Nitric oxide and endothelial permeability

FRANK HINDER, MICHAEL BOOKE, LILLIAN D. TRABER, AND DANIEL L. TRABER

Klinik und Poliklinik für Anästhesiologie und operative Intensivmedizin, der Westfälischen Wilhelms-Universität, Münster, Germany; and Department of Anesthesiology, The University of Texas Medical Branch, Galveston, Texas 77555-1091

Hinder, Frank, Michael Booke, Lillian D. Traber, and Daniel L. Traber. Nitric oxide and endothelial permeability. J. Appl. Physiol. 83(6): 1941–1946, 1997.—Nitric oxide synthase inhibition reverses systemic vasodilation during sepsis but may increase endothelial permeability. To assess adverse effects on the pulmonary vasculature, 12 sheep were chronically instrumented with lung lymph fistulas and hydraulic pulmonary venous occluders. Escherichia coli endotoxin (lipopolysaccharide; 10 ng·kg⁻¹·min⁻¹) was continuously infused for 32 h. After 24 h, six animals received 25 mg/kg of N-nitro-l-arginine methyl ester (l-NNAME), and six received saline. All sheep developed a hyperdynamic circulatory response and elevated lymph flows by 24 h of lipopolysaccharide infusion. l-NNAME reversed systemic vasodilation, increased pre- and postcapillary pulmonary vascular resistance index, pulmonary arterial pressure, and, transiently, effective pulmonary capillary pressure. Lung lymph flows were not different between groups at 24 h or thereafter. Calculated as changes from baseline, however, lung lymph flow was higher in the l-NNAME group than in the control animals, with a trend toward lower lymph-to-plasma protein concentration ratio at 25 h. Permeability analysis at 32 h by the venous occlusion technique showed normal reflection coefficients and elevated filtration coefficients without differences between groups. Reversal by l-NNAME of the systemic vasodilation during endotoxemia was associated with high pulmonary vascular resistance without evidence of impaired pulmonary endothelial barrier function.

Nitric oxide synthase inhibition; lung; hyperdynamic sepsis; lung edema; vasodilation; pulmonary endothelial permeability

NITRIC OXIDE (NO) produced by the different isoforms of NO synthase (NOS) accounts for most of the activity of the endothelium-derived relaxing factor and is a key mediator in the regulation of blood flow under many physiological conditions (33, 34). During sepsis, however, an overproduction of NO by the inducible NOS (iNOS) may occur and result in septic vasodilation and shock. iNOS is synthesized in high quantities in endothelial cells (23), vascular smooth muscle cells (1), and macrophages in response to a variety of stimuli during sepsis (19). NOS inhibitors have been administered both to septic patients (6, 27) and in the hyperdynamic animal model of sepsis (20, 21, 25, 26), characterized by a low systemic vascular resistance and an elevated cardiac output (CO), to reverse the sepsis-associated systemic vasodilation.

Blockade of NOS, however, may elicit serious side effects on microvascular permeability. Granulocytes, adhering to the endothelium of postcapillary intestinal venules, emigrated from the vessel when a NOS inhibitor was administered (17). Furthermore, inhibition of NOS led to an increase in both endothelial and epithelial intestinal permeability in normal cats (13, 15), and an increase in permeability due to ischemia-reperfusion injury was attenuated when a NO donor compound was infused (14).

Other studies indicated that NO itself may be detrimental regarding microvascular barrier function (2, 3, 10, 12, 28, 29). The generation of strong oxidants like peroxynitrite from the reaction of NO with superoxide may cause or aggravate endothelial and epithelial damage (2, 28, 29). Recently, evidence has been found for nitrogen-derived oxidants in human pulmonary tissue after acute lung injury (12). The administration of NOS inhibitors limited vascular permeability in endotoxin-induced gut mucosal injury in rats (3) and prevented permeability changes in lung alveolar injury from smoke inhalation (10).

Yet to be defined is the role endogenous NO may play in the maintenance of an intact pulmonary endothelial barrier function when NOS inhibitors are administered during a state of septic vasodilation.

This study was designed to evaluate the effect of NOS inhibition on lung microvascular permeability in a model of hyperdynamic ovine endotoxemia in awake spontaneously breathing sheep, where factors like ventilation that may alter permeability independent of NO are not present. We hypothesized that pulmonary endothelial permeability to protein would increase after inhibition of NOS.

