Original article

Effects of inhaled nitric oxide in neonatal hypoxemic respiratory failure from a multicenter controlled trial

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Keywords: *hypoxemic respiratory failure; neonate; nitric oxide; respiratory therapy*

Background Hypoxemic respiratory failure (HRF) is one of the most common causes for neonatal infants requiring aggressive respiratory support. Inhaled nitric oxide (iNO) has been established routinely as an adjunct to conventional respiratory support in developed countries. The aim of this study was to investigate effects of iNO in neonates with HRF in resource limited condition with no or limited use of surfactant, high frequency oscillatory ventilation (HFOV) and extracorporeal membrane oxygenation.

Methods A non-randomized, open, controlled study of efficacy of iNO was conducted over 18 months. Eligible term and near-term neonates from 28 hospitals with HRF (oxygenation index >15) were enrolled prospectively into two groups as either iNO or control. Oxygenation improvement and mortality as primary endpoint were determined in relation with dosing and timing of iNO, severity of underlying diseases, complications and burden. Intention-to-treat principle was adopted for outcome assessment. Response to iNO at 10 or 20 parts per million (ppm) was determined by oxygenation in reference to the control (between-group) and the baseline (within-group).

Results Compared to 93 controls, initial dose of iNO at 10 ppm in 107 treated infants significantly improved oxygenation from first hour (P=0.046), with more partial- and non-responders improved oxygenation with subsequent 20 ppm NO (P=0.018). This effect persisted on days 1 and 3, and resulted in relatively lower mortalities (11.2% vs. 15%) whereas fewer were treated with surfactant (10% vs. 27%), HFOV (<5%) or postnatal corticosteroids (<10%) in both groups. The overall outcomes at 28 days of postnatal life in the iNO-treated was not related to perinatal asphyxia, underlying diseases, severity of hypoxemia, or complications, but to the early use of iNO. The cost of hospital stay was not significantly different in both groups.

Conclusions With relatively limited use of surfactant and/or HFOV in neonatal HRF, significantly more responders were found in the iNO-treated patients as reflected by improved oxygenation in the first three days over the baseline level. It warrants a randomized, controlled trial for assessment of appropriate timing and long-term outcome of iNO.

Chin Med J 2011;124(8):1156-1163

Hypoxemic respiratory failure (HRF) is one of the most common causes for term and near-term neonatal infants requiring aggressive respiratory support, often leading to death or severe disabilities. In the past decade, inhaled nitric oxide (iNO) has been established as a routine, adjunctive respiratory therapy with conventional respiratory support, through selective dilatation of pulmonary vasculature, alleviating pulmonary hypertension associated ventilation-perfusion mismatching and hypoxemia, significantly reduced both death rate and dependence on extracorporeal membrane oxygenation (ECMO) as life support.[1] In the published randomized, controlled trials of iNO, surfactant, high frequency oscillatory ventilation (HFOV), postnatal corticosteroids, among other therapies, are generally used without restriction, ranging from 50%–90%.[1-10] However,

DOI: 10.3760/cma.j.issn.0366-6999.2011.08.006
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This study was supported by grants from Project 211 (Phase III), Ministry of Education; China-Canadian Joint Health Research Program (No. 30611120518), Program for Outstanding Medical Science Leader (No. LJ06038), and the Excellence of Institute Development in Neonatal Medicine, Shanghai Municipal Department of Health.
Disclosure of conflicts of interests: Dr. Sun B discloses that he is one of the patent inventors of the NO delivery device in part used in this study, with Children's Hospital of Fudan University as the patent owner, and he is also committed to the research and development of iNO technology in the Shanghai Noventek Inc.

it is not clear how the effects are solely by iNO as it may be used in resource limited conditions where above mentioned adjunctive respiratory therapies are not extensively used in routine practice. Furthermore, recommended maximum iNO dosage as concentration in ventilator circuit is equal to or less than 20 parts per million (ppm, vol/vol, $\times 10^{-6}$ concentration),[1-4] and follow-up studies do not show increased risk in adverse outcome of long-term neuro-motor development,[11] or lower psychomotor developmental index scores.[12] A very recent report from the European iNO registry[13] demonstrated very similar outcome in routine use of iNO as in the randomized controlled trials. No data so far have reported in the efficacy of iNO with regard to overall outcome assessment where the use of surfactant, HFOV, corticosteroids and ECMO was limited.

