An Animal Model of Response and Nonresponse to Inhaled Nitric Oxide in Endotoxin-Induced Lung Injury*

Hedwig Maurenbrecher, MD; Maurice Lamy, MD; Ginette Deby-Dupont, PhD; Philippe Frascarolo, PhD; and Göran Hedenstierna, MD

Study objective: Oxygenation may be improved in 40 to 60% of ARDS patients by inhalation of nitric oxide (NO). We have studied the response to inhaled NO in porcine acute lung injury 4 h and 6 h after onset of a 2-h endotoxin infusion (30 μg/kg/h), hypothesizing that a responder may change to a nonresponder over time and with progression of lung injury.

Design: Animal study.

Setting: Experimental laboratory in a university hospital.

Interventions and measurements: We studied eight pigs under general anesthesia (mean weight, 26.2 kg) receiving mechanical ventilation adjusted to normocapnia, with a fraction of inspired oxygen (FIO2) of 0.5 to 1.0. Blood gases, endotoxin concentration, and central hemodynamics were measured hourly, and ventilation-perfusion (V/Q) relationships were assessed by multiple inert gas elimination technique before and after inhalation of NO. NO was delivered at 40 ppm for 10 min at 4 h and 6 h of endotoxin exposure.

Results: Seven of eight pigs were responders to NO at 4 h, defined as a ≥20% increase in oxygenation index (Pao2/Fio2) [223 ± 43 to 330 ± 56 mm Hg; p = 0.001]. The same pigs exhibited a ≥20% fall in mean pulmonary artery pressure (39.4 ± 2.2 to 30.0 ± 2.1 mm Hg; p < 0.001). The response correlated to the perfusion to “normal V/Q” regions (r = 0.82) and negatively to shunt and dead space ventilation (r = 0.76 and r = 0.87, respectively). At 6 h, seven of eight pigs were nonresponders, despite unaltered hemodynamics and gas exchange. Correlations at 4 h between physiologic variables and response to NO were abolished. The logarithmic SDs of the perfusion distribution, a measure of the degree of V/Q mismatch, increased significantly from 4 to 6 h (p = 0.04).

Conclusion: Response to inhaled NO is abolished over time in endotoxin-induced ARDS pig lungs. The response seems to be related to the degree of hypoxic pulmonary vasoconstriction. (CHEST 2001; 120:573–581)

Key words: endotoxin; lung injury; nitric oxide; pig

Abbreviations: CVP = central venous pressure; EU = endotoxin units; Fio2 = fraction of inspired oxygen; HPV = hypoxic pulmonary vasoconstriction; iNOS = inducible nitric oxide synthase; logSDQ = logarithmic SDs of perfusion distribution; MAP = mean systemic arterial pressure; MIGET = multiple inert gas elimination technique; MPAP = mean pulmonary artery pressure; NO = nitric oxide; Pao2,i = oxygenation index; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; V/Q = ventilation/perfusion; Vt = tidal volume

ARDS is a clinical syndrome of multiple origin associated with a high mortality rate.1 It is characterized by acute sustained pulmonary hyperten-

*From the Department of Clinical Physiology (Drs. Maurenbrecher and Hedenstierna), University Hospital, Uppsala, Sweden; Department of Anesthesia and Intensive Care (Drs. Lamy and Deby-Dupont), University Hospital, Liège, Belgium; and Department of Anesthesiology (Dr. Frascarolo), University Hospital, Lausanne, Switzerland.

This study was supported by grants from the Swedish Medical Research council (grant 5315), the Swedish Heart and Lung Fund, the Laerdal Foundation, and the AGA Medical Fund.

Manuscript received November 30, 1999; revision accepted February 21, 2001.

