Inhaled NO reduces leukocyte-endothelial cell interactions and myocardial dysfunction in endotoxemic rats

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Inhaled nitric oxide (NO) has been shown to have some protective effect in the peripheral distal inflamed vasculature. The objective of the study was to determine whether inhaled NO would reduce endotoxin-induced leukocyte activation and myocardial contractile dysfunction. Rats were treated with either saline or endotoxin (10 mg/kg iv) and then allowed to breathe (4 h) either air or air plus NO (10 ppm). In endotoxemic rats, mesenteric venular endothelium leukocyte firm adhesion increased compared with control rats (1.15 ± 0.32 vs. 4.08 ± 0.96 leukocytes/100 µm; P < 0.05). Inhaled NO significantly attenuated endotoxin-induced venular endothelium leukocyte adhesion (4.08 ± 0.96 vs. 1.86 ± 0.76 leukocytes/100 µm; P < 0.05) and FITC-conjugated anti-intercellular adhesion molecule-1 fluorescence intensity. Endotoxin-induced myocardial dysfunction and leukocyte content increases were reduced in inhaled NO-treated rats. These observations suggest that inhaled NO reduces the degree of cardiovascular dysfunction and inflammation in endotoxemic rats.

The relevance of leukocyte adhesion and activation in mediating myocardial damage and dysfunction is illustrated by the finding (12) that in endotoxin-mediated septic shock, the reduction of leukocytes perfusing the coronary circulation preserved systolic contractility of the isolated heart.

In recent years, considerable research has focused on the physiological, biochemical, and molecular actions of NO in normal physiological processes as well as during pathological conditions. NO has been shown to modulate a number of physiological processes relating to vascular reactivity (19), platelet function (13), leukocyte-endothelial cell interactions in the microcirculation, and vascular permeability (21, 24). With regard to NO production during endotoxia and sepsis, conflicting results have been reported, i.e., overproduction of NO in some studies (3) and a reduced production in others (42). Endotoxin exposure stimulates an inducible form of NO synthase within vascular endothelial cells and smooth muscle cells in the peripheral vasculature, as well as macrophages (38). In contrast to the above studies, a number of investigators (42–44) have demonstrated that endothelin-derived NO decreased significantly following endotoxin administration or endotoxic shock. The latter observations have been supported by recent studies (42) in which NO blockade during endotoxia and sepsis was detrimental.

Exogenous inhaled NO is increasingly used in patients with acute lung injury and usually results in selective pulmonary arterial vasodilation, decreased venous admixture, and improved arterial oxygenation (37). Despite its pulmonary selectivity, inhaled NO has been recently shown (8, 36) to affect systemic hemodynamics and leukocyte function in the inflamed peripheral vasculature. Although these findings suggest that, under certain conditions, inhaled NO may exhibit extrapulmonary effects within the systemic vasculature, the effects of inhaled NO on endothelin-leukocyte-endothelial cell activation and myocardial dysfunction have not been specifically addressed. We tested whether inhaled NO would reduce endothelium-leukocyte interaction and improve myocardial depression in a rat model of sepsis. The results of this study provided new pieces of information: 1) inhaled NO reduced leukocyte-endothelial cell interaction in the peripheral septic microvasculature, and 2) inhaled NO reduced myocardial leukocyte sequestration and ame-
H1784  INHALED NITRIC OXIDE DURING ENDOTOXEMIA IN RATS

MATERIALS AND METHODS

Animal preparation. Sprague-Dawley rats (Dépré, Saint Douichard, France) (300–350 g) were housed for 6 days in groups of six in standard cages and supplied ad libitum with laboratory chow and tap water. Endotoxemia was then induced in rats by intravenous injection of 10 mg/kg body wt endotoxin, Escherichia coli 026:B6 (Sigma, Saint Quentin Fallavier, France), in 1 ml normal saline under brief ether anesthesia. As a control, other animals received an injection of an equal volume of sterile saline. The study described herein was performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and with approval from our institution's Animal Research Committee.

