Cardiopulmonary effects of inhaled nitric oxide in normal dogs and during E. coli pneumonia and sepsis

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We have developed a canine model of gram-negative pneumonia that simulates many of the cardiopulmonary changes occurring in patients with this condition. We hypothesized that the selective pulmonary vasodilation associated with inhaled NO would reduce pulmonary arterial hypertension and improve oxygenation in this canine model. By altering inspired O2 concentrations, we also investigated whether hypoxic pulmonary vasoconstriction was still present during gram-negative pneumonia and, if present, how NO might alter it.

MATERIALS AND METHODS

Study design and experimental subjects. Thirty-eight 2-yr-old purpose-bred beagles weighing 10–12 kg had permanent tracheostomies placed as previously described (9). Baseline evaluations were performed 7 days later. Using local anesthesia, we placed femoral and pulmonary arterial catheters (35). Awake animals were then studied while breathing eight different gas mixtures for 10 min each. Four fractional inspired O2 (FiO2) doses were tested (0.08, 0.21, 0.50, and 0.85) in both the presence [80 parts/million (ppm)] and absence (0 ppm) of NO. During the last 5 min of each 10-min inhalation period, a complete cardiopulmonary evaluation (see Cardiopulmonary measurements) was performed. After completion of this study, all intravascular catheters were removed. Seven days later (day 0), animals were challenged intrabronchially with E. coli pneumonic and sepsis. Three days later (day 0), animals were challenged intrabronchially with E. coli pneumonic and sepsis. Seven days later (day 0), animals were challenged intrabronchially with E. coli pneumonic and sepsis.

HYPOXEMIA AND PULMONARY hypertension contribute to the morbidity and mortality of patients with pneumonia and acute lung injury (50, 63). Hypoxemia during such injury results in large part from mismatching of perfusion and ventilation (23, 24, 63). In addition, abnormalities of hypoxic pulmonary vasoconstriction, the normal physiological response that diverts blood flow from poorly to well-ventilated lung regions, may contribute to hypoxemia (7, 24, 27–29, 45, 59).

Inhaled nitric oxide (NO), a potent pulmonary vasodilator, has been studied as adjuvant therapy for acute lung injury (4, 5, 11, 21, 50). Because inhaled NO only produces pulmonary vasodilation in well-ventilated lung regions, it can potentially improve matching of ventilation and perfusion (4, 5, 11, 21, 50). When inhaled NO reaches the pulmonary vasculature, it is thought to be rapidly bound to and inactivated by hemoglobin, which in turn produces systemic effects (31, 46, 62). Therefore, in contrast to systemic vasodilators previously used to treat pulmonary hypertension (20, 39, 44, 52), inhaled NO may selectively dilate the pulmonary vasculature without producing systemic vasodilation (5, 11, 21, 46, 50).

Inhaled NO has been shown to decrease pulmonary hypertension associated with a number of stimuli, including hypoxia, thromboxane analogs, and heparin-protamine treatment (8, 12, 14, 47, 48). However, with acute lung injury, in humans and animals, the effects of inhaled NO on pulmonary hypertension and arterial oxygenation have been variable (3–5, 11, 12, 36–38, 40, 49–51, 53, 54) and in certain settings detrimental (1). Therefore, it is possible that the effects of inhaled NO may vary depending on several factors, such as the etiology, stage, and severity of the underlying lung injury and the presence of other therapies.

We have developed a canine model of gram-negative pneumonia that simulates many of the cardiopulmonary changes occurring in patients with this condition. We hypothesized that the selective pulmonary vasodilation associated with inhaled NO would reduce pulmonary arterial hypertension and improve oxygenation in this canine model. By altering inspired O2 concentrations, we also investigated whether hypoxic pulmonary vasoconstriction was still present during gram-negative pneumonia and, if present, how NO might alter it.
with Escherichia coli 0111:B4 (infected) or 0.9% saline (noninfected) administered in a lobar or diffuse distribution (see Intrabronchial inoculation). Studies identical to those at baseline were repeated 24, 48, and 504 h after intrabronchial inoculation (day E0). These time points were selected because pilot and other prior studies indicated that mortality was maximal during the first 48 h after inoculation and that by 504 h cardiopulmonary function in surviving septic animals had returned to preseptic baseline values (13).

