Published in final edited form as:

J Pediatr. 2014 April ; 164(4): 744–748. doi:10.1016/j.jpeds.2013.11.040.

Effects of Early Inhaled Nitric Oxide Therapy and Vitamin A Supplementation on the Risk for Bronchopulmonary Dysplasia in Premature Newborns with Respiratory Failure

Monika M. Gadhia, MD¹, Gary R. Cutter, PhD², Steven H. Abman, MD^{3,4}, and John P. Kinsella, MD^{3,5}

¹Weill Cornell Medical College, Division of Neonatology, Department of Pediatrics

²Department of Biostatistics, University of Alabama, Birmingham, Alabama

³Pediatric Heart Lung Center, Department of Pediatrics, University of Colorado School of Medicine, Aurora CO

⁴Section of Pulmonary Medicine, Department of Pediatrics, University of Colorado School of Medicine, Aurora CO

⁵Section of Neonatology, Department of Pediatrics, University of Colorado School of Medicine, Aurora CO

Abstract

Objective—To assess whether the combination of early inhaled nitric oxide (iNO) therapy and vitamin A (vitamin A) supplementation lowers the incidence of bronchopulmonary dysplasia (BPD) in premature newborns with respiratory failure.

Study design—793 mechanically ventilated infants (birth weight 500–1250g) were randomized (after stratification by birth weight) to receive placebo or iNO (5 ppm) for 21 days or until extubation (500–749g, 750–999g, or 1000–1250g). 398 newborns received iNO, and of these, 118 (30%) received vitamin A according to their enrollment center. We compared patients who received iNO+vitamin A with those who received iNO alone. The primary outcome was a composite of death or BPD at 36 weeks postconceptual age.

Results—BPD was reduced in infants who received iNO+vitamin A for the 750–999g birth weight group compared with iNO alone ($p=0.01$). This group also showed reduction in the combined outcome of BPD+death compared with iNO alone ($p=0.01$). vitamin A did not change the risk for BPD in the placebo group. Overall, vitamin A use was low (229 out of 793 patients, or 29%). Combined therapy improved Bayley scores at one year compared with infants treated solely with iNO for the 500–749g birth weight group.

Conclusions—In this retrospective analysis of the nonrandomized use of vitamin A, combined iNO+vitamin A therapy in preterm infants with birth weight 750–999g reduced incidence of BPD

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Correspondence: Monika M Gadhia, MD, Address: Neonatal-Perinatal Medicine, Department of Pediatrics, 525 East 68th Street, Suite N 506, New York, NY 10065, Phone: 212-746-3530, Fax: 212-746-8608, Mog9018@med.cornell.edu.

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The authors declare no conflicts of interest.

and BPD+death, and improved neurocognitive outcomes at 1 year in the 500–749g birth weight group.

Keywords

Neurodevelopmental Outcome; Bayley Scales of Development; Preterm Neonates

In preterm infants, inhaled nitric oxide (iNO) improves gas exchange in infants with respiratory distress syndrome and persistent pulmonary hypertension^{1–4}. In animal models iNO reduces lung inflammation and oxidant stress that cause acute lung injury in preterm infants^{5–7}. Furthermore, iNO maintains surfactant activity and improves lung structure in experimental models of bronchopulmonary dysplasia (BPD)^{8–11}. Despite favorable preclinical studies, the effects of iNO for the prevention of human BPD have been variable. In two randomized control studies, iNO therapy improved pulmonary outcomes and survival in preterm newborns born <1250 grams or <34 weeks gestational age^{12, 13}. However, in a meta-analysis early iNO for premature newborns did not decrease the risk of BPD or BPD and death¹⁴. Inconsistencies in the results between clinical trials with iNO in preterm infants may be partly due to differences in dose, duration, age at time of therapy, study populations, and/or other factors. In one study, the early use of iNO in preterm newborns with respiratory failure reduced the incidence of BPD in infants between 1000 to 1250 grams birth weight, but the overall incidence of BPD was not reduced¹⁵. These findings failed to show improvement in the birth weight stratum of 750 to 1000 grams, which was the largest subgroup of preterm infants included in the study¹⁵. Factors contributing to the lack of iNO efficacy to reduce BPD or BPD and death in this subgroup are uncertain.