Another controversial issue is about initial dose of iNO. Although iNO at 2–6 ppm was tested versus the highest NO concentration (20 ppm) in various controlled trials,[5-10] no definitive conclusion has been achieved yet as to whether initial dose of iNO at low (<5 ppm) or high (20 ppm) level is more effective and safer than the other. Furthermore, when no routine use of surfactant and HFOV is provided, to achieve functional residual capacity during cyclic ventilation of the sick lungs, positive end-expiratory pressure (PEEP), mean airway pressure (MAP) and fraction of oxygen (FiO_2) may be set high to facilitate oxygenation. Thus NO diffusion may be enhanced and pulmonary vascular resistance be alleviated, thereby reducing oxygen exposure and ventilator settings.

As all the provincial and most sub-provincial tertiary pediatric centers and maternity hospitals in China have established neonatal intensive care unit (NICU) in recent years, implementation of advanced respiratory therapy is compelling and in priority, as these NICUs treat substantial numbers of patients with HRF as a population at high risk of death, the use of surfactant and HFOV was very limited, and ECMO was not available.[14,15] In order to facilitate effective technology flow to those initially well established NICUs, taken into consideration of service condition, domestic socioeconomic and cultural diversities in local perspective, we conducted a prospective, controlled study of iNO in major tertiary NICUs. We questioned whether, without ECMO and with limited surfactant or HFOV affordability, iNO may exert its therapeutic efficacy similar to that reported from the randomized controlled trials. We speculated that advantage of iNO for rescue use in HRF may be appreciated as well without being confounded by multiple factors such as adjunctive respiratory therapies and underlying pathological conditions.

METHODS

Study design

The protocol for a prospective, multicenter, non-randomized, controlled study was approved by the Ethics Committee of Children's Hospital of Fudan University according to Chinese regulation for clinical investigation. Based on the previous studies in term and near-term neonates with severe HRF dependent on both continuous positive airway pressure (CPAP) and conventional mechanical ventilation (CMV), there was an average survival rate of 70% in respiratory distress syndrome (RDS), meconium aspiration syndrome (MAS) and pneumonia/sepsis in the provincial and sub-provincial NICUs.[14,15] In our previous experience with a randomized controlled trial for testing efficacy of surfactant therapy in MAS, the control group had a survival rate of 90%.[16] It was therefore conceivable that the mortality of HRF in the target subjects might be 10%–30%, and rational to hypothesize that the effect of iNO in the improvement of survival rate through early alteration of hypoxemia without substantial impact of surfactant, HFOV and ECMO should not exceed that reported by the randomized, controlled trials. We thus estimated that using a prospective cohort study to recruit cases should be relevant for each center to adhere to their routine protocol for treatment of HRF with best available care without compromising the study protocol, and to minimize the influence of give-up during the treatment.[14,15] In this manner, we should be able to obtain mortality rate in both iNO treated and non-treated groups with the same severity of underlying diseases and timing of intervention.

HRF was defined as requiring respiratory support with CMV or HFOV when oxygenation index (OI) was higher than 15,[16] and the condition was deemed by the attending staffs to persist for at least 48 hours. OI was derived from an equation of mean airway pressure (cmH_2O) times FiO_2 times 100, divided by PaO_2 (mmHg). After informed consent by the parents or guardians was obtained, for those enrolled in iNO treatment (14 units), an initial iNO dose at 10 ppm was provided. In the same period for the units where facilities of iNO were not available (14 units), based on the same criteria and strategy for HRF, treatment was provided to conform to the study protocol. The control cases may be obtained from the unit with iNO treatment. The primary endpoint was to reduce cumulated oxygen exposure by improving OI and PaO_2/FiO_2, or to reduce mortality before discharge or was fulfilled 28 days of postnatal life. The secondary endpoint was to reduce major complications and their relative risks due to hypoxemia, hyperoxia and mechanical ventilation during neonatal period, with special emphasis on the use of surfactant, HFOV, corticosteroids, and the influence of perinatal asphyxia, initial severity of HRF, and timing of iNO. After approval of the protocol by each participating center's review board for clinical investigation, all engaged research staffs were trained to follow the study protocol, safe use of iNO, report of adverse event, and data collection in a uniform procedure.

