Continuous Positive Airway Pressure Modulates Effect of Inhaled Nitric Oxide on the Ventilation-Perfusion Distributions in Canine Lung Injury*

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Objectives: The present study was designed to evaluate if continuous positive airway pressure (CPAP) augments the effect of nitric oxide (NO) inhalation on matching between ventilation and perfusion (VA/Q) during acute lung injury.

Design: Prospective, randomized study.

Setting: A research laboratory at a university medical center.

Subjects: Ten anesthetized mongrel dogs with oleic acid-induced lung injury.

Interventions: Zero or 40 parts per million of NO in the inspiratory gas, with and without 10 cm H2O CPAP in random order.

Measurements and main results: Gas exchange was assessed by estimating the VA/Q distributions using the multiple inert gas elimination technique. Application of CPAP decreased blood flow to shunt units by 26±2 percent (mean ± SD) and increased the fraction of cardiac output to normal VA/Q units (VA/Q ratio of 0.1 to 10) by 26±2 percent (p<0.05). Inhalation of NO during CPAP accounted for a further 10±2 percent decrease in the blood flow to shunt units and an 8±2 percent increase in the fraction of the cardiac output to normal VA/Q units (p<0.05). Inhalation of NO alone had no significant effect on the VA/Q distributions. Inhalation of NO decreased mean transmural pulmonary artery pressure (Pmp) both without (Ppmp) from 30±2 to 23±2 mm Hg; PVR from 323±44 to 228±43 dynes·s·cm⁻⁵; p<0.05) and with CPAP (Ppmp from 25±2 to 20±2 mm Hg; PVR from 255±30 to 173±31 dynes·s·cm⁻⁵; p<0.05).

Conclusions: Although pulmonary vascular resistance can be lowered with NO inhalation alone, recruitment of gas exchange units with CPAP is necessary to produce a beneficial effect of NO inhalation on VA/Q matching and oxygenation. When recruitment of gas exchange units with CPAP brings gaseous NO in contact with enough pulmonary blood vessels, NO-induced vasodilation will augment VA/Q matching by a steal mechanism.

CO=cardiac output (thermodilution); CPAP=continuous positive airway pressure; dead space=percent of Vt to lung units with VA/Q ratios >100; DISP=dispersion (SD) of perfusion on a log scale; logSD=dispersion (SD) of ventilation on a log scale; low Vt=percent of Qt to lung units with VA/Q ratios from 0.005 to 0.1; NO=nitric oxide; PaCO₂=arterial carbon dioxide tension; PaO₂=arterial oxygen tension; Pao₂=alveolar oxygen tension; Ppmt=mean transmural pulmonary artery occlusion pressure; P=airway pressure; P(tot)=transmural central venous pressure; PEEP=positive end-expiratory pressure; Pes=esophageal pressure; Ppm=transmural pulmonary artery pressure; ppm=parts per million; Psa=systemic blood pressure; PVR=pulmonary vascular resistance; P(O₂)=mixed venous oxygen tension; Q=mean perfusion of pulmonary blood flow distribution; Qt=pulmonary blood flow; SaO₂=arterial oxygen saturation; SFO₂=mixed venous oxygen saturation; SVR=systemic vascular resistance; VA/Q ratio of ventilation distribution; VA/Q ratio=ventilation-perfusion ratio; VT=minute ventilation; VO₂=oxygen consumption; Vt=tidal volume.

Key words: acute lung injury; continuous positive airway pressure; nitric oxide; pulmonary gas exchange; ventilation-perfusion distribution

Acute lung injury causes alveolar collapse with decrease in lung compliance and resting lung volume, resulting in a mismatch between ventilation and perfusion (VA/Q). The VA/Q mismatch accounts entirely for the severe arterial hypoxemia observed during acute lung injury. Application of continuous positive airway pressure (CPAP) recruits additional lung units for gas exchange and improves VA/Q matching.

Inhalation of nitric oxide (NO) in concentrations ranging from 5 to 80 parts per million (ppm) has been shown to cause selective pulmonary vasodilation and to improve pulmonary gas exchange in patients with acute lung injury. Because NO is rapidly inactivated by binding to hemoglobin, inhalation of NO results in pulmonary vasodilation limited to ventilated lung areas. Inhalation of 36 ppm NO in patients with acute lung injury selectively improved perfusion of ventilated lung areas, resulting in a re-

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Supported by the Erwin Schroedinger Foundation, grant J0637-MED and J0622-MED (Dr. Putensen).

Manuscript received November 17, 1993; revision accepted January 31, 1994.

