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The Effects of Nitric Oxide Inhalation on the Pulmonary Circulation of Preterm Lambs

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ABSTRACT

This study was designed to test the hypothesis that inhalation of nitric oxide by lambs delivered prematurely would result in increased systemic arterial blood oxygen tension and decreased pulmonary vascular resistance. Eleven premature fetal lambs were delivered by cesarean section at 126–127 d gestation. One hundred min after the onset of ventilation, nitric oxide gas was added to the lambs' breathing mixture. The animals were exposed in random order to 5 ppm for 10 min, 20 ppm for 10 min, and 20 ppm for 20 min. Each treatment period was preceded by and followed by a 10-min washout period. When compared with the washout (control) periods, all three treatment periods resulted in an improvement in both the systemic arterial blood oxygen tension and the physiologic intrapulmonary shunt. Inhalation of nitric oxide also resulted in a selective decrease in pulmonary arterial blood pressure. Comparisons between the different treatment groups revealed a further improvement in blood oxygenation and pulmonary hemodynamics when using the higher concentration of nitric oxide. Interestingly, the rise in arterial blood oxygenation continued after inhaling 20 ppm nitric oxide for more than 10 min. (***Pediatr Res* 37: 35–40, 1995**)

Abbreviations

Qs/Qt, physiologic intrapulmonary shunt
PAP, mean pulmonary arterial pressure
ppm, parts per million
RDS, respiratory distress syndrome
SAP, mean systemic arterial pressure
LAP, left atrial pressure
RAP, right atrial pressure
Qp, pulmonary blood flow
PVR, pulmonary vascular resistance
Pao_2, arterial oxygen partial pressure

Nitric oxide, an inorganic gas formed by combustion processes, has been recognized for many years as an industrial pollutant. In fact, cigarette smoke contains nitric oxide gas in concentrations up to 1000 ppm (1). In both dogs (2, 3) and humans (4–6), inhalation of concentrations of more than 15 000 ppm rapidly causes a fatal condition involving severe alveolar edema, methemoglobinemia, and hypoxemia. Ironically, inhalation of concentrations less than 100 ppm has been used in an attempt to facilitate the management of several cardiopulmonary diseases such as persistent pulmonary hypertension of the newborn (7, 8), primary pulmonary hypertension (9, 10), and adult RDS (11).

Treatment of pulmonary hypertension with i.v. administered vasodilator agents has been limited because of the inability to localize the effects of these drugs to the pulmonary circulation. Nitric oxide gas seems to be an ideal drug to obviate this problem. Experience in animals (12–16) and humans (9, 17) suggests that inhaled nitric oxide can cause a decrease in PVR without causing significant systemic hypotension. The mechanisms of this selective pulmonary vasodilation are not entirely understood; however, the short half-life of nitric oxide (18, 19) and its rapid inactivation by Hb (18, 20) are likely to be involved.

The selectivity of nitric oxide inhalation extends beyond simply dilating the pulmonary vascular bed without affecting the systemic vascular bed. Mismatching of ventilation and perfusion is a common cause of hypoxemia in critically ill patients (21). An important feature of the inhalation route for drug delivery is that the drug is selectively distributed only in areas of the lung that are ventilated. Preliminary evidence suggests that nitric oxide inhalation can cause selective dilation of blood vessels in ventilated regions (11). This regional specificity of inhaled nitric oxide therefore leads to a decrease in intrapulmonary shunting and an increase in ventilation-perfusion matching.

RDS of premature infants is a condition that is complicated by pulmonary hypertension (22–27). Severe pulmonary hypertension can cause right-to-left shunting of blood across the foramen ovale and the ductus arteriosus. This shunting can exacerbate hypoxemia in premature infants (23, 28). Walther *et al.* (26) reported that right-to-left shunting across the ductus

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arteriosus and the foramen ovale is common among premature infants. They suggested that large right-to-left shunts predict an early death of infants with RDS. RDS is also complicated by intrapulmonary shunting of blood (28–35). Regional atelectasis and hyaline membrane formations probably contribute greatly to the pathophysiology of the intrapulmonary shunting associated with RDS.

