Inhaling Nitric Oxide: A Selective Pulmonary Vasodilator and Bronchodilator*

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In a number of animal models and clinical pulmonary diseases of human subjects, inhaled nitric oxide (NO) is now firmly established as a selective pulmonary vasodilator.

Selective Pulmonary Vasodilation in Lambs

We first examined the effect of inhaled NO on the normal pulmonary circulation of the awake 40 kg lamb with a previously implanted left atrial catheter.1 Inhaling 80 parts per million (ppm) NO did not alter the normal pulmonary artery pressure (PAP), cardiac output (CO), systemic arterial pressure (SAP), or systemic vascular resistance (SVR). Therefore, we proceeded to study the effects of NO inhalation upon the pharmacologically constricted pulmonary circulation. To stably constrict the pulmonary vessels we continuously infused intravenously U46619, a thromboxane analog. Sheep breathed mixtures of NO continuously mixed with low levels of NO. NO and O3 were measured by chemiluminescence. First, the inhaled NO dose-pulmonary vasodilator response was examined. As can be observed in Figure 1, the pulmonary vascular resistance (PVR) and PAP decreased during the 6 min of inhaling each NO mixture, and each time NO inhalation was ceased for 6 min, the PAP and PVR promptly increased. The SAP and SVR were unchanged. It is important to note that the pulmonary vasodilator effect occurred at low inhaled NO levels (ie, 5 ppm) and that potent vasodilation (65 percent of maximal vasodilation) occurred at 20 ppm inhaled NO which is less than the NIOSH standard for 8-h working exposures (25 ppm).

We then examined the effect of inhaling 80 ppm NO for up to 1 h during a U46619 infusion. The NO inhalation continuously lowered the PVR and PAP without evidence of tolerance to this pulmonary vasodilator. Once again, there was a rapid return of pulmonary vasoconstriction within minutes after ceasing NO administration. Tolerance to NO pulmonary vasodilation should not occur since inhaled NO (unlike nitroglycerine) does not require metabolism to the active vasodilator molecule.

Selective Pulmonary Vasodilation in the Term Newborn Lamb

Next we examined inhalation of NO by the near-term sheep fetus.2 Since pulmonary vasoconstriction is believed to be a key component of the syndrome of persis-

tent pulmonary hypertension of the newborn (PPHN), we required an animal model of this condition. We chose to study the near-term sheep fetus, since its birth weight (3.5 to 4.5 kg) approximates the human, and pulmonary hemodynamics can be readily monitored. We sought to learn if inhaled NO could dilate the muscularized pulmonary arterial circulation of the fetal lamb. We delivered the fetus by cesarean section, mechanically ventilated the lungs, occluded the umbilical circulation, and produced pulmonary vasoconstriction by inhaling a hypoxic gas mixture (FiO2 0.1). We anesthetized the fetus with potent narcotics, performed a thoracotomy, and measured pressure in the pulmonary artery, left atrium, and aorta. Flow was measured with ultrasonic flowmeter probes placed.

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Figure 1. Plots of mean PAP and PVR during a continuous infusion of U46619. Lambs breathed various levels of nitric oxide (5-80 ppm) at FIO2 0.6 for 6 min, then breathed a gas mixture at FIO2 0.6 for 6 min without nitric oxide (n = 8, mean ± SEM). (Reprinted with permission.)

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about the ductus arteriosus, descending aorta, and pulmonary artery. Hypoxic breathing (10 percent O₂) caused rapid pulmonary vasoconstriction with pulmonary hypertension and right-to-left ductal shunting. Breathing NO despite continued hypoxia produced a rapid reduction of pulmonary vascular resistance and PAP, with reversal of the direction of shunting via the ductus arteriosus. There was no change of systemic arterial pressure during NO breathing. This study demonstrated that inhaled NO promptly and selectively reversed hypoxic pulmonary vasoconstriction in the near-term sheep fetus.

Two additional findings of this study are important. First, the concomitant presence of severe respiratory acidosis (PaCO₂ 79 ± 15 mm Hg and arterial pH (pHa) 7.14 ± 0.06, mean ± SD) did not reduce the ability of inhaled NO to selectively vasodilate the hypoxic pulmonary circulation. Thus, clinical states of asphyxia in the newborn which are often combined with hypercapnia may be more effectively reversed by breathing oxygen with added NO. Second, we measured markedly elevated lung and plasma levels of cyclic guanosine monophosphate (cGMP) during NO breathing. Since NO should activate its receptor molecule, guanylate cyclase, (which contains a ferrous heme ring) to produce cGMP, we reasoned that inhaling NO should elevate the circulating and lung tissue levels of this important secondary mediator. Indeed, we learned that within 4 min of breathing 80 ppm NO, the fetal lung tissue and circulating plasma levels of cGMP were increased threefold.

