Minimizing the Inhaled Dose of NO With Breath-by-Breath Delivery of Spikes of Concentrated Gases

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Background—Pulmonary vasodilatation with a 100 ppm concentration of NO given as a short burst of a few milliliters at the beginning of each breath (NO_{min}) was compared with conventionally inhaled NO, in which a full breath of 40 ppm of NO was inhaled (NO_{CD}).

Methods and Results—NO_{min} was studied in 16 patients with severe pulmonary hypertension and in 16 isolated porcine lungs with experimentally induced pulmonary hypertension. We compared volumes of 8 to 38 mL of 100 ppm NO in N_{2} injected at the beginning of each breath with conventional inhalation of 40 ppm NO in air. NO_{CD} and NO_{min} were studied in 4 pigs after inhibition of NO synthase with N^{G}-nitro-L-arginine methyl ester (1 to 2 mg/kg IV) had raised the pulmonary vascular resistance index (PVRI) from 4.4±0.8 to 10.0±1.6 mm Hg·L^{-1}·min^{-1}·kg^{-1}. A similar comparison was made in 7 isolated porcine lungs after the thromboxane analogue U46619 (10 pmol·L^{-1}·min^{-1}) increased the mean PVRI from 4.6±0.8 to 12.2±1.3 mm Hg·L^{-1}·min^{-1}·kg^{-1}. Patients’ mean PVRI was reduced from 29.2±3.7 to 24.0±3.1 with NO_{min} and 24.5±3.3 mm Hg·L^{-1}·min^{-1}·m^{-2} (mean±SEM) with NO_{CD}. In isolated porcine lungs, there was the same reduction of PVRI for NO_{min} and NO_{CD} between 12.7% and 34.8%.

Conclusions—A small volume of NO inhaled at the beginning of the breath was equally effective as NO_{CD} but reduced the dose of NO per breath by 40-fold, which ranged from 1.2×10^{-3} (0.4 μg) to 1.6×10^{-5} mol/L (4.8 μg) compared with 5.3×10^{-7} (16 μg) to 1.2×10^{-6} mol/L (36 μg) per breath with NO_{CD}. (Circulation. 1998;98:2429-2432.)

Key Words: hypertension, pulmonary artery lung vasodilation

Inhaled NO is a selective pulmonary vasodilator, and in acute respiratory distress syndrome (ARDS), it improves gas exchange. Current methods of administration of NO are complicated and not without hazard.

NO is stored in concentrations of 100 to 10 000 ppm, with N_{2}. Safe and effective concentrations of inhaled NO range from 1 to 40 ppm, which are achieved by mixing NO with respiratory gases. The flow rate of the mixed gases is carefully matched to the rate of ventilation, preventing buildup of NO_{2}. While straightforward for ventilated patients, ambulatory patients who vary their rate of ventilation renders delivery difficult. A new method of delivery is required for them because inhaled NO can be used to treat pulmonary hypertension.

Higher doses of inhaled NO are needed to reduce pulmonary hypertension than to improve gas exchange in ARDS. Conventional delivery (NO_{CD}), which distributes inhaled NO throughout the lungs, can, however, worsen gas exchange in patients with chronic obstructive pulmonary disease (COPD). Selective delivery of NO to fast-ventilated regions of the lungs could reduce this problem.

An alternative to NO_{CD} is to inject a small volume of NO, added at the start of each breath. This has been learned from measurement of the gas transfer of carbon monoxide (TLCO) and allows NO to reach the resistance pulmonary arteries.

Methods

Experimentally Induced Pulmonary Hypertension

Sixteen pathogen-free pigs weighing 36 to 60 kg were studied as previously described. Midazolam (0.3 mg/kg IM) and droperidol (0.5 mg/kg IM) premedication was followed by anesthesia with sodium pentobarbital (25 mg/kg IV). They were ventilated (Manley Ventilator, Blease Medical) with 40% O_{2} and 60% N_{2}, a tidal volume of 10 to 12 mL/kg, and a maximum airway pressure of 10 mm Hg.