Recent advances in the treatment of RDS, such as intratracheal administration of surfactant (36) and the use of antenatal corticosteroids (37), have improved the prognosis of this disease. Despite these advancements, however, RDS remains a major component of both the morbidity and the mortality associated with prematurity. Because of the high prevalence of RDS (38–40), we believe that a safe, selective pulmonary vasodilator might significantly improve the morbidity and mortality associated with prematurity.

Premature lambs have been used extensively as models for human neonatal RDS, particularly in the development of surfactant therapy (41). Using this model of RDS, our goal was to learn whether inhaled nitric oxide could cause significant increases in the systemic arterial blood oxygen tension. Secondarily, we attempted to investigate the mechanisms of this improvement by studying the effects of nitric oxide inhalation on both the physiologic intrapulmonary shunt and the PVR.

METHODS

Preparation. The following procedures were approved by the University of Florida Animal Research Committee before the studies were conducted. Using i.v. chloralose anesthesia (initial dose 50 mg/kg followed by 10 mg/kg/h), 11 fetal lambs were delivered by cesarean section between 126 and 127 d gestation. During delivery, special care was taken to maintain the umbilical circulation intact. A tracheostomy was performed on each animal, and the tracheal tube was connected to a bag of saline so that air could not enter the lungs. A polyvinyl catheter was placed in the femoral artery for SAP and heart rate monitoring. Another catheter was placed in the femoral vein to allow continuous administration of a 10% dextrose solution at a rate of 3 mL/kg/h. Through a left lateral thoracotomy, catheters were directly placed into the left atrium and the main pulmonary artery. The LAP and the main PAP were recorded continuously. Qp was monitored using a 6-mm diameter Transonic Systems (Ithaca, NY) Doppler flow probe that was placed around the main pulmonary artery.

After placement of all the catheters and the flow probe, fluid was suctioned from the lungs and five manual breaths were given. The tracheal tube was then connected to the ventilator circuit. The animal was ventilated with a time-cycled, pressure-limited infant ventilator (Healthdyne, Marietta, GA). The ventilator was set initially to provide 45 cm H_2O of peak inspiratory pressure and 4 cm H_2O of positive end-expiratory pressure. The ventilation rate was initially set at 40 breaths per minute. The inspired gas mixture always contained 95% oxygen. The ventilatory pressures, inspiratory time, and rate were modified to maintain oxygen tension between 5.33 and 8.00 kPa and arterial blood pH between 7.10 and 7.30. The resultant ventilator settings were as follows: peak inspiratory pressure 30–45 cm H_2O, end expiratory pressure 4–5 cm H_2O, rate 40–60 breaths per minute, and inspiratory time 0.4–0.6 s. The animal's core temperature was measured rectally and maintained between 38 and 39°C using a heating pad and lamp.

Hemodynamic measurements were monitored continuously using a Gould eight-channel polygraph. The polygraph was connected to an IBM personal computer using a Kiethley 570 Data Acquisition System (Cleveland, OH). Data from the polygraph were plotted and stored in real time. Blood pH, P_{CO_2}, and P_{O_2} levels were measured using a model 288 Ciba-Corning blood gas system (Medfield, MA). Blood Hb concentrations and their respective oxygen saturation percentages were measured using a model OSM2 Radiometer hemoximeter (Radiometer, Copenhagen, Denmark).

Stock nitric oxide cylinders were purchased from Alphagaz (LaPorte, TX) at concentrations of 100 and 400 ppm with the balance being nitrogen gas. A 1:20 dilution of these gases with pure oxygen produces a gas containing 95% oxygen and either 5 or 20 ppm nitric oxide. Volumetrically calibrated flowmeters were adjusted to deliver approximately 9.5 L of oxygen plus 0.5 L of the nitric oxide/nitrogen mixture. Because the inspired oxygen concentration was monitored throughout each experiment using a model 252 Datex airway gas monitor (Helsinki, Finland), fine adjustments in the flow rates could be made to yield an inspired oxygen concentration of 95% and an approximate concentration of nitric oxide. Pure nitrogen gas was substituted for the nitric oxide/nitrogen mixture when these treatment gases were not in use.

