Randomized, Controlled Trial of Low-dose Inhaled Nitric Oxide in the Treatment of Term and Near-term Infants With Respiratory Failure and Pulmonary Hypertension

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ABSTRACT. Recent reports indicate that inhaled nitric oxide (iNO) causes selective pulmonary vasodilation, increases arterial oxygen tension, and may decrease the use of extracorporeal membrane oxygenation (ECMO) in infants with persistent pulmonary hypertension of the newborn (PPHN). Despite these reports, the optimal dose and timing of iNO administration in PPHN remains unclear.

Objectives. **To test the hypotheses that in PPHN 1) iNO at 2 parts per million (ppm) is effective at acutely increasing oxygenation as measured by oxygenation index (OI); 2) early use of 2 ppm of iNO is more effective than control (0 ppm) in preventing clinical deterioration and need for iNO at 20 ppm; and 3) for those infants who fail the initial treatment protocol (0 or 2 ppm) iNO at 20 ppm is effective at acutely decreasing OI.**

Study Design. **A randomized, controlled trial of iNO in 3 nurseries in a single metropolitan area. Thirty-eight children, average gestational age of 37.3 weeks and average age <1 day were enrolled. Thirty-five of 38 infants had echocardiographic evidence of pulmonary hypertension. On enrollment, median OI in the control group, iNO at 0 ppm, (n = 23) was 33.1, compared with 36.9 in the 2-ppm iNO group (n = 15).**

Results. **Initial treatment with iNO at 2 ppm for an average of 1 hour was not associated with a significant decrease in OI. Twenty of 23 (87%) control patients and 14 of 15 (92%) of the low-dose iNO group demonstrated clinical deterioration and were treated with iNO at 20 ppm. In the control group, treatment with iNO at 20 ppm decreased the median OI from 42.6 to 23.8, whereas in the 2-ppm iNO group with a change in iNO from 2 to 20 ppm, the median OI did not change (42.6 to 42.0). Five of 15 patients in the low-dose nitric oxide group required ECMO and 2 died, compared with 7 of 23 requiring ECMO and 5 deaths in the control group.**

Conclusion. **In infants with PPHN, iNO 1): at 2 ppm does not acutely improve oxygenation or prevent clinical deterioration, but does attenuate the rate of clinical deterioration; and 2) at 20 ppm acutely improves oxygenation in infants initially treated with 0 ppm, but not in infants previously treated with iNO at 2 ppm. Initial treatment with a subtherapeutic dose of iNO may diminish the clinical response to 20 ppm of iNO and have adverse clinical sequelae.** ***Pediatrics*** **1999;104:1089–1094;** ***persistent pulmonary hypertension of the newborn, extracorporeal membrane oxygenation, hypoxemia, respiratory failure, pulmonary vascular reactivity.***

ABBREVIATIONS. PPHN, persistent pulmonary hypertension of the newborn; iNO, inhaled nitric oxide; ECMO, extracorporeal membrane oxygenation; OI, oxygenation index; P/F, arterial partial pressure of oxygen/fraction of inspired oxygen; a/A, arterial/alveolar ratio; NO, nitric oxide; NO_2, nitrogen dioxide.

At birth, the pulmonary circulation changes dramatically. Pulmonary blood flow increases 8- to 10-fold and pulmonary arterial blood pressure decreases to less than half systemic levels in the first 24 hours of life.[1] Although increased oxygenation, ventilation,[2] establishment of an air-liquid interface,[3] and elaboration of vasoactive mediators[4–6] have been shown to play a central role in modulating the transition of the pulmonary circulation, endothelium-derived nitric oxide (NO) production is necessary for the successful transition of the pulmonary circulation.[7,8] If postnatal adaptation of the pulmonary circulation does not occur, a clinical syndrome, persistent pulmonary hypertension of the newborn (PPHN), results. PPHN is characterized by extrapulmonary shunting of blood and severe central hypoxemia that is not responsive to high concentrations of inspired oxygen.[9] PPHN is often complicated by parenchymal lung injury, such as meconium aspiration, pneumonia, and surfactant deficiency, further compromising efforts to improve oxygenation.

