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

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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\textsubscript{min}) was compared with conventionally inhaled NO, in which a full breath of 40 ppm of NO was inhaled (NO\textsubscript{CD}).

Methods and Results—NO\textsubscript{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\textsubscript{2} injected at the beginning of each breath with conventional inhalation of 40 ppm NO in air. NO\textsubscript{CD} and NO\textsubscript{min} were studied in 4 pigs after inhibition of NO synthase with N\textsuperscript{G},N\textsuperscript{G}-dimethyl-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\textsuperscript{-1}·min\textsuperscript{-1}·kg\textsuperscript{-1}. A similar comparison was made in 7 isolated porcine lungs after the thromboxane analogue U46619 (10 pmol·L\textsuperscript{-1}·min\textsuperscript{-1}) increased the mean PVRI from 4.6±0.8 to 12.2±1.3 mm Hg·L\textsuperscript{-1}·min\textsuperscript{-1}·kg\textsuperscript{-1}. Patients’ mean PVRI was reduced from 29.2±3.7 to 24.0±3.1 with NO\textsubscript{min} and 24.5±3.3 mm Hg·L\textsuperscript{-1}·min\textsuperscript{-1}·m\textsuperscript{-2} (mean±SEM) with NO\textsubscript{CD}. In isolated porcine lungs, there was the same reduction of PVRI for NO\textsubscript{min} and NO\textsubscript{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\textsubscript{CD} but reduced the dose of NO per breath by 40-fold, which ranged from 1.2×10\textsuperscript{-8} (0.4 μg) to 1.6×10\textsuperscript{-7} mol/L (4.8 μg) compared with 5.3×10\textsuperscript{-7} (16 μg) to 1.2×10\textsuperscript{-6} mol/L (36 μg) per breath with NO\textsubscript{CD}. (Circulation. 1998;98:2429-2432.)

Key Words: hypertension, pulmonary | lung | vasodilation

Inhaled NO is a selective pulmonary vasodilator,\textsuperscript{1,2} and in acute respiratory distress syndrome (ARDS), it improves gas exchange.\textsuperscript{3} Current methods of administration of NO are complicated and not without hazard.\textsuperscript{4}

NO is stored in concentrations of 100 to 10 000 ppm, with N\textsubscript{2}. Safe and effective concentrations of inhaled NO range from 1 to 40 ppm,\textsuperscript{5} 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\textsubscript{2}.\textsuperscript{6} While straightforward for ventilated patients, ambulatory patients who vary their rate of ventilation renders delivery difficult. A new method of delivery is needed for them because inhaled NO can be used to treat pulmonary hypertension.\textsuperscript{7}

Higher doses of inhaled NO are needed to reduce pulmonary hypertension than to improve gas exchange in ARDS.\textsuperscript{5} Conventional delivery (NO\textsubscript{CD}), which distributes inhaled NO throughout the lungs, can, however, worsen gas exchange in patients with chronic obstructive pulmonary disease (COPD).\textsuperscript{9,10} Selective delivery of NO to fast-ventilated regions of the lungs could reduce this problem.

An alternative to NO\textsubscript{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.\textsuperscript{11}

Methods

Experimentally Induced Pulmonary Hypertension
Sixteen pathogen-free pigs weighing 36 to 60 kg were studied as previously described.\textsuperscript{12,13} 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\textsubscript{2} and 60% N\textsubscript{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\textsuperscript{13} 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 NOmill, a device delivered a range of volumes of a mixture of 100 ppm NO/N2 at the beginning of each breath. With a solenoid valve (M8B-3E2C-6DC, Valuable Engineering Ltd), NO/N2 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 NOmin, NO was delivered in a gas mixture of 79% N2 and 21% O2 (5% CO2 was added for the isolated lungs) at a concentration of 40 ppm diluted (Pneumopac Ltd) from a mixture of 10 000 ppm NO/N2.