Patients

Infants born at more than 34 weeks of gestation who had

diagnosis of HRF were enrolled in the study. Primary causes of HRF were RDS, MAS, pneumonia/sepsis or idiopathic persistent pulmonary hypertension of the newborn (PPHN), and initial blood gas analysis and assisted ventilation indicated OI >15.[16] The decision was made by on-site attending physicians according to the study protocol whether to use iNO (INO group) or not (Control group, Con) in which no placebo was used (see below). At the time of enrollment, all patients were ventilated with either CMV or HFOV. No attempt was made to control the mode of ventilation, however, monitoring and control of exhaled tidal volume was recommended in CMV mode in order to minimize over-ventilation. Echocardiographic evidence of primary or secondary PPHN was demanded but not generally required.

Exclusion criteria were applied in those whose age was more than 28 days postnatally, who had life-threatening congenital malformations, defective cardiac anomaly other than patent ductus arteriosus or patent foramen ovale, congenital diaphragmatic hernia, uncorrected hypotension, severe intraventricular hemorrhage (IVH), pneumothorax or pulmonary hemorrhage, substantial bleeding tendency and other conditions which were defined as unstable conditions. According to the study protocol, each center developed management guidelines to optimize the care of infant at best available level before and after the enrollment. These included life sign monitoring, CMV before or after CPAP, intravascular catheters and intravenous transfusion, inotropes, alkalosis, diuretics, sedatives and corticosteroids. Administration of surfactant or HFOV was accepted before or after enrollment. All these were at attending staffs' discretion.

Administration of iNO and assessment of response

NO was supplied, by either an attached mass flow controller (Shanghai Noventek, Shanghai, China) or a build-in flow controller in ventilator, from a cylinder at a concentration of 1000 ppm balanced by pure nitrogen. Therapeutic NO gas was introduced into the afferent limb of the ventilator circuit near the humidifier, and final NO and NO_2 concentrations were monitored continuously at the afferent limb of the circuit proximal to endotracheal tube by an electrochemical monitor (NOxBOX Plus®; Bedfont Scientific, Rochester, England). The NO gas contained <10 ppm NO_2, and detected level of NO_2 in the ventilator circuit was kept less than 3 ppm when FiO_2 was 0.3–1.0.[17]

For assessment of the treatment response patterns, improvement of oxygenation was evaluated as magnitude change according to the reported trials.[1,2,8,18,19] The response to iNO was evaluated by increment of PaO_2 from baseline to 1 hour after the initiation of iNO at 10 ppm, or after alteration of NO concentration from 10 to 20 ppm for 1 hour when full response to 10 ppm had not been achieved. An increment of PaO_2 >20, 10–20, or <10 mmHg was regarded as full, partial, or no response, respectively. The dose was maintained when the infant had at least partial response after 1 hour. When the infant had no response (increase of PaO_2 ≤10 mmHg) at 20 ppm, the dose was turned back to 10 ppm. The concentration of NO was decreased by 1–2 ppm every 6 to 12 hours until weaning off as long as there was no obvious deterioration in oxygenation regardless of previous response patterns at 10 or 20 ppm of iNO. This dose regimen should be maintained for 3 days or as long as it required. The values of oxygenation in Con were derived from the initial and corresponding intervals as in INO. Values of $PaCO_2$ and pH were collected in series to evaluate the ventilation effects.

Statistical analysis

Intention-to-treat principle was applied in the final data analysis based on assumption that both INO and Con groups had the same severity of HRF irrespective of the constituent of the underlying diseases. Continuous variables for between-group or within-group differences were presented as mean±standard deviation (SD) or median and interquartile range (IQR), and assessed by Student's *t* test or Wilcoxon Mann-Whitney test where appropriate. Incidence in categorical variables was analyzed with chi-square test or Fisher's exact test. A *P* value <0.05 was considered statistically significant. Relative risk is expressed as a factor for death or major complications in both groups, with 95% confidence interval (*CI*). All statistical analyses were performed using SPSS 11.0 software (SPSS Inc., USA).

RESULTS

Baseline characteristics

From March 2007 to August 2008, a total of 200 infants were qualified for the enrollment, in which 107 were treated by iNO, and 93 were controls. Excluded cases at the end of the enrollment include one with unstable condition died 4 hours after the enrollment and 2 with OI <15 at the enrollment. Included in the final data analysis were four infants, 3 in INO and 1 in Con group, who did not completed first 48 hours of ventilation. There were no significant differences between the two groups with regard to birth weight (BW), gender, maternal complications, delivery mode, amniotic fluid status, and 1 and 5 minutes Apgar scores (Table 1). Infants in INO group had higher values of mean gestational age, more with RDS or MAS, but fewer with pneumonia/sepsis. PPHN was considered in both INO (*n*=78) and Con (*n*=33) groups, in which 6 and 3, respectively, were diagnosed as idiopathic, and the others were secondary ones.