The nitrogen-containing gases were delivered into the inspiratory limb of the breathing circuit 50 cm from the endotracheal tube. Further confirmation of the appropriate concentrations of nitric oxide gas was obtained using both a Matheson-Kitagawa 8014KA toxic gas detector system (Rutherford, NJ) and a Dasibi 2108 chemiluminescent nitrogen oxides analyzer (Glendale, CA). Samples of gas were obtained from a port in the efferent limb of the breathing circuit located 5 cm away from the endotracheal tube. The nitrogen dioxide concentrations never exceeded 0.5 ppm.

Protocol. The first phase of the experiment began after the initiation of ventilation and involved adjusting the ventilator settings based on blood gas measurements obtained every 20 min. This phase lasted 100 min and allowed the animal to acclimate to an *ex utero* environment. During this phase, ventilation was initiated and followed by ligation of both the umbilical cord and the ductus arteriosus. This order of events remained constant for each experiment and each event was separated by approximately 20 min. During the remaining 40 min, the animal continued to acclimate to "newborn" circulation although final manipulation of the ventilator settings was accomplished. After ligation of the umbilical cord, the lambs were given hourly supplementation of i.v. chloralose anesthesia (10 mg/kg).

The second phase entailed three treatment periods and four control periods. Each treatment period was preceded by and followed by a 10-min control period. The animals were exposed in random order to 5 ppm for 10 min, 20 ppm for 10 min, and 20 ppm for 20 min. During the control periods, nitrogen gas was substituted for the nitric oxide/nitrogen gas mixture.

The average PAP, LAP, SAP, and Qp during the last minute of each period was used for analysis. At the end of each period, arterial blood samples were obtained and analyzed for pH, P_{CO_2}, P_{O_2}, Hb, and oxygen saturation levels. After the first three experiments, similar measurements were obtained using blood samples taken from the pulmonary artery.

Calculations. PVR was calculated using the following formula:

$$PVR = (PAP - LAP)/Qp.$$

The physiologic shunt (Qs/Qt) was calculated using the standard shunt equation (42, 43):

$$Qs/Qt\ (\%) = (C\acute{c}O_2 - CaO_2)/(C\acute{c}O_2 - CvO_2) \times 100$$

where $C\acute{c}O_2$ represents the end-capillary blood oxygen content of the pulmonary vasculature, CaO_2 represents the systemic arterial blood oxygen content, and CvO_2 represents the mixed venous (or pulmonary artery) blood oxygen content. The oxygen content (CxO_2) of all blood samples was calculated using the following formula:

$$CxO_2\ (\text{volume } \%) = (1.34 \times Hb \times SxO_2) + (0.003 \times PxO_2)$$

where Hb represents the total Hb expressed in g/dL, SxO_2 represents the percent saturation of Hb with oxygen, 0.003 represents the solubility coefficient for oxygen, and PxO_2 represents the blood oxygen partial pressure. The $C\acute{c}O_2$ was calculated by first determining the alveolar oxygen partial pressure (P_AO_2) using an adaptation of the alveolar air equation:

$$P_AO_2 = (760 - 50) \times 0.95$$

$$- \left[\frac{0.95 + (1 - 0.95)}{0.8}\right] Paco_2 = 674.5 - (1.012 \times Paco_2)$$

where 760 represents barometric pressure in mm Hg, 50 represents water vapor pressure at 38° in mm Hg, 0.95 represents the inspired oxygen fraction, and 0.8 represents the assumed respiratory exchange quotient. Calculation of the $C\acute{c}O_2$ is calculated using the P_AO_2 (assumed to be equal to $P\acute{c}O_2$).

Statistical analysis. All data are presented as mean ± SEM. Because the different control values for each experiment were not significantly different from each other, they were averaged together. Differences between mean values recorded during the treatment periods and the control periods were assessed by a one-way repeated measures analysis of variance using Sigma STAT version 1.01 (Jandel Scientific, San Rafael, CA). A Student-Newman-Keuls test was performed to determine whether the mean values differed from each other. The Kruskal-Wallis one-way analysis of variance test was used to analyze the Pao_2 values because they did not have a normal distribution. A p value < 0.05 was considered significant.