In fetal life, vitamin A (vitamin A) is important for cellular differentiation in the lung and for surfactant synthesis. Preterm infants are deficient in vitamin A due to loss of maternally acquired vitamin A, and low administration of vitamin A in neonatal life^{16–18}. Vitamin A is metabolized into two classes of biologically active retinoids: 11-cis-retinoids and acidic retinoids¹⁹. Both activate nuclear hormone receptors which form dimers to act as transcriptional regulators for a myriad of genes¹⁹. Thus, vitamin A has widespread impacts on diverse cellular processes including growth and differentiation, immunocompetence, maintaining integrity of the epithelial lining, and retinal photosensitivity^{18, 20}. In animal models, early deficiency of vitamin A leads to tracheobronchitis, and persistent deficiency will ultimately result in squamous metaplasia of lung epithelium²⁰. As a consequence of epithelial injury, the airway undergoes striking hyperplasia of mucus-producing goblet and other cells, loss of normal water homeostasis across the tracheobronchial epithelium, impaired mucociliary transport with predisposition to infection and obstruction, and narrowing of the airway lumina with decreased distensibility leading to increased airway resistance and work of breathing²⁰.

Fetal and neonatal animal studies also show that vitamin A plays a role in enhancing surfactant production^{16, 21}. In term rats and preterm lambs vitamin A supplementation results in increased alveolarization and decreased septal thickening^{22, 23}. A multicenter randomized control study in 1999 showed that vitamin A improved the incidence of BPD in extremely low birth weight infants²⁶, and a meta-analysis of eight randomized trials in very low birth weight infants demonstrated vitamin A was associated with significant reduction in death or BPD and oxygen requirement at 36 weeks gestational age²⁷. Despite this evidence, the use of vitamin A supplementation is still not widely practiced in the United States^{28, 29} perhaps due to the small size of the effect, concerns for administering vitamin A as a parenteral therapy, and the lack of improvement in long term outcomes³⁰.

iNO and vitamin A potentially have different mechanisms for the prevention of BPD in preterm patients. We sought to determine whether the combination of iNO with vitamin A is more effective than monotherapy. Therefore, we retrospectively examined the treatment arm of the early iNO trial and compared the cohort of infants < 1000 gram birth weight that received vitamin A supplementation in addition to iNO versus the response of infants that received iNO alone. In addition, we compared the patients in the control arm that received early vitamin A supplementation to those that did not receive it.

METHODS

This study was approved by the Institutional Review Board of the University of Colorado School of Medicine. Data were collected on patients from the original study at 16 centers. Criteria for enrollment included a gestational age of 34 weeks or less, birth within the previous 48 hours, birth weight between 500 grams and 1250 grams, and respiratory failure requiring endotracheal intubation and mechanical ventilation. Patients were excluded if they had lethal congenital anomalies or congenital heart disease, active pulmonary hemorrhage, unevacuated pneumothorax, or an expected duration of ventilation of less than 48 hours.

Patients were randomized according to center and birth weight (500 to 749 grams, 750 to 999 grams, and 1000 to 1250 grams), and received masked cylinders of nitric oxide or placebo study gas. iNO at 5 ppm or nitrogen placebo was delivered for 21 days or until extubation. Patients that received vitamin A therapy did so without randomization according to the practice at the center in which they enrolled. vitamin A supplementation is generally used in preterm infants <1000 grams birth weight and it is given intramuscularly. Data were not collected about doses and intervals between dosing. All infants in the study had mechanical ventilation with standard neonatal time-cycled, pressure-limited ventilators or high-frequency devices, and specific ventilation decisions were made by the clinical team. Nutritional strategies were left to the discretion of the neonatologist.

We compared data for patients who received iNO alone, iNO plus vitamin A supplementation, placebo gas without vitamin A supplementation, and placebo gas with vitamin A supplementation. The primary outcome was the combined endpoint of death or BPD. BPD was defined as the need for supplemental oxygen or mechanical ventilation at 36 weeks of postmenstrual age, plus abnormal findings on chest radiography, assessed by a centrally read and standardized scoring system. All classifications of BPD, whether mild, moderate, or severe, were included in the primary outcome. Secondary outcomes included the incidence of intracranial hemorrhage, retinopathy of prematurity, pulmonary hemorrhage, pneumothorax, and necrotizing enterocolitis.