Protocol

Endothelial NO synthase was inhibited with N\textsuperscript{-}\textsuperscript{-}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\textsuperscript{-1} min\textsuperscript{-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\textsubscript{early} 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/N2 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\textsubscript{early} at volumes of 8 to 38 mL produced the same fall in PVRI as 100 ppm of NO\textsubscript{early} (Figure 1) in 5 animals after L-NAME increased the PVRI by 6.5 ± 1.1 mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · kg\textsuperscript{-1} and in clinical studies to body surface area (mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · m\textsuperscript{2}). The NO\textsubscript{early} caused a fall in PVRI to 66.5% of this baseline (mean ± SEM). The NO\textsubscript{early} decreased PVRI significantly (P < 0.05 compared with L-NAME, #P < 0.01 compared with L-NAME).

Calculations and Statistical Analysis

The PVRI was calculated by dividing the pressure difference across the lungs [PAP—LAP or PWP] by 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\textsuperscript{-1} · min\textsuperscript{-1} · kg\textsuperscript{-1}) and in clinical studies to body surface area (mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · m\textsuperscript{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\textsubscript{early} was a less effective vasodilator than 40 and 80 ppm of NO\textsubscript{early} (Figure 1) in 5 animals after L-NAME increased the PVRI by 6.5 ± 1.1 mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · kg\textsuperscript{-1} and in clinical studies to body surface area (mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · m\textsuperscript{2}). In 4 animals, L-NAME caused a rise of PVRI from 4.3 ± 0.8 to 10.0 ± 1.6 mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · kg\textsuperscript{-1}. The NO\textsubscript{early} 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\textsuperscript{-1} · min\textsuperscript{-1}) caused an increase in PVRI from 4.6 ± 0.8 to 12.2 ± 1.3 mm Hg · L\textsuperscript{-1} · min\textsuperscript{-1} · kg\textsuperscript{-1}. The NO\textsubscript{early} caused a fall of PVRI to 66.5%, and similar reductions occurred with NO\textsubscript{early} at volumes of 3 to 38 mL of 100 ppm NO in N2 (Figure 2).

![Figure 1. Effects of fixed concentration of NO on PVRI in 4 isolated, perfused porcine lungs induced by L-NAME (mean ± SEM). Each concentration of NO reduced PVRI significantly. Falls in PVRI caused by 40 and 80 ppm NO were not significantly different. ∗P < 0.05 compared with L-NAME, #P < 0.01 compared with L-NAME.

### 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/N2 Emitted, mL</th>
<th>Dose Given × 10\textsuperscript{-9} mol/L</th>
<th>Reduction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>8.20 ± 0.03</td>
<td>3.4</td>
<td>19.1 ± 5.1</td>
</tr>
<tr>
<td>80</td>
<td>12.40 ± 0.03</td>
<td>5.2</td>
<td>20.0 ± 5.2</td>
</tr>
<tr>
<td>160</td>
<td>21.40 ± 0.13</td>
<td>8.9</td>
<td>25.8 ± 7.8</td>
</tr>
<tr>
<td>320</td>
<td>38.30 ± 0.13</td>
<td>15.9</td>
<td>21.7 ± 9.6</td>
</tr>
<tr>
<td>1000</td>
<td>115.10 ± 0.31</td>
<td>47.9</td>
<td>34.8 ± 8.4</td>
</tr>
</tbody>
</table>

| Baseline PVRI in response to L-NAME was taken as 100%; reduced PVR is expressed as percentage of this baseline (mean ± SEM). All spiked NO reduced PVR significantly ( P < 0.01). |
Figure 2. Response to spiked NO and continuous NO inhalation in 7 isolated, perfused lungs. PVR was raised by thromboxane analogue U46619. Baseline PVR in response to U46619 is shown as 100%. Data are presented as percentage of baseline (mean ± SEM). Forty ppm NOCD caused PVR to fall to 66.5% of baseline value (*P<0.01). Similar reductions in PVR were obtained with 8 to 38 mL spikes of 100 ppm NO/N₂.