The enrollment was generally within the first 3 days after birth in both groups at a median (IQR) study entry time of 16 (8–30) hours for INO and 16 (3–32) hours for Con. INO had lower baseline PaO_2/FiO_2 and higher OI compared to Con. The initial treatment before study entry was not significantly different between the two groups, with less than 10% received surfactant and/or HFOV.

About 1/4 of all the infants received vasopressor support

Table 1. Perinatal characteristics in both groups with hypoxemic respiratory failure

Items	INO (*n*=107)	Con (*n*=93)	*P* values
Birth weight (g)	3197±685	3066±617	0.187
Gestational age (weeks)	39.2±2.2	38.2±2.2	0.002
Male (*n* (%))	75 (70.1)	76 (81.7)	0.057
Delivery (*n* (%))			0.465
Vaginal	34 (31.8)	25 (26.9)	
Elective C-section	39 (36.4)	37 (39.8)	
Mergent C-section	34 (31.8)	31 (32.3)	
Apgar score at 1 minute	8.0 (5.0–9.0)	8.0 (7.0–9.0)	0.090
Apgar score at 5 minutes	9.0 (7.0–10.0)	10.0 (8.0–10.0)	0.012
Contaminated amniotic fluid (*n* (%))			0.901
None	41 (38.3)	49 (52.7)	
I	1 (0.9)	1 (1.1)	
II	9 (8.4)	9 (9.7)	
III	40 (37.4)	21 (22.5)	
Unknown	16 (15.0)	13 (14.0)	
Maternal complications (*n* (%))			
Antepartum hemorrhage	4 (3.7)	6 (6.4)	0.380
Preeclampsia	7 (6.5)	2 (2.1)	0.135
Diabetes	4 (3.7)	4 (4.3)	0.839
Hypertension	1 (0.9)	0 (0)	0.927
Placental abruption	1 (0.9)	1 (1.1)	0.921
None	85 (79.4)	72 (77.4)	0.870

INO: inhaled nitric oxide; Con: control. Some cases may have more than one maternal complication.

Table 2. Baseline characteristics in both groups with hypoxemic respiratory failure

Variables	INO (*n*=107)	Con (*n*=93)	*P* values
Primary cause (*n* (%))			
RDS	45 (42.1)	29 (31.2)	0.112
MAS	38 (35.5)	26 (28.0)	0.253
Pneumonia or sepsis	18 (16.8)	35 (37.6)	0.001
Idiopathic PPHN	6 (5.6)	3 (3.2)	0.418
Age at admission (days)	1.0±1.2	1.1±2.2	0.316
Oxygenation index	28.4±16.5	25.8±17.1	0.079
PaO_2/FiO_2 (mmHg)	47.4±16.3	55.4±18.2	0.006
Treatment before enrollment (*n* (%))			
Volume expander	13 (12.1)	7 (7.5)	0.277
Vasopressor	29 (27.1)	21 (22.6)	0.461
Alkalosis	30 (28.0)	22 (35.5)	0.481
Surfactant	5 (4.7)	6 (6.4)	0.582
HFOV	9 (8.4)	4 (4.3)	0.240

INO: inhaled nitric oxide; Con: control. RDS: respiratory distress syndrome; MAS: meconium aspiration syndrome; PPHN: persistent pulmonary hypertension of the newborn; HFOV: high frequency oscillatory ventilation.

or alkalosis before enrollment. There was no difference in intravascular volume support and corticosteroids (Table 2). There was no difference in $PaCO_2$ and pH in the first three days after enrollment (data not shown).