Infant development was assessed using the Bayley Scales of Infant Development II Mental and Psychomotor Developmental Indexes³¹. Examiners were blinded to the patients' treatment assignments in the initial study and follow-up for the first two years following the primary trial. Our analysis of the subset of patients receiving iNO with or without vitamin A therapy compared three birth weight strata using a Bonferroni correction yielding a p value <0.017.

RESULTS

Seven hundred ninety three newborns were randomized in the original study: 398 received iNO and 395 received placebo gas. Of the patients that received iNO, 118 (30%) also received vitamin A supplementation, and 111 (28%) patients in the control group received vitamin A (p=0.64). We compared patients that received iNO plus vitamin A with those that received iNO alone. We found no significant differences between groups for patient

characteristics (Table I). There were also no differences at baseline in Apgar score ventilator requirements, levels of arterial blood gases, or use of surfactant consistent with our random assignments.

There was no difference in the overall incidence of BPD plus death for the iNO group (73.9% iNO without vitamin A versus 66.1% iNO+vitamin A, $p=0.25$). Analyses by birth weight subgroup did show a significant reduction in primary outcomes for the 750 to 999 gram birth weight infants who received iNO plus vitamin A compared with iNO without vitamin A (Table II). There was no difference in primary outcomes between the control group that received vitamin A compared with the control group that did not receive vitamin A (Table III). Secondary outcomes showed no differences between the iNO plus vitamin A and iNO without vitamin A groups for intracranial hemorrhage, retinopathy of prematurity, sepsis, pulmonary hemorrhage, pneumothorax, and necrotizing enterocolitis.

Long term follow up of these patients occurred at one year and two years of age for Bayley assessments. Two hundred thirty two patients in the original iNO group followed up at one year and 225 patients in the original iNO group followed up at 2 years. Eighty nine out of the 232 (38%) patients received iNO plus vitamin A at one year follow up, and 84 out of 225 (37%) patients received iNO plus vitamin A at two year follow up. There was an overall improvement in mental development for the one year assessment in infants in the iNO plus vitamin A group compared with those in the iNO group, and this improvement was mostly driven by the 500 to 749g birth weight stratum (Table IV).

DISCUSSION

From the original cohort in the multicenter trial of iNO therapy, we examined the effect of iNO combined with vitamin A supplementation on the incidence of BPD in premature newborns with respiratory failure. We found that combined therapy decreased the incidence of BPD and BPD plus death for the 750 to 999 gram birth weight newborns. Combined iNO therapy and vitamin A supplementation was also associated with improved mental developmental index Bayley scores at one year follow-up for the 500 to 749 gram birth weight stratum. Although iNO was previously shown to lower the risk for BPD in preterm infants above but not below 1000 grams¹⁵, this analysis suggests that combination therapy may be more effective than iNO alone in smaller birth weight infants.

The separate effects of iNO therapy and vitamin A supplementation have been studied in animal models and human studies, but no previous study has looked at the effects of the combination of both of these therapies in preterm newborns at risk for BPD. Ongoing postnatal deficiency of vitamin A in very low birth weight preterm infants may promote chronic lung disease by impairing lung healing, contributing to the loss of cilia, increasing epithelial cell metaplasia and susceptibility to infection, and decreasing the number of alveoli in lung parenchyma^{22, 24}. Although exact mechanisms by which iNO reduces risk of bronchopulmonary dysplasia are uncertain, nitric oxide decreases neutrophil adhesion in microcirculation³² which may attenuate the inflammatory cascade that contributes to lung injury and BPD in the preterm infant^{6, 33–37}. Laboratory studies suggest that iNO therapy modulates lung injury by decreasing oxidant stress, augmenting surfactant expression or directly enhancing distal lung growth^{7, 10, 11, 38}. In combination, these therapies may act through different mechanisms and this combined effect on lung development and improvement in acute lung injury may confer a greater benefit for reducing the risk of BPD than either therapy alone.

Our findings were significant for a reduction in BPD in the 750 to 999 gram birth weight stratum but not in the other groups, nor in the whole cohort. This is possibly due to the low

incidence of vitamin A use in the > 1000 gram birth weight infants and that this larger birth weight group responded significantly to iNO alone^{15, 29}. In a multicenter clinical trial of vitamin A supplementation by Tyson et al, infants over 1000 grams were not included in the study, and thus centers that use vitamin A supplementation often do so only for < 1000 gram birth weight newborns. Our sample size for infants that received both iNO and vitamin A supplementation in the 1000 to 1250 gram birth weight strata was very small compared with numbers of infants in other birth weight strata.