Three animals were studied each day: one animal with lobar pneumonia, one animal with diffuse pneumonia, and one noninfected animal. For 5 days, all animals received ceftriaxone (100 mg·kg⁻¹·day⁻¹) Ringer solution (50 ml/kg) at 6 and 12 h on day 0, in addition to a 20 ml/kg bolus followed by a 10 ml/kg continuous infusion that was administered during cardiopulmonary studies at baseline, 24, 48, and 504 h.

Intrabronchial inoculation. On day 0, using topical anesthesia (20 ml of 1% lidocaine), we introduced a bronchoscope (BF type 1T2OD; Olympus, Lake Success, NY) into the tracheal stoma. The right and left caudal and cranial lobe bronchi were identified, and a balloon-tipped pulmonary artery catheter was introduced through the injectate port of the bronchoscope. The balloon was then inflated, and a 5-ml aliquot of 0.9% saline or E. coli 0111:B4 was injected. Animals randomized to lobar pneumonia were inoculated with the total E. coli dose in one lobe and 0.9% saline in the other three lobes. Animals randomized to have diffuse pneumonia were given one-fourth of the total dose of E. coli in each of four lobes. Those randomized to be noninfected were given an equivalent amount of 0.9% saline in all four lobes. To study the effects of inhaled NO in nonlethal vs. lethal pneumonia, 9 animals were inoculated with 0.75 × 10¹⁰ colony-forming units (CFU)/kg body weight (5 lobar and 4 diffuse), and 16 animals were inoculated with 1.5 × 10¹⁰ CFU/kg body weight of E. coli 0111:B4 (50% lethal dose; n = 8 lobar and 8 diffuse animals).

Inhalation challenges. The order of the eight inhalation challenges for each animal was randomly determined at baseline, and this order was repeated on all subsequent study days. Briefly, the animals had auffed tracheostomy tube placed. The tube was then connected to a nonrebreathing gas system consisting of a 5-liter mixing chamber, a 5-liter reservoir bag, and a one-way valve to separate inspired from expired gas. By using volumetric calibrated flowmeters, varying quantities of O₂, nitrogen, and NO were delivered through the gas system to produce gas mixtures with four FIO₂ doses (0.08, 0.21, 0.50, and 0.85), each administered with or without NO (80 or 0 ppm). Inhaled NO concentration was continuously monitored and maintained constant throughout the duration of each inhalation challenge by using chemiluminescence analysis (model 14A; Thermo Environmental Instruments, Franklin, MA). The efficiency of the chemiluminescence analyzer was measured by a generator of NOₓ (model 100B; Thermo Environmental Instruments); the efficiency was found to be 98%. NO, mixed with pure nitrogen, was supplied at a concentration of 800 ppm from a tank that contained only 3.2 ppm of other nitrogen oxides such as NO₂ (Air Products and Chemicals, Allentown, PA). After cardiolungary measurements. Femoral arterial and thermodilution pulmonary arterial catheters were used to measure, at each NO and FIO₂ concentration studied, heart rate (HR), mean arterial pressure (MAP, in mmHg), central venous pressure, mean pulmonary artery pressure (MPAP, in mmHg), pulmonary artery occlusion pressure (Ppa, in mmHg), and cardiac output (in ml/min). Hemodynamic data were indexed to body weight in kilograms. Cardiac index (CI), stroke volume index (SVI), left ventricular stroke work index, systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI; in dyn·s·cm⁻⁵·kg⁻¹), O₂ delivery index, and alveolar-arterial O₂ gradient (A-aDO₂, in Torr) were calculated by using standard formulas. At baseline and at 48 and 504 h after completing all inhalation challenges, left ventricular ejection fraction (LVEF) was measured by radio-nuclide-gated blood pool scanning as previously described (35).