Inhaled NO delivery system. Experimental animals were placed to breathe spontaneously either air or NO in air (10 ppm) in a specially designed chamber (Air Liquide, Jouy en Josas, France). NO gas (Air Liquide) was delivered into the chamber at a rate necessary to achieve a concentration of 10 ppm. The final concentration of 10 ppm of NO was ensured with the use of a mixing gas system. The mixed gas was checked to remain homogenous in different areas of the exposure chamber. NO concentration was continuously monitored using an electrochemical sensing system (Draeger, Lubeck, Germany) that was calibrated at the start of each experiment using known standard gas concentrations.

Intravital observation of leukocyte adhesion in the mesenteric venules. Rats were anesthetized with 50 mg/kg ketamine xylazine intramuscularly, and the carotid artery was cannulated with a PE-50 tube connected to a pressure transducer (Kontron, Basel, Switzerland) to monitor mean arterial pressure. The abdomen was then opened via a midline laparotomy, and a segment of the distal ileum was gently exposed and mounted in an optical chamber. With the use of this design, the exposed bowel wall within the chamber was superfused with a thermostat-controlled saline solution maintained at 37°C. After a 30-min equilibration period, the mesenteric microcirculation was observed with the use of an intravital microscope. An Eclipse E800 Nikon microscope (Nikon, Tokyo, Japan) fitted with a xenon light source and an epifluorescence assembly was used with filter sets for acridine orange (excitation, 470 nm FWHM 40; emission, 540 nm FWHM 40). A Hamamatsu C2400-08 videocamera (Hamamatsu City, Japan) mounted on the microscope projected the image onto a monitor, and the images (720 × 576 pixels) were recorded for playback analysis with a digital videocassette recorder (Sony DVR 30). Magnification ×40 was used. Consequently, the final resolution was 0.5 µm per pixel.

As previously described (8, 24), single unbranched mesenteric venules (25–40 µm in diameter) were selected for the study of each animal. Red blood cell velocity (V_RBC) was measured off-line as the distance through which packed red blood cells or plasma break traveled within two subsequent video-frame time intervals of 40 ms. The leukocyte behavior in the venules was observed for a 1-min period after the injection of 1 ml of 1% wt/vol acridine orange into the carotid artery. Leukocyte rolling flux was determined off-line during playback of videotaped images by counting the number of leukocytes that stuck and remained stationary for a period >30 s per 100 µm of venule.

Visualization of microvascular expression of intercellular adhesion molecule 1 in vivo. The expression of intercellular adhesion molecule 1 (ICAM-1) in the rat mesenteric venules was examined with a fluorescence microscope (Eclipse E800, Nikon) as previously described (17). In some rats, 1 mg/kg body wt of FITC-labeled 1A29 (mouse anti-rat CD54: FITC, Serotec, Varilhes, France) was injected intravenously. Anti-r IgG1 was used as a negative control (mouse anti-rat IgG1 negative control: FITC, Serotec). Fluorescence intensity (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) was detected for 30 min, and images were recorded for playback analysis. The fluorescence images were digitally processed and analyzed using a computer-assisted image analyzer (Mocha software, J andel, San Rafael, CA). The expression of ICAM-1 in the rat mesenteric venules (n = 6 in each animal) was quantified as the area stained per venule.

Isolated and perfused heart preparation. Myocardial contractile function was studied using a modified Langendorff isolated heart preparation (28, 35). Briefly, after heparinization and ether anesthesia, the heart was rapidly excised, the ascending aorta was cannulated, and retrograde perfusion was initiated with Krebs-Henseleit solution (in mM: 120 NaCl, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.25 CaCl2, 25 NaHCO3, and 11 glucose) saturated with a 95% O2-5% CO2 gas mixture at 37°C and at a flow rate of 10 ml/min. To assess contractile function, we inserted a water-filled latex balloon through the left atrium into the left ventricle (LV) and connected it to a pressure transducer. This balloon was then adjusted to a left ventricular end-diastolic pressure (LVEDP) of 5 mmHg. The heart was paced at 300 beats/min and allowed to equilibrate for 30 min. Left ventricular developed pressure (LVDP), its first derivative (+dp/dt) and the −dp/dt), and coronary perfusion pressure (CPP) were monitored and recorded on a chart recorder (recorder and modules, Kontron Supermon, Basel, Switzerland). After baseline measurements were taken, LVDP- and LV volume-preload relationships (LVEDP from −5 to 20 mmHg) were obtained.