Oxygenation improvement and reduction of mortality

One hour after the enrollment, INO group had a significantly greater increase in the mean ± SD levels of PaO_2 (relative changes over the pre-treatment conditions, (24.3±29.3) vs. (17.7±19.1) mmHg), PaO_2/FiO_2 ((53.8±61.0) vs. (30.7±38.6) mmHg). Corresponding relative changes over the pre-treatment condition for OI over the first 3 days are shown in the Figure. Although there were no significant differences between the two groups at each time point, these changes were significantly found only in INO group. Compared to Con, more infants in INO who had partial and no response from 10 ppm of iNO responded to 20 ppm. When

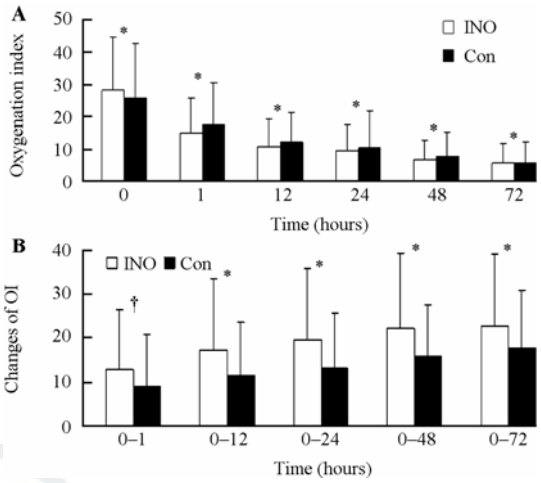

Figure. Changes of oxygenation index (OI) in both groups. **A:** Changes of OI in the first 72 hours. **B:** Relative changes of OI to the baseline level (0). Numbers of values calculated: 93–107 at 0–1 hour, 82–101 at 12 hours, 81–95 at 24 hours, 85–95 at 48 hours, and 73–81 at 72 hours. $^*P$ <0.05, $^\dagger P$ <0.01 vs. Con (in **A**) or vs. baseline level (time 0–1 in **B**)

Table 3. Response patterns in both groups with hypoxemic respiratory failure

Variables	INO (*n*=107)	Con (*n*=93)	*P* values
Change in PaO_2 (mmHg)	19.5 (7.3–34.0)	13.2 (6.0–25.0)	0.077
Change in PaO_2/FiO_2 (mmHg)	35.3 (13.1–74.0)	22.2 (7.1–38.4)	0.005
Change in oxygenation index	10.3 (6.2–16.6)	5.4 (1.9–10.6)	0.001
Response at 10 ppm (*n* (%))			0.046
Complete	49 (45.8)	22 (23.6)	
Partial	25 (23.4)	24 (25.8)	
None	33 (30.8)	47 (50.6)	
Response at 20 ppm (*n* (%))			0.018
Complete	11 (19.0)	11 (15.5)	
Partial	30 (51.7)	22 (31.0)	
None	17 (29.3)	38 (53.5)	
Combined response (*n* (%))			<0.001
Complete	60 (56.1)	33 (35.5)	
Partial	35 (32.7)	28 (30.1)	
None	12 (11.2)	32 (34.4)	

Values are acute changes or responses during the first hour of inhaled nitric oxide (INO) treatment and expressed as median (interquartile range) or number (%). The response at 20 ppm was from the subsequent hour in those from partial and none response to 10 ppm. The combined response was summarized from all those responded to either 10 or 20 ppm. Values of blood gas in the control (Con) group were obtained from the corresponding time as in INO group after enrollment.

combining both NO concentrations in INO group for response, the proportion of infants having complete and partial response was significantly higher than Con (Table 3). By post hoc analyses, the response to iNO was associated with underlying disease in those with RDS and MAS, but not in those with pneumonia/sepsis. These effects persisted on day 1 ((8.9±11.7) in Con vs. (13.0±13.3) in INO, P <0.05) and day 3 ((17.6±13.2) vs. (22.6±16.5), P <0.05), respectively, as relative changes of OI from the pre-treatment, baseline level. Twenty-six patients died before discharge in both groups without significant difference, however, those treated by HFOV in Con had significantly higher death rate (Table 4). In those whose underlying diseases were RDS, a trend of higher survival

rate was seen in INO group than in Con (Table 5).

Table 4. Primary and secondary outcomes, relative risks, and disease burden in both groups with hypoxemic respiratory failure