Effective treatment has been limited by the absence of a selective pulmonary vasodilator. Intravenous vasodilator agents can cause nonselective vasodilation, resulting in worsening of intrapulmonary shunting[10] or systemic hypotension. Recent studies have demonstrated that inhaled nitric oxide (iNO) causes selective and sustained pulmonary vasodilation in infants with PPHN. In 1992, 2 groups of investigators independently reported improved oxygenation in infants with PPHN.[11,12] Further studies have demonstrated that in infants with PPHN, iNO can decrease the need for more invasive and costly support modalities such as extracorporeal membrane oxygenation (ECMO).[13,14]

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Although the use of iNO may contribute to the successful treatment of patients with severe PPHN, the optimal dose and timing of iNO administration has yet to be established. Experimental data supports the notion that the minimally effective dose of iNO should be used. For example, at high concentrations iNO can react with oxygen to form ONOO, which has been shown in vitro to cause surfactant destruction.[15] Exposure of NO to high concentrations of inspired oxygen can yield peroxynitrite, a potent oxidant that is capable of causing tissue injury through lipid peroxidation[16] and potentiating lung injury.

To determine the effects of low-dose iNO in the treatment of near-term infants with neonatal respiratory failure compounded by pulmonary hypertension, we conducted a randomized, controlled trial comparing iNO at 2 parts per million (ppm) with iNO at 0 ppm. We hypothesized that an acute improvement in oxygenation would result with iNO treatment at 1) 2 ppm; and 2) 20 ppm. To test these hypotheses, we randomly assigned 38 infants with respiratory failure and pulmonary hypertension to receive iNO at 2 ppm or 0 ppm. Infants who did not improve with the initial assignment were subsequently treated with iNO at 20 ppm.

METHODS

Organization and Eligibility Criteria

Three clinical centers with experience in treating infants with PPHN participated in this trial. The study was approved by the institutional review board at each center. Criteria for enrollment included the following: infants ≥34 week's gestation; <7 days of age at the time of enrollment; severe respiratory failure requiring mechanical ventilation with an oxygenation index (OI) ≥25 on 2 consecutive blood gases 60 minutes apart; (OI is calculated as follows: mean airway pressure × fraction of inspired oxygen × 100 ÷ (divided by) the arterial partial pressure of oxygen, measured from a postductal blood gas sample); echocardiographic evidence of pulmonary arterial hypertension (defined as right-to-left or bidirectional shunting at the ductus arteriosus or foramen ovale, or pulmonary artery pressure more than two thirds of systemic pressure as estimated Doppler measurement of the tricuspid regurgitation jet). Exclusion criteria included: the presence of lethal congenital anomalies; head ultrasound demonstrating grade III or IV intraventricular hemorrhage; neonates who present to the neonatal intensive care unit with hemodynamic collapse and require ECMO emergently; or major structural cardiac defect.

Study Design

At time of enrollment, all patients were ventilated with either a time-cycled, pressure limited neonatal ventilator or high frequency oscillatory ventilator. No attempt was made to control the mode of ventilation. However, no changes in mode of ventilation were permitted from 1 hour before study enrollment until a minimum of 4 hours after enrollment in the study. Eligible patients with an OI ≥ 25 were randomized in a nonblinded manner to receive either 2 parts per million ([ppm]; 2-ppm group) of NO or no NO (0-ppm group). Patients were randomized through a computer generated random-number table. The minimum period of time in this initial assignment was 1 hour. Patients with an OI ≥ 35 for>1 hour after enrollment were considered treatment failures and were treated with iNO at 20 ppm. Figure 1 is a schematic representation of the study design. Patients who presented with cardiovascular collapse were not enrolled in the study and were emergently treated with ECMO as determined by the attending physician.