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CHEST / 106 / 5 / NOVEMBER, 1994 1563
distribution of blood flow from nonventilated to ventilated lung units and in improved pulmonary oxygen transfer. We hypothesized that recruitment of lung units with CPAP should augment the effect of NO inhalation on V/A/Q matching. To test this hypothesis, we examined changes in the continuous V/A/Q distributions during application of CPAP and inhalation of low concentrations of NO in dogs with oleic acid-induced lung injury.

**MATERIALS AND METHODS**

**Instrumentation**

After approval by the local laboratory animal care and use committee, 10 mongrel dogs weighing 22 to 28 kg (24.6 ± 2.4 kg; mean ± SD) were anesthetized with intravenous pentobarbital sodium, 12 mg/kg, followed by an infusion of 20 μg/kg/min. Each animal was placed in a supine position, and the trachea was intubated with a 9-mm internal diameter cuffed tracheal tube (Mallinckrodt, Argyle, NY). All dogs breathed room air spontaneously at ambient airway pressure throughout instrumentation. A catheter was inserted into the right femoral artery. A 7-French, thermistor-tipped, triple-lumen, pulmonary artery catheter (95A-131-7F, Baxter Edwards Critical-Care, Irvine, Calif) was placed through the right femoral vein.

**Cardiovascular Measurements**

Systemic blood pressure (Psa), central venous, pulmonary artery, and pulmonary artery occlusion pressures were transduced (Transpac II, Abbott Critical Care, Chicago) and recorded (TA 2600, Statham Gould, Oxnard, Calif). A horizontal plane through the shoulder was taken as zero reference point for blood pressure measurements. Cardiac output (CO) was measured with the thermal dilution technique (Oximetrix 3, Abbott Critical Care, Chicago).

**Ventilatory Measurements**

Gas flow was measured between the tracheal tube and the Y-piece of the ventilator circuit with a heated pneumotachograph (3791, Hans Rudolph, Kansas City, Mo), connected to a differential pressure transducer (DP 1060871, Validyne, Northridge, Calif). Tidal volume (VT) was derived from the integrated gas flow signal. Expiratory minute ventilation (Ve) was measured for control with a calibrated respirometer (Wright) at the outlet of the gas mixing chamber. Airway pressure (Paw) was measured at the proximal end of the tracheal tube with a differential pressure transducer (MP45-871, Validyne, Northridge, Calif). Esophageal pressure (Pes) was measured with balloon catheter (Mallinckrodt, Argyle, NY) connected to a differential pressure transducer (MP45-871, Validyne, Northridge, Calif) as described previously.11

**Physiologic Gas Analysis**

Arterial and venous blood gases and pH were determined immediately after sampling with standard blood gas electrodes (model 1303, Instrumentation Laboratories, Lexington, Mass). Oxygen saturation of the arterial (SaO2) and mixed venous (SvO2) blood, and total hemoglobin and methemoglobin concentrations were determined with a CO-oximeter (model 282, Instrumentation Laboratories, Lexington, Mass). Fractions of inspired oxygen (FIO2) and carbon dioxide and fractions of mixed expired oxygen (FEO2) and FrCO2 were determined with a scattering gas analyzer (Raman, Rascal II, Ohmeda, Louisville, Co).12

**Inert Gas Analysis**

The method for estimating the distributions of continuous V/A/Q ratios was described by Wagner et al.13,14 Six inert gases (sulfur hexafluoride, ethane, cyclopropane, ethylene, diethyl ether, and acetone) were dissolved in lactated Ringer’s solution and infused into a peripheral vein at a constant rate set at 0.05 percent of Ve for at least 40 min.15 Arterial and mixed venous blood and expired gas samples were collected during stable conditions confirmed by constancy (±5 percent) of Ve, FEO2, FrCO2, and CO. Expired gas samples were collected with an appropriate time delay from a heated mixing chamber.16 Concentrations of the inert gases were measured with a gas chromatograph (HP 5890, Hewlett-Packard, Wallingford, Mass) and blood-gas partition coefficients were determined.15

**Data Analysis**

Arterial to mixed venous (retention) and mixed expired to mixed venous (excretion) concentration ratios of the inert gases were used to obtain retention-solubility and excretion-solubility relationships.13,14 By formal mathematical analysis with enforced smoothing, these relationships were transformed into a 50-compartmental distribution plot of blood flow and ventilation against V/A/Q.14,15 Intrapulmonary shunt defined as fraction of Q perfusing essentially nonventilated alveoli (V/A/Q < 0.005), low V/A/Q as fraction of the pulmonary blood flow (Qtr) perfusing poorly ventilated lung areas (0.005 < V/A/Q < 0.1), high V/A/Q as fraction of Ve ventilating poorly perfused lung areas (V/A/Q > 100), mean V/A/Q ratio of perfusion (Q) and ventilation (V), and logarithmic standard deviations of perfusion (logSDQ) and ventilation (logSDV) were derived from the 50-compartmental model. Predicted values for PaO2 were calculated from the recovered V/A/Q distributions as described.15 The index DISPR-S* was calculated as the root mean square difference of measured retentions and excursions corrected for dead space.17 It is an overall index of V/A/Q heterogeneity with a minimum value of zero (homogeneous lung) and a maximum value of 100 (100 percent shunt, 100 percent dead space).