Measurements of heart antioxidant enzymes. Preparation and measurement of antioxidant enzymes in the heart were performed according to the method described by Brown et al. (1). Left ventricles were stored at −70°C until they were analyzed for superoxide dismutase (SOD), catalase (Cat), and glutathione reductase (GPX) activities as previously described (32). For analysis, the tissues were thawed and homogenized as a 10% wt/vol mixture in a 20 mM phosphate buffer (pH 7.4) and centrifuged at 8,000 g for 20 min at 4°C. The resulting supernatant was analyzed for SOD, Cat, and GPX activities. Inhibition of the rate of reduction of cytochrome c by superoxide radicals was used as an indirect measurement of SOD activity. SOD activity measured in this assay represents total tissue SOD activity and one unit of SOD activity is defined as the activity required to inhibit cytochrome c reduction by 50%. Disappearance of hydrogen peroxide was monitored at 240 nm as a measure of Cat activity. One unit of Cat activity is defined as 1 µmol of H2O2 degraded per minute per milligram of protein. GPX activity was determined by monitoring the reduction of glutathione (oxidized) spectrophotometrically after a coupled reaction involving the oxidation of NADPH for which the disappearance of NADPH is monitored at 340 nm. One unit of GPX activity is defined as 1 µmol of NADPH converted to NADP per minute per milligram of protein. All biochemical analyses were standardized per gram of protein content, as determined by a Bio-Rad protein assay (Richmond, CA).
Measurements of heart MPO activity. Myeloperoxidase (MPO) activity in the LV was used as an index of leukocyte infiltration. Briefly, left ventricles were placed in a 20 mM phosphate buffer (pH 7.4) at 10% wt/vol and homogenized. One milliliter of the homogenate was then adjusted to a total volume of 10 ml with 20 mM phosphate buffer (pH 7.4) and centrifuged at 6,000 g for 20 min at 4°C. The pellet was rehomogenized and sonicated for 10 s in 1 ml of 50 mM acetic acid (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide detergent. Twenty microliters of the prepared samples were used in reactions for MPO activity determined spectrophotometrically (650 nm) by measuring hydrogen peroxide-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine. One unit of MPO activity is defined as the amount of MPO that produces an absorbance change of 1.0 optical density per minute per gram of tissue at 37°C.

Heart morphometric analysis. LV tissue sections (5 mm thick) from hearts fixed in 10% formaldehyde solution were dehydrated and embedded in paraffin. Serial sections (4 µm thick) were stained with hematoxylin and eosin for myocardial leukocyte content and interstitial edema (35). The number of random fields needed to count 100 leukocytes in each group at ×400 magnification was recorded. The average number of leukocytes per ×400 field was then determined as a quantitative assessment of myocardial leukocyte content. The fractional area of interstitial edema was measured for five fields at ×400 magnification on digitalized captured images using a computer-assisted image analyzer (Mocha software, Jandel).

Experimental design. Animals were randomly assigned to one of the following groups. Control animals received an intravenous infusion of sterile saline only. Control NO-treated animals received an intravenous infusion of sterile saline and received inhaled NO at 10 ppm. Endotoxin-treated animals received an intravenous infusion of endotoxin (10 mg/kg) and received inhaled NO at 10 ppm. Endotoxemic (EDTX) animals were then exposed to a 4-h period of NO 10 ppm in air. *P < 0.05 compared with CTR animals. §P < 0.05 compared with EDTX/NO animals.

