its neuroprotective effect on behaviors measured by the Bayley scales: children in the iNO-treated group had half the incidence of having either MDI or PDI scores below 70 compared with placebo-treated children. Furthermore, this same magnitude of decrease was apparent in the incidence of isolated MDI less than 70, whereas the incidences of isolated PDI less than 70 were not different between the groups. Of note is the observation that there was no significant difference (or even a trend) in the incidence of both MDI and PDI below 70—the worst affected children—in the iNO-treated group. Thus, the primary effect of iNO treatment in this follow-up study was to decrease the number of children with mental delay.

If iNO treatment during the first week of postnatal life improves long-term neurodevelopmental outcomes in preterm infants, by what mechanisms does this neuroprotection occur? Further statistical analysis of the Chicago follow-up data sheds some light on the role played by key neonatal conditions in altering neurodevelopmental outcome. Because iNO treatment decreased the incidences of chronic lung disease and severe IVH/PVL, conditions known to adversely affect neurodevelopmental outcome,^{11,46–52} it is possible that improved neurodevelopmental outcome occurs because of an iNO-induced decrease in the incidence of these conditions. Indeed, as anticipated, the incidences of severe IVH/PVL and chronic lung disease were decreased significantly in the cohort of iNO-treated survivors compared with placebo-treated survivors. However, adjustment for the presence, individually or collectively, of severe IVH/PVL and chronic lung disease did not appreciably alter the relative risk of the incidence of abnormal neurodevelopmental outcome.

Without adjustment for any factors, the relative risk for abnormal neurodevelopmental outcome in the iNO-treated group was 0.53 (95% confidence interval: 0.33–0.87, $P = .01$). With simultaneous adjustment for chronic lung disease and severe IVH/PVL, the relative risk was 0.60 (0.38–0.96, $P = .03$). Thus, the substantial neuroprotective effect of iNO treatment in this follow-up study appears to be independent of known CNS complications of prematurity. If these findings are corroborated by other studies that follow graduates of low-dose NO trials, iNO will constitute the only postnatal intervention to date that improves neurodevelopmental outcome in preterm infants.

Two additional randomized, placebo-controlled studies of iNO treatment in preterm infants deserve examination. Ballard and colleagues,²⁸ at the Children's Hospital of Philadelphia performed a multicenter, placebo-controlled, double-blind trial of iNO versus placebo in infants weighing <1250 g. In this 21-center trial, 582 infants requiring ventilatory support were enrolled at 7 to 21 days of age and treated with iNO (beginning at 20 ppm) or placebo for a minimum of 24 days. The overall hypothesis was that prolonged therapy, ie, 24 days, might be needed to prevent increased airway resistance and muscularity, conditions associated with chronic lung disease, as well as to improve alveolarization and lung growth. The primary outcome variable was survival without chronic lung disease at 36 weeks postconceptional age. There was a significant increase in survival without chronic lung disease in the iNO-treated patients (relative benefit, 1.23 [95% confidence interval 1.01–1.51, $P = 0.04$]).

Enrollment was delayed until 7 days of age because of concerns of an interaction between iNO treatment and brain injury. Importantly, the investigators found no evidence of increased evolution of brain injury in iNO-treated infants, either during or after gas delivery. The incidences of PVL or severe IVH in the iNO- and placebo-treated groups were not reported. Information on long-term neurodevelopmental outcomes has not yet been reported.

The NICHD performed a moderately sized ($N = 420$), double-blind, placebo-controlled, randomized, multicenter trial of iNO (5–10 ppm) in preterm infants with severe respiratory failure.⁵⁴ The primary outcome was the incidence of death or chronic lung disease. Children weighing less than 1500 g who had an OI of 10 twice in succession were eligible. However, the infants in this study had, in fact, very severe respiratory disease: the mean OI in enrolled patients was 23 in the iNO-treated group and 22 in the placebo-treated group. In this study, only infants who responded to study gas with an immediate improvement in oxygenation continued to receive treatment. The median duration of therapy for the iNO-treated and responsive group was 76 hours.