In Vivo Porcine Studies

In supine animals, pulmonary artery pressure (PAP), pulmonary wedge pressure (PWP), and right atrial pressure (RAP) in 9 animals, together with right carotid artery pressure (SAP), were measured. Euthanasia was effected immediately at the end with intravenous (10 mL of 1 mol/L) potassium chloride.

Isolated, Perfused Porcine Lung Studies

Isolated, salt-perfused lungs were prepared as previously described through a median sternotomy in 7 pigs. Euthanasia was undertaken by exsanguination. Pulmonary blood flow (Q) was measured with a Doppler flow probe and meter (model 16SB185 and model T101D,
Methods of NO Delivery

For the NO_{max}, a device\(^4\) delivered a range of volumes of a mixture of 100 ppm NO/N\(_2\), at the beginning of each breath. With a solenoid valve (M8B-3E2C-6DC, Valeader Engineering Ltd), NO/N\(_2\) was released at a flow rate of 12 L/min. The volume of gas and hence the dose delivered depended on the duration of opening times, which ranged from 10 to 1000 ms. This was calibrated before each study with a water spirometer. The valve was manual or automatic and synchronized with the start of inhalation, an automatic function triggered from the airway pressure transducer.

With NO_{min}, NO was delivered in a gas mixture of 79% N\(_2\) and 21% O\(_2\) (Pneumopac Ltd). A 150-mL reservoir was interposed between the pump and the pulmonary artery. Perfusion rate of the lungs was slowly increased from 10 to 100 mL min\(^{-1}\) kg\(^{-1}\) at a concentration of 40 ppm diluted (Pneumopac Ltd). For the NO CD, 40 ppm NO was delivered in a gas mixture in triggered from the airway pressure transducer.

Protocols

Endothelial NO synthase was inhibited with \(N^\bullet\)-nitro-L-arginine methyl ester (L-NAME) (1 to 2 mg/kg IV; Sigma Chemical Company Ltd). A stable rise of pulmonary vascular resistance index (PVRI) and systemic vascular resistance index (SVRI) occurred 20 to 30 minutes after injection. In the isolated lung studies, to achieve a stable elevation of PVRI after 10 to 15 minutes, the thromboxane analogue U46619 (10 pmol L\(^{-1}\) min\(^{-1}\), Sigma Chemical Company Ltd) was infused into the isolated lung perfusate. The experiments lasted 120 minutes.

A dose response to increasing concentrations of NO_{max} was undertaken in 4 animals. Concentrations of 10, 40, and 80 ppm of NO were tested in random order after the PVRI had been increased by L-NAME. Minimal dose of 100 ppm NO/N\(_2\) was tested in 4 pigs after L-NAME infusion. The valve opening time was set at 10, 20, 40, 80, 160, 320, and 1000 ms in random order. The measurements of PVRI were made over a period of 5 minutes.

In 7 isolated lungs, the PVRI after U46619 was reduced by NO_{CD} of 40 ppm NO and NO_{max} with valve opening times set at 10, 80, 160, and 320 ms.

Clinical Study of Severe Pulmonary Hypertension

Sixteen patients with severe pulmonary hypertension were studied. The mean age of the patients was 44.2 (SD\(\pm\)13.9) years; 6 had thromboembolic pulmonary hypertension, 8 had unexplained pulmonary hypertension, 1 had pulmonary veno-occlusive disease, and 1 had pulmonary hypertension associated with sarcoidosis. All gave written consent, and the study was approved by the local hospital ethics committee. A diagnostic right heart catheter allowed measurement of mean RAP, mean PAP, and mean PWP.

Methods of NO Delivery

For the NO_{max}, the solenoid switch was operated manually at the start of inhalation. The patient inhaled from a 1-L reservoir bag, through a tightly fitting face mask (Nasal CPAP mask, Puritan-Bennet Corp) fitted with a pneumotachograph and differential manometer giving a record of the breathing pattern (PK Morgan, Maidstone). The reservoir was replenished with 79% N\(_2\) and 21% O\(_2\) (Pneumopac Ltd). For the NO_{CD}, 40 ppm NO was delivered in a gas mixture in 79% N\(_2\) and 21% O\(_2\) to a 1-L reservoir bag from which the patient breathed with the tightly fitted mask.