Arterial blood gas tensions and systemic arterial blood pressures were recorded at baseline, 30, and 60 minutes, at 2, 4, 6, 8, 12, and 24 hours, and every 12 hours for the duration of treatment in all patients. The same measurements were obtained 1 hour after the initiation of iNO at 20 ppm. Methemoglobin levels were obtained at every 6 hours on the first day of the study and at least twice daily for the duration of the study.

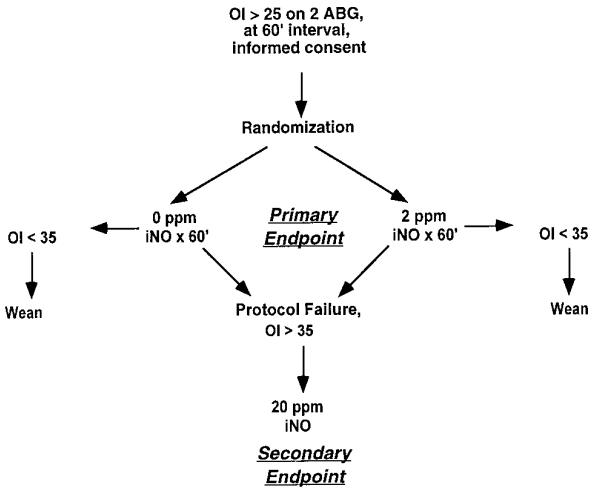

Fig 1. Schematic representation of the experimental design. Patients with an oxygenation index >25 were eligible. After enrollment, patients were randomized to receive either 0 or 2 ppm inhaled nitric oxide. Patients with an oxygenation index >35 for >1 hour after being enrolled in the study were eligible to receive inhaled nitric oxide at 20 ppm and were considered treatment failures.

Sample Size and Data Analysis

Sample size estimates and outcomes within each treatment category were based on pilot studies. It was estimated that 60 patients would be required to provide an 80% power to detect at the 0.05 level a 60% difference in the failure rates between the treatment groups. As part of study monitoring, interim blinded analysis was planned to be performed on recruitment of two thirds of the projected sample size. The interim analysis was performed blindly by the investigator in charge of the data analysis and trial monitoring (C.E.M.). This investigator had no involvement in the care of the patients. Based on this analysis, a decision was made to terminate the study early because significant differences were detected for response to 20 ppm of iNO depending on the initial assignment. Secondary analyses of differences between the treatment groups were performed after study termination.

Data are presented as mean ± SD for normally distributed variables and as median (rank) for those variables not normally distributed. To test for differences in characteristics at baseline between the treatment groups (0 ppm and 2 ppm), either the t test (normally distributed variables) or the Wilcoxon test (nonnormally distributed variables or ordinal variables) were used. Because the main outcomes of interest (OI, arterial partial pressure of oxygen/fraction of inspired oxygen [P/F], and arterial to alveolar oxygen gradient (a/A ratio), were not normally distributed, responses within each of the treatment groups were performed by the nonparametric sign test. For comparisons between the treatment groups in the responses seen, the data were analyzed by rank analysis of covariance, so as to control for the value of each variable of interest at baseline. To evaluate for differences between the treatment groups in time-to-event for different events considered of importance, the log-rank test was used and Kaplan-Meier plots were generated. A significance level of 0.05 was used for all analyses.

Monitoring

The level of NO and nitrogen dioxide (NO_2) delivered to the patient was monitored using either a chemiluminescent or an electrochemical analyzer and a microcomputer. The gas was sampled from the inspiratory limb of the ventilator circuit. Approximately 150 mL/min of gas was drawn into the analyzer. A mi-

croprocessor monitored the function of the analyzer and triggered an alarm in the event of analyzer malfunction or if the levels of NO or NO_2 were out of specified ranges. The computer recorded the levels of NO and NO_2. On initiation of the study, continuous on-line monitoring was in place.

