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Review

Inhaled Nitric Oxide and Methemoglobin in Full-Term Infants with Persistent Pulmonary Hypertension of the Newborn

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SUMMARY: Inhaled nitric oxide (iNO), a potent pulmonary vasodilator, has become a mainstay therapy for neonates with persistent pulmonary hypertension of the newborn (PPHN). However, it also oxidizes hemoglobin to methemoglobin (metHgb), thereby reducing the delivery of oxygen to tissues. Studies have suggested that elevated levels of metHgb may be avoided by limiting iNO concentration to less than 40 parts per million (ppm). However, the relationship between iNO exposure and elevated levels of metHgb (greater than 4%) has not been examined. Therefore, we studied this relationship in full term newborns with PPHN.

We reviewed the charts of twenty-eight neonates with a diagnosis of PPHN admitted to our Intensive Care Nursery between 1/92 and 10/97. Our retrospective analysis demonstrated that: (1) high metHgb levels can occur with exposure to low iNO concentration (three of eight newborns with maximum metHgb levels >4% had been exposed to no more than 40 ppm of iNO concentration); and (2) cumulative iNO (ΣiNO) exposure was the best predictor of elevated metHgb levels (seven of nine newborns receiving ΣiNO >2000 ppm × hour had maximum metHgb levels >4%).

KEY WORDS: ECMO: Extracorporeal membrane oxygenation, iNO: Inhaled nitric oxide, metHgb: methemoglobin, PPHN: Persistent pulmonary hypertension of the newborn.

INTRODUCTION

Persistent Pulmonary Hypertension of the Newborn (PPHN) is an abnormal condition of term and near term neonates characterized by elevated pulmonary arterial pressure and right-to-left shunting of deoxygenated blood across the foramen ovale or ductus arterious.[1] PPHN results from the failure of the pulmonary vasculature to transition from the intrauterine state of high resistance and low blood flow to the postnatal state of low resistance and high blood flow.[2] PPHN can occur as an isolated condition or complicate a wide variety of disorders, including Meconium Aspiration Syndrome, perinatal asphyxia, congenital diaphragmatic hernia, neonatal sepsis, and Respiratory Distress Syndrome.[3] PPHN is the most common condition in neonates who are placed on extracorporeal membrane oxygenation (ECMO).[4]

Recent understanding of the physiology of pulmonary vasodilation has led to novel approaches towards therapy.[5] Animal studies have elucidated an endogenous nitric oxide pathway as a major contributor to the normal transition of the pulmonary circulation that occurs at birth.[6,7] Chronic inhibition of the nitric oxide pathway by L-nitroarginine disrupts the normal transition and results in PPHN.[8] In newborn neonates with PPHN, administration of exogenous inhaled nitric oxide (iNO) results in improved oxygenation.[9] The advent of iNO therapy has markedly reduced the need for ECMO.[10,11]

When nitric oxide contacts blood within the circulation, it rapidly binds to hemoglobin, oxidizing the iron molecule from the ferrous (Fe^{2+}) to ferric (Fe^{3+})

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state. This transformation of the heme iron results in methemoglobin (metHgb), which prevents the red blood cell from carrying oxygen to tissues. In healthy, full-term newborns, less than 1% of the circulating hemoglobin is in the form of metHgb.[12] This low physiological level is maintained primarily by an NADH-cytochrome b5 reductase enzyme that restores the ferrous hemoglobin.[13] Impaired reduction of metHgb or excessive production can lead to elevated levels and if high enough (greater than 10%) to the clinical syndrome called methemoglobinemia.[14] This is characterized by cyanosis at rest, respiratory distress, and tachycardia. If severe enough methemoglobinemia can be fatal.[15]

Clinical studies of iNO have found that concentrations >40 parts per million (ppm) in inhaled gas mixture may result in elevated levels of metHgb.[16,17] However, in our Intensive Care Nursery, we have observed metHgb levels greater than 4% in some neonates receiving no more than 40 ppm of iNO. This suggests that prolonged exposure to iNO, even at low concentrations, may cause significant elevation of metHgb. Therefore, we reviewed our own clinical experience among full-term neonates with PPHN to identify the relationship between iNO exposure and elevated metHgb levels.