Laboratory measurements. Routine chemistries, complete blood counts, quantitative blood and sputum cultures, and endotoxin levels were obtained on each study day by using previously described techniques (35). In addition, complete blood counts were obtained at 1, 2, and 3 h on day 0. Furthermore, at each FIO₂ and NO concentration studied, arterial and mixed-venous partial pressures of O₂ (PaO₂) and a-vPO₂, respectively, in Torr), arterial partial pressure of CO₂ (PaCO₂, in mmHg), and methemoglobin concentration were measured by using a blood-gas analyzer (model 288; Radiometer, Medfield, MA). Blood lactate (in mM) was determined by using a glucose-lactate analyzer (YSI model 2300 STAT; Yellow Springs Instrument, Yellow Springs, OH). Anterior-posterior and lateral chest radiographs were also obtained on each study day before beginning inhalation studies (model SPG 5125; CGR Medical, Baltimore, MD). Chest radiographs and the severity of infiltrates were analyzed by a veterinarian radiologist unaware of the treatment group to which each animal belonged. The right and left cranial and caudal lobes, as well as the right middle and accessory lobes, were graded as to the level of opacification on a scale of 1 (normal) to 4 (severe).

Animal care. The protocol for this investigation was approved by the Animal Care and Use Committee of the Clinical Center of the National Institutes of Health. Throughout the studies, all efforts were undertaken to minimize animal pain and suffering.

Statistical methods. Hemodynamic and laboratory parameters were analyzed by using an analysis of variance (ANOVA; Ref. 52). The analyses were performed in stages. At baseline, to examine the effects of FIO₂ and inhaled NO before infection, a five-way ANOVA with FIO₂ dose (0.08, 0.21, 0.50, and 0.85), inhaled NO dose (0 and 80 ppm), group (control, diffuse, and lobar), dose of E. coli, and dog (nested within group and dose of E. coli) was performed. All higher-order interactions, except those including dog, were included in the model. All interactions that include dog were used as the error term. When interaction terms were nonsignificant, data were pooled over these terms to increase the power of analysis. When FIO₂ and NO, or FIO₂-NO interactions are reported, all higher order interactions involving these terms were not significant.

To examine the effects of E. coli challenges, changes from baseline on hemodynamic and laboratory parameters were computed. These changes were computed for each dog and each dose of FIO₂ and NO, on both days 1 and 2 postdol implantation. We performed a six-way ANOVA by using all factors above plus the additional factor of day of the study. In addition, the factor for group was decomposed into two independent factors: one for infected-noninfected and the other for the type of infection. Similar to the analyses performed on baseline data, the reporting of significant effects implies that the higher order interactions that include the reported term are all nonsignificant. Multiple comparisons associated with FIO₂ effects were controlled by using a Tukey test.
Table 1. Pulmonary hemodynamic and gas exchange variables measured 7 days before intrabronchial inoculation during inhalation of 4 different FIO₂ levels with and without NO

<table>
<thead>
<tr>
<th>Variable/Group</th>
<th>FIO₂ 0.08 NO, ppm</th>
<th>FIO₂ 0.21 NO, ppm</th>
<th>FIO₂ 0.50 NO, ppm</th>
<th>FIO₂ 0.85 NO, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ± 80</td>
<td>0 ± 80</td>
<td>0 ± 80</td>
<td>0 ± 80</td>
</tr>
<tr>
<td>MAP, mmHg C</td>
<td>26 ± 1</td>
<td>21 ± 0.5</td>
<td>21 ± 0.6</td>
<td>21 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>27 ± 0.7</td>
<td>22 ± 0.6</td>
<td>21 ± 0.7</td>
<td>21 ± 0.5</td>
</tr>
<tr>
<td>PpaO, mmHg C</td>
<td>10 ± 0.5</td>
<td>10 ± 0.6</td>
<td>11 ± 0.5</td>
<td>11 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>10 ± 0.5</td>
<td>10 ± 0.4</td>
<td>10 ± 0.3</td>
<td>10 ± 0.8</td>
</tr>
<tr>
<td>PVRI, dyn·s·cm⁻⁵·kg⁻¹ C</td>
<td>4.0 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3.5 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>PaO₂, Torr C</td>
<td>31 ± 1</td>
<td>31 ± 1</td>
<td>101 ± 1</td>
<td>248 ± 19</td>
</tr>
<tr>
<td></td>
<td>32 ± 1</td>
<td>106 ± 1</td>
<td>106 ± 2</td>
<td>261 ± 6</td>
</tr>
<tr>
<td>A-aD O₂, Torr</td>
<td>0.2 ± 0.2</td>
<td>0 ± 0</td>
<td>6 ± 2</td>
<td>66 ± 20</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.2</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>53 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. NO, nitric oxide; ppm, parts/million; FIO₂, fractional inspired O₂; MAP, mean pulmonary artery pressure; PpaO, pulmonary artery occlusion pressure; PVRI, pulmonary vascular resistance index; PaO₂, partial pressure of arterial O₂; A-aD O₂, alveolar-arterial O₂ gradient; C, controls; I, animals that later would be intrabronchially inoculated with Escherichia coli.

