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Original Article

A prospective clinical study on inhaled nitric oxide therapy for neonates in Japan

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Abstract

Background: This is the first report about a prospective clinical investigation to study the efficacy and safety of nitric oxide (NO) inhalation in infants with persistent pulmonary hypertension of the newborn (PPHN) in Japan.

Methods: Patients in the present study had to meet the following entry criteria: (i) they had to be younger than 7 days of age; (ii) they had to have evidence of PPHN as defined by echocardiograph; (iii) they had to have severe systemic hypoxemia under mechanical ventilation at 100% oxygen supplementation; and (iv) they had to have a failure to respond to conventional therapies. Patients were excluded from this trial if they had any of the following: hypoplastic lung, structural cardiac lesions or severe multiple anomalies.

Results: Nitric oxide inhalation therapy was performed in 68 infants who had severe PPHN at 18 hospitals between May 1995 and May 1997. At birth, 21 of 68 infants (31%) weighed less than 1500 g and 39 infants weighed more than 2500 g. The diagnoses associated with PPHN were as follows: 27 infants had meconium aspiration syndrome, 15 infants had dry lung syndrome, nine infants had congenital diaphragmatic hernia, six infants had respiratory distress syndrome, three infants had pneumonia and eight infants had other diagnoses. The mean oxygenation index (OI) before NO inhalation therapy in 68 infants was 43.2; 55 infants (81%) had good responses.

Conclusions: These results may be valuable for further randomized controlled and double-blind trials in Japan to evaluate whether NO inhalation therapy is more effective than conventional therapy in infants with severe PPHN.

Key words clinical study, inhalation therapy, low-birth weight infant, nitric oxide, persistent pulmonary hypertension of the newborn.

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Nitric oxide (NO) is an endogenous vasodilator produced by the endothelium in response to both chemical and physical stimuli.[1,2] Inhaled NO is a selective pulmonary vasodilator that does not alter systemic vascular resistance. This occurs because NO is inactivated immediately upon entering the blood by binding to hemoglobin. The pulmonary vasodilator

effect of inhaled NO has been well established in animals and in patients with pulmonary hypertension.[3–6] There have been many clinical trials that have used inhaled NO therapy to treat severe hypoxic respiratory failure with or without persistent pulmonary hypertension of the newborn (PPHN). Presently, in Japan, NO gas itself is not a licensed medical gas. The first conference of the Neonatal Inhaled NO Therapy Study Group was held in August of 1994. Our goal was to design a self-controlled system that permitted the use of NO gas in hospitals filled with some fixed indispensable criteria. The present paper is the first report about a prospective multicenter clinical study of neonatal inhaled NO therapy in Japan.

Methods

Goal of the study

This trial was designed as a prospective multicenter clinical investigation to study the efficacy and safety of NO inhalation in infants with PPHN in Japan.

Entry criteria for hospitals and patients

The eligibility criteria to participate in this trial were as follows: (i) the study had to be approved by the ethics committee of the participating hospital; (ii) informed consent had to be obtained from the parent(s); (iii) inhaled NO and nitrogen dioxide (NO_2) concentrations should be monitored continuously; (iv) blood methemoglobin levels should be measured frequently; and (v) exhaled gases from the breathing circuit should be scavenged to minimize contamination of the environment. Patients were enrolled into the present study if they had a primary or secondary PPHN with severe hypoxic respiratory failure. They also had to meet the following entry criteria: (i) they had to be younger than 7 days of age; (ii) they had to have evidence of PPHN defined by echocardiograph (right to left or bi-directional shunting across the patent ductus arteriosus and/or foramen ovale); (iii) they had to have severe systemic hypoxemia ($P_aO_2 < 80$ mmHg for more than 3 h) under mechanical ventilation at 100% oxygen supplementation; and (iv) they had to have a failure to respond to conventional therapies, such as intravenous vasodilators, high-frequency oscillatory ventilation and/or hyperventilation to improve oxygenation (the conventional therapies should have been tried before inhaled NO therapy). Patients were excluded from this trial if they had any of the following: hypoplastic lung (excluding hypoplastic lung with congenital diaphragmatic hernia), structural cardiac lesions (other than a patent ductus arteriosus) or severe multiple anomalies.