Transmural central venous (Pcvmn), pulmonary artery (Ppmvn), pulmonary artery occlusion pressures (Pvomn), were derived by subtracting Pes from the blood pressures at end-expiration. Systemic vascular resistance (SVR) was calculated as 80 × (map-Pcvmn)/CO; pulmonary vascular resistance (PVR) as 80 × (map-Pvomn)/CO, and oxygen consumption (VO2) as (VI × FIO2) - (Ve × FEO2).

**Administration and Measurement of Nitric Oxide**

Nitric oxide was obtained as a mixture of 900 ppm NO balanced with N2 (Matheson, Rutherford, NJ). The NO/N2 and N2 source were connected via a soda lime absorber that scavenged nitrogen dioxide (NO2)18 to a gas blender (5100, Bird, Palm Springs, Calif) which allowed the titration of the inspiratory NO concentration. The output of the gas blender delivered a NO/N2 gas mixture through the air supply inlet to the high-pressure servo valve of the ventilator that under normal conditions regulated air entry. The two high-pressure servo valves, one for NO/N2 and the other for oxygen, were controlled by the original microprocessor of the ventilator. They opened proportionally, depending on the VT, flow rate, inspiratory-to-expiratory time ratio, ventilator rate, and FIO2 settings. Nitric oxide and NO2 were measured with a chemiluminescence-type NO detector (model 14B/E, Thermo Electron Corp, Franklin, Mass).

**Experimental Procedure**

After the instrumentation, dogs remained supine. Pulmonary artery blood temperature was kept at 38°C with a heating pad.
Adequate hydration and energy supply (25 kcal/kg/d) was ensured with an infusion of 5 percent dextrose and lactated Ringer's solution, to achieve a PaO₂ of 8 mm Hg.

Acute lung injury was induced by injection of 0.05 ml/kg of purified oleic acid (J.T. Baker Inc, Phillipsburg, NJ) into the right atrial catheter over 15 min. Additional 0.2-ml increments of oleic acid were administered every 30 min until PaO₂ was between 50 and 60 mm Hg while breathing room air at ambient airway pressure. Then, FlO₂ was set to 0.3 and the lung injury was allowed to stabilize for 90 min.

Dogs then received, in random order, zero or 40 ppm of NO in the inspiratory gas, with and without 10 cm H₂O CPAP. Forty minutes of equilibration were allowed before measurements. To restore lung history, the animals' lungs were inflated manually to an airway pressure of 30 cm H₂O for 10 s after each measurement, before either the inhaled NO concentration or the CPAP level was changed.

Statistical Analysis

Results are expressed as mean±standard error of the mean (SE). Data were normally distributed and analyzed by using an analysis of variance for repeated measures to determine if inhaled NO concentration and the CPAP level had significant effects on the measured and calculated variables. The relationship between measured and predicted PaO₂ was assessed with a linear regression analysis; p<0.05 was considered to be statistically significant.

Results

Hemodynamic Effects

The effects of NO inhalation and CPAP on cardiovascular function are shown in Table 1. Mean Ppa and PVR decreased during inhalation of 40 ppm NO with and without CPAP. In the absence of NO inhalation, mean Ppa and PVR decreased during CPAP compared to ambient airway pressure. Inhalation of NO (p<0.05), CPAP (p<0.05), and the combination of CPAP and NO inhalation (p<0.05) decreased mean Ppa and PVR significantly. Heart rate, mean PaO₂, SVR, CO, Pcv, and Pao remained unchanged.

Respiratory Effects

Minute ventilation and respiratory rate were lowered by CPAP (p<0.05) (Table 2). Application of CPAP (p<0.05), the combination of CPAP and NO inhalation (p<0.05) but not NO inhalation alone, increased PaO₂ and PVaO₂ significantly (Table 2). The highest PaO₂ was observed during NO inhalation with CPAP. Arterial pH, PaCO₂, and methemoglobin remained essentially unchanged.