Items	INO (*n*=107)	Con (*n*=93)	Relative risks (95% *CI*)	*P* values
Death (*n* (%))	12 (11.2)	14 (15.0)	0.7 (0.3–1.6)	0.421
Surfactant (died)	11 (3)	25 (2)	4.3 (0.6–30.7)	0.123
HFOV (died)	8 (1)	16 (5)	0.3 (0.03–3.3)	0.005
Corticosteroids (died)	9 (2)	8 (2)	0.8 (0.1–8.1)	0.893
Complications (*n* (%))				
Air leak	10 (9.3)	13 (14.0)	0.6 (0.3–1.5)	0.306
Pulmonary hemorrhage	7 (6.5)	3 (3.2)	2.1 (0.5–8.4)	0.283
Pneumonia/sepsis	40 (37.4)	35 (37.6)	1.3 (0.8–2.3)	0.316
PDA	0 (0)	1 (1.1)		0.915
IVH III-IV	1 (0.9)	0 (0)		0.927
LOT (days)	10.1±5.7	10.6±10.6	–	0.466
LOV (days)	5.6±3.1	5.8±6.8	–	0.527
LOS (NICU) (days)	13.8±8.4	11.9±11.5	–	0.010
COS (NICU) $\times 10^3$	23.7±27.8	20.7±19.5	–	0.119
LOS (hospital) (days)	17.1±8.8	17.4±11.7	–	0.666
COS (hospital) $\times 10^3$	25.8±27.9	23.7±19.0	–	0.680

Values are number (%) or means ± SD; relative risks denote either death (overall or with additional therapies as listed) or complications between the two groups. COS: cost of stay in Chinese Yuan (CNY); HFOV: high frequent oscillation ventilation; IVH: intraventricular hemorrhage; LOS: length of stay; LOT: length of oxygen therapy; LOV: length of mechanical ventilation; PDA: patent ductus arteriosus.

Mortality, complications, relative risks and burden

Among the survivors, there were no differences between the two groups with regard to the length of respiratory support (oxygen therapy or mechanical ventilation), or the incidence of complications, such as air leak, pneumonia/sepsis and pulmonary hemorrhage (Table 4). Length and cost of NICU stay in INO group were higher than in Con, but these differences were not significant for the length and cost of hospital stay. Meanwhile, the use of surfactant after enrollment was low in both groups, but significantly more surfactant was used in Con. There were no differences between the two groups in HFOV, corticosteroid, sedation, intravenous volume, alkalosis, vasopressor, corticosteroids, analgesia or neuromuscular blockade (data not shown). When comparing all those treated with surfactant in both groups, those from INO had lower Apgar score (6.9±2.7 vs. 7.7±2.1, *P*=0.090), higher baseline OI (28.4±16.5 vs. 25.8±17.1, *P*=0.123) and better improvement of OI at 1 and 72 hours (13.0±13.3 vs. 8.9±11.7, *P*=0.001; 22.6±16.5 vs. 17.6±13.2, *P*=0.026, relative to the respective baseline OI values). The dosage of surfactant in both groups after the enrollment was 100 mg/kg. These suggest that they had much severe condition at the enrollment and better but transient response to iNO treatment. However, their outcome was not optimal.

By further comparison of the outcomes between the two groups (Table 5), no significant differences were found with regard to the relative risk of the mortalities in subgroups with primary disease diagnosis, baseline OI, perinatal asphyxia, timing of enrolment, and surfactant use, as assessed by stratified values. The subgroup analysis of the outcomes, disease burden and response to iNO in both groups stratified by disease severity as reflected by OI and age at the enrolment were also performed. Baseline OI between 20–25 tended to have significantly longer length of ventilation, oxygen therapy and length and cost of NICU stay in INO compared to Con, but such influence was not shown in those whose OI was 15–20 or >25, though cost of NICU was significantly high in INO. It also showed that for those enrolled in 24–48 hours, length of ventilation or NICU stay was significantly longer in INO than in Con, whereas such difference was not found in those enrolled before 24 hours.

There was no premature discontinuation of iNO as no adverse effect was reported. Detected NO_2 in ventilator circuit was kept below 3 ppm. Methemoglobin level was not routinely determined as very short period (60 minutes) of 20 ppm was administered and most of the time iNO

Table 5. Death rate and its relative risks associated with enrollment in both groups (*n/N* (%))