Correspondence to: Göran Hedenstierna, MD, Department of Clinical Physiology, University Hospital, S-75185 Uppsala, Sweden.
soluble guanylate cyclase, which increases intracellular concentration of guanosine 3',5'-cyclic monophosphate in the smooth-muscle cell and causes relaxation.\textsuperscript{3} Endogenous NO, produced by the endothelium by a constitutive NO synthetase, contributes to the basic vascular tone. Inducible NO synthase (iNOS) is stimulated by endotoxin and cytokines, producing large amounts of NO and inhibiting hypoxic pulmonary vasoconstriction (HPV).\textsuperscript{4,5}

Inhaled NO, by its unique route of administration and extremely short half-life, selectively dilates vessels in ventilated lung units. This results in redistribution of lung blood flow, reduction of shunt with improvement of arterial oxygenation, and a decrease in mean pulmonary artery pressure (MPAP).\textsuperscript{6} However, the initial enthusiasm for its clinical application in patients with ARDS has been tempered by its inconsistent efficacy. Clinical studies\textsuperscript{7–10} have shown an effectiveness of 40 to 60% in terms of improvement in arterial oxygenation. Attempts to identify predictive factors for the gas exchange response to inhaled NO in patients with ARDS, such as the level of shunt, oxygenation index (PaO2\textsubscript{i}) [PaO2/fraction of inspired oxygen (FiO2)], pulmonary vascular resistance (PVR), and cardiac output prior to NO inhalation, have yielded inconclusive results.\textsuperscript{10} However, septic shock is a condition associated with less response to inhaled NO.\textsuperscript{8,11}

The knowledge about the mechanisms of nonresponse is limited. Most animal studies\textsuperscript{12–13} testing the effect of inhaled NO have had an observation period of 3 to 4 h, and the nonresponse phenomenon has not been reported except in two studies\textsuperscript{13,14} of oleic acid-induced ARDS, where the histologic analysis of the nonresponder animals revealed more severe and diffuse lung damage. Hyposensitivity in terms of a decrease in MPAP has been reported in isolated lung.\textsuperscript{15}

Based on these observations and of the clinical experience that NO inhalation is often introduced in a later phase of ARDS, we tested the degree of response to inhaled NO delivered at two different times (4 h and 6 h) after the induction of lung injury by endotoxin. In previous experimental studies,\textsuperscript{12–13} animals were found to be responders in an early phase (3 to 4 h). We hypothesized that the progression of the injury will lead to nonresponse at 6 h. We therefore investigated the question of whether the response to NO inhalation in an endotoxin porcine model changed over time with regard to hemodynamic and blood gas parameters and to ventilation-perfusion (V/Q) distribution.

### Materials and Methods

#### Animals

After approval of the local Animal Research Ethical Committee, eight pathogen-free pigs (mixed Hampshire, Yorkshire, and landrace breeds) of either sex (mean weight, 26.2 ± 1.0 kg), submitted to regular health checks, were studied.

#### Anesthesia

The animals were brought to the laboratory in a heated car the morning of the experiment. General anesthesia was induced with IM atropine, 0.04 mg/kg; tiletamine-zolazepam, 6 mg/kg; and xylazine, 2.2 mg/kg. After loss of the eyelid reflex, the pig was placed in a supine position on the operation table, a venous line was introduced into an ear vein, and the trachea was intubated with a 7-mm inner-diameter tube. IV fentanyl, 200 μg, and pancuronium, 0.2 mg/kg, were administered, and mechanical ventilation was started in a volume-cycled mode (Servo 900C, Siemens-Elema AB; Lund, Sweden). At baseline, the ventilator settings were as follows: tidal volume (VT) of 12 mL/kg at 20 breaths/min, inspiration to expiration ratio of 1:2, and positive end-expiratory pressure of 5 cm H\textsubscript{2}O. The FiO\textsubscript{2} was 0.5 in five animals and 1.0 in three animals throughout the experiment. The VT was adapted during the experiment to attempt normoventilation, as guided by the end-tidal CO\textsubscript{2} tension (35.9 ± 5.2 mm Hg) and by intermittent PaCO\textsubscript{2} measurements (45.9 ± 0.9 mm Hg). Anesthesia was maintained by continuous infusion of clomethiazole, 400 mg/h; fentanyl, 150 μg/h; and pancuronium, 2.5 mg/h. Additional fentanyl and pancuronium were administered if needed. Ringer’s lactate at body temperature (1,000 mL) was infused prior to baseline measurements, and the infusion was thereafter adjusted to yield a stable systemic arterial pressure and hemoglobin concentration (8.43 ± 0.11 g/dL).

#### Catheterization

An arterial line was inserted into the left carotid artery together with a thermistor-tipped catheter, which was advanced into the descending aorta for measurement of cardiac output. A Swan-Ganz catheter and an 18-gauge catheter were introduced into the right jugular vein, and their positions were confirmed by the corresponding pressure curves. A silicone-coated balloon catheter (Ch 20; Riisch AG; Kernen, Germany) was inserted into the bladder, and peritoneal fluid was drained by a multihole catheter.