RESULTS

Comparability of Treatment Groups Based on Initial Assignment

Data were analyzed from a total of 38 newborn infants enrolled in this trial. Clinical characteristics at enrollment for the study groups did not differ in terms of age, weight, severity of illness, duration of mechanical ventilation (Table 1), or diagnosis (Table 2). There were no significant differences between the group randomized to treatment with 0 ppm of iNO ($n = 23$) and the group randomized to treatment with 2 ppm iNO ($n = 15$) in any of their baseline characteristics (all $P > .1$), an indication that the randomization procedure produced comparable treatment groups.

Response to Initial Assignment

After randomization, the OI, P/F, or a/A ratio did not change from baseline in either of the treatment groups (Fig 2). There was no significant difference between the treatment groups in the responses seen. Thus, treatment with iNO at 2 ppm did not induce any detectable acute effects. Of the 23 infants treated with 0 ppm of gas, 20 were considered treatment failures and progressed to treatment with 20 ppm of iNO. Of the 15 infants treated with 2 ppm of iNO, 13 were considered treatment failures and progressed to treatment with 20 ppm of iNO. There was no statistically significant difference in the failure rate between the treatment groups. However, there was a marked difference between the treatment groups in the time elapsed to treatment failure (Fig 3), with all but 1 of the failures occurring before the second hour of treatment in the group treated with 0 ppm. The median time to treatment failure in the 0-ppm group was 1 hour compared with 2 hours in the 2 ppm group ($P < .01$).

TABLE 1. Characteristics of the Study Participants at the Time of Enrollment Into the Study*

	0 ppm NO ($n = 23$)	2 ppm NO ($n = 15$)
Age (h)	15.0 (1.8–113.3)	15.6 (1–87)
Gestational age (wk)	37.57 ± 2.39	38.46 ± 2.50
Male:female	16:7	10:5
1-minute Apgar	6 (1–9)	6 (1–8)
5-minute Apgar	7 (1–10)	8 (1–9)
Oxygenation index	33.9 (25–114)	36.9 (25–91)
Arterial/alveolar oxygen ratio	0.075 (0.04–0.13)	0.068 (0.03–0.23)
P/F	48 (25–81)	46 (21–151)
Duration of ventilation (h)	8.8 (1–77)	9.0 (1–92)
Surfactant treatment	59%	46%

Abbreviation: P/F, arterial partial pressure of oxygen/fraction of inspired oxygen.
* Values are expressed as the mean ± SD or as the median (range). There was no difference between these groups in any of the measured parameters.

TABLE 2. Diagnosis of Study Participants Enrolled Into the Study

	0 ppm NO ($n = 23$)	2 ppm NO ($n = 15$)
Respiratory distress syndrome	4	4
Meconium aspiration syndrome	5	3
Pneumonia	3	1
Sepsis	4	2
Congenital diaphragmatic hernia	5	4
Idiopathic (PPHN)	2	1

Abbreviation: PPHN, persistent pulmonary hypertension of the newborn.

Response to 20 ppm After Failure of Initial Assignment

The group initially treated with 0 ppm demonstrated a significant improvement in OI, P/F, and a/A ratio after the start of iNO at 20 ppm. In contrast, oxygenation did not improve in the group initially treated with 2 ppm (Fig 4). There was no acute improvement in oxygenation as assessed by OI, P/F, and a/A ratio. In the control group, treatment with iNO at 20 ppm resulted in a decrease of the median OI from 42.6 to 23.8 ($P < .01$), whereas in the 2-ppm iNO group with a change in iNO concentration from 2 to 20 ppm, OI did not change (42.6 to 42.0; P = NS). The differences in the responses between the treatment groups were statistically significant for the 3 variables measured ($P = .01$ for OI, $P = .049$ for P/F, $P = .049$ for a/A). Despite the initial response to iNO at 20 ppm in the 0-ppm iNO group, 5 infants progressed to ECMO and 7 died. Of the infants initially treated with 2 ppm of iNO, 5 progressed to ECMO, and 2 died. There was no difference between the 2 treatment groups with respect to the rate of progression to ECMO or death (Fig 5). In addition, initial treatment with iNO at 2 ppm did not decrease the total duration of mechanical ventilation, the total number of hours on iNO at 20 ppm, or the total exposure to NO in dose-hours (all $P > .1$, compared with the control group).