METHODS

Animal preparation. Twelve adult ewes (43.8 ± 2.5 kg) were instrumented for a chronic study with femoral arterial and venous catheters as well as a Swan-Ganz thermodilution catheter (model 93A 131 7F, American Edwards Laboratories, Santa Ana, CA) under halothane anesthesia. A bilateral thoracotomy in the sixth intercostal space was performed. The efferent vessel of the caudal mediastinal lymph node was cannulated with Silastic medical-grade tubing (0.025 in. ID, 0.047 in. OD, Dow Corning, Midland, MI) by using a modification of the technique described by Staub and colleagues (31). Several steps were taken to avoid systemic contamination of the lung lymph: the distal part of the caudal mediastinal lymph node was ligated, and the vessels approaching this node from the diaphragm and the posterior aspects of the right hemithorax were stained with Evans blue and were subsequently cauterized. Hydraulic occluders were attached to all pulmonary veins, and a Silastic catheter (0.062 in. ID, 0.125 in. OD, Dow Corning) was placed in the left atrium for the measurement of left atrial pressure (LAP). After the surgical procedure was completed, the animals were moved into metabolic cages where they were allowed to recover for a period of 5 days, with free access to food and water.

Data collection. The catheters were connected to pressure transducers (Statham Gould P23 1D, Oxnard, CA) with continuous flushing devices on the day before the experiment. Hemodynamic pressures were read from physiological recorders.
ers (Honeywell OM) 9, Electronics for Medicine, Pleasantville, NY). Zero calibration of the catheters was taken at the level of the hip joint of the front leg while the animal was standing. All hemodynamic variables were later recorded with the animals standing. Pulmonary vascular pressure (Pmv) was derived from pulmonary capillary wedge pressure (PCWP) tracings according to the technique of Holloway et al. (8). CO was measured with the thermal dilution technique by using a CO computer (model 9520 or 9530, American Edwards Laboratory). Cardiac index (CI) and systemic and pulmonary vascular resistance indexes (SVRI and PVRI, respectively) were calculated by using standard formulas. The body surface area was derived from body weight by using an equation described by Guyton et al. (7). Distribution of pulmonary vascular resistance was calculated as described in the following equations

Precapillary PVRI = (PAP − Pmv)·79.9/Ci

Postcapillary PVRI = (Pmv − PCWP)·79.9/Ci

where PAP is pulmonary arterial pressure. Lung lymph was collected in graduated cylinders over a period of 15 min. Lung lymph flow (QL) is presented both in milliliters per hour and as changes from baseline. The lymph was then transferred to heparinized sample tubes. Lymph (Clp) and plasma (Cp) protein concentrations were determined by using the biuret technique. The colloid osmotic pressures of lymph (πl) and plasma (πp) were directly measured through the semipermeable membrane of a colloid osmometer (model 4100, Wescor, Logan, UT) with a pore diameter of 0.0004 µm.

Permeability analysis. The reflection coefficient to protein (σ) was determined by using the pulmonary venous occlusion technique described by Isago et al. (9). In short, all pulmonary venous occluders were inflated with saline to increase Pmv, which was maintained for at least 2 h. In this state, Clp/Cp is assumed to be filtration independent and can be used to calculate the reflection coefficient to protein by using the following equation

σ = 1 − Clp/Cp

The relationship among the pressures effective for the rate of transvascular fluid flux (Jv) is expressed in Eq. 4, derived from Starling (30) and Landis and Pappenheimer (18)

Jv = Kr[(Pmv − P)] − σ(πp − πl)]

The following assumptions were made regarding the variables in Eq. 4: 1) QL = Jv for a particular region of the lung; 2) the interstitial oncotic pressure πl, equals πp; and 3) the interstitial hydrostatic pressure (P) is stable and equals the mean alveolar pressure = 0 mmHg.

The filtration coefficient (Kf) was calculated by using Eq. 5 after translation of Eq. 4 and changing of the variables according to assumptions 1–3

Kf = QL[(Pmv − σ(πp − πl))]

Permeability analysis were performed at baseline (48 h before the endotoxin infusion was started) and at the end of the experiment (between 32 and 35 h of the endotoxin infusion). The delay between the first permeability analysis and the beginning of the endotoxin infusion is necessary in this model to allow the interstitial pulmonary edema due to the pulmonary venous occlusion to resolve. The edema was assumed to have resolved when QL and Clp had normalized again.

