

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

JULY 27, 2006

VOL. 355 NO. 4

Inhaled Nitric Oxide in Preterm Infants Undergoing Mechanical Ventilation

Roberta A. Ballard, M.D., William E. Truog, M.D., Avital Cnaan, Ph.D., Richard J. Martin, M.D., Philip L. Ballard, M.D., Ph.D., Jeffrey D. Merrill, M.D., Michele C. Walsh, M.D., David J. Durand, M.D., Dennis E. Mayock, M.D., Eric C. Eichenwald, M.D., Donald R. Null, M.D., Mark L. Hudak, M.D., Asha R. Puri, M.D., Sergio G. Golombek, M.D., Sherry E. Courtney, M.D., Dan L. Stewart, M.D., Stephen E. Welty, M.D., Roderic H. Phibbs, M.D., Anna Maria Hibbs, M.D., Xianqun Luan, M.S., Sandra R. Wadlinger, M.S., R.R.T., Jeanette M. Asselin, M.S., R.R.T., and Christine E. Coburn, M.S.N., for the NO CLD Study Group*

ABSTRACT

BACKGROUND

Bronchopulmonary dysplasia in premature infants is associated with prolonged hospitalization, as well as abnormal pulmonary and neurodevelopmental outcome. In animal models, inhaled nitric oxide improves both gas exchange and lung structural development, but the use of this therapy in infants at risk for bronchopulmonary dysplasia is controversial.

METHODS

We conducted a randomized, stratified, double-blind, placebo-controlled trial of inhaled nitric oxide at 21 centers involving infants with a birth weight of 1250 g or less who required ventilatory support between 7 and 21 days of age. Treated infants received decreasing concentrations of nitric oxide, beginning at 20 ppm, for a minimum of 24 days. The primary outcome was survival without bronchopulmonary dysplasia at 36 weeks of postmenstrual age.

RESULTS

Among 294 infants receiving nitric oxide and 288 receiving placebo birth weight (766 g and 759 g, respectively), gestational age (26 weeks in both groups), and other characteristics were similar. The rate of survival without bronchopulmonary dysplasia at 36 weeks of postmenstrual age was 43.9 percent in the group receiving nitric oxide and 36.8 percent in the placebo group ($P=0.042$). The infants who received inhaled nitric oxide were discharged sooner ($P=0.04$) and received supplemental oxygen therapy for a shorter time ($P=0.006$). There were no short-term safety concerns.

CONCLUSIONS

Inhaled nitric oxide therapy improves the pulmonary outcome for premature infants who are at risk for bronchopulmonary dysplasia when it is started between 7 and 21 days of age and has no apparent short-term adverse effects. (ClinicalTrials.gov number, NCT00000548.)

From Children's Hospital of Philadelphia (R.A.B., A.C., P.L.B., A.M.H., X.L., S.R.W., C.E.C.) and the University of Pennsylvania School of Medicine (J.D.M.) — both in Philadelphia; the University of Missouri, Kansas City (W.E.T.); Case Western Reserve University, Cleveland (R.J.M., M.C.W.); Children's Hospital and Research Center, Oakland, Calif. (D.J.D., J.M.A.); the University of Washington School of Medicine, Seattle (D.E.M.); Harvard Medical School, Boston (E.C.E.); the University of Utah Medical Center, Salt Lake City (D.R.N.); the University of Florida Health Science Center, Jacksonville (M.L.H.); the University of California Los Angeles School of Medicine, Los Angeles (A.R.P.); New York Medical College, Valhalla (S.G.G.); Long Island Jewish Health System, New Hyde Park, N.Y. (S.E.C.); the University of Louisville School of Medicine, Louisville, Ky. (D.L.S.); Ohio State University School of Medicine and Public Health, Columbus (S.E.W.); and the University of California at San Francisco, San Francisco (R.H.P.). Address reprint requests to Dr. Ballard at Children's Hospital of Philadelphia, 3535 Market St., Suite 1584, Philadelphia, PA 19104, or at ballard@email.chop.edu.

*Additional members of the Nitric Oxide (to Prevent) Chronic Lung Disease (NO CLD) Study Group are listed in the Appendix.

N Engl J Med 2006;355:343-53.

Copyright © 2006 Massachusetts Medical Society.

SURVIVAL AMONG PRETERM INFANTS HAS improved markedly in recent decades, in large part because of the ability to enhance fetal-lung maturation with antenatal corticosteroids and manage the respiratory distress syndrome. Chronic lung disease, or bronchopulmonary dysplasia,^{1,2} is now the most important long-term pulmonary complication in these infants and is associated with prolonged hospitalization and long-term pulmonary and neurodevelopmental problems.³⁻⁵ Severe bronchopulmonary dysplasia is associated with inflammation,⁶ pulmonary hypertension,⁷ and increased airway resistance,^{8,9} as well as abnormalities of lung growth, including disturbed angiogenesis and alveolarization.^{2,6,9,10}

Inhaled nitric oxide is an effective treatment for pulmonary hypertension in term infants¹¹⁻¹³; however, its efficacy in preterm infants is controversial. Kinsella et al.¹⁴ initially found neither a benefit nor adverse effects of inhaled nitric oxide in preterm infants but demonstrated a trend toward decreased bronchopulmonary dysplasia in treated infants. In a single-center, randomized trial, Schreiber et al.¹⁵ found a decrease in the combined outcome of death or bronchopulmonary dysplasia, as well as improved neurodevelopmental outcome at two years of age¹⁶ among infants who received a seven-day course of inhaled nitric oxide. However, a trial by the National Institute of Child Health and Human Development Neonatal Network,¹⁷ which used brief exposure to inhaled nitric oxide to treat presumed pulmonary hypertension in newborn infants with severe respiratory failure, found no overall benefit; the rates of complications and death increased with such therapy among infants weighing less than 1000 g. Earlier pilot studies of inhaled nitric oxide treatment for infants with established, severe bronchopulmonary dysplasia suggested improved outcomes and safety.^{18,19}