#### Measurements

Mean systemic arterial pressure (MAP), MPAP, central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP) values were recorded at the end of expiration. Cardiac output was measured by injection of 8 mL of cold glucose 5%. The bolus was injected into the right atrium at the end of expiration. The dilution curves for temperature were recorded in the aorta with the thermistor-tipped catheter connected to a cardiac output computer. Two measurements were made and averaged; three measurements were made and averaged if the first two measurements differed by > 10%.

Arterial and mixed venous blood samples were analyzed for pH, PO\textsubscript{2}, PCO\textsubscript{2}, O\textsubscript{2} saturation, hemoglobin, and methemoglobin.
Arterial blood samples were obtained for cell counts and measurement of albumin and plasma endotoxin concentrations; the blood samples at 4 h and 6 h were drawn just before NO inhalation. For endotoxin measurements, blood was drawn into pyrogen-free Falcon tubes (Becton Dickinson; Franklin Lakes, NJ), and the assay was performed with a quantitative end point pyrogen-free Falcon tubes (Becton Dickinson; Franklin Lakes, NJ), and the assay was performed with a quantitative end point detection was 0.06 EU/mL. The V˙/Q˙ distribution was determined in endotoxin units (EU) per milliliter, and the lowest limit of detection was 0.06 EU/mL. The V˙/Q˙ distribution was determined at 4 h and 6 h after induction of lung injury by the multiple inert gas elimination technique (MIGET). This technique is based on the elimination of six inert gases according to their different blood-gas solubility coefficients (sulfur hexafluoride, ethane, cyclopropane, enflurane, diethyl ether, acetone). At steady state, after 40 min of continuous IV infusion (2 to 3 mL/min, depending on minute ventilation) of these gases diluted in glucose 5%, arterial and mixed venous blood samples were collected together with an expired gas sample and analyzed by gas chromatography (5890, series II; Hewlett-Packard; Wilmington, DE). These data allowed the construction of a virtually continuous distribution of gas elimination technique (MIGET). This technique is based on the elimination of six inert gases according to their different blood-gas solubility coefficients (sulfur hexafluoride, ethane, cyclopropane, enflurane, diethyl ether, acetone). At steady state, after 40 min of continuous IV infusion (2 to 3 mL/min, depending on minute ventilation) of these gases diluted in glucose 5%, arterial and mixed venous blood samples were collected together with an expired gas sample and analyzed by gas chromatography (5890, series II; Hewlett-Packard; Wilmington, DE). These data allowed the construction of a virtually continuous distribution of gas flow and ventilation against different V˙/Q˙ ratios: shunt (V˙/Q < 0.005), low V˙/Q (0.005 < V˙/Q < 0.1), normal V˙/Q (0.1 < V˙/Q < 10), and high V˙/Q areas (10 < V˙/Q < 100), and dead space (V˙/Q > 100). The dispersion of V˙/Q ratios is expressed as the logarithmic SDs of perfusion distribution (logSDQ) and describes the degree of V˙/Q mismatch (not including shunt).

End-tidal CO₂ and Fio₂ were monitored continuously (Capnomac Ultima; Datex Instrumentarium; Helsinki, Finland). Compliance and resistance of the total respiratory system were determined during an inspiratory-hold maneuver (approximately 4 s). Resistance was calculated as the difference between peak airway pressure and the pressure at 2 s of the end-inspiratory phase, divided by the inspiratory flow. Compliance was calculated as Vt divided by end-inspiratory pressure minus end-expiratory pressure. Airway pressures and flow were measured in the ventilator on the inspiratory side and recorded with a personal computer for on-line signal processing, taking into account the gas compression within the ventilatory circuit. The mean of two inspiratory-hold maneuvers was used for statistical analysis (LabVIEW 3.1; C-O Sjöberg Engineering; National Instruments). The same monitoring was used for Vt, minute ventilation, respiratory rate, and airway pressures.