RESULTS

Comparison of pulmonary and systemic hemodynamic variables at baseline. At baseline, there were no significant [P = not significant (NS)] differences in pulmonary or systemic hemodynamic variables among study groups (Tables 1 and 2), except that all animals (both those designated to be noninfected controls or infected animals) from the high-dose bacteria experiments (E. coli dose of 1.0 × 10¹⁰ CFU) at FIO₂ 0.85 had higher PaO₂ (470 ± 5 vs. 434 ± 5 Torr, mean ± SE) and lower A-aD O₂ (95 ± 4 vs. 129 ± 5 Torr), compared with all animals from the high-dose bacteria experiments (E. coli dose of 1.0 × 10¹⁰ CFU) (both P = 0.0001). Inhaled NO had similar (P = NS) effects on all pulmonary and systemic hemodynamic variables measured in all groups at baseline (Tables 1 and 2).

Clinical manifestations of pneumonia and survival. After intrabronchial E. coli challenges, all infected animals had signs of pneumonia, including lethargy, tachypnea, and production of purulent sputum. After intrabronchial saline challenges, all noninfected animals appeared healthy throughout.

All animals receiving 0.75 × 10¹⁰ CFU E. coli intrabronchially survived to 21 days. Five of eight animals receiving 1.5 × 10¹⁰ CFU E. coli in one pulmonary lobe and four of eight receiving this dose equally divided among four pulmonary lobes died (Fig. 1).

Pulmonary and systemic hemodynamic effects of pneumonia. Animals with diffuse and lobar E. coli challenges had similar pulmonary and systemic hemodynamic measurements at all FIO₂ doses studied with or without inhaled NO. Therefore we averaged over this.

Table 2. Systemic hemodynamic and left ventricular variables measured 7 days before intrabronchial inoculation during inhalation of 4 different FIO₂ levels with and without NO

<table>
<thead>
<tr>
<th>Variable/Group</th>
<th>FIO₂ 0.08 NO, ppm</th>
<th>FIO₂ 0.21 NO, ppm</th>
<th>FIO₂ 0.50 NO, ppm</th>
<th>FIO₂ 0.85 NO, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ± 80</td>
<td>0 ± 80</td>
<td>0 ± 80</td>
<td>0 ± 80</td>
</tr>
<tr>
<td>MAP, mmHg C</td>
<td>133 ± 4</td>
<td>128 ± 3</td>
<td>128 ± 4</td>
<td>125 ± 4</td>
</tr>
<tr>
<td></td>
<td>138 ± 2</td>
<td>135 ± 2</td>
<td>127 ± 2</td>
<td>133 ± 3</td>
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<tr>
<td></td>
<td>126 ± 6</td>
<td>124 ± 6</td>
<td>118 ± 8</td>
<td>117 ± 7</td>
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<td>139 ± 4</td>
<td>143 ± 5</td>
<td>128 ± 4</td>
<td>135 ± 5</td>
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<td>356 ± 35</td>
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<td>422 ± 20</td>
<td>381 ± 21</td>
<td>397 ± 17</td>
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<td>31 ± 3</td>
<td>30 ± 3</td>
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<td>28 ± 3</td>
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<td></td>
<td>27 ± 1</td>
<td>26 ± 1</td>
<td>27 ± 2</td>
<td>27 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SVRI, systemic vascular resistance index.
variable to increase our ability to find significant effects (Tables 1-4, Fig. 2). Unless specified, animals with either lobar or diffuse E. coli pneumonia are referred to as infected animals.