Inert Gas Data

Changes reflecting the Vₐ/Q distributions are summarized in Table 3 and illustrated for a representative dog in Figure 1. Application of CPAP decreased blood flow to shunt units and increased the fraction of CO to units with a normal Vₐ/Q (0.1 <Vₐ/Q <10) ratio. Inhalation of NO with CPAP accounted for a further 10±1 percent decrease in the blood flow to shunt units and an 8±1 percent...
increase in the fraction of the CO to normal VA/Q units. Application of CPAP (p<0.05), the combination of CPAP and NO inhalation (p<0.05) but not NO inhalation alone, had a significant effect on the blood flow to shunt and normal VA/Q units. Dead space ventilation was lowered by CPAP (p<0.05). Blood flow distribution curves shifted to the left and their dispersions were above the upper normal limit (logSDQ >0.6). Ventilation distributions were shifted to the right, while dispersions of the curves were increased (logSDV >0.6). Application of CPAP decreased V̇. No effect was observed on Q̇, logSDQ, and log SDV. The index DISPr-e* decreased during CPAP and was lowest with simultaneous NO inhalation. Predicted PaO₂ was close to measured PaO₂ for all conditions (Table 4).

**DISCUSSION**

This study was designed to evaluate if CPAP modulates the effects of NO inhalation on pulmonary gas exchange in a canine oleic acid lung injury model. We found that NO inhalation in the absence of CPAP did not influence VA/Q matching in this setting, but significantly augmented the improvement effected by the application of CPAP. A decrease in mean Ppₐₐm and PVR was consistently seen during NO in-
halation.

Our observations were made in dogs with severe oleic acid-induced lung injury indicated by a unimodal V_A/Q distribution with 48±2 percent of the pulmonary blood flow perfusing shunt units. Distributions of V_A/Q observed in dogs with oleic acid-induced lung injury,10,20 are comparable to those in humans with acute lung injury, where blood flow is distributed to either shunt or normal V_A/Q units.2 High PaCO_2 in conjunction with low pH in our spontaneously breathing dogs may have contributed to the V_A/Q inequality.10 However, hypercapnia and respiratory acidosis remained unchanged and therefore are not responsible for the observed changes in the V_A/Q distributions. The small differences between predicted and measured PaO_2 values throughout the experiment indicate complete alveolar-end-capillary oxygen equilibration. Therefore, observed changes in pulmonary gas exchange can be almost entirely explained by the measured V_A/Q mismatch. A small amount in the variation in PaO_2 may be explained by factors other than V_A/Q mismatch that directly or through their effects on PVO_2 govern PaO_2.

In this study, inhalation of 40 ppm NO, regardless of the administration of CPAP, lowered the elevated mean Ppa and PVR. Inhalation of NO at concentrations of 5 to 80 ppm previously has been shown to produce selective pulmonary vasodilation in subjects with pulmonary hypertension secondary to global hypoxemia, primary pulmonary hypertension, and adult respiratory distress syndrome.6-10,21-24 However, inhalation of NO did not affect mean Psa and SVR in our dogs. This is in agreement with the concept of a selective dilator effect of inhaled NO on the pulmonary vasculature.6-8,10,23 The absence of systemic vascular effects is explained by high-affinity binding of NO to hemoglobin.25 Compatible with previous observations,6,7,24 this inactivation of NO did not significantly affect blood levels of methemoglobin.

Our results show that inhaled NO has pulmonary vasodilator effects, even when PVR had already been lowered by restoration of lung volume with CPAP. This finding was expected, because NO inhalation dilates selectively blood vessels in ventilated lung areas.9,10 Application of CPAP during acute lung injury has been shown to recruit additional lung units for gas exchange3,4 and thereby may dilate additional blood vessels previously not exposed to the NO present in the gas phase. Apparently, changes in lung volume altering the gas exchange surface area are sufficient to optimize the selective pulmonary vasodilator effect of inhaled NO.

Although inhalation of NO in the absence of CPAP caused significant pulmonary vasodilation, pulmonary gas exchange remained essentially unchanged. This contrasts with observations made during administration of intravenous vasodilators that have been reported to relieve regional hypoxic pulmonary vasoconstriction, increase pulmonary blood flow to shunt units, and reduce arterial blood oxygenation.5,26 In the absence of CPAP, pulmonary vasodilation during NO inhalation was apparently not sufficient to divert pulmonary blood flow from nonventilated to well-ventilated lung units. Apparently, the lack of well-ventilated lung areas limited the effect of NO inhalation alone on the V_A/Q mismatch.

