Safety and efficacy of nitric oxide in chronic lung disease

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Background: Therapies for neonatal chronic lung disease (CLD) of prematurity have had limited success. Aims: To determine whether inhaled nitric oxide (INO) administered to very low birthweight infants with developing CLD might improve oxygenation without adverse effects.

Methods: Subjects were 10–30 days of age, birth weight <1250 g, with developing or established CLD, and requiring mechanical ventilation with mean airway pressure ≥7 cm H₂O and FIO₂ ≥0.40. We monitored changes in oxygenation and FIO₂ requirement during treatment with INO (initial dose 20 ppm). Tracheal aspirate samples obtained before, during, and after treatment were analysed for interleukin 1β (IL-1β), IL-8, 8-epi-prostaglandin F₂α (8-epi-PGF₂α), laminin, and endothelin 1 (ET-1) to assess any potential effects of INO on markers of inflammation peroxidation, basement membrane injury, or vasoactivity.

Results: Thirty three patients met entry criteria. Mean gestational age was 25 (SD 2) weeks; birth weight was 736 (190) g; age of study infants was 19 (6) days (range 9–29). Mean FIO₂ decreased from baseline (0.75) to 0.58 at 72 hours. Duration of therapy was seven days. Tracheal aspirate concentrations of IL-1β, IL-8, 8-epi-PGF₂α, ET-1, and laminin were unchanged between baseline and 48 hours of INO, and 48 hours after discontinuation of INO. No new cases of, nor extension of, intraventricular haemorrhage occurred. Four infants died.

Conclusion: INO (≤20 ppm) improved oxygenation in most infants with early CLD, without inducing changes in markers of inflammatory or oxidative injury.

METHODS
The Pediatric Institutional Review Board of the University of Missouri–Kansas City and the Children’s Mercy Hospital approved the study protocol.

Subjects
All patients admitted to the Intensive Care Nursery from June 1997 to June 1999 were reviewed for eligibility. Parental consent was obtained for all eligible candidates identified, with no parental refusals. Inclusion criteria included birth weight <1250 g; age <30 days; but ≥10 days; need for assisted ventilation; need for FIO₂ ≥0.4 without fluctuations of >0.25 in the preceding 24 hours; and clinical course and radiographic findings compatible with CLD.

Exclusion criteria included: initiation of systemic corticosteroid or inhaled β agonist therapy within the preceding 48 hours; new diagnosis of sepsis (two blood cultures yielding growth of a single pathogenic organism) within the preceding 48 hours; thrombocytopenia (<100 000/mm³); progressive intraventricular haemorrhage; obviously lethal congenital anomaly; and complex congenital heart disease. The clinical course and chest radiographs of potential subjects were reviewed prior to enrolment by at least two investigators not serving as the patient’s attending physician.

Intervention
Inhaled nitric oxide was administered initially at 20 ppm. Arterial oxygen saturation was monitored by pulse oximetry, and transcutaneous levels of CO₂ and O₂ were measured over the next three hours to ascertain response. FIO₂ was adjusted to keep SpO₂ at 89–96%. No change in mean airway pressure occurred during this period. At the end of the initial three hour treatment period, a decision was made by the investigators regarding continuation of INO. If there was evidence of efficacy (FIO₂ decreased by ≥0.10, SpO₂ increased by ≥10%, or TcPo₂ improved by 1.3 kPa), INO was continued at 20 ppm.

Abbreviations: CLD, chronic lung disease; CMV, conventional mechanical ventilation; ELISA, enzyme linked immunosassay; HFOV, high frequency oscillatory ventilation; INO, inhaled nitric oxide; IVH, intraventricular haemorrhages; PCA, postconceptional age; PVR, pulmonary vascular resistance; sSC-IgA, soluble secretory component of IgA; VLBW, very low birthweight.
After 36 hours, the dose of INO was reduced to 15 ppm. The dose was further reduced every 12 hours by 2–3 ppm to a dose of 2 ppm as long as there was no worsening of oxygenation. A trial of cessation of INO was then undertaken. A reduction of INO followed by an absolute decrease of SpO₂ of >10% or need for increase in FIO₂ of ≥0.2 to sustain the previous SpO₂ reading within ≤30 minutes of changing the INO dose was considered an indication of lack of tolerance to the change, and the prior dose was reinstituted. Treatment with INO was discontinued by seven days if no evidence of dependency was detected. If INO dependency had developed, INO was continued with repeated trials of discontinuation every 24–48 hours.

All patients were treated with assisted ventilation with time cycled, pressure limited, and/or patient triggered ventilation (Dräger Babylog 8000), or high frequency oscillatory ventilation (Sensormedics 3100A). Changes from one mode of ventilation to another were allowed, but were made in only one case between 3 and 72 hours. Tracheal aspirate fluid samples were collected by standard techniques for analysis of pulmonary inflammatory cytokines (interleukin 1β (IL-1β), IL-8); a marker of peroxidative injury (8-epi-prostaglandin E₂ (8-epi-PGF₂α)); a proliferative and vasoconstrictor substance (endothelin 1 (ET-1)); and a marker of basement membrane injury (laminin). Samples were immediately frozen at −70°C until batch analysis was carried out.