The smallest birth weight infants have the highest risk of BPD, yet we cannot strongly conclude that combined iNO therapy and vitamin A supplementation has no effect on BPD in this birth weight cohort because this study was originally powered to look at a different intervention. The sample size of the 500 to 749 gram birth weight cohort in this study may be too small to detect an effect of combined therapy or the impact of mortality overwhelmed the benefit that might be present. Given that we did not control for or collect specifics on vitamin A dosage or administration at the centers which used vitamin A, future prospective randomized controlled studies that are adequately powered to account for more rigorous birth weight or gestational age effects may reveal an improvement in BPD for these tiny newborns.

Bayley Mental Developmental index scores at one year were improved in the group treated with both iNO and vitamin A, largely seen in the 500 to 749 gram birth weight group, and these survivors are a smaller proportion of the randomized cohort. Further, there were no differences in rates of intracranial hemorrhage between groups in the strata. Both iNO and vitamin A have important roles in the developing brain, and no previous study has investigated the effects of both of these therapies on long term developmental outcomes in preterm patients. In a study by Mestan et al that evaluated long term neurodevelopmental follow up of preterm infants treated with early iNO therapy, there was a significant improvement in Bayley mental developmental index scores at two years⁴⁰, and newborns who received early iNO had lower rates of death or neurodevelopmental impairment compared with patients who received placebo gas⁴¹. Extrapulmonary effects of iNO may play a role; its anti-inflammatory properties or its action on neuronal maturation may contribute to improved neurodevelopmental outcomes long term⁴², however mechanisms by which iNO could improve neurodevelopmental outcome remain unclear. Long term follow up studies of early vitamin A supplementation for the < 1000 gram birth weight infants did not show any neurodevelopmental advantage at 18 to 22 months³⁰.

In conclusion, early iNO therapy and vitamin A supplementation potentially reduces the risk of bronchopulmonary dysplasia in premature newborns with a birth weight of 750 to 999 grams compared with iNO therapy alone. Combined therapy may also improve the Bayley mental developmental index scores at one year compared with infants treated solely with iNO therapy. We speculate that these therapies act through distinct but complementary pathways to improve the effects of either therapy alone. Future studies examining the combination of iNO and vitamin A should be prospective and powered to explore effects of these therapies on < 750 gram and > 1000 gram birth weight groups.

Acknowledgments

Supported by National Heart, Lung, and Blood Institute (UO1-64857 and NHLBI-SCOR-P50-57144), National Institutes of Health (HL68702 and HL085703), National Center for Advancing Translational Sciences (Colorado CTSI UL1 TR000154), and the National Center for Research Resources (M01-RR00069).

List of Abbreviations

iNO	Inhaled nitric oxide
BPD	Bronchopulmonary dysplasia

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Table 1

Baseline Characteristics of Patients*

	iNO without vitamin A	iNO + vitamin A	P value (iNO groups)	Control without vitamin A	Control + vitamin A	P value (control groups)
Birth weight- g	800±19	810±18	0.46	790±18	810±19	0.53
Birth weight strata no./total no. (%)			0.73			0.64
500–749 g	123/253 (48)	52/118 (44)		122/256 (47)	47/111 (42)	
750–999 g	89/253 (35)	46/118 (39)		92/256 (36)	44/111 (39)	
1000–1250 g	41/253 (16)	20/118 (17)		42/256 (16)	20/111 (18)	
Gestational age- weeks	25.74±1.79	25.65±1.62	0.70	25.70±1.87	25.70±1.69	0.95
Male sex- no. (%)	46	47	0.86	49	41	0.19
Maternal Age (years)	26.68±45	27.33±6.11	0.37	26.30±6.22	26.86±6.65	0.43
Maternal race [†] no./total no. (%)			0.28			0.93
White	152/253 (60)	78/118 (66)		148/253 (58)	66/111 (59)	
Black	35/253 (14)	6/118 (5)		43/253 (17)	4/111 (3)	
Hispanic	56/253 (22)	33/118 (28)		51/253 (20)	38/111 (34)	
Other	10/253 (4)	1/118 (1)		11/253 (4)	3/111 (2)	
Antenatal corticosteroids- no. (%)	78	86	0.22	74	77	0.15

* Values are presented as means ± standard deviation. Percentages may not total 100 because of rounding.

[†] Race was self reported by parent or guardian.