**Bronchodilation in Guinea Pigs**

Since inhaled NO can diffuse through gas and pulmonary tissue to reach upstream pulmonary artery smooth muscle cells causing them to dilate, this suggested that inhaled NO might also dilate constricted airways. However, airways have a thicker epithelium than the alveoli and a mucus coating, and both should impede the diffusion of NO. Thus, we were not confident that even a highly lipid soluble substance like NO with a low aqueous solubility (about half that of oxygen) would reach airway smooth muscle in a sufficient concentration to effectively reduce airway tone. We chose for our model the anesthetized open chest guinea pig with a tracheostomy. These small animals can be placed within a constant temperature plethysmograph, and accurate measurements of breath-to-breath airway pressure and tidal volume can be obtained using a computer monitor. We stably constricted the airways of the guinea pig with an intravenous methacholine infusion. Nitric oxide at various concentrations was added to the inhaled gas.

We noted that a stable state of increased airway tone could be produced by infusing methacholine. This was characterized by a threefold increase of pulmonary resistance (Rl) and a reduced dynamic compliance (Cdyn). Adding 5 to 300 ppm NO produced a rapid and dose-related reduction of Rl and increased Cdyn. Inhaling 15 ppm NO reduced the increased Rl by 50 percent. Thus,

![Figure 2. Nitric oxide (NO) 40 ppm was inhaled during hypoxia by 9 normal volunteers. This figure illustrates the effects on mean PAP and wedge (PCWP) pressures, PVR, PaO₂, and PaCO₂, as well as right (RVSWI) and left (LVSWI) ventricular stroke work indexes. All data mean ± SEM. Asterisk = p<0.01, value differs from first control breathing air. (Reprinted with permission)].
we discovered that inhaled NO could promptly reduce airway tone in the guinea pig given methacholine. Our initial studies characterized the inhaled NO dose-bronchodilator response, and we thereafter examined the effect of breathing NO for 1 h during a continuous infusion of methacholine. Analogous to our studies of inhaling NO to produce pulmonary vasodilation, breathing NO for 1 h stably reduced the increase of airway tone, but within minutes of ceasing NO inhalation, the bronchoconstriction recurred, and subsequently ceased after stopping the methacholine infusion. Additional studies have demonstrated inhaled NO can reverse bronchoconstriction in guinea pigs due to an intravenous infusion of leukotriene D₄, histamine, or neurokinin A. The bronchodilator effects of NO are produced locally, and there was no systemic vasodilation or hypotension due to inhaling NO.

**CLINICAL STUDIES OF NO INHALATION**

**Adult Human Hypoxic Pulmonary Vasoconstriction**

Following our pulmonary circulatory studies in sheep with inhaled NO, we examined short-term exposures of humans to NO. First we studied normal volunteers to learn if NO could reverse human hypoxic pulmonary vasoconstriction. Nine healthy subjects were monitored with a Swan-Ganz catheter, arterial line, and a pulse oximeter. By allowing the subjects to breathe at FIO₂ 0.12, the mean oxygen saturation measured by pulse oximetry (SaO₂) was reduced to 86 percent, and PaO₂ was near 50 mm Hg. Hypoxia elevated both the PVR and PAP. Breathing 40 ppm NO for 10 min immediately reduced the PVR and PAP to the baseline values breathing air before hypoxia (Fig 2). In two volunteers, breathing 10 ppm NO during hypoxia completely reversed pulmonary vasoconstriction. There was no change in the systemic blood pressure or vascular resistance, and methemoglobin levels remained below 1 percent. Cessation of NO breathing during hypoxia for 10 min was followed by the slow return of a minor level of hypoxic pulmonary vasoconstriction.

**Adult Respiratory Distress Syndrome**

We reasoned that if NO selectively dilated the pulmonary circulation, it might locally dilate ventilated regions of the lung in the adult respiratory distress syndrome (ARDS), selectively reducing vascular tone and augmenting blood flow. This might occur only in ventilated regions and not in collapsed or fluid filled lung areas contributing to the venous blood shunt. Inhaled NO should then cause a steal or diversion of pulmonary artery blood flow towards well ventilated lung. We could obtain evidence of such a salutary NO effect by noting a reduction of pulmonary vascular resistance and PAP, as well as an increase PaO₂ due to reduced intrapulmonary shunting of venous blood. Such an effect would be in marked contrast to the effects of intravenous administration of a conventional vasodilator (such as nitroprusside or prostacyclin, PGL₂). These intravenous agents lower PA pressure, however, by vasodilating shunting lung regions they augment blood flow to nonventilated areas, increasing shunting of venous blood and thereby reducing the PaO₂.

We first examined the effects of inhaling 18 and 36 ppm NO in nine patients with severe ARDS and contrasted these results with an infusion of prostacyclin.7
The NO inhalation rapidly reduced the PAP as well as the level of shunt (Qs/Qt), measured with the multiple inert gas technique, while increasing the PaO₂ in these patients. This contrasted with a prostacyclin infusion which reduced the PAP but markedly increased Qs/Qt (Fig 3). There was no change of SVR or SAP during NO inhalation.

