Original article

The use of nitric oxide in premature neonates: a 15-year retrospective chart review

Kristen Haakons, Susan Ireland, Yogavijayan Kandasamy

Department of Neonatology, The Townsville Hospital, Douglas, Queensland, Australia

Abstract

Aim: We aim to describe some characteristics of premature neonates which may predict response to inhaled nitric oxide (iNO).

Methods: Neonates < 37 weeks without congenital abnormalities who received a single episode of iNO between January 2002 to December 2016 were included in this retrospective chart review. For the purposes of this study, we defined a response to iNO as being any reduction in fraction of inhaled oxygen (FiO_2) at the time of iNO weaning.

Results: 52 infants with a 57.7% overall survival were included. There was no significant difference in survival between gender or birth weight groups. Responders were found to be of older gestational age (p = 0.045), had a longer duration of iNO treatment (p = 0.004), longer time to weaning (p = 0.014) and earlier initiation of treatment (p = 0.010). Infants < 1,000 g were less likely to respond to iNO therapy (p = 0.006) and had a higher FiO_2 at weaning (p = 0.037). Gender had no effect on response to iNO therapy (p = 0.176). Infants with preterm premature rupture of membranes (PPROM) were treated for longer prior to weaning (p = 0.025), treated for longer overall (p = 0.005) and had a lower FiO_2 at weaning (p = 0.018). There was no significant correlation between methaemoglobin level and duration of iNO (R = -0.08; p = 0.57).

Conclusions: We found that premature infants with birth weight > 1,000 g and older gestational age were more likely to respond to iNO therapy. Our findings also suggest that infants with PPROM may benefit from the use of iNO for respiratory distress. As a result, these findings support the individualized use of iNO in select premature infants whose premorbid characteristics deem them more likely to have a positive response.

Keywords

Inhaled nitric oxide, premature rupture of membranes, preterm infants, pulmonary hypertension, respiratory disease, respiratory distress.

Corresponding author

Dr. Kristen Haakons, Department of Neonatology, The Townsville Hospital, 100 Angus Smith Dr, Douglas, Queensland 4814, Australia; tel.: 07 4433 1111; fax: 07 4433 2981; email: kristen.haakons@health.qld.gov.au.

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Introduction

Nitric oxide (NO) has long been known to reduce blood vessel resistance in the human body, such as during the transition from fetal to infant respiratory physiology [1]. The effect of NO has been extensively studied in animal models as well with pulmonary vascular resistance being regulated both exogenously and endogenously by NO in fetal animals [2, 3]. It has also been shown to reduce neutrophil-mediated lung inflammation and enhance lung growth and development [4, 5]. Findings in animal models also suggest that inhaled nitric oxide (iNO) reduces pulmonary hypertension without affecting systemic circulation [6-9]. Therefore iNO could be of benefit to premature infants with respiratory distress from a variety of aetiologies.

The efficacy of iNO in treating respiratory distress and its ease of administration as a gaseous agent has lead to a vast amount of research in the use of iNO for respiratory distress in newborns. As a result, some large placebo-controlled trials have shown that iNO reduced the risk of death and the need for extracorporeal membrane oxygenation in term or near-term infants with hypoxic respiratory failure [10]. However, less data exists for premature infants < 34 weeks and is even more limited in the extremely premature population.

Meta-analysis of 17 randomised controlled trials of iNO treatment in premature infants failed to show a positive effect on pulmonary outcomes, survival or neurodevelopmental outcomes [11, 12]. Inclusion and exclusion criteria, as well as patient characteristics, duration of treatment and end-point measurements, even definitions of prematurity and a positive response to iNO have varied between studies, thus making it difficult to draw conclusions about the possible benefits of iNO in infants < 34 weeks [11, 12]. The current National Institutes of Health consensus is that there is no indication for its generalized use in infants < 34 weeks [13].

In this retrospective chart review we identified the clinical situations in which iNO was used by neonatologists in our department and the characteristics and demographics of the babies on whom this treatment was deemed successful.