From baseline to day 2, infected animals had significantly greater decreases in mean LVEF compared with noninfected animals (−.17 ± 0.09 vs. −0.06 ± 0.05, P = 0.0001). Infected animals, from baseline to days 1 and 2 alike, had significant increases in mean MPAP (P = 0.0005), Ppao (P = 0.01), PVRI (P = 0.0014), and A-adO_2 (P = 0.0001); significant decreases in mean Pao_2 (P = 0.0001), MAP (P = 0.0002), CI (P = 0.02, data not shown), SVI (P = 0.002, data not shown), and left ventricular stroke work index (P = 0.0001, data not shown); and no significant changes in mean lactate, HR, and SVRI (P = NS) compared with noninfected animals.

Effect of varying FIO_2 (0.08, 0.21, 0.50, and 0.85) on the pulmonary and systemic hemodynamic effects of pneumonia. At all FIO_2 doses studied on days 1 and 2 alike, infected animals had higher mean Ppao and lower MAP compared with noninfected animals (Fig. 2, Tables 3 and 4). However, this difference was significantly greater at FIO_2 0.08 for both parameters P < 0.0008 compared with other FIO_2 doses. Furthermore, infected animals at all FIO_2 doses studied on days 1 and 2 alike had lower Pao_2 and higher A-adO_2 compared with noninfected animals, and this difference was progressively and significantly greater with increasing FIO_2 doses (0.08 ≤ FIO_2 ≤ 0.85; for both parameters, P = 0.0001). On days 1 and 2, at FIO_2 ≥ 0.21, mean HR was higher in infected compared with noninfected animals, whereas at FIO_2 < 0.21 it was lower in infected compared with noninfected animals (P = 0.0001). Finally, infected animals challenged with low-dose E. coli (0.75 × 10^{10} CFU), at FIO_2 ≤ 0.21, compared with FIO_2 > 0.21, had on days 1 and 2 alike greater increases in mean PVRI compared with noninfected animals (data not shown; P = 0.01).

On days 1 and 2, infected animals, similarly at all FIO_2 doses studied, had higher mean MPAP (P = 0.0001), PVRI (P = 0.02), and arterial lactate (P = 0.008) but lower SVRI (P = 0.09) compared with noninfected animals. Furthermore, on days 1 and 2 alike at FIO_2 ≤ 0.21, infected animals and noninfected animals had higher MPAP (P = 0.0001), PVRI (P = 0.02), and arterial lactate levels (P = 0.0001) than at FIO_2 doses > 0.21.

Effect of inhaled NO (80 ppm) at varying FIO_2 on the pulmonary and systemic hemodynamic effects of pneumonia. On days 1 and 2 alike, in infected animals, with increasing FIO_2 doses (0.08–0.85), NO produced progressively greater increases in mean Pao_2 and decreases in mean A-adO_2 and MAP, whereas in noninfected animals, the effects of NO were significantly different and opposite (all P < 0.05), such that NO produced progressively greater decreases in mean Pao_2 and increases in mean A-adO_2 and MAP (Figs. 3 and 4). In infected animals on days 1 and 2 alike, with increasing FIO_2 doses (0.08–0.85), NO produced no significant (P = NS) changes in mean HR, whereas in noninfected animals, the response was significantly different (all P < 0.05) such that NO produced progressive increases in HR.

In infected and noninfected animals alike on days 1 and 2, inhaled NO at FIO_2 doses of 0.08 and 0.21, but not at FIO_2 doses of 0.50 and 0.85, significantly decreased MPAP (P = 0.05; Fig. 5). In addition, at FIO_2 < 0.21, in infected animals and noninfected animals alike, inhaled NO significantly decreased mean Ppao and PVRI (all P < 0.001). At FIO_2 0.85, in infected animals and noninfected animals alike, inhaled NO significantly increased mean arterial lactate (P = 0.03).