Protocol

After a stabilization period of 1 h and baseline measurements, lung injury was induced by endotoxin infusion (30 μg/kg/h; Escherichia coli lipopolysaccharide O111:B4; Sigma-Aldrich; Stockholm, Sweden) administered via a peripheral venous line over 2 h. The dose of 30 μg/kg/h over 2 h that was used in the study is based on prior experiments in pigs. The animals were submitted to an observation period of 6 h with two challenges of inhaled NO of 10 min each: the first challenge at 4 h and the second challenge at 6 h after the start of endotoxin infusion.

NO was delivered from a 1,000 ppm gas tank (AGA Gas AB; Lidingö, Sweden) together with air/oxygen from a low-flow air-oxygen blender (AGA AB; Sundbyberg, Sweden) into the low-pressure gas-flow inlet of the ventilator. The NO delivery was adjusted by means of a calibrated flowmeter yielding an inspiratory concentration of 40 ppm, which was controlled by an NO chemiluminescence analyzer (9841 NOx; Lear Siegler Measurement Controls Corporation, Englewood, CO) connected to the inspiratory limb of the respiratory circuit. After 10 min of NO inhalation, circulatory and ventilatory measurements were made as described above. At the end of the experiment, the pig was killed with an IV injection of potassium chloride (20 mg).

Central hemodynamics and some ventilatory data are presented in Table 1, and gas exchange and V˙/Q results are shown in Table 2. At baseline, all variables were within normal limits as compared to values from previous studies in healthy pig lungs.

The changes in MPAP and PaO₂ over time are illustrated in Figure 1. Other effects on lung function at 4 h and 6 h after induction of lung damage are shown in Tables 1, 2. There were significant increases in MPAP and PVR, whereas cardiac output, MAP, and systemic vascular resistance remained unaltered. PaO₂ and pH were significantly reduced, and PaCO₂ was significantly increased 4 h after induction of endotoxin lung damage. The MIGET data showed the presence of a >20% shunt, whereas perfusion of low V˙/Q regions was minor at 4 h; dead space ventilation was approximately 50% of the total ventilation, and there was no ventilation to high V˙/Q areas. logSDQ, a measure of V˙/Q ratio dispersion, was increased compared to measurements in normal pigs in previous studies.

Compliance fell and resistance increased after induction of the lung injury (Table 1).

There were no significant differences in any variable between the measurements made at 4 h and 6 h.

Statistical Analysis

Data were given as mean ± SEM if not otherwise indicated. For each parameter, the normality was tested by the Wilcoxon-Shapiro test. One-way analysis of variance for repeated measurements was performed to assess the effect of endotoxin over time. A paired Student’s t test, or Wilcoxon’s signed rank test if appropriate, was applied to detect differences due to inhaled NO and to compare the effects of endotoxin at 4 and 6 h. Bonferroni correction for multiple comparison was then applied. Simple regression analysis was carried out with the Pearson coefficient to explain the difference in response to inhaled NO at 4 h and 6 h. All p values are given as raw data, and p < 0.05 was considered the level of significance.

RESULTS

Baseline Data and Effects of Endotoxin Infusion

Central hemodynamics and some ventilatory data are presented in Table 1, and gas exchange and V˙/Q results are shown in Table 2. At baseline, all variables were within normal limits as compared to values from previous studies in healthy pig lungs.

The changes in MPAP and PaO₂ over time are illustrated in Figure 1. Other effects on lung function at 4 h and 6 h after induction of lung damage are shown in Tables 1, 2. There were significant increases in MPAP and PVR, whereas cardiac output, MAP, and systemic vascular resistance remained unaltered. PaO₂ and pH were significantly reduced, and PaCO₂ was significantly increased 4 h after induction of endotoxin lung damage. The MIGET data showed the presence of a >20% shunt, whereas perfusion of low V˙/Q regions was minor at 4 h; dead space ventilation was approximately 50% of the total ventilation, and there was no ventilation to high V˙/Q areas. logSDQ, a measure of V˙/Q ratio dispersion, was increased compared to measurements in normal pigs in previous studies.

Compliance fell and resistance increased after induction of the lung injury (Table 1).