Blood methaemoglobin and inspired nitrogen dioxide were continually measured. Surveillance was conducted for haemorrhage in any organ, infection, thrombocytopenia, necrotising enterocolitis, and retinopathy of prematurity. Bloodstream infections were defined as clinical deterioration plus blood culture yielding a pathogenic organism. Pneumonia was defined as deterioration of respiratory status, concomitant with tracheal aspirate material yielding growth of a pathogenic organism. Cranial ultrasound examinations were performed before initiation of INO, during treatment if clinically indicated, and after discontinuation of INO therapy.

**Echocardiographic analysis**
Echocardiography was performed within the 48 hours preceding INO whenever possible. Elevation in pulmonary vascular resistance was considered to be present if there was a detectable tricuspid valve regurgitation jet.

**Respiratory monitoring**
The FIO₂, ventilator pressures (peak inflating pressure and positive end expiratory pressure for patients on conventional ventilation, and mean airway pressure and amplitude for those on high frequency oscillatory ventilation (HFOV)), tidal volume, minute ventilation, and rates were recorded hourly. The SpO₂ and the most recent blood gas tensions and pH were recorded. All variables were averaged from hourly readings for the six hours before for baseline measurements, and six hours before and six hours after the 24 and 72 hour time points.

**Tracheal aspirate analysis**
Tracheal aspirate samples were assayed for IL-1β, IL-8, ET-1, and 8-epi-PGF₂α utilising available enzyme linked immunoassay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, Minnesota). Laminin concentrations were assayed by competitive inhibition ELISA (Chemicon International, Temecula, California). The soluble secretory component of IgA (sSC-IgA) was assayed by methods established in our laboratory. All samples were assayed at least in duplicate and the results averaged. Coefficients of variation for each of these assays is <10% in our laboratory.

**Statistical analysis**
Statistical testing for continuous variables was performed by paired t test with Bonferroni correction for multiple comparisons. For non-parametric data comparison, Wilcoxon rank sign test was performed. Correlation coefficients were tested for significance by Spearman and/or Kendall tau beta tests and linear regression where appropriate. SPSS 8.0 statistical software was used for the statistical computations (SPSS Inc., Chicago, Illinois).

**RESULTS**

**Patients**
Trials were performed in 33 consecutive infants who met the criteria for entry. Twenty were male; 21 were black, 11 white, and one Hispanic. Mean birth weight was 736 g (range 509–1250 g); mean gestational age was 25.3 weeks (range 23–29); mean age at enrolment was 19 days (range 9–29). Twenty one infants were treated with antenatal steroids, 18 with antenatal magnesium sulphate; 14 were delivered by caesarean section and 29 received surfactant treatment.

**Pulmonary gas exchange**
All infants tolerated the initial three hour trial with evidence of improvement. However, two infants did not continue to receive INO beyond 48 hours as there was no evidence then of a decrease of FIO₂ needs. For the remaining group of 31 infants, there was a significant reduction in FIO₂ at 3 hours and at 72 hours of therapy (at which time the median dose of INO was 10 ppm) compared with baseline values (p < 0.05 for each comparison, paired t test and Wilcoxon rank sign test). Mean FIO₂ decreased from 0.75 (0.23) to 0.63 (0.22) at 3 hours and to 0.58 (0.18) at 72 hours of INO therapy. The calculations include data on all 33 patients, including the two infants not receiving INO at 72 hours. The mean FIO₂ at 72 hours was also lower than that at 3 hours (p < 0.05, Wilcoxon rank sign test). The reduction in FIO₂ occurred even as mean SpO₂ showed an upward trend. The SpO₂ was 90 (6)% at baseline, 91 (4)% at 3 hours, and 93 (5)% at 72 hours. In spite of the reduced dose of INO by 72 hours, 14 of the 33 trials (45%) resulted in an absolute reduction in FIO₂ at 3 hours of therapy (yielding a pathogenic organism). Cranial ultrasound examinations were performed before initiation of INO, during treatment if clinically indicated, and after discontinuation of INO therapy.

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Echocardiographic analysis
Echocardiography was performed on 27 infants within 48 hours preceding initiation of treatment with INO. Eleven showed indirect evidence of increased pulmonary vascular resistance (PVR), in the form of a measurable tricuspid regurgitation jet. There was no correlation between the presence or absence of echocardiographic evidence of raised PVR and the magnitude of response to INO.

serial tracheal aspirate sample analysis
No difference was found in concentrations of IL-1β, IL-8, 8-epi-PGF_2α, ET-1, and laminin when values of each substance were compared between baseline and during INO or after INO therapy (figs 1, 2, and 3). There was wide variation in interpatient concentrations, but not in intrapatient concentrations, over time. When comparing concentrations across time in patients with a history of air leak (pulmonary interstitial emphysema or pneumothorax, n = 5), there again was no difference.