Table 3. Pulmonary hemodynamic variables measured on days 1 and 2 after intrabronchial inoculation during inhalation of 4 FIO_2 levels with and without NO

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>FIO_2 0.08 NO ppm</th>
<th>FIO_2 0.21 NO ppm</th>
<th>FIO_2 0.50 NO ppm</th>
<th>FIO_2 0.85 NO ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>C</td>
<td>26 ± 0.6</td>
<td>21 ± 0.5</td>
<td>20 ± 0.6</td>
<td>19 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>31 ± 0.7</td>
<td>25 ± 0.7</td>
<td>25 ± 0.6</td>
<td>22 ± 0.6</td>
</tr>
<tr>
<td>Ppao, mmHg</td>
<td>C</td>
<td>11 ± 0.6</td>
<td>9 ± 0.4</td>
<td>10 ± 0.6</td>
<td>9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>16 ± 0.8</td>
<td>15 ± 0.7</td>
<td>12 ± 0.4</td>
<td>12 ± 0.4</td>
</tr>
<tr>
<td>PVRI, dyn·s·cm^{-1}·kg^{-1}</td>
<td>C</td>
<td>3.9 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4.8 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE calculated from data obtained on both days 1 and 2.
On days 1 and 2, in infected and noninfected animals alike, at all FIO2 doses studied, NO significantly decreased mean SVRI \(-1.13 \pm 0.46 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5} \cdot \text{kg}^{-1}\); \(P < 0.014\). Finally, on days 1 and 2, there were no significant effects of NO at any FIO2 studied on methemoglobin levels \((P = \text{NS}; \text{data not shown})\).

Endotoxia, microbiology, radiology, blood counts, and routine chemistry. Infected animals vs. noninfected animals had higher mean endotoxin levels at 6 h \((5 \pm 3 \text{ vs. } 0.05 \pm 0.03 \text{ EU/ml})\) and at 24 h \((4.2 \pm 0.2 \text{ vs. } 0.4 \text{ EU/ml})\) \((P = 0.008)\). Infected animals vs. noninfected animals also had greater numbers of \(E. \text{ coli}\) in sputum cultures at 24 h \((0.44 \pm 0.12 \text{ vs. } 0.10 \pm 0.10 \text{ colonies per plate})\) and at 48 h \((0.32 \pm 0.10 \text{ vs. } 0.10 \pm 0.10 \text{ colonies per plate}; \text{both } P = 0.02)\). Infected animals had significantly more severe \((P < 0.05)\) pulmonary infiltration in the right cranial, right caudal, right middle, and left caudal lobes on chest radiograph \(\text{(data not shown)}\). In addition, on chest radiograph, animals with diffuse pneumonia had more severe infiltration in the left cranial lobe compared with lobar pneumonia animals and noninfected animals \((P < 0.05)\). For the first 24 h, infected animals had lower white blood cell numbers and, for the first 48 h, had lower platelet counts compared with noninfected animals \((\text{both } P = 0.0001; \text{data not shown})\). In addition, after intrabronchial inoculation, infected animals compared with noninfected animals had greater increases in triglycerides, bilirubin, hemoglobin, and hematocrit as well as greater decreases in albumin and calcium \((\text{all } P < 0.05, \text{data not shown})\).

Recovery. When infected and noninfected survivors were compared 10 days after intrabronchial inoculation, the effects of varying FIO2 dose and inhaling NO on all pulmonary and systemic hemodynamic variables were similar to those at baseline, except that on day 10, infected animals had lower lactate levels at all FIO2 doses compared with noninfected animals \(\text{(data not shown), } P = 0.007)\).

**DISCUSSION**

Intrabronchial \(E. \text{ coli}\) challenge in canines produced pulmonary infiltrates, purulent sputum, hypoxemia, pulmonary arterial hypertension, cardiovascular dysfunction, and death. In noninfected and infected animals with increasing FIO2 doses, inhaled NO modestly
altered oxygenation. In animals with pneumonia, inhaled NO progressively decreased A-aD O2 and increased PO2 with increasing FIO2 doses, whereas in noninfected animals, inhaled NO increased A-aD O2 and decreased Pa O2. Under hypoxic and normoxic conditions in both noninfected and infected animals, inhaled NO decreased pulmonary arterial pressures, but had no significant effect on increases in pulmonary arterial pressures related to sepsis alone. Lastly, inhaled 80 ppm NO had modest effects on systemic pressures, HR, and arterial lactate levels. When noninfected and infected animals were compared, these effects were significantly different and opposite.