There were no significant differences in any variable between the measurements made at 4 h and 6 h.
Table 1—Hemodynamics and Ventilatory Parameters Over Time and Effect of Inhaled NO at 4 h and 6 h*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Endotoxin (4 h)</th>
<th>NO Trial 1 (4 h)</th>
<th>Endotoxin (6 h)</th>
<th>NO Trial 2 (6 h)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4h</td>
<td>6h</td>
<td>4h</td>
<td>6h</td>
<td>Baseline vs EXN</td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>15.1 ± 0.6</td>
<td>39.4 ± 2.2</td>
<td>30.0 ± 2.1</td>
<td>36.4 ± 3.4</td>
<td>32.7 ± 4.2</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>81.9 ± 3.3</td>
<td>76.6 ± 7.7</td>
<td>74.1 ± 7.2</td>
<td>79.3 ± 8.7</td>
<td>75.3 ± 9.6</td>
<td>0.556</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>107.8 ± 5.0</td>
<td>153.1 ± 6.3</td>
<td>154.3 ± 6.9</td>
<td>157.0 ± 11.7</td>
<td>163.4 ± 8.5</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>4.17 ± 0.34</td>
<td>4.23 ± 0.46</td>
<td>4.15 ± 0.38</td>
<td>4.32 ± 0.60</td>
<td>5.19 ± 0.64</td>
<td>0.948</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>5.1 ± 0.4</td>
<td>10.0 ± 1.9</td>
<td>11.5 ± 0.9</td>
<td>0.048</td>
<td>0.002†</td>
<td>0.552</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>4.5 ± 0.4</td>
<td>9.0 ± 1.4</td>
<td>11.6 ± 2.2</td>
<td>0.014†</td>
<td>0.023</td>
<td>0.129</td>
</tr>
<tr>
<td>PVR, dyne·s·cm⁻⁵</td>
<td>200 ± 12</td>
<td>597 ± 75</td>
<td>775 ± 344</td>
<td>0.007†</td>
<td>0.147</td>
<td>0.529</td>
</tr>
<tr>
<td>SVR, dyne·s·cm⁻⁵</td>
<td>1.599 ± 157</td>
<td>1.380 ± 129</td>
<td>1.480 ± 298</td>
<td>0.212</td>
<td>0.448</td>
<td>0.578</td>
</tr>
<tr>
<td>Ventilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p Value</td>
</tr>
<tr>
<td>Compliance, mL/cm H₂O</td>
<td>27.84 ± 1.00</td>
<td>11.23 ± 0.83</td>
<td>10.61 ± 1.49</td>
<td>&lt; 0.001†</td>
<td>&lt; 0.001†</td>
<td>0.529</td>
</tr>
<tr>
<td>Resistance, cm</td>
<td>12.61 ± 0.46</td>
<td>28.75 ± 3.25</td>
<td>31.85 ± 3.77</td>
<td>&lt; 0.001†</td>
<td>0.002†</td>
<td>0.173</td>
</tr>
<tr>
<td>H₂O·s·L⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Paw, cm H₂O</td>
<td>18.64 ± 4.90</td>
<td>35.53 ± 16.98</td>
<td>37.68 ± 17.74</td>
<td>&lt; 0.001†</td>
<td>0.020</td>
<td>0.392</td>
</tr>
</tbody>
</table>

*EXN 4h = 4 h after the start of endotoxin infusion; EXN 6h = 6 h after the start of endotoxin infusion; diffNO1 = difference between pre-NO levels and values at 10 min of inhaled NO at 4 h; diffNO2 = difference between pre-NO levels and values at 10 min of inhaled NO at 6 h; HR = heart rate; SVR = systemic vascular resistance; Paw = airway pressure.

†p values significant with Bonferroni correction for multiple comparisons.
after induction of lung damage, except for a further worsening of the matching of ventilation and blood flow, expressed as logSDQ (Table 2).

The endotoxin concentration, leukocyte count, and fraction of neutrophils are shown in Figure 2. The endotoxin concentration increased rapidly after the start of the endotoxin infusion, reaching a plateau between 1 and 2 h in parallel with a major initial fall in neutrophil count. The endotoxin concentration then decreased and regained almost baseline values at 6 h, whereas the neutrophil count increased (64 ±5% at 4 h and 75 ±4% at 6 h; p ≤ 0.001). The

Table 2—Blood Gas and V˙/Q˙ Parameters Over Time and Effect of Inhaled NO at 4 h and 6 h