Protocol

The maximal fall in PVR with the vasodilator intravenous prostacyclin (Epoprostenol, PGI2)\(^6\) was compared with NO_{CD}. For the NO_{max}, the duration of the spike of NO/N\(_2\) (100 ppm) was varied until a minimum dose caused a fall in PVR of \(\pm 10\%\).

Calculations and Statistical Analysis

The PVR was calculated by dividing the pressure difference across the lungs (PAP–LAP or PWP/pulmonary blood flow (Q), and SVR (SAP–RAP)/Q). The PVRI and SVRI were standardized to body weight in the experimental studies (mm Hg·L\(^{-1}\)·min\(^{-1}\)·kg\(^{-1}\)) and in clinical studies to body surface area (mm Hg·L\(^{-1}\)·min\(^{-1}\)·m\(^{-2}\)).

The mean values for PVRI were calculated with standard errors (SEM). ANOVA and Fisher’s test for multiple comparison were undertaken to compare treatments. Paired Student’s t tests were performed to compare baseline and post–L-NAME or post-U46619 PVRI values.

Results

Porcine Studies

The 10 ppm NO_{CD} was a less effective vasodilator than 40 and 80 ppm of NO_{CD} (Figure 1) in 5 animals after L-NAME increased the PVRI by 6.5\(\pm\)1.1 mm Hg·L\(^{-1}\)·min\(^{-1}\)·kg\(^{-1}\). In 4 animals, L-NAME caused a rise of PVRI from 4.3\(\pm\)0.8 to 10.0\(\pm\)1.6 mm Hg·L\(^{-1}\)·min\(^{-1}\)·kg\(^{-1}\). The NO_{min} at volumes of 8 to 38 mL produced the same fall in PVRI as did 115 mL (Table 1), but the SVRI was unaffected by NO.

The thromboxane analogue U46619 (10 pmol L\(^{-1}\)·min\(^{-1}\)) caused an increase in PVRI from 4.6\(\pm\)0.8 to 12.2\(\pm\)1.3 mm Hg·L\(^{-1}\)·min\(^{-1}\)·kg\(^{-1}\). The NO_{min} caused a fall of PVRI to 66.5%, and similar reductions occurred with NO_{max} at volumes of 3 to 38 mL of 100 ppm NO in N\(_2\) (Figure 2).

### Table 1. Reduction of PVR by Spiked NO on Induced Pulmonary Hypertension After L-NAME Infusion in Pigs

<table>
<thead>
<tr>
<th>Opening Time of Spiked NO, ms</th>
<th>Volumes of NO/N(_2), ml</th>
<th>Dose Given(\times)10(^{-9}), mol/L</th>
<th>Reduction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>8.20(\pm)0.03</td>
<td>3.4</td>
<td>19.1(\pm)5.1</td>
</tr>
<tr>
<td>80</td>
<td>12.40(\pm)0.03</td>
<td>5.2</td>
<td>20.0(\pm)5.2</td>
</tr>
<tr>
<td>160</td>
<td>21.40(\pm)0.13</td>
<td>8.9</td>
<td>25.8(\pm)7.8</td>
</tr>
<tr>
<td>320</td>
<td>38.30(\pm)0.13</td>
<td>15.9</td>
<td>21.7(\pm)9.6</td>
</tr>
<tr>
<td>1000</td>
<td>115.10(\pm)0.31</td>
<td>47.9</td>
<td>34.8(\pm)8.4</td>
</tr>
</tbody>
</table>