Experimental protocol. The animals were randomly assigned to one of two groups. After baseline data had been collected, all sheep were started on a continuous infusion of Escherichia coli endotoxin (10 ng·kg⁻¹·min⁻¹) in NaCl (0.9%), which was maintained for 35 h. After 24 h of endotoxemia, six animals received a bolus injection of the NOS inhibitor L-NAME in NaCl (0.9%), whereas the other group (control, n = 6) was given an equivalent volume of saline. The experiments were accomplished according to the guidelines of the National Institutes of Health and the American Physiological Society and were approved by the Animal Care and Use Committee of The University of Texas Medical Branch.

Data analysis. Results are presented as means ± SE. The data were analyzed by analysis of variance for repeated measures with post hoc Scheffe’s F-test for differences from 0 to 24 h. Analysis of variance for factorial analysis was applied to test for statistical differences between groups. Differences were regarded as statistically significant when P < 0.05.

RESULTS

Both groups of sheep developed a hyperdynamic response characterized by a significant decrease in SVRI and an elevated CI after 24 h of endotoxemia (Fig. 1). Mean arterial pressure (MAP) showed a trend to lower values than at baseline at 24 h. These changes in systemic circulatory variables remained stable in the control group until 32 h. Administration of the NOS inhibitor L-NAME reversed the pronounced vasodilation. SVRI and MAP rose to values that were even higher than at baseline and at 24 h and higher than in the control group 1 h after L-NAME had been given (P < 0.05). At 32 h, neither SVRI nor CI nor MAP of the animals treated with L-NAME was different from baseline values, but they were still significantly different from 24-h values.

PVRI was not changed during the hyperdynamic phase of endotoxemia in the control animals (Fig. 2). After inhibition of NOS, PVRI increased and remained elevated until 32 h. PVRI was also analyzed for its distribution between the pre- and postcapillary pulmonary vascular bed (Table 1). Precapillary PVRI accounted for approximately two-thirds of the total PVRI in both groups at baseline. Precapillary PVRI of the control group showed a trend to higher levels than at baseline at 24 and 25 h and was significantly elevated at 32 h (Fig. 2). Postcapillary PVRI of these animals did not change significantly at the observed time points. Precapillary PVRI had markedly increased 1 h after administration of L-NAME (P < 0.05 vs. 0-h, 24-h, and control values) and remained significantly elevated until 32 h. The distribution of PVRI did not change significantly during the marked increase in PVRI (Table 1). Accordingly, postcapillary PVRI rose 1 h after the administration of L-NAME (P < 0.05 vs. 0- and 24-h values; Fig. 2) and was still higher than at baseline at 32 h.

PAP increased during endotoxemia in both groups (P < 0.05) and remained elevated in the control group throughout the experimental period (Fig. 3). PAP increased significantly 1 h after administration of L-NAME. The delay between the first permeability analysis and the beginning of the endotoxin infusion is necessary in this model to allow the interstitial pulmonary edema due to the pulmonary venous occlusion to resolve. The edema was assumed to have resolved when QL and Clp had normalized again.
L-NAME (P < 0.05 vs. 0-h, 24-h, and control values). Pmv was elevated in the L-NAME-treated animals at 25 h compared with 0 and 24 h. Both PAP and Pmv had returned to control group levels by 32 h. Neither PCWP nor LAP changed significantly over time (Table 1), nor were there significant differences between experimental groups in these variables.

QI was different between groups, both when the baseline reflection coefficient was determined (control group: 7.9 ± 1.6 ml/h vs. L-NAME group: 4.6 ± 1.1 ml/h) and 48 h later, before the infusion of endotoxin was begun (control group: 7.6 ± 1.1 ml/h vs. L-NAME group: 3.5 ± 0.7 ml/h) (Fig. 4). Clp/Cpp was almost equal between the two groups at these time points.