Clinical Study

The mean PVR1 was 29.2±3.7 (mean±SE) mm Hg · L⁻¹ · min⁻¹ · m⁻², which fell to 24.0±3.1 with NOCD (8 to 38 mL of 100 ppm) and to 24.5±3.3 with 40 ppm of NOCD (Table 2). The average tidal volume varied from 320 to 730 mL. PGI₁ caused a comparable fall of mean PVR1 to 20.0±2.6 mm Hg · L⁻¹ · min⁻¹ · m⁻². Unlike inhaled NO, PGI₁ 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 NOCD. Conventional delivery required 5.3×10⁻⁸ to 1.2×10⁻⁶ mol/L per breath to achieve comparable pulmonary vasodilatation as 1.2×10⁻⁸ 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.17–19 The use of a small volume of CO added at the start of the inhalation provides an equivalent measure of TLCO.11 The rate of diffusion of CO (or NO) from this inhaled bolus is slower, for example, 4×10⁻⁴ · mL⁻¹ · min⁻¹ (400 ppb/min at 37.0°C)20 than the rate of convective flow of the inhaled air into the lungs, for example, 7500 mL/min.21 Little change in the concentration of NO (or CO) in the bolus is expected to occur until the alveoli are reached.

The practical advantage of NOCD is that the dose of inhaled NO depends on the frequency of breathing, for example, increasing with exercise. With NOCD, it is not possible to match the flow rate of the gas mixture of NO to the patient’s rate of ventilation unless the patient is supported by a mechanical ventilator. The concentration of NO in the spike never will exceed that in the cylinder. It is not necessary to mix with respiratory gases or to monitor the concentrations of inhaled NO. Furthermore, by comparison with NOCD, slow diffusion from the spike reduces the oxidation of NO to NO₂, therefore careful monitoring for a buildup of NO₂ is not needed.

The delivery of a spike of NO greatly reduces the gas waste compared with continuous inhalation, in which 50% of the gas is lost during expiration. We estimate that for a 10-mL spike of NO/N₂ to be delivered, in each breath only 216 L of NO/N₂ would be needed each day. A small container the size of a hip flask could be easily carried by the patient, considerably reducing the daily volume of gas needed to treat primary pulmonary hypertension.

The lowest effective vasodilatory concentration of inhaled NO remains to be determined. The NOCD systems give concentrations of NO in the inspirate of 1 to 120 ppm.22 To relax pulmonary arteries in vitro requires 2 ppm of NO gas.23 Most of the inhaled NO combines with oxyhemoglobin of red blood cells to form methemoglobin.24 This indicates a considerable redundancy in the NOCD dose.

Finally, by inhaling NO as a bolus at the beginning of inspiration, it is distributed only to fast-ventilated regions of the lungs.25 Slow-filling lung units will receive less NO than with NOCD. This should avoid the worsening gas exchange in COPD seen with NOCD, as with inhaled bronchodilators.25 This could extend treatment with inhaled NO to patients with COPD.

In summary, we describe a means of reducing the effective dose per breath of NO by 40-fold when compared with conventional delivery and that lessens potential toxicity as well as offering a safe means of treating ambulatory patients. It should overcome the problems of worsening V̇ₐ/Q inhomogeneity, to allow inhaled NO to be used for COPD.

Acknowledgments

This work was supported by the HC Roscoe award of the BMA and the British Heart Foundation grant PG93/94043.

References


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

<table>
<thead>
<tr>
<th>PGI₁</th>
<th>SVRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm Hg · L⁻¹ · min⁻¹ · m⁻²</td>
<td>mm Hg · L⁻¹ · min⁻¹ · m⁻²</td>
</tr>
<tr>
<td>Baseline 1</td>
<td>29.2±3.7</td>
</tr>
<tr>
<td>Spike 100 ppm</td>
<td>24.0±3.1*</td>
</tr>
<tr>
<td>Continuous 40 ppm</td>
<td>24.5±3.3*</td>
</tr>
<tr>
<td>Baseline 2</td>
<td>28.5±3.6</td>
</tr>
<tr>
<td>PGI₂</td>
<td>20.0±2.6†</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.


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Circulation. 1998;98:2429-2432
doi: 10.1161/01.CIR.98.22.2429

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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