Adverse Events

No acute adverse events were associated with iNO treatment. Methemoglobin levels were 0.8 ±.3% on enrollment, 0.9 ± 0.4% after 1 hour, 1.3 ± 0.5% at 6 hours, and 1.3 ± 0.4% at 24 hours. Methemoglobin levels were not different between study groups. In no patient was the study discontinued as the result of an elevated methemoglobin level. Chronic lung disease (defined as oxygen requirement at 28 days of age) was 11% for the entire study group. There was no difference between study groups.

DISCUSSION

We report that in term and near term infants with respiratory failure and pulmonary hypertension, treatment with iNO at 2 ppm did not cause an acute improvement in oxygenation. iNO at 2 ppm had no acute effect on oxygenation and did not prevent clinical deterioration, but did attenuate the rate of clinical deterioration in these infants. The observation that iNO at 20 ppm caused an acute improvement in oxygenation is consistent with other reports in the literature.[11–14] However, infants that had been ran-

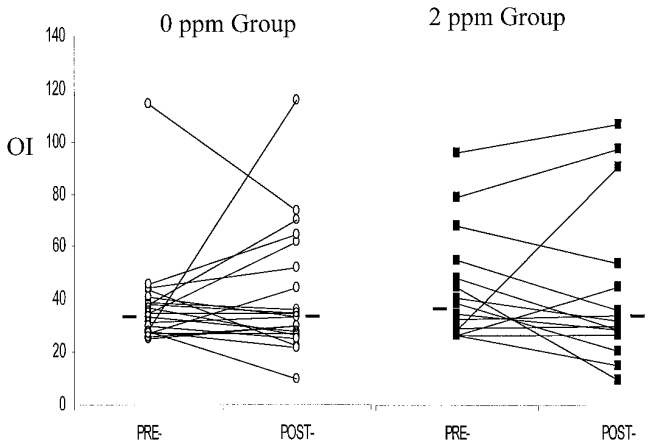

Fig 2. Responses in oxygenation index seen in the treatment groups after randomization. After 1 hour of treatment, no significant changes in the median oxygenation index (bar) were seen in both groups, with no significant differences seen in the responses between the treatment groups ($P > .1$).

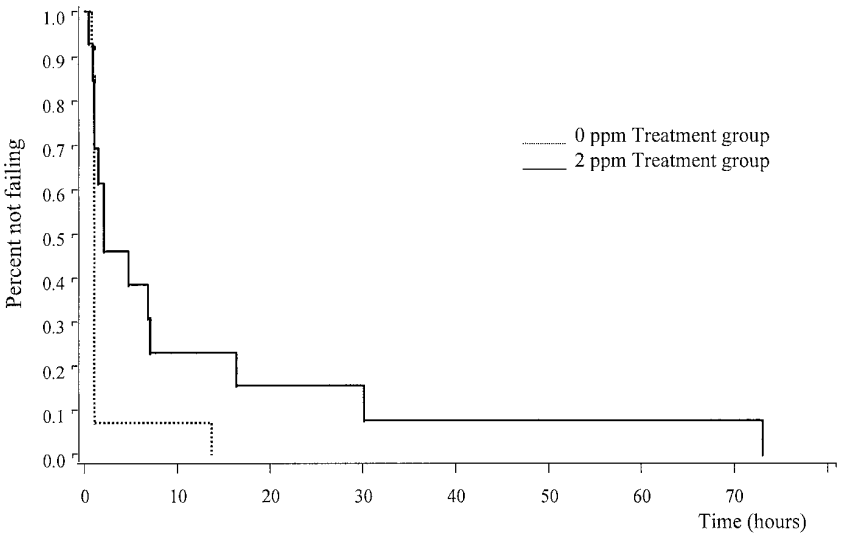

Fig 3. Kaplan-Meier plot of the time to treatment failure for both the 0- and 2-ppm treatment groups. There was a significant difference ($P < .02$) between the groups in their rate of clinical deterioration, with all but 1 of the patients initially treated with 0 ppm failing before the second hour of treatment.

domized to receive 2 ppm of iNO had no improvement in oxygenation after an increase in iNO to 20 ppm. This observation is significant because it implies that administration of a subtherapeutic dose of iNO may adversely affect the clinical response to a therapeutic dose of iNO.