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (4 h)</th>
<th>Endotoxin (4 h)</th>
<th>NO Trial 1 (4 h)</th>
<th>Endotoxin (6 h)</th>
<th>NO Trial 2 (6 h)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood gases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.52 ± 0.01</td>
<td>7.30 ± 0.03</td>
<td>7.31 ± 0.03</td>
<td>7.29 ± 0.05</td>
<td>7.27 ± 0.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>453.9 ± 25.0</td>
<td>223.0 ± 43.7</td>
<td>330.4 ± 56.3</td>
<td>178.7 ± 45.8</td>
<td>202.8 ± 49.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>PvO₂, mm Hg</td>
<td>45.9 ± 1.3</td>
<td>41.6 ± 2.0</td>
<td>45.1 ± 1.9</td>
<td>40.0 ± 4.1</td>
<td>40.8 ± 3.8</td>
<td>0.154</td>
</tr>
<tr>
<td>PacO₂, mm Hg</td>
<td>36.5 ± 1.4</td>
<td>49.7 ± 2.7</td>
<td>48.6 ± 2.8</td>
<td>52.6 ± 4.7</td>
<td>56.3 ± 4.7</td>
<td>0.004</td>
</tr>
<tr>
<td>BE</td>
<td>6.15 ± 0.40</td>
<td>-1.96 ± 0.98</td>
<td>-1.74 ± 1.26</td>
<td>-3.04 ± 2.18</td>
<td>-3.17 ± 2.43</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hemoglobin, mm Hg</td>
<td>8.0 ± 0.3</td>
<td>8.2 ± 0.3</td>
<td>8.8 ± 0.6</td>
<td>7.43 ± 0.255</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>metHb, %</td>
<td>0.089 ± 0.15</td>
<td>0.45 ± 0.16</td>
<td>0.7 ± 0.11</td>
<td>0.38 ± 0.17</td>
<td>0.77 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>MIGET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shunt, fraction of cardiac output</td>
<td>0.228 ± 0.048</td>
<td>0.153 ± 0.046</td>
<td>0.239 ± 0.043</td>
<td>0.237 ± 0.044</td>
<td>0.963</td>
<td>0.012</td>
</tr>
<tr>
<td>Normal V˙/Q˙, fraction of cardiac output</td>
<td>0.759 ± 0.048</td>
<td>0.843 ± 0.048</td>
<td>0.750 ± 0.045</td>
<td>0.760 ± 0.045</td>
<td>0.615</td>
<td>0.004</td>
</tr>
<tr>
<td>logSDQ</td>
<td>0.514 ± 0.077</td>
<td>0.764 ± 0.066</td>
<td>0.908 ± 0.101</td>
<td>0.557 ± 0.009</td>
<td>0.035</td>
<td>0.044</td>
</tr>
<tr>
<td>Dead space, fraction of V˙e</td>
<td>0.490 ± 0.023</td>
<td>0.478 ± 0.020</td>
<td>0.510 ± 0.039</td>
<td>0.483 ± 0.012</td>
<td>0.504</td>
<td>0.502</td>
</tr>
</tbody>
</table>

*pVO₂ = mixed venous O₂ tension; metHb = methemoglobin; BE = base excess; V˙e = minute ventilation; see Table 1 for definitions of other abbreviations not defined in the text.
thrombocyte count and albumin concentration decreased significantly between baseline and 6 h (389 ± 30 × 10^3 to 164 ± 10 × 10^3, and 21.7 ± 0.6 to 11.2 ± 1.1 g/L, respectively), with a significant difference between 4 h and 6 h (p = 0.024 and p = 0.035, respectively).

**Effects of Inhaled NO**

*Effect of NO at 4 h:* All pigs but one (n = 7) responded to the 10-min inhalation of 40 ppm of NO 4 h after induction of lung damage with a > 20% increase in PaO\textsubscript{2}. This was the effect of decreased shunt and reduced dispersion of V/Q ratios (logSDQ). Inhalation of NO also resulted in a significant decrease in MPAP (Fig 1, Table 1).

*Effect of NO at 6 h:* One pig died 6 h after the induction of lung damage, just before the second NO trial, which was thus conducted in seven pigs. Only one pig responded to the second NO trial, according to the definition given above. Thus, at 6 h, NO inhalation had no significant effect either on PaO\textsubscript{2} or on shunt. However, MPAP fell, though only to a minor extent, from 36 to 33 mm Hg. The MAP fell from 79 to 75 mm Hg (p = 0.06; Tables 1, 2).