In this trial, iNO treatment did not reduce the incidence of the primary outcome across the study population. Post hoc analysis showed a decrease in the primary outcome among children with birth weights more than 1000 g. More concerning, though, is the finding that infants in the iNO group having birth weights less than 1000 g had higher mortality rates and increased rates of severe IVH. The relative risk for severe IVH in the iNO-treated infants was 1.40 (95% confidence interval 1.03–1.88, $P = .03$). It is important to note that the rates of death or chronic lung disease in both groups (80% iNO and 82% placebo) were far higher than those reported in the Chicago study (51% and 65%) or the Philadelphia study (56% and 64%). Furthermore, the OIs in the NICHD trial were far higher than those of the cohorts in Chicago and Denver studies, and indicate how ill the infants were in this NICHD study. More importantly, because the protocol required that oxygenation improve in order for study gas to be continued, infants received study gas for variable times, some for as little as 30 minutes. Even infants who responded to study gas with an improvement in oxygenation were weaned from study gas after 10 to 14 hours, if tolerated. Thus, the duration of study gas therapy was highly variable and far shorter than any of the other reports. Although the increases in mortality and severe intraventricular hemorrhage rates in infants with birth weights less than 1000 g are concerning, it should be noted that these infants had extremely severe respiratory disease, quite a different population from those studied by the Chicago or Philadelphia group.

When the survivors of this trial were studied at 18 to 22 months corrected age,⁵⁵ investigators reported that iNO did not reduce the combined rate of death or neurodevelopmental impairment (defined as moderate to severe cerebral palsy, blindness, deafness, or a Bayley scale [MDI or PDI] less than 70). In infants weighing less than 1000 g at birth, the combined incidence of death or cerebral palsy was reported to be higher in the iNO-treated group. However, because the study investigators believed that it was crucial to encompass patient outcomes from the time of randomization rather than focus on patients who survived to discharge,⁵⁵ all deaths that occurred during the initial hospitalization were included in the follow-up data set. Evaluation only of children who survived the initial hospitalization, an analysis that can be directly compared with other follow-up studies, shows no significant increase in neurodevelopmental impairment or isolated delay either across the study population or in those children who weighed less than 1000 g at birth. Thus, although it is likely prudent to withhold iNO therapy from desperately ill infants weighing less than 1000 g, there remains no evidence that long-term neurodevelopmental outcome is worsened by iNO therapy in any weight group. In fact, in children who are not desperately ill, the evidence for a marked improvement in neurodevelopmental outcome is hopeful.

A relatively small (N = 108), nonblinded, randomized, placebo-controlled, 34-center trial performed in the United Kingdom and Europe⁵⁶ provides little additional data on whether iNO improves neurodevelopmental outcome in preterm infants. The mean gestational age was 27 weeks. Because an infant was enrolled only if the attending physician was unsure whether the infant would benefit from iNO, enrollment was highly skewed toward infants thought likely to die. (Presumably, infants believed by the physician to likely benefit from iNO were treated with open-label iNO and thus not enrolled.) Enrolled infants had extremely severe respiratory disease, with a median OI of 32. Half of the infants received iNO for less than 48 hours, and half received iNO for more than 3 days. At one-year corrected age, there was no difference in the incidence of death or severe neurologic disability (defined as minimal head control or inability to sit or developmental quotient less than 50) between iNO- and placebo-treated groups.

Recently, Tanaka and colleagues,⁵⁷ compared the incidence of cerebral palsy in 61 consecutive preterm infants with documented persistent pulmonary hypertension (PPHN) over two periods —1988 to 1993 (pre-iNO) and 1993 to 1999 (iNO era). After 1992, iNO was given to all infants with PPHN. Inhaled NO was started initially at 10 ppm and titrated to clinical (oxygenation) response to a maximum of 30 ppm. Median duration of iNO treatment was 19.8 hours (interquartile range: 29.5–56.0 hours). Mean gestational age was 25.5 weeks, with mean birth weight about 820 g. Follow-up examinations were performed at 3 years of age, at which time infants exhibiting abnormal muscle tone in more than one extremity as well as abnormal movement or posture were diagnosed with cerebral palsy. In the period after iNO treatment was initiated, the incidence of cerebral palsy was 12.5%, significantly lower than the 46.7% observed during the pre-iNO period. This retrospective study suffers from the drawbacks inherent in any historical retrospective study. First, it is impossible to differentiate between effects stemming from changes in neonatal care other than iNO during the two periods and iNO itself; the absence of a contemporaneous control group makes direct comparisons difficult. Second, there was a death rate of more than 50% within 3 years of birth. Third, neurologic assessments were performed by multiple nonneurologist pediatricians without prespecification of criteria for cerebral palsy. Finally, the study is quite small, so that generalization of results to other populations is difficult.