RESULTS

The measurements obtained directly from blood samples are summarized in Table 1. Neither the arterial nor the mixed venous blood pH were affected by exposure to nitric oxide. The Pao_2 rose with all doses of nitric oxide. Figure 1*A* illustrates the changes in Pao_2 with the various doses of nitric oxide. Inhaling 20 ppm NO for 20 min caused a 139.8% ± 37.9% increase in the Pao_2 when compared with values for the control periods. After inhaling the gas for 10 min, 20 ppm caused a greater rise in the Pao_2 than did 5 ppm. A greater rise in Pao_2 was evident after inhaling 20 ppm of nitric oxide for 20 min when compared with inhalation of the same concentration for only 10 min. Figure 1*B* illustrates the effects of nitric oxide inhalation on Qs/Qt. The Qs/Qt decreased by 29.4 ± 6% after inhaling 20 ppm for 20 min.

The hemodynamic data for all experiments are summarized in Table 2. PVR decreased during inhalation of all nitric oxide doses. Ten min after beginning inhalation of the gas, 20 ppm produced a greater hemodynamic effect than 5 ppm. Although PVR seemed to decrease further when using 20 ppm for 20 min as opposed to 10 min, the difference was not significant. Using 20 ppm for 20 min reduced PAP by 29.5 ± 2.7% and PVR by 30.9 ± 4.0%. The PAP/SAP ratio decreased by 21.6 ± 4.9%. Figure 1*C* illustrates a decrease in the PAP/SAP ratio.

At the end of each experiment, the thoracic organs of each lamb were inspected and proper placement of catheters and ligatures was confirmed. The mass of the lambs averaged 2.72 ± 0.05 kg. In all animals, gross examination of the chest organs revealed symmetrical lung inflation and patchy areas of atelectasis. Occasionally, grossly identifiable pulmonary interstitial emphysema was noted on the lung surfaces.

Table 1. *PH, blood gas, and oxygen saturation data*

Parameter	Control	5 ppm/10 min	20 ppm/10 min	20 ppm/20 min
pHa	7.15 ± 0.03	7.15 ± 0.03	7.17 ± 0.03	7.15 ± 0.03
pHv	7.12 ± 0.02	7.12 ± 0.03	7.12 ± 0.02	7.11 ± 0.02
$Paco_2$ (kPa)	9.27 ± 0.72	8.75 ± 1.00	8.24 ± 0.67	8.20 ± 0.68
$Pvco_2$ (kPa)	10.77 ± 0.81	10.55 ± 1.19	10.31 ± 0.87	10.17 ± 0.66
Pao_2 (kPa)	6.40 ± 0.53	9.32 ± 1.61*	11.56 ± 1.81†	14.40 ± 1.8‡
Pvo_2 (kPa)	3.49 ± 0.23	3.72 ± 0.19	4.20 ± 0.57	4.23 ± 0.35
Sao_2 (%)	82.7 ± 3.2	90.4 ± 3.1*	96.0 ± 1.0*	95.6 ± 1.6*
Svo_2 (%)	58.0 ± 5.2	63.0 ± 3.9	66.2 ± 7.3	68.1 ± 6.4

Values are presented as mean ± SEM. $n = 11$ animals for arterial measurements and $n = 8$ for venous measurements. pHa, arterial pH; pHv, venous pH; $Paco_2$, arterial carbon dioxide partial pressure; $Pvco_2$, venous carbon dioxide partial pressure; Pvo_2, venous oxygen partial pressure; Sao_2, arterial oxygen saturation; Svo_2, venous oxygen saturation.

* Treatment group is significantly different from control group ($p < 0.01$).

† Treatment group is significantly different from control and 5 ppm/10 min treatment group ($p < 0.05$).

‡ Treatment group is significantly different from all other groups ($p < 0.05$).