METHODS

We performed a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of inhaled nitric oxide in improving survival without bronchopulmonary dysplasia among infants at 36 weeks of postmenstrual age. Because our target population was infants with developing lung disease, we studied the efficacy of inhaled nitric oxide treatment initiated between 7 and 21 days among preterm infants undergoing

mechanical ventilation who were at very high risk for bronchopulmonary dysplasia. The study was approved by the institutional review boards at each participating institution, and written informed consent was obtained from the parents of all enrolled infants.

ELIGIBILITY CRITERIA

Preterm infants born at 32 weeks of gestation or less were eligible if they weighed 500 to 1250 g at birth and were receiving mechanical ventilation for lung disease (not apnea) between 7 and 21 days of age. Infants with a birth weight of 500 to 799 g who were being treated with nasal continuous positive airway pressure were also eligible, since many such infants subsequently require intubation and ventilation. Infants could be enrolled if they had previously been extubated temporarily but required reintubation. All infants underwent ultrasonography of the head before being enrolled, and those with normal findings, grade 1 or 2 intraventricular hemorrhage, or unilateral grade 3 or 4 intraventricular hemorrhage were eligible. Exclusion criteria included life-threatening conditions, such as complex congenital anomalies and other conditions deemed by the attending neonatologist as likely to result in death (see the Supplementary Appendix, available with the full text of this article at www.nejm.org); preexisting bilateral grade 4 intraventricular hemorrhage; or previous receipt of inhaled nitric oxide therapy.

STUDY DESIGN AND RANDOMIZATION

The trial was conducted at 21 infant intensive care units. Initial visits were made to each hospital by the principal investigator and a study coordinator to reach a consensus on all aspects of the trial and to ensure the hospital's compliance with long-term follow-up. The site investigators agreed to general guidelines for ventilatory management, the treatment of patent ductus arteriosus, and criteria for administration of postnatal corticosteroids. Randomization of infants occurred at the data coordinating center in Philadelphia and was stratified according to birth weight (500 to 799 g and 800 to 1250 g) and site with the use of permuted blocks, with equal assignment to the group receiving nitric oxide or the placebo group within blocks. If more than one infant from a multiple gestation was entered into the trial, only the first sibling enrolled underwent randomization. On the basis of parental requests in previous trials, any

additional infants from that pregnancy who met entry criteria were assigned to the same therapy as the randomized sibling with the use of the same blinded delivery system.

ADMINISTRATION OF STUDY GAS

Inhaled nitric oxide (INOMax, INO Therapeutics) and the delivery system (INOvent, Datex-Ohmeda) were provided to all sites by INO Therapeutics. INO Therapeutics was not involved in the study design, safety monitoring, data analysis or interpretation, or manuscript preparation. All of the physicians and nursing staff, as well as the parents, were unaware of infants' treatment assignment; only the respiratory therapist who administered the study gas was aware of infants' gas assignment. The study gas tanks were shrouded to prevent identification of contents (inhaled nitric oxide or nitrogen), and the delivery system had software that prevented the display of actual levels of nitric oxide or nitrogen dioxide on the screen. Infants initially received 20 ppm of study gas for 48 to 96 hours, and the doses were subsequently decreased to doses of 10, 5, and 2 ppm at weekly intervals, with a minimum treatment duration of 24 days.

Since few of these infants had indwelling arterial catheters for the monitoring of the partial pressure of arterial oxygen (PaO_2), it was not possible to use the oxygenation index for an evaluation of the severity of respiratory disease. (The oxygenation index was calculated as the mean airway pressure times the fraction of inspired oxygen [FiO_2] times 100, with the result divided by the PaO_2 .) A simplified severity score consisting of the mean airway pressure multiplied by the FiO_2 was used. Infants were treated with an oxygen-saturation goal of 88 to 94 percent and were expected to have a PaO_2 range of 40 to 70 mm Hg. Accordingly, a severity score of 3.5 was equivalent to an oxygenation index between 5 and 9.

HYPOTHESES AND OUTCOMES

We hypothesized that the administration of inhaled nitric oxide to infants would increase survival without bronchopulmonary dysplasia at 36 weeks of postmenstrual age (primary outcome). Infants who required ventilatory support or were unable to maintain oxygen saturation above 88 percent while breathing room air were classified as having bronchopulmonary dysplasia. The oxygen requirement at 36 weeks of postmenstrual

age was evaluated for infants receiving an effective concentration of less than 30 percent oxygen²⁰ by a stepwise reduction in oxygen delivery to the lowest tolerated oxygen concentration. Secondary outcomes included the duration of oxygen therapy and the duration of hospitalization. In addition, we prospectively evaluated the need for hospitalization and respiratory support, including mechanical ventilation, continuous positive airway pressure, and oxygen supplementation at 40, 44, 52, and 60 weeks of postmenstrual age.