Pooled data from the two NO trials showed that the increase in PaO\textsubscript{2} on inhalation of NO was correlated to the logarithmic endotoxin concentration (p = 0.006), and that the decrease of MPAP on NO inhalation was negatively correlated to the logarithmic endotoxin concentration (p = 0.029; Fig 3).

**Correlations Between Pulmonary Function and Effect of Inhaled NO**

*Effect of NO at 4 h:* The fall in MPAP during inhalation of NO 4 h after the onset of endotoxin infusion correlated to the fraction of lung perfusion that was going to normally ventilated and perfused lung regions (‘normal V/Q’; Fig 4). The fall in MPAP was negatively correlated to the magnitude of shunt and to the dead space ventilation (Table 3). Thus, when taking these correlations together, the results suggest that inhalation of NO had a better effect on MPAP when V/Q mismatch was less marked.

Four hours after induction of lung damage, there was a significant correlation between increase in PaO\textsubscript{2} and decrease in MPAP during inhalation of NO (Table 3, Fig 5). There was also a tendency toward higher PVR levels in those pigs, in which PaO\textsubscript{2} increased the most on inhalation of NO (p = 0.06). The fall in MPAP on inhalation of NO
correlated negatively to the total respiratory resistance; there was no correlation to compliance (Table 3).

**Effect of NO at 6 h:** None of the correlations observed at 4 h after induction of lung damage by endotoxin infusion remained at 6 h (Figs 4, 5; Table 3); therefore, when there was no longer a significant increase in oxygenation in response to inhaled NO, there was also no correlation between the severity of lung dysfunction and the effect of inhaled NO. This loss of correlation was noted despite the fact that the degree of lung damage in terms of PaO₂i, shunt, low V˙/Q˙ areas, and dead space ventilation were the same at 4 h and 6 h (Table 2).

**DISCUSSION**

**Response to Endotoxin**

This study presents a sepsis-induced lung injury model with the animal changing from responder to inhaled NO at 4 h of endotoxin infusion (PaO₂i increase ≥ 20%) to a nonresponder at 6 h (absence or negative response to NO). The functional lung injury was characterized by shunt, V˙/Q˙ mismatch, and increased dead space ventilation. Respiratory mechanics were impaired, with decreased compliance and increased resistance. A capillary leak syndrome (decreased albumin concentration) with generalized edema was present.

Endotoxins, lipopolysaccharide components of Gram-negative bacteria, cause an inflammatory injury mediated by activation of alveolar macrophages and neutrophils in the lungs18–20 and other organs. This stimulates iNOS to produce endogenous NO.4 Sepsis is one of the situations related to nonresponse, and we therefore chose an endotoxin infusion for inducing an acute lung injury. The differential cell count in the present study reflected a Gram-negative sepsis with leukopenia and thrombocytopenia, similar to that seen in humans. The blood endotoxin concentrations, measured to validate the model,

<table>
<thead>
<tr>
<th>Dependent vs Independent Parameter</th>
<th>EXN 4h</th>
<th>EXN 6h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>No. of Pigs</td>
</tr>
<tr>
<td>dMPAP vs:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dPaO₂i</td>
<td>0.838</td>
<td>8</td>
</tr>
<tr>
<td>Shunt</td>
<td>0.762</td>
<td>8</td>
</tr>
<tr>
<td>Q normal V˙/Q˙</td>
<td>−0.822</td>
<td>8</td>
</tr>
<tr>
<td>V normal V˙/Q˙</td>
<td>−0.857</td>
<td>8</td>
</tr>
<tr>
<td>V dead space</td>
<td>0.568</td>
<td>8</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.710</td>
<td>8</td>
</tr>
<tr>
<td>Compliance</td>
<td>−0.350</td>
<td>8</td>
</tr>
</tbody>
</table>

*V = ventilation; P = perfusion; see legends for Figures 3 and 4 and Table 1 for definition of other abbreviations.