QI was elevated at 24, 25, and 32 h in both groups (Fig. 4) without differences between the two groups. However, if QI was calculated as increase from baseline (QIrel), QIrel rose significantly after administration of L-NAME to a level higher than in the control group at 25 h (P < 0.01). The values of the control and L-NAME-treated animals were no longer significantly different 32 h after the infusion of lipopolysaccharide had begun. Clp/Cpp decreased by 24 h when QI was elevated and returned to control values in both groups. Clp/Cpp values in the L-NAME group showed a trend to lower values than in the control at 25 h. Clp/Cpp values of both groups were similar again at 32 h.

The osmotic reflection coefficient to protein was at baseline level after 32 h of endotoxemia (Fig. 5). There was no difference between groups at either baseline or after 32 h. Filtration coefficients of both groups were elevated (P < 0.05). Again, there was no difference between groups.

**DISCUSSION**

Model of hyperdynamic endotoxemia. In this study the sheep developed a hyperdynamic circulatory response to the infusion of endotoxin, characterized by pronounced systemic vasodilation and a supranormal...
CI. The hyperdynamic circulatory state induced by the continuous infusion of endotoxin is consistent with the results of other studies performed in this ovine model (20, 21, 25, 26). This model is clinically relevant because it mimics the hemodynamic pattern observed in septic patients (4) and healthy volunteers challenged with a bolus of endotoxin (32). Thus it seemed logical that we could reasonably evaluate changes in microvascular permeability in the same model by using the chronic lung lymph fistula in sheep that was originally described by Staub et al. (31) and later modified to the present venous occlusion technique by Isago et al. (9).

L-NAME reversed the hyperdynamic state for the rest of the study period. The use of L-NAME in this model and the reversal of the hemodynamic response to endotoxin have been described in other reports (21, 22). L-NAME was again administered at 24 h in this study because, by that time, systemic vasodilation had occurred, which is the rationale for administering L-NAME in a model of sepsis. The use of NOS inhibitors in septic patients has been marked by controversy. Thus it seemed appropriate to use this model to test the potential adverse effects of NOS inhibitors on the pulmonary microvasculature.

### Table 1. Left atrial pressure, pulmonary capillary wedge pressure, and ratio of precapillary pulmonary vascular resistance to total pulmonary vascular resistance during 32 h of continuous endotoxin infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time, h</th>
<th>LAP, mmHg</th>
<th>PCWP, mmHg</th>
<th>PVRI, precap/total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>0</td>
<td>6 ± 0</td>
<td>12 ± 1</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.0 ± 1</td>
<td>12 ± 1</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>8 ± 1</td>
<td>11 ± 1</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>8 ± 1</td>
<td>10 ± 1</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td><strong>L-NAME</strong></td>
<td>0</td>
<td>7 ± 1</td>
<td>12 ± 1</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8 ± 1</td>
<td>12 ± 1</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7 ± 1</td>
<td>11 ± 1</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>5 ± 1</td>
<td>10 ± 1</td>
<td>0.73 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 animals in control (saline) group and 6 animals in N-nitro-L-arginine methyl ester (L-NAME) group. LAP, left atrial pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; precap, precapillary.

In this preparation, it has been demonstrated that the reflection coefficient to protein is at the baseline level after 24 h of endotoxemia (25).

L-NAME reversed the hyperdynamic state for the rest of the study period. The use of L-NAME in this model and the reversal of the hemodynamic response to endotoxin have been described in other reports (21, 22). L-NAME was again administered at 24 h in this study because, by that time, systemic vasodilation had occurred, which is the rationale for administering L-NAME in a model of sepsis. The use of NOS inhibitors in septic patients has been marked by controversy. Thus it seemed appropriate to use this model to test the potential adverse effects of NOS inhibitors on the pulmonary microvasculature.

L-NAME and pulmonary hemodynamics. PAP was elevated at 24 h, due to the high flow state and possibly due to a higher pulmonary blood volume, without evidence of pulmonary vasoconstriction. Both PCWP and LAP were available for the calculation of PVRI. PCWP was preferred because it was measured by the...
same catheter as PAP. LAP was determined by a different catheter inserted into the left atrium. The position of the atrial catheter relative to the Swan-Ganz catheter is likely to vary between different animals and would therefore disturb calculations of the distribution of pulmonary vascular resistance. Because both LAP and PCWP data are presented, the reader has the additional information when the contribution of postcapillary resistance to total resistance may be under- or overestimated.