Intravital microscopy of the rat mesenteric venule. Table 1 summarizes the effects of inhaled NO and endotoxemia in all experimental groups (n = 8 in each group) on shear rate and leukocyte rolling flux and adherence. Shear rate decreased in endotoxin-treated rats compared with that in saline-treated rats, whereas inhaled NO had no effects on saline- and endotoxin-treated rats. Compared with saline treatment, endotoxemia was associated with increases in the number of rolling and adherent leukocytes on the mesenteric venular endothelium. Leukocyte rolling and adhesion on the mesenteric venule were reduced by NO inhalation in endotoxin-treated rats. The expression of ICAM-1 in the rat mesenteric venules (n = 6 in each animal) was quantified as the area stained per venule. No stained areas were observed in control animals, i.e., saline-treated rats (n = 2). The fluorescent stained area increased to 500 ± 80 µm² in endotoxin-treated rats (n = 2). Inhaled NO reduced the fluorescent stained area in endotoxin-treated rats (n = 2) (500 ± 80 vs. 80 ± 40 µm²).

In another series of experiments (n = 4 in each group), we inhibited NO locally within the mesentery by superfusing l-NAME (100 µM, 60 min). l-NAME superfusion was associated with increases in leukocyte rolling compared with baseline (20 ± 5 to 60 ± 6 cells/min; P < 0.05) and adhesion (1 ± 1 to 12 ± 2 cells/100 µm; P < 0.05). Furthermore, NO inhalation (10 ppm) in air for 4 h prevented the ability of l-NAME to affect the peripheral vasculature, we devised some experiments in which rats inhaled either 0 or 10 ppm NO in air for 4 h. The mesentery was then exposed for intravital microscopy while being locally superfused with the NO inhibitor N-nitro-l-arginine methyl ester (l-NAME) hydrochloride (100 µM for 60 min) (Sigma), which has been shown to increase leukocyte rolling and adhesion.

Table 1. Mean arterial blood pressure, circulating leukocyte counts, mesenteric venular shear rate, and mesenteric venule leukocyte rolling and adhesion of control, endotoxemic, inhaled NO-treated control rats, and inhaled NO-treated endotoxemic rats

<table>
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<tr>
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<th>CTR</th>
<th>EDTX</th>
<th>CTR/NO</th>
<th>EDTX/NO</th>
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<tbody>
<tr>
<td>Mean arterial</td>
<td>80±2</td>
<td>72±8</td>
<td>83±4</td>
<td>77±6</td>
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<tr>
<td>pressure,</td>
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<tr>
<td>mmHg</td>
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<tr>
<td>Circulating</td>
<td>9.4±0.9</td>
<td>2.2±0.3*</td>
<td>7.9±0.9</td>
<td>2.1±0.6*</td>
</tr>
<tr>
<td>leukocytes, 10³/liter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shear rate, s⁻¹</td>
<td>623±80</td>
<td>157±22*</td>
<td>678±52</td>
<td>143±29*</td>
</tr>
<tr>
<td>Leukocyte rolling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flux, cells/min</td>
<td>15±7</td>
<td>78±9*</td>
<td>20±6</td>
<td>27±8</td>
</tr>
<tr>
<td>Adherent leuko-</td>
<td>1.15±0.32</td>
<td>4.08±0.96*</td>
<td>1.31±0.96</td>
<td>1.87±0.76§</td>
</tr>
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<td>cyte, cells/10⁶µm</td>
<td></td>
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Results are presented as means ± SE (n = 8 in each group). Control (CTR) animals received 1 ml iv saline. Endotoxemic (EDTX) animals received 10 mg/kg iv endotoxin. CTR/nitric oxide (NO) and EDTX/NO animals were then exposed to a 4-h period of NO 10 ppm in air. *P < 0.05 compared with CTR animals. §P < 0.05 compared with EDTX/NO animals.
to increase leukocyte rolling compared with control (50 ± 6 vs. 25 ± 3 cells/min) and adhesion (60 ± 6 vs. 32 ± 6 cells/100 μm; \( P < 0.05 \)).