**Figure 5.** Regression of change in PaO₂i vs change in MPAP on inhalation of NO (values at 10 min of NO inhalation minus pre-NO values) at 4 h (p = 0.009) and 6 h (p = 0.562). See legends for Figures 3, 4 for definition of abbreviations.
confirm the efficacy of the infusion as well as of the hepatic clearing of the toxin. The 2 h of endotoxin infusion established a hemodynamically stable model at 4 h and 6 h, and seven of the eight pigs survived the 6-h study period. The animals exhibited lung damage of variable severity (shunt between 8% and 39%, \( P_{aO_2} \) between 63 mm Hg and 435 mm Hg) despite the fact that we chose young and pathogen-free animals to avoid endotoxin-primed lungs. It is well-known, however, that the extent of the injury in the lungs as well as in other organs is subject to interindividual and interspecies differences. The pigs that received ventilation with \( FIO_2 \) of 1.0 (\( n = 3 \)) seemed to be more severely affected; thus, an additional insult by \( O_2 \) toxicity was possible in these animals.

**Response to Inhaled NO**

The aim of using a high NO concentration (40 ppm) was to elicit a response (and avoid false-negative responses) during a short period of inhalation (10 min) in order not to create an impact on the second NO trial 2 h later, although this cannot be fully excluded. The correlation 4 h after the start of endotoxin infusion between the fall in MPAP induced by NO inhalation and the amount of perfusion and ventilation going to normal \( V/Q \) areas may suggest that inhaled NO must reach a certain amount of lung tissue to create a response. logSDQ had increased further from 4 h to 6 h, indicating a larger dispersion of \( V/Q \) ratios. This finding, without a change in other variables, such as cardiac output, PVR, \( P_{aCO_2} \), and mixed venous \( O_2 \) tension between 4 h and 6 h, may indicate a decline in the strength of HPV. Moreover, at 6 h, there was no longer a response to inhaled NO, and the close relationship between a decrease in MPAP and an increase in \( P_{aO_2} \) induced by inhaled NO that was observed at 4 h and that has been reported from clinical studies was also abolished. Thus, is possible that blunted HPV is a cause of nonresponse to inhaled NO; loss of vascular tone should reasonably reduce the effectiveness of a vasodilator.

Another observation in the present study was that the response to inhaled NO correlated to the endotoxin concentration in the blood. This finding may have been coincidental and should be interpreted with caution. However, Gust and coworkers reported that a low dose of endotoxin added to an oleic acid-induced acute lung injury model created a responder to inhaled NO, whereas oleic acid alone resulted in a nonresponse. This was attributed to increased release of prostacyclin, an effect of endotoxin that has been demonstrated earlier.

There are also other potential mechanisms underlying the nonresponse to inhaled NO. The endogenous production of NO, stimulated by activation of iNOS via endotoxin and cytokines as well as by hypoxia itself, as shown for bovine endothelial cells in culture, may counteract the hypoxic vasoconstrictor stimulus and contribute to the decreased response to exogenous NO. Free radicals produced by inflammatory cells, especially activated neutrophils and alveolar macrophages, may scavenge the inhaled NO before it has reached the target cell. The presence of generalized edema with albumin loss might have thickened the alveolar-capillary membrane and prevented NO from reaching the vascular smooth muscle.

The loss of effectiveness of inhaled NO might also be due to obstruction of blood vessels by microthrombi or by architectural changes in the alveolar-capillary membrane, reducing the area of the vascular bed available for gas exchange, as observed in other studies. These observations are in concordance with our findings that the response to inhaled NO was related to the amount of normally perfused and ventilated lung.

**Conclusion**

In this animal model of endotoxin-induced acute lung injury, maintained for 6 h, the reaction to inhaled NO changed from a positive response 4 h after induction of lung damage to a nonresponder situation at 6 h. We demonstrated that the response was related to the amount of lung parenchyma accessible for ventilation and perfusion and to the endotoxin concentration in blood. The increased dispersion of \( V/Q \) ratios in the nonresponders suggests that attenuation of HPV may be another determinant of the nonresponse phenomenon. Extended studies with comparisons of response and nonresponders concerning endotoxin levels, vasoactive inflammatory mediators, and histologic changes in responders and nonresponders are required to elucidate this problem further.

**References**


Laboratory and Animal Investigations
22 Doering EB, Hanson CW, Reily DJ, et al. Improvement in oxygenation by phenylephrine and nitric oxide in patients with adult respiratory distress syndrome. Anesthesiology 1997; 87:18–25