SAFETY MONITORING

Infants were monitored for complications of prematurity, including sepsis, patent ductus arteriosus, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, and retinopathy of prematurity, as well as any other adverse events occurring during their hospitalization. Injury to the central nervous system was evaluated by ultrasonography of the head, which was performed before enrollment and either during or after the administration of study gas. Deaths that occurred while infants were receiving study gas were reported within 72 hours to the institutional review boards, the data coordinating center, and subsequently, to the Food and Drug Administration. Blood methemoglobin concentrations were measured at baseline and within the first 24 hours after the initiation of study gas. Concentrations of nitrogen dioxide were monitored, and the alarm on the delivery system was set to go off if they exceeded 3 ppm. The therapist who administered the study gas regularly calibrated the machine in an unblinded fashion and recorded actual levels of nitric oxide and nitrogen being delivered. The data and safety monitoring board reviewed the safety data after the enrollment of each group of 60 infants. The protocol called for survivors to be followed for pulmonary and neurodevelopmental outcomes through two years of age.

STATISTICAL ANALYSIS

Assuming that 50 percent of the eligible infants would survive free of bronchopulmonary dysplasia,²¹ we estimated that 544 infants would need to undergo randomization for the study to have a statistical power of 80 percent to detect an absolute increase of 12.5 percent in the group given nitric oxide. The protocol included two interim analyses after 25 percent and 60 percent of the outcome data had become available, to detect ei-

ther the efficacy or futility of the therapy. We conducted the analyses using the O'Brien–Fleming stopping rule and the Lan–DeMets use function, while maintaining an overall alpha of 0.05,²² according to the intention-to-treat principle. Eligible twins and triplets within the same family were assigned to the same treatment. Because outcomes among siblings may be correlated, in analyses of all treated infants it was not appropriate to assume that the outcomes among siblings were independent. The use of generalized estimating equations,^{23,24} allowing for correlation between siblings, was not appropriate, since estimation of the common correlation was weak (7 percent of the study population included multiple siblings) and because study outcomes were associated with the size of the cluster. Therefore, we analyzed the data with the use of a multiple outputation approach with 1000 repeats.²⁵ Under the null hypothesis, the P values calculated with this approach followed a uniform distribution (0,1). Baseline characteristics were compared with the use of Fisher's exact test for dichotomous variables and the Wilcoxon rank-sum test for continuous outcomes.

Analyses of the primary outcome, complications, and post hoc analyses were done with the use of a chi-square test on the basis of a univariate logistic model. Interaction terms for subgroup analyses were tested in a multiple logistic-regression model. We compared the status of the infants at 40 and 44 weeks with the use of a Monte Carlo simulation of the exact Wilcoxon two-sample rank test.²⁶ We compared the durations of hospitalization and oxygen supplementation with the use of the log-rank test and assumed the maximum possible duration of each in the analysis of death. All reported P values are two-sided. We also analyzed the data after the exclusion of infants who, as part of a multiple gestation did not undergo randomization but, rather, were assigned to the same treatment as a randomized sibling.

RESULTS

Of 5129 infants with a birth weight of 500 to 1250 g who were admitted to participating centers, 465 died (9.1 percent) and 3109 were ineligible (60.6 percent); 253 were transferred to another hospital, 2212 were extubated, 114 had life-threat-

ening conditions, 119 had bilateral grade 4 intraventricular hemorrhage, 121 had previously received inhaled nitric oxide therapy, and 290 had other medical complications. Of 1555 eligible infants between the ages of 7 and 21 days, 587 (37.7 percent) were enrolled between May 2000 and April 2005. Five infants were withdrawn after randomization (three before treatment and two because of the withdrawal of parental consent). The 582 remaining infants had been delivered by 547 mothers (35 infants were assigned to a sibling's treatment).

Table 1 gives the baseline characteristics and status at entry of the 582 infants in the study. There were no significant differences between groups in gestational age, birth weight, sex, race or ethnic group, or incidence of preexisting conditions, including pneumothorax, pulmonary hemorrhage, sepsis, necrotizing enterocolitis, patent ductus arteriosus, or unilateral grade 3 or 4 intraventricular hemorrhage. The median age at entry was 16 days, and the median respiratory severity score was 3.5 for both groups. There was no significant difference between groups in the number of infants receiving nasal continuous airway pressure, as compared with mechanical ventilation.

In the group that was assigned to receive inhaled nitric oxide, 129 of 294 infants survived to 36 weeks of postmenstrual age without bronchopulmonary dysplasia (43.9 percent), as compared with 106 of 288 infants in the placebo group (36.8 percent) (relative benefit, 1.23; 95 percent confidence interval, 1.01 to 1.51; $P=0.04$) (Table 2). The number needed to treat for one improved outcome was 14. The rate of survival without bronchopulmonary dysplasia was similar in both birth-weight strata; the study was not powered to detect significant differences within weight groups. The infants who were treated with inhaled nitric oxide were discharged sooner ($P=0.04$ by the log-rank test) and received supplemental oxygen therapy for a shorter period ($P=0.006$ by the log-rank test) than did the controls.