Guidelines for management

Nitric oxide gas was administered into the inspiratory limb of the breathing circuit of a neonatal ventilator. Either intermittent mandatory ventilation or high-frequency oscillatory ventilation mode were available in this trial. The concentrations of NO and NO_2 were monitored continuously by either a chemiluminecence or an electrochemical analyzer. In addition, methemoglobin levels were measured frequently. The starting dosage of inhaled NO was 10 p.p.m., which was weaned down to the minimum necessary dosage to sustain a clinically significant response. Dosages above 80 p.p.m. NO were not used. Before starting inhaled NO, arterial blood gases, blood pressure and ventilatory settings were measured and recorded on data sheets. These parameters were periodically measured at 15 min and 1, 12, 24 and 72 h after commencing inhaled NO therapy and after the settings of the ventilator were changed.

Outcomes and statistical analysis

Responses to inhaled NO therapy were classified into one of three groups according to changes from baseline in the oxygenation index (OI) after the initial exposure to NO gas, which was calculated as:

$$\mathrm{OI} = 100 \times \text{mean airway pressure} \times F_iO_2/P_aO_2$$

where F_iO_2 is the fractional inspired concentration of oxygen and P_aO_2 is the post-ductal partial pressure of arterial oxygen. An early response was defined as the OI falling to below 20 within 1 h after commencing inhaled NO therapy. A late response was defined as the OI falling to below 20 within 1–24 h after commencing inhaled NO therapy. These two groups of early and late responses were taken as a good response to inhaled NO therapy. A poor response was defined as the OI remaining > 20 during the first 24 h after commencing inhaled NO therapy. For comparing responses to NO inhalation therapy, Fisher's exact probability test was used. Differences were considered statistically significant when $P < 0.05$.

Results

Nitric oxide inhalation therapy was performed in 68 infants who had severe PPHN at 18 hospitals of the total of 59 hospitals in the Japanese Neonatal Inhaled Nitric Oxide Therapy Study Group between May 1995 and May 1997. Sixteen infants weighed less than 1000 g at birth, five infants weighed between 1000 and 1500 g, six infants weighed between 1500 and 2000 g, two infants weighed between 2000 and 2500 g and 39 infants weighed more than 2500 g. The diagnoses associated with PPHN were as follows:

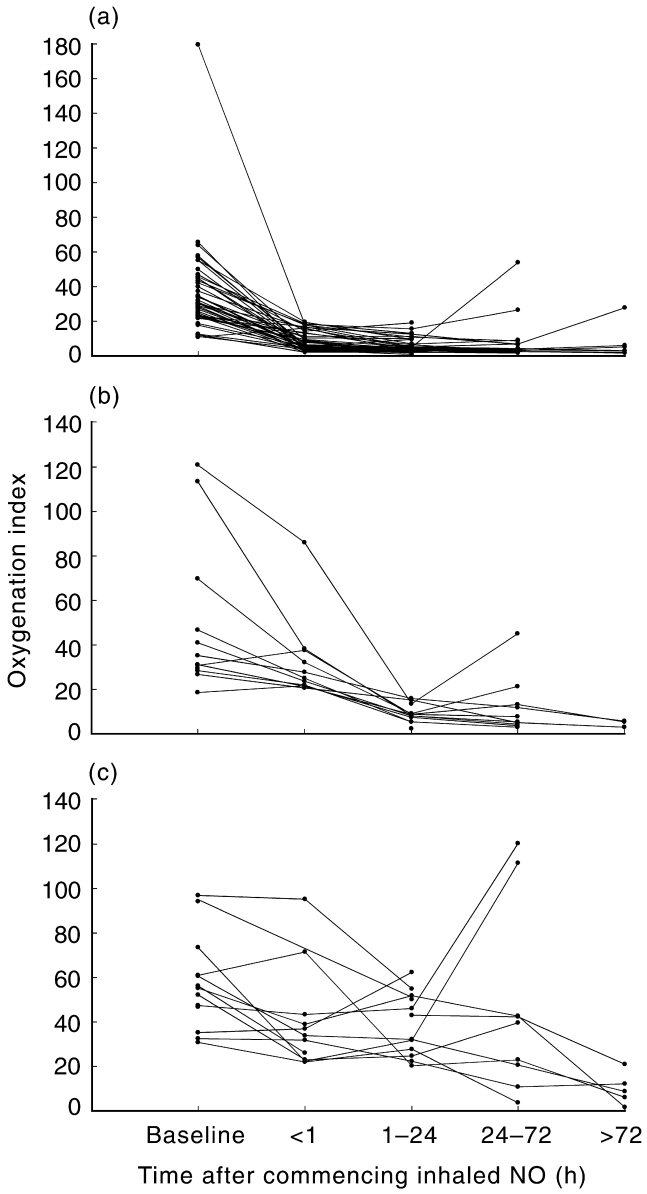

Fig. 1 Responses to inhaled nitric oxide (NO) therapy were classified into one of three groups according to changes in the oxygenation index (OI) from baseline. (a) An early response was defined as the OI falling to below 20 within 1 h after commencement of inhaled NO. (b) A late response was defined as OI falling to below 20 within 1–24 h after commencing inhaled NO. (c) A poor response was defined as OI being above 20 within 24 h after commencement of inhaled NO.

27 infants had meconium aspiration syndrome (MAS), 15 infants had dry lung syndrome (DLS), nine infants had congenital diaphragmatic hernia (CDH), six infants had respiratory distress syndrome (RDS), three infants had pneumonia, two infants had primary PPHN, two infants had sepsis, two infants had hydrops fetalis, one infant had congenital cystic adenomatoid malformation (CCAM) and one infant had lung hemorrhage.

Table 1 Responses to inhaled nitric oxide therapy according to diagnoses associated with persistent pulmonary hypertension of the newborn

Diagnosis	Good response		Poor response
	Early	Late	
MAS (*n*)	15	5	7
DLS (*n*)	12	3	0
CDH (*n*)	4	1	4
RDS (*n*)	5	1	0
Pneumonia (*n*)	2	1	0
Primary PPHN (*n*)	2	0	0
Sepsis (*n*)	0	1	1
Hydrops fetalis (*n*)	1	0	1
CCAM (*n*)	1	0	0
Lung hemorrage (*n*)	1	0	0

MAS, meconium aspiration syndrome; DLS, dry lung syndrome; CDH, congenital diaphragmatic hernia; RDS, respiratory distress syndrome; CCAM, congenital cystic adenomatoid malformation; PPHN, persistent pulmonary hypertension of the newborn.

The mean (± SD) starting age of inhaled NO therapy was 22.2 ± 28.1 h. Forty-eight infants (71%) started NO therapy within 24 h after birth and 62 (91%) infants started within 48 h after birth. The mean oxygenation index (OI) before NO inhalation therapy was 43.2 ± 27.8; five infants (7%) had an OI > 80, 25 infants (37%) had an OI between 80 and 40, 31 infants (46%) had an OI between 40 and 20 and seven infants (10%) had an OI between 20 and 10. Responses to NO inhalation therapy were as follows; 43 infants (63%) had early responses, 12 infants (18%) had late responses and 13 infants (19%) had poor responses (Fig. 1). In 29 of 43 infants with early responses, the OI fell below 10 within 1 h after the inhalation of NO. In this study, 16 infants died in hospital, of whom four (9%) were early responders, three (25%) were late responders and nine (69%) were poor responders. Seven infants also received extracorporeal membrane oxygenation (ECMO), of whom three were early responders (one of three died), one was a late responder (one survived) and three were poor responders (three of three died).

The various responses to NO inhalation therapy according to the diagnosis associated with PPHN are shown in Table 1. All infants with DLS, RDS, pneumonia and primary PPHN had good responses. Twenty of 27 infants with MAS had good responses. Five of nine infants with CDH had good responses. Infants with sepsis or hydrops fetalis had poor responses. All infants were further divided into one of two groups on the basis of the pathologic categories of PPHN, such as the vascular disease alone (primary PPHN and DLS) and mixed disease (all diseases

except the former two) with vascular plus interstitial, alveolar and/or airway disease. All 17 infants with vascular disease had good responses, while 38 of 51 infants with mixed disease had good responses. Table 2 shows that infants with vascular disease had significantly better responses than those with mixed disease to NO inhalation therapy according to pathologic category ($P < 0.01$).

Responses to NO inhalation therapy according to birth weight are shown in Table 3. All 29 infants weighing less than 2500 g at birth had good responses and 23 infants (79%) had early responses. However, only 26 of 39 full-term infants (67%) had good responses. There was a significant difference between infants weighing < 2500 g at birth and the full-term infants ($P < 0.01$).