Methods

We performed a retrospective chart review using information collected for the local neonatal intensive care unit (NICU) database at The Townsville Hospital. It is a tertiary level teaching hospital that caters for more than 10,000 births per year with a 50-bed Neonatal Unit consisting of a NICU and a special care nursery. It is the only level six tertiary referral centre, the highest level of care for neonates, north of Brisbane and as a result all high-risk deliveries in Northern Queensland are referred to the department.

The neonatal database was reviewed with The Townsville Hospital's Human Research Ethics Committee approval and any infant < 37 weeks who received iNO therapy between January 2002 to December 2016 was included. Corresponding medical charts were reviewed for details on iNO initiation and duration. Patient data such as birth weight, gestational age, time to commencement of iNO, maximal iNO dose and fraction of inhaled oxygen (FiO_2) were recorded. Any infants with major congenital abnormalities were excluded from final analysis. In addition, babies with multiple episodes of treatment with iNO were not included.

For the purposes of this study, we defined a response to iNO as being any degree of reduction in FiO_2 at the time of iNO weaning compared to immediately before initiation. These FiO_2 values were available for all individuals in this study except eight.

Statistical analyses were performed using SPSS® Statistics (Version 23, IBM® Analytics, Armonk, New York). A Shapiro-Wilk Normality Test was performed on all continuous numerical variables, including subgroup distributions. Data are presented as median (Interquartile Range) or percentage of total for categorical variables. Mann-Whitney U test, Fisher's Exact and X^2 test were used where appropriate for comparisons between sub-groups with a p-value of < 0.05 being considered significant.

Results

A total of 60 infants < 37 weeks gestation were treated with iNO between January 2002 and December 2016. Three of these infants were diagnosed with diaphragmatic hernias while an additional 5 infants received two separate episodes of iNO and were therefore excluded from final analysis. Of the 52 infants meeting inclusion criteria, 24 (46.2%) were male and 14 (26.9%) were outborn. The median gestational age was 28.1 (25.5-31.1) weeks and the median birth weight was 1,110.0 (775-1,601) grams. The most common indications for iNO are listed in **Tab. 1**. Other less common indications for commencing iNO included asphyxia, hypercarbaemia, pneumothorax, right ventricular failure and pulmonary hypoplasia. Overall, 57.7% (30 babies) survived their NICU admission and 27.4% (14 babies) of infants suffered from periventricular leukomalacia (PVL) or an intraventricular haemorrhage (IVH) of any grade of severity. The mean starting dose of iNO was 20.3 ± 0.3 mean starting dose. There was no significant difference in the maximum iNO dose used in the infants who survived versus those who died.

Infant characteristics, physiological measurements and treatment parameters were compared between subgroups such as gender, extremely low birth weight (ELBW, ≤ 1,000 g) and preterm premature rupture of membranes (PPROM). Between genders, we found no significant differences in patient demographics, total admission (p = 0.720) or intubation time (p = 0.112), need for inotropes (p = 0.756), iNO therapy parameters, response to iNO therapy (p = 0.176), combined rate of IVH or PVL (p = 0.375), or survival (p = 0.634) between male and female infants.

Survival was 50.0% (11/22) in ELBW babies. **Tab. 2** shows that ELBW babies received a shorter duration of iNO (44.5 [15.3-68.3] versus 79.5 [53.8-111] hours total; p = 0.015) compared to babies weighing 1,000 grams or more at birth. However, ELBW babies also had a significantly higher FiO_2 requirement at wean (0.52 [0.42-0.89] versus 0.40 [0.29-0.51]; p = 0.037) and poorer response to iNO treatment (52.9% versus 89.3% responders; p = 0.006). There was no significant difference in the combined rate of IVH or PVL or in survival between the two groups.

Table 1. Most common indications for inhaled nitric oxide (iNO) therapy (n = 52).