MATERIALS AND METHODS

Selection of charts

We performed a chart review of all newborns admitted to our Intensive Care Nursery between January 1992 to October 1997. Charts were selected based on a discharge diagnosis of PPHN. Eligibility criteria for review included: (1) a gestational age between 37 and 42 weeks; (2) confirmation of pulmonary hypertension by echocardiogram and/or preductal versus postductal oxygen saturation difference $\geq 10\%$; (3) need for mechanical ventilation; (4) treatment with iNO; and (5) ≥ 3 metHgb measurements during iNO therapy recorded. Neonates who received ECMO generally had an abbreviated course of iNO and therefore were excluded from the review. The clinical characteristics, details of iNO administration, and blood gas values (including metHgb measurements) were abstracted from each chart.

Data analysis

For the purpose of this study, we defined elevated metHgb as a level greater than 4% of total hemoglobin, since this value represents two standard deviations above normal.[18] Neonates were divided into two groups: low maximum metHgb (levels $\leq 4\%$) and high maximum metHgb (levels >4%). Continuous variables (e.g. hours of iNO, maximum iNO concentration) are expressed as means $\pm$ standard deviation and were analyzed between groups of neonates using unpaired t tests. Non-continuous variables (e.g. race, gender, underlying disease) were analyzed by chi squared analysis, using Fisher's exact test when appropriate. P values of 0.05 or less (two-sided) were considered to represent statistical significance.

To identify a possible relationship between iNO exposure and metHgb level, we performed simple linear regression analyses between various measures of iNO exposure and maximum metHgb levels.

Table 1 Clinical characteristics

	Maximum metHgb $\leq 4\%$ (n = 20)	Maximum metHgb >4% (n = 8)
Gender (M:F)*	6:14	6:2
Birth Weight (g)	3238 $\pm$ 750	3277 $\pm$ 558
Gestational age (week)	39 $\pm$ 3	40 $\pm$ 1
R to L Shunt by electrocardiogram	17	8

* Statistically significant ($P<0.05$)

RESULTS

Twenty-eight charts of approximately 4700 total Intensive Care Nursery admissions fulfilled the study design criteria. Table 1 indicates the clinical characteristics of these patients. An echocardographic confirmation of a right to left shunt was documented in twenty-five cases. In three cases, echocardiograph confirmation reports were missing from the medical charts, but all three patients had a preductal versus postductal oxygen saturation difference greater than 10%. Meconium Aspiration Syndrome and Respiratory Distress Syndrome were the two most commonly (10 newborns and 6 newborns, respectively) associated conditions. Other miscellaneous diagnoses included arteriovenous malformation, presumed sepsis, and polycythemia (Table 2). iNO therapy was initiated within 21 ± 20 h of age with an iNO duration of 78 ± 60 h, a maximum iNO of 48 ± 30 ppm, and an average iNO concentration of 25 ± 16 ppm (Table 3). The maximum iNO dosage ranged from 10 to 80 ppm. The average number of metHgb measurements per neonate was 20 ± 16.

The relationship between the iNO concentration being given at the time maximum metHgb was measured level is displayed in (Fig. 1). There is no apparent relationship between iNO concentration and maximum metHgb levels. In fact, most neonates with elevated maximum metHgb levels received no more than 38 ppm of iNO at any time ($r^2 = 0.0079$).

The relationship between maximum iNO concentration and the maximum metHgb is displayed in

Table 2 Associated conditions of PPHN

Diagnosis	Maximum metHgb ≤4% (n=20)	Maximum metHgb >4% (n=8)
Meconium Aspiration Syndrome	8	2
Respiratory Distress Syndrome	6	0
Trisomy 21	1	2
Pneumothorax	2	0
Congenital Diaphragmatic Hernia	2	0
Idiopathic PPHN	0	1
Other	1	3

Table 3 Summary of iNO therapy

	Maximum metHgb ≤4% (n=20)	Maximum metHgb >4% (n=8)
Age at initiation (h)	26 ± 22	15 ± 12
Duration of therapy (h)	70 ± 60	97 ± 59
Maximum iNO concentration (ppm)	35 ± 22	64 ± 21
Average iNO concentration (ppm)*	20 ± 12	38 ± 18
Maximum metHgb level (%)	2 ± 1	6 ± 1

* Time weighted average.