Items	INO (*n*=107)	Con (*n*=93)	Relative risks (95% *CI*)	*P* values
Asphyxia before enrollment				
Yes	4/41 (9.8)	3/27 (11.1)	0.86 (0.18–4.21)	0.857
No	4/46 (8.7)	7/54 (13.0)	0.64 (0.18–2.34)	0.497
Unknown	4/20 (20.0)	4/12 (33.3)	0.50 (0.10–2.54)	0.399
Age at enrollment (hours)				
≤24	8/73 (11.0)	10/58 (17.2)	0.59 (0.22–1.61)	0.300
24–48	0/20 (0)	1/24 (4.2)	1.15 (0.07–19.60)	0.932
48–72	2/9 (22.2)	3/11 (27.3)	0.76 (0.10–5.96)	0.795
Primary diagnosis				
RDS	3/45 (6.7)	6/29 (20.7)	0.27 (0.06–1.20)	0.072
MAS	6/39 (15.4)	5/26 (19.2)	0.76 (0.21–2.82)	0.685
Pneumonia/sepsis	3/17 (17.6)	2/34 (5.9)	3.43 (0.52–22.84)	0.183
Idiopathic PPHN	0/6 (0)	0/3 (0)	–	–
Oxygenation index at enrollment				
15–20	3/41 (7.3)	7/47 (14.9)	0.45 (0.11–1.87)	0.264
20–25	2/16 (12.5)	4/18 (22.2)	0.56 (0.09–3.49)	0.435
≥25	7/50 (14.0)	3/28 (10.7)	1.36 (0.32–5.72)	0.677
Surfactant use after enrollment				
Yes*	3/11 (27.3)	2/25 (8.0)	4.31 (0.61–30.67)	0.123
No	9/96 (9.4)	12/68 (17.6)	0.48 (0.19–1.22)	0.118

Values are death number/total number (%).*Only infants received surfactant after the enrollment were included regardless whether surfactant had been used before the enrollment or not.

was kept below 10 ppm.

DISCUSSION

In the present study, we succeeded in evaluation of efficacy of iNO in term and near-term infants with HRF through the prospective cohort study. Our major findings suggest that iNO at 10 (and 20) ppm improved oxygenation during early postnatal life as in the previously reported randomized, controlled studies [1-10] or a routine use of iNO from the European iNO Registry.[13] There was no significant increase in the major complications or reduction of mortality in iNO treated infants. These NICUs were generally representative of high service level and capacity at provincial and sub-provincial tertiary centers, however physician- or nurse-to-patient ratio remained lower (data not shown) compared to their work load, limited facilities and experience for respiratory and intensive care, along with lower health insurance coverage of the infants,[14,15] in comparison with the NICU conditions in the developed countries. Several factors are likely involved in the overall efficacy of iNO in this cohort.

Mortality

The mortality in both groups was lower than what we anticipated during the study design, whereas the average values of baseline OI in INO and Con at the study enrollment was 28.4 and 25.8, respectively, similar to that reported by Davidson et al[4] and the European iNO Registry,[13] lower than those by NINOS or others,[1-3] but higher than that of Konduri et al's.[9] As shown in Table 4, very small proportions of neonates in both groups were treated by surfactant, HFOV or corticosteroids. Their influence to the final outcome should be unlikely significant despite the baseline OI was relatively low compared to the data from randomized, controlled trials.[1-4] We also encountered relatively low rate of give-up negotiation (data not shown), which was different from our previous observational studies with severe cases of HRF.[14,15] We therefore speculate that, as the patients were enrolled in the investigation, they might have been under more deliberate care of the attending staffs in contrast to those not subjected to such studies.[14,15] As OI is a dynamic parameter for clinical assessment of ventilator strength in oxygenation, it suggests that in both groups most of the patients tolerated mechanical ventilation, as corroborated by the stable levels of $PaCO_2$ and pH over time, so that exacerbation of the underlying diseases may be avoided through alleviation of disease severity, thereby reducing the risks of ventilator-associated lung injury, hyperoxemia or hypoxemia. In the randomized, controlled trials,[1-4] the severity of underlying diseases (OI around 40) should have accounted for the different outcome despite surfactant and/or HFOV were given to most of the patients prior to or post enrollment of iNO.

Oxygenation improvement and dosing

So far the lowest initial concentration of iNO, at which optimal oxygenation improvement may be achieved, has not been determined yet.[4,6,7,20-23] Although previous trials claimed 20 ppm of NO would be safe in long-term follow up as more than half of infants responded well,[1,18] Finer et al[6] and Cornfield et al[7] found contradictory results using iNO at 2 ppm unless 10 ppm was used. Following these evidences, we used 10 ppm as initial iNO concentration to treat HRF infants whose OI >15. The results showed significant improvement of PaO_2/FiO_2, and a higher proportion of infants had effective improvement of PaO_2 in INO than in Con (Table 3). These were consistent with the results from Finer et al's[6] and Konduri et al's[9] studies. Goldman et al[19] found an effective rate of 92% in NO-treated infants with GA >35 weeks and OI >25 at study entry, however, no control was reported in the study. Kinsella et al[3] found a 100% response rate, but the proportion of HFOV was used up to 58% and they claimed that overall effectiveness of a combined iNO and HFOV was better than iNO alone. In our current study, it had very low use rate of HFOV, therefore it may not be compared with above mentioned studies, but provided evidence of similar efficacy in neonatal HRF by iNO without significant confounding influence of surfactant, HFOV or corticosteroids.