Persistent pulmonary hypertension	16 (30.8%)
Increased ventilator requirements	11 (21.2%)
Increased oxygen requirements	4 (7.7%)
Septicaemia	4 (7.7%)
Hyaline membrane disease	4 (7.7%)
Bronchopulmonary dysplasia	3 (5.8%)
Respiratory failure	3 (5.8%)
Other	7 (13.5%)

Table 2. Difference in demographics and inhaled nitric oxide (iNO) treatment between babies with extremely low birth weight (ELBW, ≤ 1,000 g) and those > 1,000 g.

	ELBW (n = 22)	> 1,000 g (n = 30)	p-value
Birth weight (g)	775 (731-887.5)	1,550 (1,284.3-2,301.5)	
Gestational age (weeks)	25.3 (24.2-25.9)	30.2 (28.2-30.2)	< 0.001
Days in hospital (days)	97 (48.8-139.5)	22 (11.8-54.8)	0.083
Age at initiation (hours)	22.7 (12.5-255.8)	7.2 (3.6-28.6)	0.092
Total iNO (hours)	44.5 (15.3-68.3)	79.5 (53.8-111)	0.015
Time to decrease iNO (hours)	22 (8.8-32.8)	38 (19.3-79.8)	0.083
Difference in FiO_2 between start and decrease iNO	0.22 (-0.26-0.59)	0.45 (0.29-0.6)	0.111
FiO_2 at start	0.96 (0.63-1)	0.96 (0.8-1)	0.651
FiO_2 at decrease iNO	0.52 (0.42-0.89)	0.40 (0.29-0.51)	0.037
Maximum methaemoglobin	1.2 (1-1.35)	1.2 (1.05-1.5)	0.109
Total days intubation	6 (3-10)	11 (6-21)	0.070
Response to iNO	9/17 (52.9%)	25/28 (89.3%)	0.006
IVH/PVL	5/22 (22.7%)	9/29 (31.0%)	0.510
Survival	11/22 (50.0%)	19/30 (63.3%)	0.336

Results presented as median (IQR) or number (percentage) as appropriate.
ELBW: extremely low birth weight; iNO: inhaled nitric oxide; FiO_2: fraction of inhaled oxygen; IVH: intraventricular haemorrhage; PVL: periventricular leukomalacia.

We also compared individual characteristics and treatment parameters of the 21 babies with PPROM versus those without. Infants in the PPROM group were 1,131 (813-1,742) grams with a gestational age of 29.2 (25.9-31.8) weeks. 10 (47.6%) infants in the PPROM group were male. Although the demographics were not significantly different between groups, babies with PPROM were treated with a longer total duration of iNO (duration of 80.5 [56.5-94.5] versus 55.0 [23.0-88.5] hours; p = 0.005) and were treated for longer before weaning commenced (31.0 [20.3-101.8] versus 25.5 [8.75-54.3] hours; p = 0.025). Babies with PPROM also had a lower FiO_2 at decrease compared to non-PPROM babies (0.33 [0.27-0.4] versus 0.52 [0.42-0.9]). However, there were no significant differences in survival or response to iNO therapy between groups.

Characteristics of responders were compared with non-responders in **Tab. 3**. Responders were found to be of older gestational age (29.4 [27.4-32.7] versus 25.6 [24.4-27.6] weeks) and had iNO initiated more quickly after birth (8.1 [3.4-22.7] versus 57.8 [21-361.3] hours). Responders also received a longer duration of iNO (71 [54.5-97.5] versus 17.5 [8.5-61] hours) and were maintained on treatment doses for longer (32.5 [20.5-64] versus 9.5 [5-22] hours) prior to weaning. We did not find any significant correlation between methaemoglobin level and duration of iNO (R= -0.08; p = 0.57).