LV contractile function. Table 2 summarizes the effects of inhaled NO and endotoxemia in all experimental groups (\( n = 7 \) in each group) on heart function. LVDP and its first derivatives, i.e., \( +dP/dt \) and \( -dP/dt \), were decreased in endotoxin-treated rats compared with saline-treated rats. Inhaled NO treatment did not affect LVDP and its first derivatives in saline-treated rats but was associated with increases of LVDP and its first derivatives in endotoxin-treated rats (Table 2). No statistically significant difference in CPP was found among the groups (Table 2). Endotoxin-treated rats exhibited LVDP-preload relationships that were shifted downward and to the right of those for saline-, inhaled NO + saline-, and inhaled NO + endotoxin-treated rats (Fig. 1), in the direction of improved contractile performance. Inhaled NO treatment was associated in both saline- and endotoxin-treated rats with an LV volume-preload relationship shift upward and to the left, in the direction of increased LV compliance (Fig. 1).

Heart antioxidant enzyme and MPO activities. Biochemical analyses of the antioxidant status of the myocardial tissue in all experimental groups (\( n = 5 \) in each group) are shown in Fig. 2. After endotoxin infusion, heart SOD and GPX activities were unchanged. In the endotoxin-treated rats, inhaled NO-reduced heart Cat activity increased, whereas heart SOD and GPX activities were unchanged (Fig. 2).

MPO activity of the myocardial tissue was increased 4 h after endotoxin infusion (4.86 ± 0.24 vs. 6.29 ± 0.26 U/g of protein, \( P < 0.05 \)). In the endotoxin-treated rats, inhaled NO-reduced heart MPO activity increased (5.35 ± 0.56 vs. 4.52 ± 0.26 U/g of protein, \( P < 0.05 \)).

Morphometric analysis of the LV myocardium. Quantitative histological assessment analyses of the LV myocardium in all experimental groups (\( n = 5 \) in each group) are shown in Fig. 3. The number of leukocytes and the area of fraction of interstitium in the myocardium in endotoxin-treated rats increased 4 h after endotoxin infusion compared with saline-treated rats, whereas inhaled NO reduced both parameters in endotoxin-treated rats.

**Table 2.** Myocardial function of CTR, EDTX, inhaled NO-treated control rats, and inhaled NO-treated EDTX rats

<table>
<thead>
<tr>
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<th>CTR</th>
<th>EDTX</th>
<th>CTR/NO</th>
<th>EDTX/NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP, mmHg</td>
<td>75 ± 15</td>
<td>48 ± 4*</td>
<td>77 ± 7</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>35 ± 5</td>
<td>34 ± 7</td>
<td>36 ± 2</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>1,687 ± 207</td>
<td>841 ± 160*</td>
<td>1,502 ± 138</td>
<td>1,466 ± 225</td>
</tr>
<tr>
<td>-dP/dt, mmHg/s</td>
<td>881 ± 35</td>
<td>401 ± 57*</td>
<td>799 ± 103</td>
<td>833 ± 84</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE (\( n = 7 \) in each group). CTR animals received 1 ml iv saline. EDTX animals received 10 mg/kg iv endotoxin. CTR/NO and EDTX/NO animals were then exposed to a 4-h period of NO 10 ppm in air. LVDP, left ventricular developed pressure; CPP, coronary perfusion pressure; \( +dP/dt \) and \( -dP/dt \), LVDP first derivatives. *\( P < 0.05 \) compared with control animals.

**DISCUSSION**

Taken together, these observations demonstrate for the first time that administration of inhaled NO during endotoxemia in rats provided significant reduction of adhesive interactions between leukocytes and venular endothelial cells and improved myocardial contractile dysfunction. We used the intravital mesenteric venule preparation as a surrogate of vascular inflammation to assess endotoxin-induced leukocyte-venular endothelial cell inflammation. Indeed, leukocyte-mesenteric venular endothelium activation is a hallmark of the
endotoxin-induced generalized malignant vascular inflammation, and a number of factors that govern the interactions between leukocytes and endothelium have been described in the postcapillary mesentery venule preparation (11, 24). Using this intravital preparation, our observations suggest that inhaled NO decreased endothelium leukocyte adhesion and ICAM-1 expression during endotoxemia. Finally, we observed that inhaled NO improved endotoxin-induced myocardial dysfunction in the isolated and perfused heart preparation.