When no response was encountered at 10 ppm of iNO, Cornfield assessed 20 ppm but found no further improvement in PaO_2.[7] Finer et al[6] showed that there was no difference between lower and higher concentration of NO. Although in the initial NINOS study[1] iNO from 20 to 80 ppm resulted in 29% of full response in contrast to 8% in the control, it was associated with very high risk of methemoglobinemia,[4] and an iNO dose >20 ppm was precluded in neonatal HRF. In the present study, some of the infants not fully responded to 10 ppm NO further did in 20 ppm with better oxygenation improvement, indicating individual variation towards different range of iNO concentration, supposedly due to limitation of diffusion property of NO in different lung pathology, suggesting an optimal dosage for response assessment should be considered in guiding local practice of iNO administration. Put together, our results ensured a regimen of iNO at 10–20 ppm as initial dose for rescue treatment of neonatal HRF in conditions where availability of surfactant and/or HFOV was poor.

Severity of underlying diseases and timing

Previous trials proved that early iNO treatment could prevent the disease from getting worse and improve the clinical outcome. Konduri et al[9] treated neonatal HRF with GA >34 weeks and OI between 15 and 25, and found that early treatment resulted in a better improvement rate (73% vs. 37%) whereas more control patients became deteriorated (OI >40) and required ECMO to survive. Day et al[18] found that infants with focal change in the lungs by chest radiograph had the best response to iNO in contrast to those with moderate to severe diffusive lung pathology. Though both used iNO at

2 ppm, patients in Cornfield et al's study[5] had OI >30 but did not have significant improvement in oxygenation, while those in the Finer et al's[6] had OI >20 and oxygenation improved well. In the European iNO Registry study,[13] the responders were initially treated later (4 days in average) than the non-responders (2 days) without significant difference in the outcome (death or ECMO). In contrast, our stratified analyses revealed a trend of better response to iNO in favor of early (<24 hours) treatment while OI was at 15–20. Their implication of clinical relevance remains to be verified.

Limitation of the study

There were disparities at study entry in the proportions of the underlying diseases of HRF, PPHN, gestational age, gender, Apgar score at 5 minutes, and PaO_2/FiO_2 values. However, other perinatal-neonatal conditions, disease severity, and respiratory support were about the same which warranted initial iNO treatment modalities and assessment of strength and overall outcome of respiratory support. All the enrolled patients fulfilled the diagnostic criteria for HRF and the general treatment should have followed the protocol as the experiences and limitations for management of PPHN[24] were taken into consideration in the study protocol to minimize ventilator associated lung injury. Data from ventilation and monitoring of lung mechanics were incomplete and tidal volume measurement was not uniformly agreed upon and carried out. Therefore this part of the information was not provided, making determination of ventilation associated lung complications (such as pneumonia/sepsis from underlying diseases) difficult. However, no major parameters indicate that iNO is inferior to the concurrent therapies. Finally, many babies were not subjected to health insurance coverage for the cost of stay in NICU and hospitalization,[14,15] in addition, a high chance to encounter withdrawal from the study still existed. Thus to perform a completely randomized, controlled study would be a challenge in view of local conditions. Hence current study design and management using prospective, cohort study may be an alternative solution appropriate for evaluation of efficacy of respiratory therapies in these HRF infants, thereby deriving relevant parameters as endpoints for design of a randomized, controlled trial.

In conclusion, these results of response pattern to iNO and outcome of neonatal HRF, obtained through our NICU network study from respiratory support and resource limited conditions, were associated with improvement of oxygenation but not with the reduction of neonatal mortality. We anticipate that, with more cautious use of rescue iNO for neonatal HRF/PPHN, it should achieve better outcome in NICU from the emerging regions where the facilities and standard for neonatal special care are improving. It warrants further investigation to assess efficacy, safety and cost-effectiveness of iNO in neonatal HRF in these NICUs.

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(Received November 1, 2010)
Edited by CHEN Li-min