At 40 and 44 weeks of postmenstrual age, a significantly lower proportion of infants receiving inhaled nitric oxide than of infants in the placebo group remained in the hospital and required mechanical ventilation, nasal continuous positive airway pressure, or supplemental oxygen ($P=0.01$ at 40 weeks and $P=0.03$ at 44 weeks); bronchopulmonary dysplasia, when it occurred,

Table 1. Baseline Characteristics of the Infants.*

Characteristic	Inhaled Nitric Oxide (N=294)	Placebo (N=288)	P Value†
Birth weight			
Mean — g	766±161	759±155	0.45
500–799 g — no. (%)	197 (67.0)	197 (68.4)	0.60
800–1250 g — no. (%)	97 (33.0)	91 (31.6)	
Gestational age — wk	26±1.5	26±1.5	0.38
Male sex — no. (%)	155 (52.7)	162 (56.2)	0.37
Mother's race or ethnic group — no. (%)‡			0.22
White	170 (57.8)	145 (50.3)	
Black	76 (25.9)	90 (31.3)	
Hispanic	32 (10.9)	43 (14.9)	
Other	16 (5.4)	10 (3.5)	
Antenatal corticosteroids — no. (%)	243 (82.7)	229 (79.5)	0.23
Surfactant — no. (%)	288 (98.0)	277 (96.2)	0.25
Vitamin A — no. (%)	154 (52.4)	160 (55.6)	0.58
Age at entry			
Median (interquartile range) — days	16 (12–19)	16 (13–19)	0.68
7–14 days at entry — no. (%)	112 (38.1)	115 (39.9)	0.71
Respiratory severity score at entry — no. (%)§			0.60
<3.5	162 (55.1)	149 (51.7)	
3.5 to <10	120 (40.8)	126 (43.8)	
≥10	12 (4.1)	13 (4.5)	
Clinical complications — no. (%)¶			
Pneumothorax or pneumomediastinum	34 (11.6)	32 (11.1)	0.76
Patent ductus arteriosus	192 (65.3)	194 (67.4)	0.84
Necrotizing enterocolitis	12 (4.1)	11 (3.8)	0.83
Sepsis	70 (23.8)	58 (20.1)	0.36
Grade 3 or 4 intraventricular hemorrhage	35 (11.9)	45 (15.6)	0.13
Type of ventilation — no. (%)			
Conventional	202 (68.7)	191 (66.3)	0.66
High frequency	65 (22.1)	74 (25.7)	
Nasal continuous positive airway pressure	27 (9.2)	23 (8.0)	

* Plus–minus values are means ±SD.

† P values correspond to multiple outputation analysis.

‡ Race or ethnic group was self-reported by the parents of the patient.

§ The respiratory severity score was calculated as the fraction of inspired oxygen multiplied by the mean airway pressure (in centimeters of water). The median value for infants in this trial was 3.5, which represents less severe disease.

¶ Patent ductus arteriosus was reported only if echocardiography was performed and treatment with either indomethacin or surgical closure was provided. Necrotizing enterocolitis was diagnosed by the presence of pneumatosis, hepatobiliary gas, or pneumoperitoneum on radiography, plus one or more of the following symptoms: bilious gastric aspirate or emesis, abdominal distension, or occult or gross blood in the stool not secondary to a fissure. Sepsis was diagnosed by a positive culture of blood or cerebrospinal fluid.

Table 2. Incidence of the Primary Outcome.

Outcome	Inhaled Nitric Oxide no./total no. (%)	Placebo no./total no. (%)	P Value	Relative Benefit (95% CI)*
Overall population			0.04	1.23 (1.01–1.51)
Survival without chronic lung disease	129/294 (43.9)	106/288 (36.8)		
Death or survival with chronic lung disease	165/294 (56.1)	182/288 (63.2)		
Chronic lung disease	149/294 (50.7)	164/288 (56.9)		
Death	16/294 (5.4)	18/288 (6.3)		
Birth weight of 500–799 g			0.14	1.20 (0.94–1.54)
Survival without chronic lung disease	85/197 (43.1)	74/197 (37.6)		
Death or survival with chronic lung disease	112/197 (56.9)	123/197 (62.4)		
Chronic lung disease	99/197 (50.3)	108/197 (54.8)		
Death	13/197 (6.6)	15/197 (7.6)		1.01 (0.96–1.07)
Birth weight of 800–1250 g			0.14	1.30 (0.91–1.87)
Survival without chronic lung disease	44/97 (45.4)	32/91 (35.2)		
Death or survival with chronic lung disease	53/97 (54.6)	59/91 (64.8)		
Chronic lung disease	50/97 (51.5)	56/91 (61.5)		
Death	3/97 (3.1)	3/91 (3.3)		1.00 (0.95–1.06)

* CI denotes confidence interval.

was less severe among infants receiving inhaled nitric oxide (Table 3). By 52 weeks, most infants in both groups either no longer needed respiratory support or had been discharged. Of the 582 enrolled infants, 46 died (7.9 percent), with 3 deaths occurring after discharge from the hospital. A total of 21 infants (9 who received inhaled nitric oxide and 12 who received placebo) died while receiving study gas. When the nonrandomized siblings were excluded, differences between groups in the primary outcome remained significant (relative benefit, 1.25; 95 percent confidence interval, 1.01 to 1.53; $P=0.035$).

Among the infants receiving inhaled nitric oxide, none had significant elevations of methemoglobin. There were also no significant differences between groups in the incidence of complications of prematurity (Table 4) — including sepsis, necrotizing enterocolitis, patent ductus arteriosus requiring therapy, retinopathy of prematurity, or the evolution of neurologic findings on ultrasonography — either during or after the administration of study gas.

We conducted post hoc analyses on the basis of the age at study entry, the severity of lung disease at study enrollment, and race or ethnic group

(Table 5). We observed a significant interaction between the age at study entry and treatment ($P=0.006$). Nitric oxide treatment yielded a significant benefit in the group of infants between the ages of 7 and 14 days at enrollment, but not in the group of infants between the ages of 15 and 21 days. For the subpopulation of infants who entered the study at 7 to 14 days of age, the group given inhaled nitric oxide and the placebo group were similar with regard to baseline characteristics (data not shown). There was no interaction between the severity score at study entry and treatment ($P=0.20$). The effect of inhaled nitric oxide appeared to differ according to race or ethnic group ($P=0.05$ for the interaction between race and treatment). There were no significant differences in the response to inhaled nitric oxide according to sex or exposure to postnatal corticosteroids (data not shown).