PVRI was at baseline level after 24 h of endotoxemia but increased markedly after the administration of L-NAME. This increase occurred on both the pre- and postcapillary side of the pulmonary circulation. In a consideration of the trend to elevated PCWP at 25 h with the LAP remaining stable, the increase in postcapillary PVRI was still underestimated. The higher postcapillary PVRI may have contributed to the simultaneous elevation in Pmv after inhibition of NOS.

Analysis of pulmonary transvascular fluid flux and permeability. QI differed between groups at the two baseline measurements that were 48 h apart. Because QI was stable and concomitant Clp/Cpp was practically equal in both groups, the higher QI is likely to have resulted from causes such as different lengths of the lymphatic catheters, different heights of the catheter outlet relative to the point of its insertion, and different volumes of lung drained. If the elevated QI had been due to a real increase in fluid filtration, lymph protein would have been washed down, which obviously was not the case. The trend to higher filtration coefficients in the control group can be explained by the fact that this variable is calculated by an equation that contains QI. Finally, the baseline values in both groups were well within the limits of published baseline values in recent investigations (5, 24). To unmask possible effects of L-NAME, which may have been hidden otherwise, QI was presented both as raw data and as increases from baseline (i.e., QIrel).

A high QI indicated an elevated rate of transvascular fluid flux at 24 h. QI did not differ between both groups after the administration of L-NAME. QIrel in the L-NAME-treated sheep, however, surpassed those of the control animals at 25 h, but inhibition of the NOS did not affect the reflection coefficient to protein or filtration coefficient at 32 h.

The increase in QIrel occurred during a period when Pmv was elevated. Both variables returned to their former levels thereafter. Moreover, inhibition of NOS has been shown to prevent the negative chronotropic and inotropic effects that NO has on the spontaneous contraction of isolated lymphatics (36). Therefore, a more efficient lymphatic pump may have contributed to the higher QIrel in the L-NAME-treated animals. The higher QIrel compared with the control group was associated with a trend of Clp/Cpp to decline in the L-NAME group. The fact that Clp/Cpp tended to decrease during the elevated QIrel suggests a washdown effect on the lymph protein.

The venous occlusion technique is generally accepted as a method that allows for the evaluation of the lung microvascular permeability to protein in conscious animals. For interpretation of our data, several issues must be considered. Studies that had shown an increase in systemic microvascular permeability to protein after the administration of L-NAME in normal cats reported that the changes in permeability occurred within the first hour after inhibition of NOS and were associated with a fourfold increase in QI (15). We did not occlude the pulmonary veins before 8 h after the administration of L-NAME but followed QI and Clp/Cpp during the 8 h period. Thus the lymph data represent early changes in transvascular fluid flux, whereas the reflection and filtration coefficients represent late changes in pulmonary microvascular permeability.

Even if the venous occlusion technique was not performed before 8 h after L-NAME had been given, it can be concluded that, if an increase in microvascular permeability to small particles had occurred early after L-NAME, it would have been of minor impact. Our data are consistent with those of Kavanagh et al. (11), which did not find an increase in the filtration coefficient when a NOS inhibitor was added to the buffer that perfused an isolated rabbit lung with oxidant-induced injury.

Still, a clear picture regarding the role of endogenous NO in the maintenance of an intact barrier function under physiological and pathological conditions cannot be drawn. There are data supporting both protective (13–16, 35) and detrimental effects (2, 3, 10, 12, 28, 29) of NO.

In conclusion, administration of NOS inhibitor reversed the hyperdynamic response to the continuous infusion of endotoxin. Inhibition of NOS was associated with a transient increase in Pmv and QIrel. There was no evidence of an elevated lung microvascular permeability.
ability to protein or a further increase in filtration coefficient after administration of L-NAME in this model. Further studies must be performed to elucidate the impact of different species and models on the role of NO in the maintenance of endothelial barrier function.

Address for reprint requests: D. L. Traber, The Univ. of Texas Medical Branch, Dept. of Anesthesiology, Investigational Intensive Care Unit, 610 Texas Ave., Galveston, TX 77555-1091 (E-mail: traber@beach.utmb.edu).

Received 20 August 1996; accepted in final form 25 July 1997.

REFERENCES