Recent studies have demonstrated that iNO causes a significant improvement in oxygenation in some neonates with severe PPHN. Previous investigators have shown acute improvement in oxygenation using iNO in doses ranging from 5 to 80 ppm.[11,17,18] This is the first study to evaluate the effect of iNO at 2 ppm. We chose to evaluate iNO at 2 ppm because there is data to suggest that the naturally occurring level of NO is in the parts per billion range.[19] Moreover, work in animal models of acute lung injury suggest that iNO at 2 ppm is sufficient to cause significant pulmonary vasodilation.[20,21] Given the in vitro data that NO can cause surfactant destruction[15] and evidence that iNO can combine with oxygen to form molecules that can damage pulmonary epithelium,[16,22] we attempted to determine the minimally effective dose of iNO.

This study provides evidence that treatment with iNO at 2 ppm: 1) does not cause an acute improvement in oxygenation; and 2) may compromise the response to iNO at 20 ppm. Interestingly, although iNO at 2 ppm did not acutely improve oxygenation, it did attenuate the rate of clinical deterioration. This suggests that even in the absence of an acute effect on oxygenation, iNO at 2 ppm likely had a biological effect that was not directly demonstrated by the outcomes measured in this trial. iNO at 2 ppm might sufficiently stimulate the pulmonary vascular smooth muscle to prevent further pulmonary vasoconstriction, but insufficient to cause vasorelaxation.

Although several previous studies have demonstrated that iNO at >5 ppm acutely increases oxygenation,[26] this is the first to suggest that exposure to a subtherapeutic dose of iNO might diminish the efficacy of iNO at >5 ppm. The observation that iNO at 20 ppm had no acute effect on oxygenation in the group initially randomized to receive iNO at 2 ppm; whereas the group of infants initially randomized to receive iNO at 0 ppm had a marked improvement in oxygenation may have significant clinical implications. First, this data suggests that optimal iNO treatment in this patient population includes administration of iNO at a dose that exceeds 2 ppm. Although further study is necessary to establish a minimum

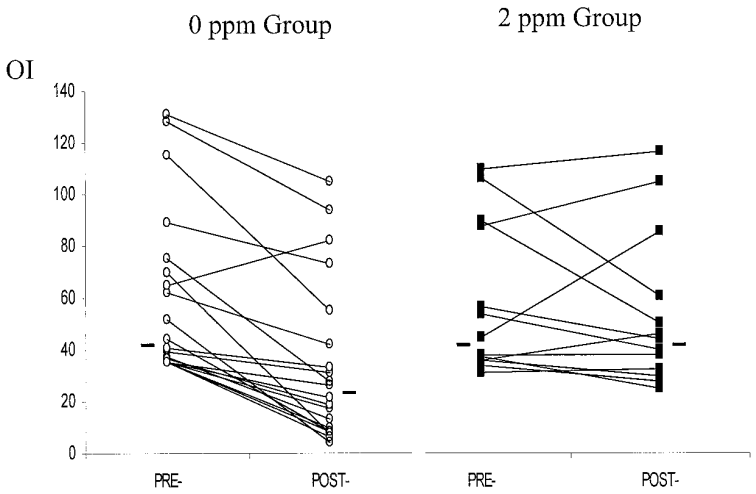

Fig 4. Responses seen in the treatment failures in both groups after the administration of inhaled nitric oxide (iNO) at a dose of 20 ppm. A significant decrease in median oxygenation index (bars) occurred in the group initially treated with 0 ppm of iNO (P = .001), but not in the group initially treated with iNO at 2 ppm. The difference in the response between groups was significant (P < .01).