DISCUSSION

In this multicenter, randomized trial of inhaled nitric oxide among premature infants undergoing mechanical ventilation, we found that treatment for 24 days significantly improved the like-

Table 3. Outcome According to the Severity of Disease at 40 and 44 Weeks of Postmenstrual Age.*

Outcome	500–799 g		800–1250 g		All Infants		P Value†
	Nitric Oxide (N=197)	Placebo (N=197)	Nitric Oxide (N=97)	Placebo (N=91)	Nitric Oxide (N=294)	Placebo (N=288)	
	<i>number (percent)</i>						
Severity of disease at 40 wk							0.01
Discharged	82 (41.6)	60 (30.5)	43 (44.3)	38 (41.8)	125 (42.5)	98 (34.0)	
Hospitalization without support‡	45 (22.8)	44 (22.3)	21 (21.6)	12 (13.2)	66 (22.4)	56 (19.4)	
Hospitalization with oxygen only	40 (20.3)	55 (27.9)	26 (26.8)	29 (31.9)	66 (22.4)	84 (29.2)	
Hospitalization with mechanical ventilation	15 (7.6)	21 (10.7)	3 (3.1)	9 (9.9)	18 (6.1)	30 (10.4)	
Death	15 (7.6)	16 (8.1)	4 (4.1)	3 (3.3)	19 (6.5)	19 (6.6)	
Unknown	0	1 (0.5)	0	0	0	1 (0.3)	
Severity of disease at 44 wk							0.03
Discharged	151 (76.6)	129 (65.5)	82 (84.5)	70 (76.9)	233 (79.3)	199 (69.1)	
Hospitalization without support‡	6 (3.0)	15 (7.6)	2 (2.1)	4 (4.4)	8 (2.7)	19 (6.6)	
Hospitalization with oxygen only	19 (9.6)	26 (13.2)	8 (8.2)	9 (9.9)	27 (9.2)	35 (12.2)	
Hospitalization with mechanical ventilation	5 (2.5)	8 (4.1)	1 (1.0)	4 (4.4)	6 (2.0)	12 (4.2)	
Death	16 (8.1)	16 (8.1)	4 (4.1)	4 (4.4)	20 (6.8)	20 (6.9)	
Unknown	0	3 (1.5)	0	0	0	3 (1.0)	

* Percentages may not total 100 because of rounding.

† P values reflect the comparison of status of respiratory disease between nitric oxide and placebo with the use of a Monte Carlo simulation of the exact Wilcoxon two-sample test. All P values are based on multiple outputation analysis. Discharged infants were discharged to home and included some infants discharged with supplemental oxygen. The status of one infant at 40 weeks and three infants at 44 weeks was unknown.

‡ This category refers to hospitalization without ventilatory support or supplemental oxygen.

Table 4. Incidence of Clinical Complications after Study Entry.

Variable	Inhaled Nitric Oxide (N=294)	Placebo (N=288)	Relative Risk (95% CI)*	P Value
	<i>no. (%)</i>			
Sepsis	121 (41.2)	118 (41.0)	0.98 (0.80–1.20)	0.91
Necrotizing enterocolitis	23 (7.8)	19 (6.6)	1.17 (0.64–2.13)	0.63
Necrotizing enterocolitis requiring surgery	10 (3.4)	8 (2.8)	1.20 (0.46–3.13)	0.84
Patent ductus arteriosus treated	54 (18.4)	55 (19.1)	0.96 (0.68–1.35)	0.85
Retinopathy of prematurity	246 (83.7)	236 (81.9)	1.00 (0.93–1.07)	1.00
Retinopathy of prematurity requiring surgery	72 (24.5)	68 (23.6)	0.97 (0.72–1.31)	0.95
Neurologic evolution†	13 (5.0)	10 (4.1)	1.21 (0.53–2.76)	0.67

* CI denotes confidence interval.

† Evolution of a neurologic lesion on ultrasonography of the head was defined as the occurrence of a new grade 3 or 4 intraventricular hemorrhage, periventricular leukomalacia, or hydrocephalus requiring a shunt during or after administration of study gas, when there was either no or only grade 1 or 2 intraventricular hemorrhage before study entry (as occurred among 259 infants receiving inhaled nitric oxide and 243 infants receiving placebo). Retinopathy of prematurity was defined as stage 1 to 4 disease by ophthalmologic examination. Treatment of patent ductus arteriosus was defined as the administration of indomethacin or surgical ligation.

Table 5. Post Hoc Subgroup Analyses of Survival without Chronic Lung Disease at 36 Weeks of Postmenstrual Age.