Since these results of inhaling NO in ARDS were so promising, we proceeded to treat seven severely ill patients with ARDS for long periods with NO inhalation. Several patients had severe lung disease, and they were also treated with extracorporeal membrane oxygenation (ECMO). Seven patients with severe ARDS inhaled 5 to 20 ppm NO for periods of 3 to 53 days, six of these seven patients survived and were eventually discharged from the hospital. The NO inhalation was stopped for a brief period each day, and the changes in Qs/Qt, PaO₂, PAP, and PVR were recorded (Fig 4). When NO was withdrawn, there was a prompt increase of PAP, PVR, and Qs/Qt with a reduced PaO₂/FlO₂ ratio, and the opposite effect when NO was once again administered. Believing that the reduction of PAP would help reduce pulmonary edema formation, and since the decreased Qs/Qt allowed a 10 to 15 percent reduction of FlO₂, we treated each of these patients with low levels of NO inhalation for prolonged periods, until their lung injury had improved.

Blood levels of methemoglobin always remained below 1.3 percent.

**Congenital Heart Disease**

Since children with congenital heart disease (CHD) can develop severe pulmonary artery hypertension, we studied the pulmonary hemodynamic effects of inhaling NO, and compared these effects with those produced by breathing oxygen. We studied ten children from 3 months to 6.5 years with chronic pulmonary hypertension due to diverse congenital heart defects. Each spontaneously breathed 20 to 80 ppm NO for 10 min during a cardiac catheterization. We found that inhaling NO produced a prompt reduction of PAP and PVR without causing systemic vasodilation. The reduction of PAP and PVR produced by inhaling NO was greater than that produced by breathing 100 percent oxygen. Indeed, breathing NO further reduced the PAP and PVR of children with CHD who were breathing 90 percent O₂.

Inhaled NO may provide a selective pulmonary vasodilator for children with CHD, especially during the perioperative period after cardiac surgery when endogenous NO production is probably reduced. In addition, the effects of prolonged periods of NO breathing should be examined to learn if NO prevents pulmonary vascular
muscularization and vascular obliteration in children with pulmonary hypertension.

**Persistent Pulmonary Hypertension of the Newborn**

Based upon studies of near-term fetal sheep, it was clear that inhaled NO was a selective pulmonary vasodilator of the ovine perinatal pulmonary circulation. We therefore treated seven patients with PPHN with brief periods of NO inhalation to learn if NO would augment their PaO₂. We chose not to invasively measure pulmonary hemodynamics in these critically ill newborns since the persistence of ductal shunting makes newborn pulmonary hemodynamics difficult to interpret, and we did not wish to delay a possibly lifesaving treatment. In each of the seven newborns, there was a prompt and significant increase of preductal SpO₂, and in five infants, an increased postductal PaO₂ while breathing 80 ppm NO. The increased PaO₂ persisted while breathing NO, and in one infant, the PaO₂ remained elevated after NO breathing ceased. The six others were treated with ECMO. Kinsella et al reported treating nine babies with inhalation of 10 to 20 ppm NO for periods of up to 24 h. All nine babies improved their O₂ exchange during NO inhalation, and six infants were treated with 6 ppm inhaled NO for 24 h with sustained clinical improvement. The PaO₂ remained improved after the withdrawal of NO inhalation. The NO inhalation is presently being studied in a blinded randomized fashion in PPHN by two multicenter trials.

**NO Inhalation in Primary Pulmonary Hypertension and COPD**

Pepke-Zaba et al reported a study of 10-min inhalation periods of 40 ppm NO in air in eight patients with primary pulmonary hypertension. Apparently these patients breathed from previously prepared bags of mixed gas, and NO₃ levels were not reported. They noted PVR was reduced but did not report PAP or CO. Frattaci et al have recently reported the results of inhaled NO in patients with COPD and pulmonary hypertension. They reported that breathing 40 ppm NO reduced the PVR and PAP and slightly increased the PaO₂.

**The Future of NO**

Inhaled NO is now firmly established as a selective pulmonary vasodilator in a number of animal models and clinical pulmonary diseases of humans. Much remains to be learned. In our opinion, several important areas remain to be studied:

1. To define the toxicity of inhaled NO. For how long and at what levels is it safe for humans to breathe NO? Is it safe for the newborn to breathe NO? Is NO breathing safe for the acutely injured lung? Is genotoxicity a problem?

2. If NO breathing is safe, then in which respiratory diseases can NO cost effectively produce important benefits? PPHN? CHD? ARDS?

3. If proven safe, will prolonged NO breathing prevent additional lung injury or even save lives in the treatment of chronic pulmonary vascular diseases such as CHD? COPD? Chronic pulmonary hypertension?

4. Will NO be a useful human bronchodilator?

**References**


**Nitric Oxide Inhalation**

**Effects on the Ovine Neonatal Pulmonary and Systemic Circulations**

Vincent DeMarco, Ph.D.; Jeffrey Skimming, M.D.; Tamir M. Ellis, Ph.D.; and Sidney Cassin, Ph.D.

These experiments were designed to test the hypothesis that inhaled nitric oxide (NO), an endothelium-derived relaxing factor, causes a decrease in pulmonary arterial pressure and pulmonary vascular resistance (PVR) in the normal and constricted ovine neonatal pulmonary circulation (N = 7; age = 13.9 ± SD 3.3; mass = 5.7 ± SD 0.8 kg).

Using aseptic surgery, catheters were placed in the

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