Mechanisms of Neuroprotection from Inhaled Nitric Oxide

What are the mechanisms underlying abnormal neurodevelopmental outcomes in preterm infants, and how might iNO alter these mechanisms? White matter injury is the hallmark of preterm brain injury. Evidence is accumulating in rodent models that a primary mechanism of white matter injury involves maturation-dependent vulnerability to ischemic injury of progenitors of oligodendrocytes.^{58,59} Such white matter injury can lead to PVL and cerebral palsy. Rodent models have also implicated ischemic injury to subplate neurons that occurs during development, such that pervasive abnormalities of cortical development occur, leading to cognitive and sensory deficits.⁶⁰

Nitric oxide is a ubiquitous, endogenous, intercellular signaling molecule with autocrine and paracrine activities (reviewed in⁶¹). Nitric oxide regulates vascular tone in the pulmonary and systemic circulation and mediates neurotransmission and neurosecretion.⁶¹ Nitric oxide is produced by nitric oxide synthase (NOS); in the presence of oxygen, NOS catalyzes the conversion of L-arginine to L-citrulline plus NO. Three primary isoforms of NOS have been identified: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Neuronal and eNOS are constitutively expressed and depend on Ca²⁺-calmodulin for activation. In the central nervous system, nNOS is localized to neurons in multiple brain regions, as well as in astrocytes and cerebral blood vessels.⁶² Endothelial NOS is expressed in cerebral endothelial cells. Nitric oxide interacts with soluble guanylyl cyclase to increase levels

of cyclic guanosine monophosphate and has also been reported to interact with heme oxygenase 1⁶³ and cyclooxygenase.⁶⁴

Inhaled NO may decrease lung-derived inflammatory mediators leading to brain injury.^{65–67} However, if iNO provides neuroprotection at sites distal to the lung, it is unlikely that neuroprotection occurs through direct activity on neurons or pre-oligodendrocytes. There is considerable uncertainty regarding whether NO, even at pharmacologic doses, can be transported to distal vascular beds, let alone across multiple diffusion barriers to leave the capillary.

Although clinical evidence suggests that exogenous iNO provides neuroprotection in preterm infants, many studies using models of hypoxic–ischemic injury indicate that endogenous cytosolic NO produced in neurons results in neuronal death.^{68–72} A key mechanism underlying hypoxic–ischemic neuronal death is the induction of an increase in cytosolic calcium concentration^{73,74} mediated by overactivation of *N*-methyl-D-aspartate receptors.^{75–77} This calcium increase activates calmodulin, which activates NOS.^{78,79} The resultant NO increase, combined with an increase in reactive oxygen species production, leads to formation of peroxynitrite and subsequent neuronal injury and death.^{69,80,81} Accordingly, if iNO were to reach immature subplate neurons and oligodendrocytes, the likely effect would be an increase in reactive nitrogen stress and peroxidative injury.

Clinical use of NO as a pulmonary vasodilator has been predicated on the fact that the action of NO on vascular smooth muscle is restricted to the pulmonary vasculature. This local restriction occurs because inhaled NO, once having crossed into the pulmonary circulation, reacts with molecules in plasma and erythrocytic hemoglobin.⁸² As reviewed by Schechter and Gladwin,⁸² NO is destroyed rapidly by its reaction with iron-containing heme groups of oxyhemoglobin. The reaction of NO with hemoglobin occurs at diffusion-limited rates, resulting in methemoglobin and nitrate (from reaction with oxyhemoglobin) and iron-nitrosyl hemoglobin (from reaction with deoxyhemoglobin).⁸³ Accordingly, exogenous iNO decreases pulmonary vascular resistance without altering systemic blood pressure and, presumably, systemic vascular tone.