The NO-related changes in oxygenation that were seen in canines with gram-negative pneumonia are consistent with clinical studies of acute respiratory distress syndrome, showing that inhaled NO may improve oxygenation without observable changes in pulmonary arterial pressures (15). It has been postulated that the increased oxygenation with NO in this setting results from redistribution of blood flow rather than from reductions in global pulmonary pressures with enhancement of total pulmonary perfusion. The small improvements we observed in oxygenation with NO were unexpectedly dependent on a high FIO2 dose. These changes may reflect a direct effect of high FIO2 doses on the measurement of PaO2 and A-aDO2. It is also possible, however, that the effects of inhaled NO are augmented by increasing O2 concentration (10). In vitro studies evaluating alterations in the activity of guanylate cyclase, the primary receptor for NO, suggest a mechanism by which O2 could produce this effect. In these studies, carbon monoxide and hydrogen peroxide, both of which could increase with hyperoxia, facilitate the activity of guanylate cyclase (34, 57). Therefore, during hyperoxia, the effects of NO on guanylate cyclase might be augmented by either of these molecules. Many studies in humans and animals with lung injury report improvements in oxygenation with NO in subjects administered increased FIO2 doses (8, 15, 37, 41, 43, 50, 53, 54, 60, 61). Our data suggest that these changes may be less pronounced if the effects of NO on oxygenation were examined at lower FIO2 doses. Of note, our findings suggest that exogenous NO may minimally but significantly antagonize mechanisms maintaining optimal ventilation and perfusion matching in the normal lung.

Gram-negative pneumonia with sepsis, which our model was designed to simulate, is associated with increases in both PVR resistance and pulmonary arterial pressures. Mechanisms for these increases may be related to endothelial injury, with a subsequent fall in endogenous vasodilators such as NO, or to a reduction in the responsiveness of the endothelium to such media-

**Fig. 4.** Increases or decreases (means ± SE) in mean arterial pressure (ΔMAP; A), heart rate (ΔHR; B), and arterial lactate (ΔLactate; C) produced by inhaled NO (80 ppm) with 4 different FIO2 doses (0.08, 0.21, 0.50, and 0.85) after intrabronchial inoculation with E. coli (infected animals; solid bars) or saline (noninfected controls; open bars). In infected animals, with increasing FIO2 doses (0.08 to 0.85), NO produced progressively greater decreases in MAP, whereas in noninfected animals, the effects of NO were significantly different and opposite (all P < 0.05), producing progressively greater increases in MAP. In infected animals, with increasing FIO2 doses (0.08 to 0.85), NO produced no significant (P = NS) changes on mean HR, whereas in noninfected animals, the response was significantly different (all P < 0.05) such that NO produced progressive increases in HR. At FIO2 0.85, in infected and noninfected animals alike, inhaled NO significantly increased mean arterial lactate (P = 0.03).
tors. In endotoxemic dogs without pneumonia, treatment with NO synthase inhibitors demonstrated that NO was critically important in maintaining a low level of pulmonary vascular tone (6). Conversely, in dogs with pneumonia, administration of inhaled NO in a dose sufficient to reduce increases in pulmonary pressures related to hypoxia had no significant effect on increases in pulmonary pressures related to gram-negative pneumonia. These findings suggest that insufficient production of or responsiveness to NO is not the sole or predominant mechanism for the increases in pulmonary artery pressures observed during pneumonia. Other mechanisms, such as obstruction to blood flow by intravascular cellular elements or excessive production of endogenous vasoconstrictors may also play important roles in some types of lung injury (33).