Complications of NO inhalation therapy are shown in Table 4. Potential side effects include the formation of methemoglobin and higher NO_2 concentrations. Two patients developed more than 5% methemoglobinemia and high NO_2 levels, more than 1 p.p.m., were measured in four infants. Three infants suffered from intracranial hemorrhage during the inhalation of NO or after the discontinuation of NO inhalation. One infant had a small hemorrhage in the bilateral thalamus during NO inhalation, one had a grade 4 intraventricular hemorrhage and one had a subependymal hemorrhage after the discontinuation of NO inhalation. One patient had a lung hemorrhage during NO inhalation.

Table 2 Response to inhaled nitric oxide therapy according to pathologic categories of persistent pulmonary hypertension of the newborn

Pathologic category	Good response		Poor response
	Early	Late	
Vascular disease (*n*)	14	3	0
Mixed disease (*n*)	29	9	13

Vascular disease includes dry lung syndrome and primary persistent pulmonary hypertension of the newborn. Mixed disease includes meconium aspiration syndrome, congenital diaphragmatic hernia, respiratory distress syndrome, pneumonia, sepsis, hydrops fetalis, congenital cystic adenomatoid malformation and lung hemorrhage.

Table 3 Response to inhaled nitric oxide therapy according to birth weight

Birth weight	Good response		Poor response
	Early	Late	
Pre-term (*n*)	23	6	0
Full-term (*n*)	20	6	13

Pre-term infants weighed less than 2500 g at birth; full-term infants weighed more than 2500 g at birth.

Discussion

Because the present prospective multicenter clinical study had no restrictions on gestational age and birth weight in the entry criteria for patients, 31% of all infants enrolled in the present study weighed less than 1500 g at birth and 24% weighed less than 1000 g. This presents a great difference when compared with most random multicenter controlled trials in Europe or the US. In those studies, infants born at less

Table 4 Complications of inhaled nitric oxide therapy

	Gestational age	Birth weight (g)	[NO] (p.p.m.)	Time complications occurred
High methemoglobin level (> 5%)				
Maximum methemoglobin (%)				
7.5	38 weeks 4 days	1412	10	104 h after starting iNO
6.4	38 weeks 4 days	3000	10–40	9 days after starting iNO
High NO_2 level (> 1 p.p.m.)				
Maximum NO_2 (p.p.m.)				
1.6	38 weeks 5 days	2924	10	11 h after starting iNO
1.4	36 weeks 4 days	2568	6–10	1 h after starting iNO
1.3	42 weeks 2 days	3394	6–30	5 h after starting iNO
1.2	36 weeks 5 days	2570	10–56	4 days after starting iNO
Bleeding disorders				
Lung hemorrhage	38 weeks 4 days	3000	10–40	84 h after starting iNO
Thalamus hemorrhage	41 weeks 2 days	3170	10	36 h after starting iNO
Ventricles hemorrhage	28 weeks 0 days	984	10–30	24 h after stopping iNO
Subependymus hemorrhage	26 weeks 1 day	837	8	2 days after stopping iNO

iNO, inhaled nitric oxide therapy.

than 34 weeks gestation were ineligible for entry into the study.[7–10] It seems to be impossible that conventional treatment with high fractions of inspired oxygen, mechanical ventilation and intravenous vasodilator drugs save the lives of some premature infants with PPHN, but they are excluded from ECMO therapy. For these reasons, we included premature infants in the present study.

The randomized multicenter controlled trial of the Neonatal Inhaled Nitric Oxide Study Group demonstrated that inhaled NO therapy reduced the use of ECMO, but had no apparent effect on mortality in full-term and nearly full-term infants with hypoxic respiratory failure.[8] Also reported in this paper[8] is the finding that 54% of 176 infants with pulmonary hypertension detected by echocardiography had improved oxygenation. This improvement was defined as an increase in the P_aO_2 of more than 20 mmHg with the administration of 20 p.p.m. NO at 30 min, as an initial response to the inhalation of NO. Roberts *et al*. have reported that NO inhalation therapy successfully caused systemic oxygenation in 16 of 30 full-term infants (53%) with severe hypoxemia and PPHN.[7] In contrast, conventional therapy without NO inhalation increased oxygenation in only two of 28 infants (7%). In this paper,[7] a successful treatment was defined as an increase in the P_aO_2 of more than 55 mmHg and a decrease in the OI to less than 40. In the present study, good responses to NO inhalation therapy were defined as a decrease in the OI to below 20 within 24 h after NO inhalation began. Fifty-five of 68 infants (81%) had good responses and the OI in 43 of these 55 infants decreased to below 20 within 1 h after the inhalation of NO.