It should be noted that, in our study, NO inhalation was started at the time of endotoxin administration and continued for 4 h. The dose of 10 ppm NO in air was tested because many clinical studies (3, 37, 42) generally use <40 ppm inhaled NO. Indeed, inhaled NO shows promising results in the treatment of early onset lung injury associated with clinical conditions such as septic shock and endotoxemia (37). In this clinical context, it has been proposed (3, 42) that exogenous NO could be beneficial by limiting sepsis-induced organ dysfunction. Thus NO inhalation could be a valuable supportive therapy in acute sepsis in patients.

Systemic and physiological effects of inhaled NO. When inhaled NO reaches the pulmonary vasculature, it is rapidly bound to the Hb, which in turn limits its systemic effects. In vitro and in vivo studies (18, 40) have shown, however, that a biologically active form of NO can circulate in plasma while reversibly bound to serum proteins. Thus if inhaled NO can enter this circulating pool of protein-bound NO, it might cause changes in both systemic and pulmonary vasculature (39). Indeed, recent work from our group (16) and from other investigators (8, 36) suggests that, under some conditions, exogenous as well as inhaled NO may be able to alter the cardiovascular response to ischemia-reperfusion and sepsis.

In the present study, we directly determined that inhaled NO was reaching the peripheral microcirculation. We observed that increases in leukocyte rolling and adhesion on the mesenteric venule related to L-NAME superfusion were prevented in animals breathing 10 ppm NO in air for 4 h. This observation has been previously reported (8) in a similar experimental design in which inhaled NO was administered at an 80 ppm dose. Although the focus of this study was not to identify the molecule that can deliver NO to the periphery, these findings suggest that inhaled NO is reaching the peripheral vascular beds.

Inhaled NO reduced leukocyte adhesion in the septic microvasculature. Growing evidence suggests that endogenously produced endothelium-derived NO and exogenous NO may prevent organ injury related to acute inflammation by modulating neutrophil functions. First, administration of NO donors has been shown (22, 34) to attenuate tissue injury during reperfusion of posts ischemic tissue. Second, superoxide-induced leukocytic influx could be altered by NO donors (9). Third, it has been reported (27) that isolated vascular strips har-
vested from postischemic tissue supported less adhesion in the presence of NO donors. Furthermore, exogenous NO may exhibit some protective effects in various shock models, including improvement of organ blood flow (8), inhibition of platelet aggregation (14), and decreases in cytokine-induced endothelial activation (4).

Using intravital microscopy of the rat mesentery venule, we showed that inhaled NO therapy reduced leukocyte rolling and adhesion in endotoxic rats. There are several possible mechanisms by which NO donors could reverse endotoxin-induced leukocyte sequestration, including a direct effect on leukocyte rolling and/or adhesion, as well as improved blood flow, i.e., increased shear rates that oppose leukocyte adhesion (11). In our study, the administration of inhaled NO attenuated endotoxin-induced leukocyte adherence but did not prevent the reduction of erythrocyte velocity and thus venular wall shear rate. This finding suggests that inhaled NO does not interfere with the physical forces that modulate leukocyte behavior within the microvasculature.

A likely mechanism of action of inhaled NO may be the prevention of endothelial dysfunction induced by acute inflammation. First, NO markedly attenuates leukocyte rolling along the endothelium by inhibiting the expression of P-selectin on the vascular endothelium (23). Second, NO inhibits the firm adherence of leukocytes to the endothelium, partially by the inhibition of ICAM-1 and vascular cell adhesion molecule-1 (4). Using intravital microscopy of the endotoxemic mesentery venule, we consistently showed that inhaled NO decreased fluorescence image intensity associated with the infusion of anti-rat monoclonal antibody anti-ICAM-1.