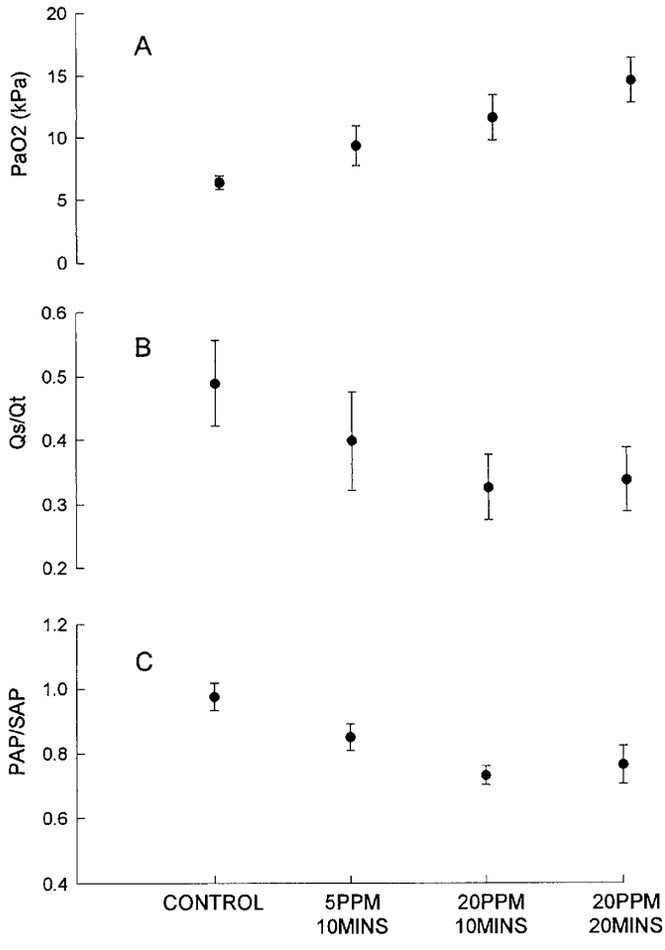

Figure 1. *A* illustrates the concentration- and exposure duration–dependent change in Pao_2. All treatment groups differ significantly from the control group ($p < 0.01$). All treatment groups differ from each other ($p < 0.05$). *B* illustrates the changes in Qs/Qt. All treatment groups differ significantly from the control group ($p < 0.01$). Except for the two groups that included different durations of exposure to 20 ppm, all treatment groups are significantly different ($p < 0.05$). In *C*, all treatment groups differ significantly from the control group ($p < 0.01$). A concentration-dependent (but not exposure duration–dependent) change in PAP/SAP ($p < 0.05$) is illustrated.

DISCUSSION

Our results show that nitric oxide inhalation causes a dose-dependent increase in the arterial blood oxygenation of premature lambs with RDS. In an earlier study (submitted for publication), we showed that the Pao_2 of term newborn lambs with hypoxia-induced pulmonary hypertension was not affected by nitric oxide inhalation. In both studies, the ductus arteriosus had been ligated and Qp remained unchanged with nitric oxide inhalation. Our study also illustrates a significant decrease in Qs/Qt associated with nitric oxide inhalation. This finding suggests that the increase in Pao_2 relates to a shifting of blood flow from poorly ventilated regions to well-ventilated regions of the lung. A similar relationship has been suggested to explain changes in Pao_2 that occur in patients with adult respiratory syndrome who are treated with nitric oxide (11).

The results of this study confirm the suggestion by Dawes *et al.* (44) that premature lambs, unlike term newborn lambs, fail to adequately dilate their pulmonary vasculature after the onset of ventilation. Full dilation of the pulmonary vascular bed of term newborn lambs is nearly complete within minutes after the onset of ventilation (44, 45). The results of this study suggest that nitric oxide inhalation can cause pulmonary vasodilation of preterm lambs. The decrease in PAP/SAP illustrates the selectivity of nitric oxide for the pulmonary vascular bed.

The recognized responses of our premature lambs to nitric oxide were all dependent on the concentration of the inhaled nitric oxide. Although the Pao_2 continued to improve after 10 min of exposure to nitric oxide, the PVR and the Qs/Qt did not. With a much larger sample size, demonstrating a significant difference between 10 and 20 min of exposure to 20 ppm may have been possible. However, an unrecognized mechanism for improving Pao_2 besides a reduction in right-to-left intracardiac shunting or improved ventilation-perfusion matching may have occurred.