Discussion

From our chart review of 52 premature infants receiving single episodes of iNO over a 15-year period, we found that premature babies who were of older gestational age were significantly more likely to respond to iNO therapy. We also found that responders had iNO initiated more quickly after birth suggesting that early initiation may play a role in response to iNO.

iNO is a selective pulmonary vasodilator which also reduces ventilation-perfusion mismatch and improves overall oxygenation of infants [4-9]. As a result, iNO is frequently used in term infants with hypoxic respiratory distress; however, its use in premature babies remains a contentious issue. An Australian cross-sectional survey of NICU directors found a wide variation in the protocol and the clinical indicators used to initiate and monitor response to treatment with iNO [14]. It is this heterogeneity which has made it difficult to identify potential characteristics or indications for which iNO may be beneficial in premature infants [11-13].

Despite the conflicting evidence and lack of clear guidelines, all tertiary perinatal centres in Australia continue to use iNO in premature infants who have failed other therapies [11]. In fact, the use of iNO in premature infants increased 6-fold in the United States between 2000 and 2008 with the largest increase occurring in infants between 23 and 28 weeks of age [15]. From our retrospective audit, we identified that iNO is being used by our department as early rescue therapy for a variety of clinical indications similar to other centres across the country [14]. We also noted that our department typically used a higher starting dose of 20 ppm iNO compared to the majority of Australian centres [14]. This was justified by a study in full-term and nearly full-term infants which found

Table 3. Difference in demographics and inhaled nitric oxide (iNO) treatment between babies who responded to iNO treatment and those who were non-responders.

	Responders (n = 34)	Non-responders (n = 11)	p-value
Birth weight (g)	1,355 (1,046-1,857.5)	875 (775-1,149)	0.139
Gestational age (weeks)	29.4 (27.4-32.7)	25.6 (24.4-27.6)	0.045
Days in hospital (days)	41 (14.5-89.5)	33 (19.5-107.5)	0.895
Age at initiation (hours)	8.1 (3.4-22.7)	57.8 (21-361.3)	0.010
Total duration iNO (hours)	71 (54.5-97.5)	17.5 (8.5-61)	0.004
Time to decrease iNO (hours)	32.5 (20.5-64)	9.5 (5-22)	0.014
Maximum methaemoglobin	1.2 (1-1.5)	1.3 (1.1-1.6)	0.326
Total days intubation	10 (5.5-16.5)	7 (1.5-25.5)	0.632
IVH/PVL	10/34 (29.4%)	2/11 (18.2%)	0.699
Survival	22/34 (64.7%)	6/11 (54.5%)	0.722

Results presented as median (IQR) or number (percentage) as appropriate.
iNO: inhaled nitric oxide; IVH: intraventricular haemorrhage; PVL: periventricular leukomalacia.

that infants who received 20 ppm of iNO had significantly greater improvement in PaO_2 and oxygenation index without increasing mortality [16]. Furthermore, we found no indication of toxicity in the form of maximum methaemoglobin at this level of iNO therapy.

Sub-group analysis of all premature babies receiving iNO in our department over the past 15 years found that ELBW infants were significantly less likely to respond to iNO therapy. These infants also required a shorter duration of treatment than infants weighing > 1,000 g at birth, likely due to the fact that they were deemed to be non-responsive clinically. We also found that premature infants who responded to iNO were of significantly more advanced gestational age. This is concordant with findings of previous studies in which infants > 1,000 g treated with iNO had a significantly lower rate of death than a placebo group [17-19]. Previous retrospective chart reviews have also found that infants with higher birth weight and more advanced gestational age were more likely to respond to iNO treatment [20, 21]. One study also found that earlier initiation of iNO leads to increased likelihood of a positive response [20]. This suggests that early initiation of iNO may increase the likelihood of a positive response to therapy.

These results suggest that response to iNO treatment may be dependent upon the development of NO-responsive pulmonary vasculature at later stages of gestation. The pre-acinar branching pattern of fetal lungs arises by the 20th week of gestation while the development of intra-acinar arteries and veins begin to form at approximately 28 weeks and even after birth [22, 23]. In addition, vasodilation is mediated, at least partially, by NO release from pulmonary nerves which increase in number with age [22]. Our results suggest that the intra-acinar vasculature is the primary location at which iNO produces a vasodilatory response. Therefore, iNO treatment may only be beneficial in infants who have the physiological capability to respond to therapy.