Variable	Inhaled Nitric Oxide (N = 294)	Placebo (N = 288)	Relative Benefit (95% CI)*	P Value†
	<i>no./total no. (%)</i>			
Age at entry				0.006
7–14 days			1.81 (1.27–2.59)	
Survival without chronic lung disease	55/112 (49.1)	32/115 (27.8)		
Death	11/112 (9.8)	13/115 (11.3)		
15–21 days			0.99 (0.77–1.27)	
Survival without chronic lung disease	74/182 (40.7)	74/173 (42.8)		
Death	12/182 (6.6)	10/173 (5.8)		
Severity score at entry				0.20
<3.5			1.26 (1.00–1.58)	
Survival without chronic lung disease	92/162 (56.8)	69/149 (46.3)		
Death	8/162 (4.9)	9/149 (6.0)		
≥3.5			1.10 (0.74–1.64)	
Survival without chronic lung disease	37/132 (28.0)	37/139 (26.6)		
Death	15/132 (11.4)	14/139 (10.1)		
Survival without chronic lung disease				0.05‡
Race or ethnic group				
White	59/170 (34.7)	50/145 (34.5)	1.04 (0.76–1.43)	
Black	43/76 (56.6)	32/90 (35.6)	1.66 (1.16–2.37)	
Hispanic	21/32 (65.6)	17/43 (39.5)	1.62 (1.04–2.53)	
Other	6/16 (37.5)	7/10 (70.0)	0.57 (0.27–1.20)	
Age of 7–14 days at entry				
White	24/60 (40.0)	16/52 (30.8)	1.37 (0.81–2.32)	
Nonwhite	31/52 (59.6)	16/63 (25.4)	2.32 (1.43–3.77)	
Age of 15–21 days at entry				
White	35/110 (31.8)	34/93 (36.6)	0.89 (0.60–1.33)	
Nonwhite	39/72 (54.2)	40/80 (50.0)	1.13 (0.83–1.54)	
Severity score <3.5 at entry				
White	38/82 (46.3)	28/67 (41.8)	1.14 (0.78–1.67)	
Nonwhite	54/80 (67.5)	41/82 (50.0)	1.37 (1.05–1.80)	
Severity score ≥3.5 at entry				
White	21/88 (23.9)	22/78 (28.2)	0.88 (0.52–1.50)	
Nonwhite	16/44 (36.4)	15/61 (24.6)	1.52 (0.84–2.76)	

* CI denotes confidence interval.

† Interaction terms for subgroup analyses were tested in a multiple logistic-regression model.

‡ The P value is for the comparison between white infants and nonwhite infants.

likelihood of survival without bronchopulmonary dysplasia at 36 weeks of postmenstrual age. As compared with infants who received placebo gas, infants who were treated with inhaled nitric oxide were hospitalized for fewer days, needed supplemental oxygen for a shorter period, and had less severe disease. We found no evidence of short-

term deleterious clinical effects of nitric oxide therapy.

This trial differed in design from the study by Kinsella et al.,²⁷ which appears elsewhere in this issue of the *Journal*, and from previously reported trials of inhaled nitric oxide,^{14,15,17,28,29} which focused on preterm infants with respiratory failure

shortly after birth with the intention of treating pulmonary hypertension or reducing inflammation. In our study, therapy was started later (between 7 and 21 days, as compared with during the first 24 to 48 hours after birth^{14,15,17}) and was continued for a longer period (24 days vs. 76 hours to 14 days). We hypothesized that prolonged therapy with inhaled nitric oxide might be needed to prevent increased airway resistance and muscularity,^{8,9} to attenuate hyperoxic injury,³⁰ and to improve surfactant function,³¹ lung growth, angiogenesis, and alveolarization, as has been reported in studies in animals.³²⁻³⁴

We delayed enrollment in the study until infants were seven days of age because of concern about a possible interaction between inhaled nitric oxide and brain injury. We did not find any evidence of increased evolution of brain injury in treated infants. We entered infants when they were 7 to 21 days of age in an effort to include all infants at high risk for bronchopulmonary dysplasia. Very preterm infants frequently have initial respiratory disease that improves, often with extubation at approximately days 4 to 7, and later worsens, requiring reintubation. Moreover, our earlier pilot trial demonstrated the benefit of inhaled nitric oxide for infants with severe disease who are older than 28 days of age.¹⁸

Infants in the current trial had greater total exposure to inhaled nitric oxide, in terms of both the duration (24 days) and the peak and total dose, than did infants in other trials. In the National Institute of Child Health and Human Development Neonatal Network trial,¹⁷ only infants who had a response to treatment (defined as an immediate improvement in oxygenation) continued to receive treatment (mean duration, 76 hours). In that trial, the benefit of treatment was limited to infants weighing more than 1000 g at birth. In a study by Schreiber et al.,¹⁵ infants were treated for a maximum of seven days, and only those with less severe disease at enrollment benefited. Kinsella et al.,²⁷ who administered 5 ppm of inhaled nitric oxide and discontinued therapy on extubation, found that this approach had respiratory benefit only among infants with a birth weight of more than 1000 g. In our trial, the effect of inhaled nitric oxide on survival without bronchopulmonary dysplasia appeared to be similar among infants in the birth-weight strata of 500 to 799 g and 800 to 1250 g, although results were not significantly different in individual

subgroups; only 55 infants weighed more than 1000 g.

The mechanism of the beneficial effect of inhaled nitric oxide may include a decrease in airway resistance, resulting over time in a decreased need for supplemental oxygen and ventilatory support, with less oxidative stress. Results of post hoc analyses suggest that inhaled nitric oxide may be less beneficial when initiated later (after 14 days), indicating that infants receiving such treatment may already have sustained lung damage secondary to oxidative stress and volutrauma. Inhaled nitric oxide also appeared to have less benefit among white infants, as compared with nonwhite infants; some investigators have suggested the possibility of racial differences in responsiveness to inhaled nitric oxide. However, these results must be viewed as hypothesis-generating; the study was not powered to address such subgroups.³⁵

In conclusion, prolonged inhaled nitric oxide therapy that is initiated between 7 and 21 days of age in preterm infants undergoing mechanical ventilation significantly improved survival without bronchopulmonary dysplasia without short-term adverse effects. Definitive recommendations regarding the use of inhaled nitric oxide among infants at high risk for bronchopulmonary dysplasia await further long-term neurodevelopmental follow-up in the completed trials.