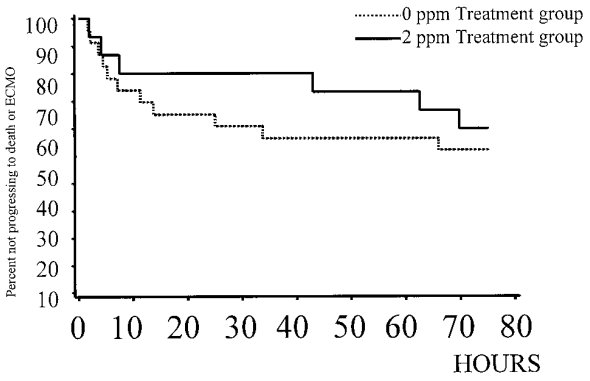

Fig 5. Kaplan-Meier plot of the time to extracorporeal membrane oxygenation or death, whichever occurred first, as a combined outcome for both the 0- and 2-ppm treatment groups. There was no significant difference in the experience of the 2 treatment groups with respect to progressing to either extracorporeal membrane oxygenation or death. (P = .5).

threshold for effective iNO administration, the experience of Finer and coworkers[18] suggests that iNO at 5 ppm may represent an acceptable minimum dose.

Consideration of the reasons that underlie the diminished response to 20 ppm of iNO after treatment with iNO at 2 ppm may provide insight into the physiologic effect of iNO in infants with PPHN. First, administration of a subtherapeutic dose of iNO may allow for increased production of a pulmonary vasoconstrictor agent such as endothelin. Because endothelin production is increased in infants in PPHN,[5,6] it is possible that treatment with a subtherapeutic dose of iNO allows for enhanced production of an endogenous pulmonary vasoconstrictor agent. Alternatively, there is evidence suggesting that endogenous vasodilator activity may be decreased after prolonged exposure to iNO.[23] Thus, administration of a subtherapeutic dose of iNO may attenuate the response of the pulmonary circulation to endothelial-derived vasodilator agents such as endogenous NO or prostacyclin. Finally, the attenuated response may be the result of altered phosphodiesterase activity in the pulmonary vascular smooth muscle, leading to accelerated rate of cyclic 3′,5′-guanosine monophosphate breakdown and diminished pulmonary vasodilation.[24,25]

There was no difference in terms of outcome associated with the 2 treatment groups. The rate of failure, defined as neonates having an OI <35 1 hour after enrollment, was the same. There was no difference between the 2 groups in terms of infants going on to ECMO or death. Whereas treatment with iNO at 2 ppm attenuated the acute response to iNO at 20 ppm, it is not possible, based on the data presented in this study, to determine the degree, if any, that the biologic response to iNO was attenuated. It is important to note that this study was not designed to address the questions surrounding the efficacy of iNO relative to placebo. Another limitation of this study is that the clinicians caring for these children were aware of the initial assignment to the 0- or 2-ppm groups. To mitigate against this potential bias, a change in the mode of ventilation was not allowed during the initial 4 hours of the study. Interestingly, the conclusions of this study are unchanged even if infants with congenital diaphragmatic hernia are excluded from the data analysis. This fact increases the likelihood that the present conclusions are biologically relevant, as opposed to being the result of studying a nonrepresentative population.

CONCLUSION

In summary, this study provides evidence that iNO at 20 ppm causes an acute improvement in oxygenation in infants with PPHN. Although iNO at 2 ppm may attenuate the rate of clinical deterioration, it does not cause an acute improvement in oxygenation. Interestingly, infants receiving iNO at 2 ppm have an attenuated response to the subsequent administration of iNO at 20 ppm. Although the reasons for the diminished response to iNO remain incompletely understood, this observation has important implications for the treatment strategies used in the clinical treatment of infants with PPHN. Further studies are necessary to establish the minimal effective treatment dose of iNO in infants with PPHN and the physiologic alterations that underlie the attenuated response to iNO at 20 ppm in infants previously treated with iNO at 2 ppm.

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