Table 2

Incidence of BPD or death at 36 weeks postmenstrual age in the treatment group*

	iNO + VA, n=118	iNO without VA, n=253	P value	RR (CI)
BPD	<i>no./total no. (%)</i>			
500–749grams	36/45 (80.0)	76/98 (77.6)	0.74	1.03 (0.86–1.24)
750–999grams	22/43 (51.2)	60/82 (73.2)	0.01	0.70 (0.51–0.96)
1000–1250grams	9/19 (47.4)	8/36 (22.2)	0.06	2.13 (0.98–4.62)
Death				
500–749grams	9/52 (17.3)	32/123 (26.0)	0.21	0.67 (0.34–1.29)
750–999grams	3/46 (6.5)	8/89 (9.0)	0.75	0.73 (0.20–2.61)
1000–1250grams	1/20 (5)	5/41 (12.2)	0.65	0.41 (0.05–3.28)
Death+ BPD				
500–749grams	43/52 (82.7)	103/123 (83.7)	0.86	0.99 (0.85–1.14)
750–999grams	25/46 (54.4)	67/89 (75.3)	0.01	0.72 (0.54–0.97)
1000–1250grams	10/20 (50.0)	13/41 (31.7)	0.17	1.58 (0.84–2.95)

* CI denotes confidence interval. Percentages may not total 100 because of rounding.

Table 3

Incidence of BPD or death at 36 weeks postmenstrual age in the control group*

	Control + vitamin A, n=111	Control without vitamin A, n=256	P value	RR (CI)
BPD	<i>no./total no. (%)</i>			
500–749 g	29/36 (80.6)	71/96 (74.0)	0.43	1.09 (0.89–1.33)
750–999 g	25/40 (65.5)	51/80 (63.8)	0.89	0.98 (0.73–1.31)
1000–1250 g	11/20 (55.0)	23/37 (62.2)	0.6	0.88 (0.55–1.41)
Death				
500–749 g	13/47 (27.7)	30/122 (24.6)	0.68	1.12 (0.64–1.96)
750–999 g	5/44 (11.4)	16/92 (17.4)	0.36	0.65 (0.26–1.67)
1000–1250 g	0/20 (0.0)	6/42 (14.3)	0.16	NA [†]
Death+ BPD				
500–749 g	42/47 (89.4)	97/122 (79.5)	0.13	1.12 (0.98–1.28)
750–999 g	29/44 (66.0)	63/92 (68.5)	0.76	0.96 (0.75–1.24)
1000–1250 g	11/20 (55.0)	28/42 (66.7)	0.37	0.83 (0.53–1.29)

* CI denotes confidence interval. Percentages may not total 100 because of rounding.

[†] Value could not be calculated due to incidence of 0 in one of the groups

Table 4

Long-Term Developmental Follow-Up of iNO with or without vitamin A *

	iNO + vitamin A, n=89	iNO without vitamin A, n=140	P value
Bayley scores PDI-1	80.8±19.3	80.3±22.0	0.89
500–749 g	77.1±17.3	73.2±21.8	0.41
750–999 g	80.1±20.1	83.8±19.7	0.54
1000–1250 g	89.9±19.6	91.7±24.0	0.49
Bayley scores PDI-2	83.9±23.1	79.1±21.7	0.13
500–749 g	81.7±22.7	75.0±22.0	0.19
750–999 g	83.5±23.1	83.2±21.3	0.93
1000–1250 g	89.4±24.9	78.5±20.4	0.2
Bayley scores MDI-1	92.3±19.4	86.9±18.3	0.02
500–749 g	91.6±20.4	81.5±17.9	0.01
750–999 g	92.1±18.7	90.8±16.3	0.44
1000–1250 g	94.3±19.6	91.3±22.3	0.82
Bayley scores MDI-2	83.0±21.8	79.3±22.5	0.21
500–749 g	79.7±21.6	72.9±21.9	0.11
750–999 g	83.1±21.6	84.2±20.5	0.94
1000–1250 g	89.8±22.3	85.1±27.7	0.84

* Values are presented as means ± standard deviation.

Percentages may not total 100 because of rounding. PDI-1 refers to psychomotor developmental index scores at one year follow up, MDI-1 refers to mental developmental index scores at one year follow up, PDI-2 refers to psychomotor developmental index scores at two year follow up, MDI-2 refers to mental developmental index scores at two year follow up.