Supported by grants (U01-HL62514, P50-HL56401, P30-HD26979, MRDDRC-P30, and HD26979) from the National Institutes of Health and grants (M01-RR00240, M01-RR00084, M01-RR00425, M01-RR001271, M01-RR00064, and M01-RR00080) from the General Clinical Research Centers Program.

Dr. R. Ballard reports having received grant support from INO Therapeutics for an MRI follow-up study of infants in the trial; Dr. Walsh, support from INO Therapeutics to fund a research nurse coordinator for a follow-up of this trial; Dr. Durand, grant support from Bunnell for a trial of jet ventilation; Dr. Hudak, support from Inhibitex for the Veronate Study follow-up; Dr. Courtney, lecture fees from INO Therapeutics and Viasys; Drs. Golombek and Stewart, lecture fees from INO Therapeutics; and all investigators in the trial, support from the National Heart, Lung, and Blood Institute for enrolling patients in the trial. Dr. Courtney also reports having served on the advisory board of Discovery Labs. No other potential conflict of interest relevant to this article was reported.

This article is dedicated to the memory of Thomas Hazinski, M.D., a distinguished scientist and valued colleague and friend, who served as chairman of the data and safety monitoring board.

We are indebted to Beverly Banks Randall, M.D., Ph.D., for doing the initial pilot trials; to all the neonatal nurses, residents, fellows, and respiratory therapists who made this study possible; to Carol Dennis for help with the manuscript; to INO Therapeutics for providing study equipment and gas; and to the families and infants who participated in the study.

APPENDIX

In addition to the authors, the following members of the NO CLD Study Group participated in this study: **Centers** — Alta Bates Summit Medical Center, Berkeley, Calif., and Children's Hospital and Research Center Oakland, Oakland, Calif. — L. Pacello, R. Ratcliff, V. Daly, A. Espinosa; Brigham and Women's Hospital and Children's Hospital, Boston — K. Puopolo, A. Hansen, T. Cimini, D. Beadles, C. Pantano, C. Martin; Cedars-Sinai Medical Center, Los Angeles — W. Bunuan, A. Verne, J. Raber, S. Sehgal; Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania, Philadelphia — L. Corcoran, J. Fricko, S. Zirin, A. Hedgman, K. Kelly, B. Hubble, K. Mooney, L. Brown, R. Scarborough, J. Bernbaum, H. Hurt; Children's Hospital and Regional Medical Center and University of Washington Medical Center, Seattle — C. Gleason, S. Jacques, H. Meo, F. Bennett; Children's Mercy Hospital, Kansas City, Mo. — I. Ekekezie, C. Castor, P. Johnson, K. Meinert, D. Taylor, H. Kilbride; Columbus Children's Hospital, Columbus, Ohio — S. Farley, T. Preston, C. Timan; Kosair Children's Hospital, Louisville, Ky. — S. Daugherty, J. Foos, K. Sheeley, S. Polston, S. Wilkerson; North Shore–Long Island Jewish Health System and Schneider Children's Hospital, New Hyde Park, N.Y. — A. Steele, D. Potak, B. Wilkens, S. Pollard, A. Adesman; Primary Children's Medical Center and the University of Utah Hospital and Clinics, Salt Lake City — R. Milley, S. Baker, L. Cole, K. Hillier, L. Hiatt, A. Bodnar; Rainbow Babies and Children's Hospital, Cleveland — A. Zadell, M. Tracy, J. Di Fiore, M. Hack, D. Costello; University of Florida Wolfson Children's Hospital at Baptist Medical Center and Shands Jacksonville Medical Center, Jacksonville — S. Osbeck, A. Kellum, L. Hogans, D. Childers; University of California at San Francisco Medical Center, San Francisco — S. Sehring, J. Imamura-Ching, N. Newton, C. Kelly, R. Piecuch; Westchester Medical Center, Valhalla, N.Y. — L. Parton, N. Dweck, J. Weissleder, J. Kase; **Data Coordinating Center** — T. Alvarado-Taylor, J. Valliyil, M. Davis, K. Gibbs; **Data and Safety Monitoring Committee** — Vanderbilt University School of Medicine, Nashville — T. Hazinski (chair, deceased); New York Academy of Medicine, New York — A. Fleischman; Children's Hospital of Buffalo, Buffalo, N.Y. — F. Morin; Women and Infants Hospital, Brown University School of Medicine, Providence, R.I. — B. Vohr; Emmes, Rockville, Md. — S. Carter; National Heart, Lung, and Blood Institute, Bethesda, Md. — M. Berberich, N. Geller, C. Hunt, G. Zheng; **Clinical Steering Committee** — R. Ballard, W. Truog, R. Martin, P. Ballard, J. Merrill, M. Walsh.