Importantly, some reactions of NO with hemoglobin do not destroy NO. The presence of iron-nitrosyl-hemoglobin (produced from NO-deoxyhemoglobin interaction) and S-nitrosyl-hemoglobin (produced by reaction of NO with the cysteine at position 93 of the hemoglobin beta chain), while at low concentrations, raises the possibility that hemoglobin in red cells may act as a carrier of gas in circulation.⁸² Indeed, one hypothesis states that S-nitrosohemoglobin, produced in tissues at high O₂ tensions, decomposes at low O₂ tensions, releasing NO and increasing blood flow to relatively hypoxic tissues.⁸⁴ Competing studies argue that S-nitrosohemoglobin is at much lower concentrations in plasma than previously believed, and quite unstable.^{85,86} Other molecules that may conserve NO activity in the plasma for delivery to distant vascular beds include nitrite ion (from reaction with oxygen) and iron-nitrosyl hemoglobin.⁸³ Nitrite ions can be converted to NO enzymatically, via xanthine oxidoreductase and heme proteins.⁸³ Although many of these pathways are likely to have negligible effects at physiologic levels of endogenous NO, they may serve as NO reservoirs under such pharmacologic NO levels as exist during iNO delivery.⁸³

If iNO treatment does result in increased delivery of NO to the brain, how might this NO mediate neuroprotection during postnatal life in preterm infants? Animal and in vitro studies using isoform-specific NOS inhibitors, as well as mice in which single NOS-isoforms have been genetically knocked out, have found a differential role for NO, depending on which isoforms produce it and the tissue to which the isoforms are localized.^{87,88} Thus, global inhibition of all NOS isoforms in an animal model of brain hypoxia-ischemia results in

decreased infarct size, whereas eNOS blockade increases infarct size, indicating that eNOS is protective. Neuronal NOS-specific blockade also decreases infarct size, indicating that nNOS over-activity is neurotoxic.⁸⁷ Similar results are seen using eNOS- and nNOS-knockout mice.⁸⁸ Providing additional NO with iNO to cerebrovascular beds supplying vulnerable pre-oligodendrocytes and neurons, therefore, could result in improved blood flow during hypoxic episodes and neuroprotection.

A closely related hypothesis to explain a potential role for increased NO delivery to the cerebrovascular bed focuses on the inhibition of eNOS activity during hypoxia. Hypoxia-induced inhibition of eNOS is mediated by decreases in eNOS mRNA half-life and occurs within several hours after hypoxia.⁸⁹ Implicated in this mRNA down-regulation is Rho-kinase, a serine-threonine kinase. Rho-kinase also decreases eNOS activity via posttranslational mechanisms within minutes of activation.⁹⁰ Rho-kinase is activated in animal models of cerebral ischemia.⁹¹ Thus, under conditions of hypoxia, eNOS can be down-regulated, leading to dysregulation of cerebral blood flow and worsening injury. Delivery of NO via iNO treatment to cerebral vasculature could, therefore, overcome down-regulated eNOS and improve cerebral blood flow and reducing ischemic injury.

Summary

Preterm infants are at high risk for neurodevelopmental impairment, including blindness, deafness, cerebral palsy, and global cognitive delay. Despite an increasing understanding of the risk factors leading to abnormal neurodevelopmental outcomes, little progress has been made in treatment. Recent data suggest that for preterm infants with moderately severe respiratory distress syndrome, iNO decreases the incidence of severe acute brain injury and improves long-term neurodevelopmental outcomes. Although iNO-mediated decreases in chronic lung disease and severe intraventricular hemorrhage/periventricular leukomalacia undoubtedly contribute to improved neurodevelopmental outcomes, iNO may have an independent neuroprotective effect. Although these data are encouraging, additional studies are required before recommending the routine use of iNO for neuroprotection in preterm infants.

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