Small intraatrial shunts can be difficult to evaluate, particularly when bidirectional shunting is present. In one of our animals, Doppler echocardiography was performed. The results of this study demonstrated a very small amount of unidirectional atrial left-to-right shunting. The shunt was too small to be evaluated quantitatively. The administration of nitric oxide did not appear to change this shunt qualitatively. In another animal, we attempted to measure a pressure gradient across the atrial septum by measuring the mean RAP and LAP. The RAP and LAP were nearly equal, so a gradient could not be clearly detected. The administration of nitric oxide had no obvious effect on the mean atrial pressures. Technical factors prohibited our ability to obtain echocardiograms and measure RAP on each lamb.

The presence of right-to-left shunting of blood in association with neonatal RDS is well documented (22–35). Several investigators have suggested that the right-to-left shunting in infants with RDS occurs predominantly within the lungs (29, 46, 47). What remains unclear is whether human premature infants have a reactive pulmonary vascular bed that can respond to nitric oxide inhalation. Premature lambs have been widely used in studies of RDS (41, 48); however, the model is not perfect. The maturation of ovine fetal lungs is notably different from that of human fetal lungs. Comparing fetal lung maturation of the two species is not easily accomplished. One study that used measurements of surface tension properties of lung extracts found that 126-d gestation lambs were similar to 28-wk gestation human infants (49).

The impact of tying the ductus arteriosus on our ability to model accurately the effects of nitric oxide inhalation in human infants with RDS is not clear. A decrease in PVR is likely to cause a decrease in any right-to-left (and an increase in any left-to-right) ductal or intracardiac shunting. In the presence of a patent ductus arteriosus, a decrease in PVR could therefore cause a significant increase in Qp. This shift in blood flow could be deleterious either by causing "stealing" of blood flow from the body or by causing "volume overloading" of the left ventricle.

This study was not designed to evaluate the toxic effects of nitric oxide. It should be remembered, however, that the side effects of this gas are likely to be different in premature newborns compared with mature newborn and adult individu-

Table 2. *Hemodynamic data*

Parameter	Control	5 ppm/10 min	20 ppm/10 min	20 ppm/20 min
Qp (mL/min)	150 ± 25	143 ± 25	141 ± 22	138 ± 23
PAP (mm Hg)	33.4 ± 1.6	28.0 ± 1.8*	25.3 ± 1.2†	23.4 ± 1.0†
LAP (mm Hg)	2.9 ± 0.3	3.1 ± 0.4*	2.9 ± 0.3†	3.0 ± 0.3†
PVR (mm Hg/mL/min)	0.304 ± 0.055	0.244 ± 0.047*	0.227 ± 0.052†	0.201 ± 0.036†
SAP (mm Hg)	35.0 ± 2.2	33.6 ± 2.5	35.0 ± 1.8	32.7 ± 3.0

Values are expressed as mean ± SEM. For all values, $n = 11$ animals.

* Treatment group is significantly different from control group ($p < 0.01$).

† Treatment group is significantly different from both control and 5 ppm/10 min treatment group ($p < 0.05$).

als. Maturational differences in Hb, coagulation, free radical scavenging mechanisms, and surfactant production may be important considerations when using inhaled nitric oxide therapeutically. Infants may also be protected from the toxicities of nitric oxide because of their ability to generate new lung tissue for several years after birth.

We conclude that nitric oxide inhalation causes a concentration-dependent increase in the blood oxygenation of 126- to 127-d gestation newborn lambs. The maximal change in Pao_2 did not occur within 10 min of initiating the nitric oxide treatment. Nitric oxide inhalation also causes a concentration-dependent decrease in both PAP/SAP and Qs/Qt. Clinical trials involving human infants with RDS will be necessary to find out whether nitric oxide inhalation can reduce morbidity and mortality associated with this disease.

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Announcement

1995 Annual Meetings

The American Pediatric Society, The Society for Pediatric Research, and The Ambulatory Pediatric Association will hold their annual meetings May 8–11, 1995, at the San Diego Convention Center, San Diego, CA.

For further information, contact: APS/SPR Association Headquarters, 141 Northwest Point Blvd., P.O. Box 675, Elk Grove Village, IL 60009-0675, (708) 427-0205, Fax: (708) 427-1305 or Ambulatory Pediatric Association, 6728 Old McLean Village, McLean, VA 22101, (703) 556-9222, Fax: (703) 556-8729.