REFERENCES

- Northway WH Jr, Rosan RC, Porter DY. Pulmonary disease following respiratory therapy of hyaline-membrane disease: bronchopulmonary dysplasia. *N Engl J Med* 1967;276:357-68.
- Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723-9.
- Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med* 2005;352:9-19.
- Vohr BWL, Hack M, Aylward G, Hirtz D. Follow-up care of high-risk infants. *Pediatrics* 2004;114:Suppl:1377-9.
- Laptook AR, O'Shea TM, Shankaran S, Bhaskar B. Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents. *Pediatrics* 2005;115:673-80.
- Coalson JJ. Pathology of new bronchopulmonary dysplasia. *Semin Neonatol* 2003;8:73-81.
- Berman W Jr, Katz R, Yabek SM, Dillon T, Fripp RR, Papile LA. Long-term follow-up of bronchopulmonary dysplasia. *J Pediatr* 1986;109:45-50.
- Martin RJ, Mhanna MJ, Haxhiu MA. The role of endogenous and exogenous nitric oxide on airway function. *Semin Perinatol* 2002;26:432-8.
- Bland RD, Albertine KH, Carlton DP, MacRitchie AJ. Inhaled nitric oxide effects on lung structure and function in chronically ventilated preterm lambs. *Am J Respir Crit Care Med* 2005;172:899-906.
- Martin RJ, Walsh MC. Inhaled nitric oxide for preterm infants — who benefits? *N Engl J Med* 2005;353:82-4.
- Roberts JD Jr, Fineman JR, Morin FC III, et al. Inhaled nitric oxide and persistent pulmonary hypertension of the newborn. *N Engl J Med* 1997;336:605-10.
- Clark RH, Kueser TJ, Walker MW, et al. Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. *N Engl J Med* 2000;342:469-74.
- The Neonatal Inhaled Nitric Oxide Study Group. Inhaled nitric oxide in full-term and nearly full-term infants with hypoxic respiratory failure. *N Engl J Med* 1997;336:597-604. [Erratum, *N Engl J Med* 1997;337:434.]
- Kinsella JP, Walsh WF, Bose CL, et al. Inhaled nitric oxide in premature neonates with severe hypoxaemic respiratory failure: a randomised controlled trial. *Lancet* 1999;354:1061-5.
- Schreiber MD, Gin-Mestan K, Marks JD, Huo D, Lee G, Srisuparp P. Inhaled nitric oxide in premature infants with the respiratory distress syndrome. *N Engl J Med* 2003;349:2099-107.
- Mestan KK, Marks JD, Hecox K, Huo D, Schreiber MD. Neurodevelopmental outcomes of premature infants treated with inhaled nitric oxide. *N Engl J Med* 2005;353:23-32.
- Van Meurs KP, Wright LL, Ehrenkranz RA, et al. Inhaled nitric oxide for premature infants with severe respiratory failure. *N Engl J Med* 2005;353:13-22.
- Banks BA, Seri I, Ischiropoulos H, Merrill J, Rychik J, Ballard RA. Changes in oxygenation with inhaled nitric oxide in severe bronchopulmonary dysplasia. *Pediatrics* 1999;103:610-8.
- Clark PL, Ekekezie II, Kaftan HA, Castor CA, Truog WE. Safety and efficacy of nitric oxide in chronic lung disease. *Arch Dis Child Fetal Neonatal Ed* 2002;86:F41-F45.
- Walsh MC, Yao Q, Gettner P, et al. Impact of a physiologic definition on bronchopulmonary dysplasia rates. *Pediatrics* 2004;114:1305-11.
- Ballard RA, Ballard PL, Cnaan A, et al. Antenatal thyrotropin-releasing hormone to prevent lung disease in preterm infants. *N Engl J Med* 1998;338:493-8.
- Jennison C, Turnbull BW. Group sequential methods with applications to clinical trials. Boca Raton, Fla.: Chapman & Hall/CRC Press, 2000.
- EaST, version 3.0, software for advanced clinical trial design, simulation, and monitoring: user's manual. Cambridge, Mass.: Cytel Statistical Software Services, 2003.
- Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13-22.
- Follmann D, Proschan M, Leifer E. Multiple output: inference for complex clustered data by averaging analyses from independent data. *Biometrics* 2003;59:420-29.
- StatXact 7: statistical software for exact nonparametric inference. Cambridge, Mass.: Cytel, 2005.
- Kinsella JP, Cutter GR, Walsh WF, et al. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med* 2006;355:354-64.
- Field D, Elbourne D, Truesdale A, et al. Neonatal ventilation with inhaled nitric oxide versus ventilatory support without inhaled nitric oxide for preterm infants with severe respiratory failure: the INNOVO multicentre randomised controlled trial (ISRCTN 17821339). *Pediatrics* 2005;115:926-36.
- Hascoet JM, Fresson J, Claris O, et al. The safety and efficacy of nitric oxide therapy in premature infants. *J Pediatr* 2005;146:318-23.
- Cotton RB, Sundell HW, Zeldin DC, et al. Inhaled nitric oxide attenuates hyperoxic lung injury in lambs. *Pediatr Res* 2006;59:142-6.
- Ballard PL, Gonzales LW, Godinez RI, et al. Surfactant composition and function in a primate model of infant chronic lung disease: effects of inhaled nitric oxide. *Pediatr Res* 2006;59:157-62.
- Lin YJ, Markham NE, Balasubramanian V, et al. Inhaled nitric oxide enhances distal lung growth after exposure to

- hyperoxia in neonatal rats. *Pediatr Res* 2005;58:22-9.
33. Tang JR, Markham NE, Lin YJ, et al. Inhaled nitric oxide attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L344-L351.
34. McCurnin DC, Pierce RA, Chang LY, et al. Inhaled NO improves early pulmonary function and modifies lung growth and elastin deposition in a baboon model of neonatal chronic lung disease. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L450-L459.
35. Bloche MG. Race-based therapeutics. *N Engl J Med* 2004;351:2035-7.
Copyright © 2006 Massachusetts Medical Society.