Complications
Five subjects showed intraventricular haemorrhages (IVH) of grade 3 (n = 3) or grade 4 (n = 2) at baseline. All other infants had grade 2 or less IVH prior to enrolment. No cases of new intracranial haemorrhage nor extension of old haemorrhage occurred. No subject experienced a clinically evident pulmonary haemorrhage. There were no sudden or unexplained decreases in haemoglobin or haematocrit, nor were there any unexplained episodes of thrombocytopenia. No concentration of methaemoglobin above 2.8% was detected at any time during INO use. Bloodstream infection occurred in five patients (coagulase negative staphylococci in three, Candida sp. in two) during or following therapy with INO. Five patients developed pneumonia (ureaplasma/mycoplasma species in three, Candida sp. in one, and coagulase negative staphylococci in one) during or following INO.

Outcome
Four of the 33 infants died before discharge (see table 1). No additional infants died during mean period of 22 months (range 15–36). At 36 weeks postconceptional age (PCA), one child continued to be treated with assisted ventilation, two were treated with continuous positive airway pressure, and 22 were treated with nasal cannula supplemental oxygen. Three infants tolerated breathing room air. By 44 weeks PCA, only three infants remained hospitalised, two of whom were treated with nasal cannula oxygen; 10 infants were breathing room air, and 19 were utilising nasal cannula oxygen. At six months PCA, 25 infants had accessible records available for evaluation. Ten infants continued treatment with supplemental oxygen by nasal cannula; 15 were breathing room air.

DISCUSSION
Inhaled NO acutely improved pulmonary oxygen uptake in most infants with early CLD. This is the first report of administration of INO in premature infants without evidence of increasing airway inflammation, peroxidation, or basement membrane breakdown. We studied a specific but important population of infants. These extremely low birthweight infants had very significant but early CLD, probably characterised by substantial pulmonary inflammation. Use of INO has not been reported in this specific patient population, yet the pulmonary morbidity and mortality for 750 g infants still requiring assisted ventilation at 19 days of age with a mean FIO_2 of 0.75 would be predicted to be considerable.
Neonatal CLD appears to represent the effects of mechanical distortion of the lung, oxidative injury, and resultant inflammatory response in immature lungs. Evidence for early inflammatory changes in CLD has been documented. Increased concentrations of IL-1β and IL-6 have been found in bronchoalveolar lavage fluid from patients with CLD, and IL-1β has been suggested as a participant in the development of CLD. The neutrophil chemotactic cytokine, IL-8, may participate in CLD, and both its production and that of IL-1β may be regulated directly or indirectly by NO and by peroxynitrites. Our findings of no effect of INO on tracheal aspiration concentrations of IL-1β and IL-8 suggest that proinflammatory lung cytokine production was not worsened by INO administration. Inhaled NO could exert anti-inflammatory effects by means of its effect on cytokines and neutrophil migration. Inhaled NO has also been shown to reduce neutrophil accumulation in the lung.

There is evidence that 8-isoprostanes such as 8-epi-PGF_2α, which are generated by oxidation of membrane phospholipids, serve as markers for pulmonary oxidative injury, and may themselves contribute to pulmonary hypertension. Concentrations of the profibrotic and constrictor substance ET-1 have been found to be increased in tracheal aspirate fluid of infants developing CLD. Tracheal aspirate concentrations of 8-epi-PGF_2α and ET-1 were unchanged with NO treatment. Concentrations of laminin, an indicator of basement membrane disruption, were also unchanged with use of INO in our patients. Thus, with relatively short term use of INO we found no evidence of additional peroxidative or inflammatory damage or of matrix disruption.

Microvascular obliteration and disorganised vasculogenesis in CLD may lead to pulmonary hypertension and contribute to poor matching of ventilation and perfusion, leading to hypoxaemia, hypercarbia, and exacerbation of pulmonary hypertension. Indirect evidence suggests that PVR is increased in anaemia, hypercarbia, and exacerbation of pulmonary hypertension. Longterm efficacy cannot be inferred from our study. We terminated NO usage, by design, at 7 days when possible, and had decreased the dose to 10 ppm by 3 days. It is possible that a more substantial reduction in FiO₂ could have been achieved with continuation of the starting dose of 20 ppm for 72 hours or more, or the same reduction achieved by starting at 10 ppm without lowering the dose.

Chronic lung disease is now recognised as an independent contributor to poor neurodevelopmental outcome. In addition, diminished long term pulmonary function remains a major long term sequela of CLD. Our findings support the performance of a definitive trial of INO in early or evolving CLD, but do not offer sufficient support to warrant widespread use of INO in infants remaining on assisted ventilation for early CLD.

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