Baseline PVR in response to L-NAME was taken as 100%; reduced PVR is expressed as percentage of this baseline (mean\(\pm\)SEM). All spiked NO reduced PVR significantly (\(P<0.01\)).
start of the inhalation provides an equivalent measure of diffusion. The use of a small volume of CO added at the pulmonary acini. Inhaled NO must therefore reach the alveolar arteries, located anatomically within the precapillary arteries, located anatomically within the pulmonary acini. Inhaled NO must therefore reach the alveolar region at a sufficient concentration. We can learn from the measurement of gas diffusion (TLCO) with carbon monoxide. Being similar to CO, NO is also used to measure the measurement of gas diffusion (TLCO) with carbon monoxide. Being similar to CO, NO is also used to measure pulmonary acini. Inhaled NO must therefore reach the alveolar region at a sufficient concentration. We can learn from the measurement of gas diffusion (TLCO) with carbon monoxide. Being similar to CO, NO is also used to measure diffusion. The use of a small volume of CO added at the start of the inhalation provides an equivalent measure of TLCO. The rate of diffusion of CO (or NO) from this inhaled bolus is slower, for example, 4 × 10⁻⁴ · mL⁻¹ · min⁻¹.

**Clinical Study**

The mean PVR was 29.2 ± 3.7 (mean ± SE) mm Hg · L⁻¹ · min⁻¹ · m⁻², which fell to 24.0 ± 3.1 with NO cocain (8 to 38 mL of 100 ppm) and to 24.5 ± 3.3 with 40 ppm of NO cocain (Table 2). The average tidal volume varied from 320 to 730 mL. PGI₂ caused a comparable fall of mean PVR to 20.0 ± 3.2 mm Hg · L⁻¹ · min⁻¹ · m⁻². Unlike inhaled NO, PGI₂ also caused a fall in SVRI (Table 2). The volumes of gas delivered by the solenoid valve ranged from 3 to 115 mL (Table 1).

**Discussion**

A small volume of NO/N₂ of up to 38 mL, delivered at the beginning of the breath, was as effective a vasodilator as a 40-fold higher dose of NO CD. Conventional delivery required 5.3 × 10⁻⁷ to 1.2 × 10⁻⁶ mol/L per breath to achieve comparable pulmonary vasodilatation as 1.2 × 10⁻⁷ to 1.6 × 10⁻⁷ mol/L of the spike.

Pulmonary hypertension is reduced by inhaled NO acting on the precapillary arteries, located anatomically within the pulmonary acini. Inhaled NO must therefore reach the alveolar region at a sufficient concentration. We can learn from the measurement of gas diffusion (TLCO) with carbon monoxide. Being similar to CO, NO is also used to measure diffusion. The use of a small volume of CO added at the start of the inhalation provides an equivalent measure of TLCO. The rate of diffusion of CO (or NO) from this inhaled bolus is slower, for example, 4 × 10⁻⁴ · mL⁻¹ · min⁻¹.

**TABLE 2. Response of Spiked NO, Continuous NO, and PGI₂ Infusion in Patients With Severe Pulmonary Hypertension**

<table>
<thead>
<tr>
<th></th>
<th>PVRI mm Hg · L⁻¹ · min⁻¹ · m⁻²</th>
<th>SVRI mm Hg · L⁻¹ · min⁻¹ · m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 1</td>
<td>29.2 ± 3.7</td>
<td>48.4 ± 3.6</td>
</tr>
<tr>
<td>Spike 100 ppm</td>
<td>24.0 ± 3.1*</td>
<td>45.0 ± 3.4</td>
</tr>
<tr>
<td>Continuous 40 ppm</td>
<td>24.5 ± 3.3*</td>
<td>45.4 ± 3.1</td>
</tr>
<tr>
<td>Baseline 2</td>
<td>28.5 ± 3.6</td>
<td>47.0 ± 3.4</td>
</tr>
<tr>
<td>PGI₂</td>
<td>20.0 ± 2.6†</td>
<td>35.7 ± 3.0†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Spike of 8 to 38 mL of NO of 100 ppm NO/N₂ at the beginning of the breath achieved the same reduction in PVR as did a full breath of 320 to 730 mL of 40 ppm of NO. Similar falls in PVR are seen with PGI₂. *P<0.05 compared with baseline 1, †P<0.01 compared with baseline 2.

**References**


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