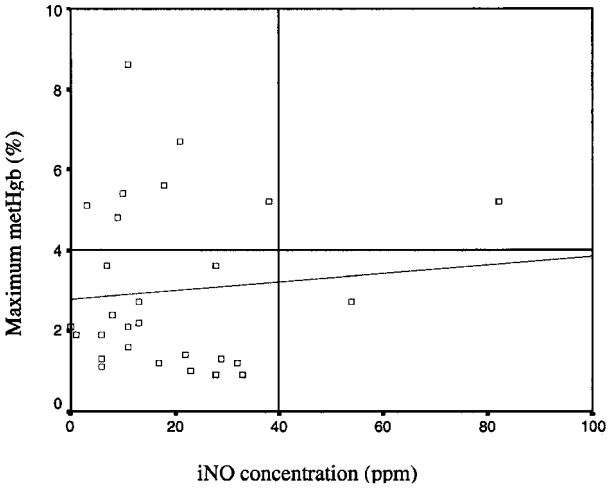

Fig. 1. iNO concentration at the time of maximum metHgb levels $r^2 = 0.0079$.

(Fig. 2). If one selects 40 ppm iNO as the upper bound of a cutoff (i.e. ≤40 ppm) then five of nine newborns receiving concentrations higher than this had maximum metHgb levels 4%, while three newborns who received no more than 40 ppm had maximum metHgb levels 4%. On the other hand, if one selects 40 ppm iNO as the lower bound of a cutoff (i.e. ≥40 ppm) then only one of thirteen newborns who received

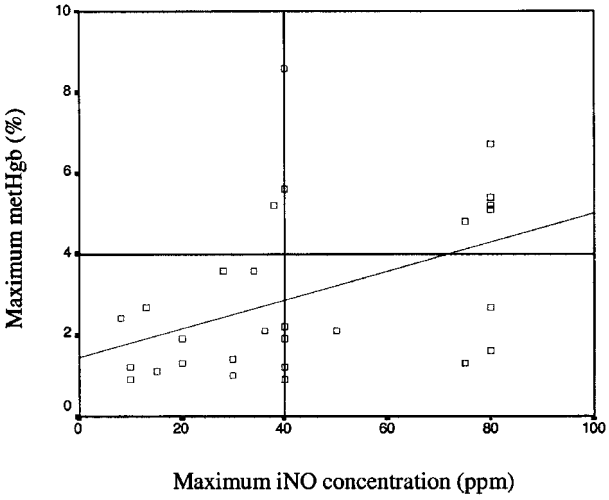

Fig. 2. Maximum metHgb plotted against maximum iNO concentrations in ppm. $r^2 = 0.1930$.

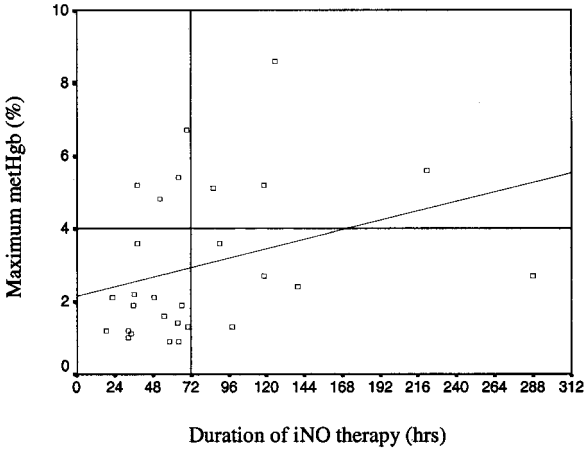

Fig. 3. Maximum metHgb plotted against the duration of iNO therapy in hours. $r^2 = 0.0999$.

concentrations less than this had maximum metHgb levels 4%.

The relationship between duration of iNO exposure and the maximum metHgb is displayed in (Fig. 3). Only four of nine newborns with iNO duration >72 h had maximum metHgb levels >4%, while four newborns with iNO duration of <72 h had maximum metHgb levels >4%.

The relationship between cumulative iNO exposure (ΣiNO; ppm × h) and the maximum metHgb is displayed in (Fig. 4). Seven of nine newborns receiving ΣiNO >2000 (e.g. 20 ppm for 100 h) developed maximum metHgb levels >4%, while only one of nineteen infants with a ΣiNO ≤2000 developed a maximum metHgb levels >4%.