Our findings differ from other models of lung injury such as challenges with endotoxin, oleic acid, thromboxane analogs, and saline lavage in which inhaled NO reduced pulmonary arterial hypertension (12, 14, 22, 37, 38, 41, 51, 53, 60, 61). It is important to note, however, that our model and study design were directed at assessing the effects of NO during the later stages of lung injury associated with intrapulmonary infection. In contrast to our model, the effects of NO in other models cited were assessed immediately or shortly after administration of challenge. Insufficient production of or unresponsiveness to NO may have a role in early pulmonary arterial hypertension with these types of acute lung injury, but not necessarily over the full time course of all forms of lung injury.

The dose of inhaled NO (80 ppm) used in this study was based in part on dose-response studies we had performed in normal animals breathing hypoxic O2 concentrations. These studies were consistent with other published studies showing that this dose of inhaled NO (80 ppm) would decrease pulmonary arterial pressures and PVR more than lower doses would (10, 53, 54, 57). It is possible, however, that with lung injury and hypoxemia related to pneumonia, lower doses of inhaled NO may have produced a more selective effect in well-ventilated lung regions and produced greater improvements in arterial oxygenation (13).

It is also important to note that our model evaluated the effects of inhaled NO in spontaneously breathing canines. Mechanical ventilation in such a model requires the use of additional drugs (e.g., sedatives and paralytics) that may have confounded the effects of NO in the setting of gram-negative pneumonia. However, many patients with gram-negative pneumonia and severe hypoxemia are supported with positive pressure mechanical ventilation and positive end-expiratory pressure. Application of inhaled NO with this mode of ventilation might have augmented its effects and resulted in greater increases in arterial oxygenation in our pneumonia model (41, 43).

Both infected and noninfected animals during inhalation of hypoxic gas mixtures had significant increases in pulmonary pressures that were reversible with NO. In contrast, other studies have shown that hypoxic pulmonary vasoconstriction is blunted or absent after intravenous endotoxin (19, 30, 59) or bacteria challenge (17, 18, 24, 33). These disparate results may relate to type of challenge (intrabronchial vs. intravenous), or the fact that measurements were made either earlier (within hours, Refs. 18 and 59) or later (7–10 days; Refs. 17 and 33) than those in our study. Nonetheless, our data suggest that hypoxic pulmonary vasoconstriction may be preserved in some cases of bacterial pneumonia, even when abnormalities of systemic hemodynamics are pronounced and/or death occurs.

During pneumonia, inhaled NO with increasing FIO2 doses modestly decreased blood pressure, but in noninfected animals it increased blood pressure and HR. Inhaled NO increased serum lactate in both noninfected and infected animals. Similarly, in all animals, inhaled NO also modestly decreased SVRI. Adverse effects of NO on blood pressure have been noted in a porcine model of oleic acid-induced lung injury (53). These data suggest that under some conditions inhaled NO may be able to alter systemic hemodynamics. In vitro and in vivo studies have shown that a biologically active form of NO can circulate in plasma while reversibly bound to serum proteins (S-nitrosylated protein) like albumin (16, 55, 56). If inhaled NO can enter this circulating pool of protein-bound NO, it might cause changes in systemic vasomotor tone (16, 55). Alterations of this mechanism by sepsis or by changing O2 concentrations may explain the differential effects we observed of NO with increasing FIO2, in infected and noninfected animals. The effect of inhaled NO on systemic hemodynamics, however, may also be dose dependent, as suggested by others (53). Therefore, reducing its concentration may limit its potential adverse hemodynamic effects.

In summary, intrabronchial E. coli inoculation in dogs resulted in pulmonary infiltration, purulent spum, hypoxemia, changes in pulmonary and systemic hemodynamics, and death. In this model of pneumonia, inhaled NO reversed hypoxic pulmonary vasoconstriction, but had only modest effects on arterial oxygenation and did not affect increases in PVR related to pneumonia. Extrapolated clinically, these findings suggest that inhaled NO may have a limited therapeutic role in acute lung injury associated with gram-negative bacterial pneumonia. Further studies to evaluate the effects of lowered concentrations of inhaled NO in combination with mechanical ventilation and positive end-expiratory pressure may still be necessary to define a role for inhaled NO during gram-negative pneumonia.

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