Our sub-group analysis also found that infants with PPROM were treated with iNO for significantly longer and had to be weaned later compared to their peers. While these therapeutic differences did not lead to a statistically significant difference in response rate, we would have predicted that iNO would be particularly beneficial in PPROM infants due to the effects of oligohydramnios on lung development and pulmonary vasculature. This leads to further alveolar hypoplasia, ventilation-perfusion mismatch and, subsequently, persistent pulmonary hypertension of the newborn which may be partially reversible with iNO therapy [24-28]. Previous studies found that low birth weight infants with PPROM and oligohydramnios had a significantly higher PaO_2 after 2 hours of iNO therapy compared to a non-iNO group [25]. In a separate study, infants with oligohydramnios and PPROM treated with iNO had better oxygenation and reduced rates of death with no effect on rates of severe IVH or PVL [28]. Interestingly, our PPROM subgroup of patients did have significantly lower FiO_2 requirements at weaning of iNO suggesting that treatment with iNO was potentially beneficial in these babies.

One major difficulty in justifying the use of iNO in premature infants lies in the definition of a "clinical response". We chose to define response as any decrease in FiO_2 at the time of weaning compared to initiation of therapy due to inherent limitations in a retrospective review. Currently, there is no consensus on how a responder should be defined and multiple definitions have been used in previous studies as a proxy. Oxygenation index was used in many studies while others used pre-defined increases in PaO_2 as an indicator of response [16, 18, 20, 21]. A recent retrospective cohort study chose a reduction in FiO_2 of greater than 20% within 1 hour of treatment as a clinical definition of response [29]. Finally, some studies have used composite endpoints such as chronic lung disease with death as a way of determining the effects of iNO treatment on premature babies [8, 17-19, 21, 30]. While composite endpoints provide an estimate of the long-term effects of iNO, a lack of state-wide digital hospital records makes it impractical to use long-term measures as a referral centre.

Other limitations in our study are inherent to the retrospective study design and the nature of our NICU as a major referral centre for a geographically large area. Having such a large number of referred infants prohibited assessment of important sequelae of iNO treatment such as neurodevelopmental outcome. A lack of written protocol within our department or a national guideline for iNO therapy also created additional heterogeneity within our study which made interpretation of some results difficult. There was also variation in the method of delivery of iNO in our population. Although all babies

were intubated, the method of ventilation varied between infants. However, previous studies found no significant difference in the rate of death between types of ventilation used to deliver iNO; therefore, this is unlikely to have had a major effect on our analysis [30].

An early uncontrolled study of the use of iNO in preterm babies showed a high rate of IVH [31]. iNO has also been found to increase bleeding time in term infants, reduce platelet aggregation, limit venous thrombosis and inhibit cytokines which may contribute to the pathogenesis of IVH and PVL [32-38]. Both of these pathologies are thought to be the primary cause of serious long-term neurological deficits in this population. Although we were unable to collect long-term data on neurological outcome, our acute combined incidence of IVH and PVL was similar to a randomized, double-blind placebo-controlled study which showed no significant differences in IVH and PVL incidence [30]. This provides reassurance that, despite early studies, the use of iNO in preterm infants does not appear to increase the risk of neurological deficits.

Conclusion

This chart review agrees with previous studies which have found that premature infants of higher birth weight and older gestational age have better outcomes with iNO therapy. This may be due to the development of the pulmonary vasculature and therefore the ability for iNO to promote selective vasodilation. Our findings also suggest that infants with PPROM may benefit from the use of iNO for respiratory distress. While these findings should be noted cautiously, they lend support for the individualized use of iNO in select premature infants.

Abbreviations

ELBW: extremely low birth weight

FiO_2: fraction of inhaled oxygen

iNO: inhaled nitric oxide

IVH: intraventricular haemorrhage

NICU: neonatal intensive care unit

NO: nitric oxide

PPROM: preterm premature rupture of membranes

PVL: periventricular leukomalacia

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Declaration of interest

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