DISCUSSION

To our knowledge, our observations are the first to study the relationship between cumulative iNO

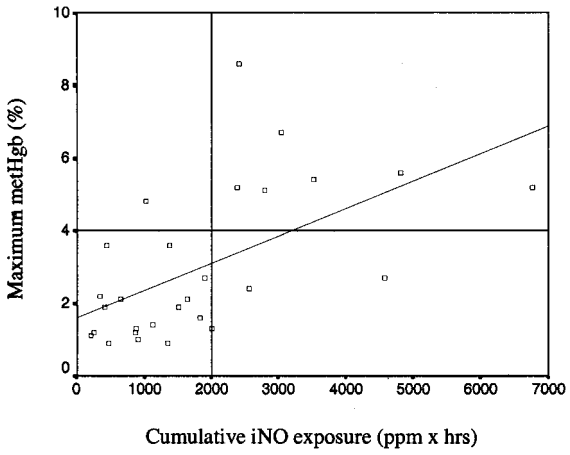

Fig. 4. Maximum metHgb plotted against cumulative iNO exposure in ppm × h. $r^2 = 0.3315$.

Table 4 Measures of iNO exposure as predictors of metHgb greater than 4%

	Sensitivity (%)	PPV (%)*	Specificity (%)
Duration >72 h	50	44	75
Maximum iNO >40 ppm	63	55	80
Maximum iNO ≥40 ppm	88	46	60
ΣiNO >2000 ppm × h	88	78	90

* Positive predictive value.

exposure and metHgb levels in full term neonates with PPHN. We found that ΣiNO (defined as concentration times duration) was the best predictor of subsequent elevated metHgb levels and ΣiNO greater 2000 ppm × h predicted maximum metHgb levels >4%.

The concentration of iNO alone was a poor predictor of metHgb levels. Sixteen percent of neonates receiving maximum iNO ≤40 ppm developed maximum metHgb levels >4%. In contrast, only 40% of neonates receiving maximum iNO >40 ppm developed maximum metHgb levels >4%. Since several infants had maximum iNO of exactly 40 ppm, the sensitivity of maximum iNO in predicting maximum metHgb levels can be significantly altered depending on whether one chooses to include or exclude the value of 40 ppm. However, if one choose to increase the sensitivity by doing so, one will significantly worsen the specificity of this parameter (Table 4). On the other hand, cumulative iNO >2000 had both high sensitivity and specificity.

The fact that maximum metHgb levels increase linearly with cumulative iNO exposure suggest that there is a rate limiting step in metHgb removal. We know that metHgb removal is dependent on the activity of NADH-cytochrome *b*5 enzyme reductase. Further, we know that this enzyme activity is low in the perinatal period, increasing the risk of methemoglobinemia in newborns. Theoretically, if the activity of this enzyme can be manipulated, it may be possible to safely extend the cumulative iNO exposure in young infants requiring iNO therapy.

Our observations in full term neonates may not be applicable to pre-term neonates, older children or adults. Pre-term neonates have an increased vulnerability to the development of metHgb because the delicate skin is more permeable to absorbing oxidizing agents.[19,20] Furthermore, the metHgb NADH-cytochrome *b*5 reductase activity increases substantially beyond twelve weeks of age.[21] Therefore, older children and adults may have a greater tolerance to iNO exposure and metHgb oxidation.

A second limitation of our study is the relatively narrow range of maximum iNO concentrations used to treat the newborns in our review. The maximum iNO concentration ranged from 10 to 80 ppm. However, the majority of our newborns with high cumulative iNO exposure tended to also have a high maximum iNO concentration. Therefore, we cannot be certain that low iNO concentration for a long duration would yield significant metHgb elevation. For example, it would be interesting to observe whether a prolonged exposure (e.g. 5 ppm for 400 h) carries the same risk for elevated metHgb levels as an abbreviated exposure to a high dose (e.g. 40 ppm of iNO for 50 h).

Interestingly, in our patient population, males were more likely to develop maximum metHgb levels >4%. However, male newborns also had higher ΣiNO (2549 ± 1764 versus 1341 ± 1211 ppm × h). Whether there is a gender specific predisposition to developing PPHN or elevated levels of metHgb is unclear and could not be readily addressed in our small, observational study.

Although we found a relationship between cumulative iNO exposure and metHgb, none of our cases had levels greater than 8%; classic methemoglobinemia is not known to occur with levels less than 10%.[22] However, it is possible that even long-term effects may result when metHgb levels increase above the normal physiological level. This could not be addressed in our small, retrospective analysis and should be studied.

In summary we found that cumulative iNO exposure was better than either maximum iNO concentration or duration of iNO therapy in predicting elevated metHgb levels, and that significant elevation of metHgb can occur even when iNO is limited to a maximum concentration of 40 ppm. We therefore recommend that cumulative iNO exposure be followed closely on infants receiving this therapy.

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