The results of the present study show that inhalation of NO causes an increase in the P_aO_2 of preterm infants with RDS. This finding was consistent with previously published reports that inhaled NO improves oxygenation levels in prematurely delivered lambs.[11,12] It is possible that the inhalation of NO decreases pulmonary vascular resistance and intrapulmonary shunting due to regional atelectasis and hyaline membrane formation associated with RDS. Infants with DLS had similar clinical findings, with an early onset of oligohydramnios due to prolonged rupture of membranes and pulmonary insufficiency requiring high ventilatory pressures.[13,14] Most of these cases received inhaled NO during high-frequency oscillatory ventilation and showed good responses to the treatment. Two infants with primary PPHN were extremely low-birth weight infants without known causative factors for the pulmonary hypertension. It is, however, presumed that the cause of their severe hypoxemia was hypoxic pulmonary vasoconstriction. This is thought to be the cause because both infants had good responses to inhaled NO.

In contrast, infants with poor responses had not only pulmonary hypertension but also mixed pathologic factors, such as vascular disease plus interstitial, alveolar and/or airway disease for the etiology of severe hypoxemia. Infants with MAS often have obstructive sites in the bronchiolar passage and parenchymal lung disease. These can lead to the blockage of NO diffusion to the alveoli and ventilation/perfusion mismatch. Therefore, the response to NO inhalation therapy in infants with MAS depends on the severity of the airway and/or parenchymal lung disease.[15–19] Infants with CDH also had variable responses to NO. This was because some had pulmonary hypoplasia that is related to an unresponsiveness to NO.[9,20] It is difficult to estimate the response to NO in infants with sepsis because we had only two patients with this diagnosis. Endotoxins cause increased NO synthase activity and enhanced NO production. Recent laboratory observations have demonstrated that symptoms of septic shock may be due, in part, to the excessive endogenous production of NO. Therefore, patients with septic shock may not improve with NO therapy.[21,22] In the present study, all low-birth weight infants had good responses to inhaled NO compared with 67% of full-term infants. This finding was partly explained by the fact that there were no low-birth weight infants with mixed pathologic disease, such as MAS or CDH.

Finally, we mention the complications found following NO inhalation therapy. The majority of inhaled NO reacts with hemoglobin in the blood, producing methemoglobin. Therefore, methemoglobinaemia is a potential side effect of this treatment. Two patients showed increases in their methemoglobin levels of 7.5 and 6.4%. However, these elevated methemoglobin levels decreased to normal after the concentration of NO was reduced. Another potential toxic effect of this treatment is an increased production of NO_2 in the NO delivery system. Nitric oxide reacts with oxygen to form NO_2, which is known to damage the pulmonary epithelium and, furthermore, causes pulmonary edema in animals.[23,24] Four infants had NO_2 concentrations of more than 1 p.p.m.; however, these elevated NO_2 levels rapidly decreased to normal after the concentration of inhaled NO was reduced.

Inhaled NO has been shown to moderately prolong bleeding time in animals and adults due to interference with platelet adhesion and aggregation functions. This could contribute to bleeding disorders in such infants.[25–27] Four infants in the present study suffered from bleeding disorders. One had a lung hemorrhage and three had intracranial hemorrhages. However, it is unclear whether inhaled NO is directly associated with the initiation or extension of these hemorrhages. This is because none of the infants in the present study had any detailed information about cranial ultrasound findings or laboratory data on blood coagulation before or during the NO inhalation. Nitric oxide inhalation therapy, especially in premature infants, causes a rapid decrease in pulmonary vascular resistance and an increase in left-to-right shunts through the ductus arteriosus. This may

result in marked increases in pulmonary blood flow and lethal pulmonary hemorrhage.

We conclude that the results of the present study may be valuable for further randomized controlled and double-blind trials in Japan to evaluate whether NO inhalation therapy is more effective than conventional therapy in infants with severe PPHN.

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