Application of CPAP resulted in a marked decrease in blood flow to shunt units that was most pronounced in the presence of inhaled NO. The observed decrease in intrapulmonary shunt may be explained by two mechanisms: (1) shunt units were recruited and became ventilated, thereby converting to low or normal V_A/Q units, or (2) perfusion of shunt units was reduced by redistribution of the blood flow to normal V_A/Q, high V_A/Q, or previously nonperfused areas. Unfortunately, the results of the inert gas measurements only quantify the effects of CPAP and NO inhalation on the V_A/Q distributions and do not allow us to distinguish between the two potential mechanisms.

Alveolar recruitment with CPAP is supported by previous studies demonstrating decrease in intrapulmonary shunt and increase in end-expiratory lung volume with application of positive end-expiratory pressure (PEEP) during mechanical ventilation in humans and experimental animals with severe lung injury.2,20,27 Although end-expiratory lung volumes were not measured in our dogs, it is well accepted that CPAP improves pulmonary gas exchange during acute lung injury by increasing end-expiratory lung volumes and by recruiting poorly ventilated lung units.3,5 Increased perfusion of high V_A/Q and nonperfused units during CPAP is unlikely,28,29 and

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*Values are mean±SE.
would be expected to produce changes of mean \( V_{A}/Q \) of the blood flow distribution and its dispersion (logSDv), which we did not see. Instead, decrease in mean \( V_{A}/Q \) of the ventilation distribution, reduction in dead space ventilation, and the absence of changes in the dispersion of the ventilation distribution (logSDv) and in high \( V_{A}/Q \) units, indicate that CPAP redistributed ventilation to previously nonventilated or poorly ventilated lung areas.

Previous experimental\(^9\) and clinical\(^9\) observations suggest that NO inhalation improves overall \( V_{A}/Q \) matching by redistributing blood flow from essentially nonventilated to ventilated lung units. Decrease in blood flow to shunt units without changes in the dispersion of ventilation distribution (logSDv) and in high \( V_{A}/Q \) units during CPAP with NO inhalation support that blood flow was distributed from nonventilated to well-ventilated lung areas. This effect has been attributed to selective vasodilation in ventilated lung regions.\(^9\)\(^,\)\(^10\) It has been suggested that inhaled NO by its lipophilic properties diffuses directly into the smooth muscle of the pulmonary resistance vessels in the proximity of alveoli, and activates soluble guanylate cyclase.\(^10\) This increases intracellular guanosine 3',5'-cyclic monophosphate (cGMP) and causes smooth muscle relaxation.\(^30\)\(^,\)\(^31\) Simultaneously, inactivation of NO in the blood seems to be fast enough to prevent an effect on hypoxic pulmonary vasoconstriction in nonventilated lung areas.\(^9\)\(^,\)\(^10\)

Application of CPAP with and without NO inhalation converted shunt units directly to normal \( V_{A}/Q \) units without creating regions of low \( V_{A}/Q \). Similar phenomena have been observed previously when PEEP was applied\(^2\) or NO was added in the inspiratory gas\(^2\) in mechanically ventilated patients with adult respiratory distress syndrome. However, in this study, inhalation of NO did not significantly affect the distribution of pulmonary blood flow and \( V_{A}/Q \) matching in the absence of CPAP. These findings are not in conflict with previous clinical\(^9\) observations that NO inhalation decreases perfusion of shunt units by redistribution of the blood flow to well-ventilated lung units. However, in patients with adult respiratory distress syndrome, inhalation of NO was always provided during mechanical ventilation with 10 to 15 cm H\(_2\)O of PEEP. Our results indicate that adequate recruitment of gas exchange units is essential to get the gaseous NO in contact with enough pulmonary blood vessels and to cause vasodilation affecting significantly \( V_{A}/Q \) matching.

The effect of NO inhalation on \( V_{A}/Q \) distributions also has been attributed to relaxation of bronchial smooth muscles.\(^18\) However, bronchodilation, as inferred from measurements of Paw, Pes, and corresponding gas flow, was not noted in our dogs with oleic acid lung injury. Furthermore, dispersion of pulmonary blood flow (logSDv) did not indicate lower \( V_{A}/Q \) inequality of below normal and low \( V_{A}/Q \) areas, as might be expected during NO-induced bronchodilation.\(^32\)

Recent development in the treatment of acute lung injury has introduced NO inhalation to improve pulmonary gas exchange by better \( V_{A}/Q \) matching and to decrease PVR. The results of this study demonstrate that although PVR can be lowered with NO inhalation alone, recruitment of gas exchange units with CPAP is necessary to produce an effect of NO inhalation on \( V_{A}/Q \) matching and oxygenation. A clinical study is necessary to evaluate the validity of these results in critically ill patients.

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