Inhaled NO improved contractile dysfunction in the septic heart. Leukocytes, and particularly neutrophils, are retained in the heart early in the systemic inflammatory response to endotoxin (10). In animals infused with endotoxin, leukocyte transit time across the coronary capillary bed and intramyocardial capillary obstruction is dramatically increased. Leukocytes may contribute to endotoxin-induced endothelial cell damage leading to impaired regulation of microvascular blood flow (10). In addition, leukocytes may cause a decrease in ventricular contractility by producing significant myocardial damage, including interstitial edema, zonal contraction bands, and contraction band necrosis, via the generation of reactive oxygen species (35). The relevance of leukocyte adhesion and activation in mediating myocardial dysfunction is illustrated by the finding that 1) the reduction of circulating leukocytes, 2) anti-leukocyte serum, and 3) antiadhesion therapies including anti-CD18 and anti-ICAM-1 antibodies attenuate myocardial injury in various experimental shock models (26). Hence, the reduction of leukocyte activation might represent a possible therapeutic approach in the prevention of endotoxin-induced cardiovascular injury.

On the basis of these observations, we considered that inhaled NO could improve endotoxin-induced myocardial dysfunction by reducing leukocyte adhesive interactions in the distally inflamed microvasculature. This contention is based on work by a number of laboratories (8, 9, 15, 16) reporting that NO donors reduce leukocyte sequestration and improve LV contractile and diastolic dysfunction in postischemic preparations. Indeed, in our study, NO administration reduced leukocyte sequestration in the septic myocardium. However, quantification of the number of extravasated leukocytes by either a histological approach or MPO activity provide some discrepancies. A 50% increase in MPO was noted in hearts of endotoxin-treated animals, whereas direct quantification of the number of extravasated leukocytes with the use of a histological approach indicated a 10-fold increase. It should be pointed out that the histological approach is an estimate based on direct observation of leukocytes, whereas MPO activity is an indirect estimate and can be used only as a guide for changes in leukocyte accumulation. Our data are, however, internally consistent in that MPO activity and the histological approach show the same changes in leukocytes, i.e., an increase following endotoxin treatment and a decrease following NO inhalation.

NO has been shown (2) to inhibit leukocyte function by attenuating the release of reactive oxygen species by activated leukocytes. The role that reactive oxygen species play in septic myocardial dysfunction has not been directly and extensively determined. Previous studies (1) demonstrated that endotoxin increases endogenous myocardial Cat activity, which has been proposed as an indirect marker of the release of reactive oxygen species. Consistently, we observed that heart Cat activity was increased in endotoxemic animals. Reactive oxygen species come from several sources and could have several effects in the septic hearts. Activated leukocytes are important sources of reactive oxygen species and leukocytes accumulated in the heart after endotoxin administration. Because filtering leukocytes from the coronary vasculature (12) and intracellular antioxidants (35) prevents the decrease in myocardial contractility, it is reasonable to postulate that leukocytes mediate much of their myocardial depressant effects via reactive oxygen species. Consistently, we observed that inhaled NO therapy reduced endotoxin-induced increases in heart leukocyte accumulation and Cat activity. However, changes in septic heart Cat activity related to endotoxin and inhaled NO therapy were only minor. Hence, it is possible that the prevention of leukocyte accumulation in the septic heart by NO inhalation has beneficial effects by other means than the release of reactive oxygen species.

In summary, administration of inhaled NO during endotoxemia in rats can provide a significant reduction of adhesive interactions between leukocytes and vascular endothelial cells and improve myocardial contractile function. Because our study examined isolated heart contractile function and leukocyte-endothelium interactions in a rat model of sepsis, extrapolation of the data to the human sepsis must be approached with caution. However, these observations may provide an opportunity for the exploitation of NO therapy as a
beneficial immunomodulatory approach in patients at high risk for developing gram-negative sepsis.

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