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

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1Réanimation Médicale, Hopital Calmette, 2EA 2689 Institut National de la Santé et de la Recherche Médicale, and 3Service de Biochimie, Hopital Huriez, Centre Hospitalier Universitaire Lille 59037, France

Nevière, Rémi, Benoît Guery, Serge Mordon, Farid Zerimech, Stéphane Charré, Francis Wattel, and Claude Chopin. Inhaled NO reduces leukocyte-endothelial cell interactions and myocardial dysfunction in endotoxemic rats. Am J Physiol Heart Circ Physiol 278: H1783–H1790, 2000.—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 either with 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 endotoxemia 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 endothelium-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 endotoxemia 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 extrapolungary effects within the systemic vascular system, the effects of inhaled NO on endotoxin-induced 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 reduces leukocyte-endothelial cell interaction in the peripheral septic microvasculature, and 2) inhaled NO reduced myocardial leukocyte sequestration and ame-
riorated endotoxin-induced heart contractile dysfunc-
tion.

MATERIALS AND METHODS

Animal preparation. Sprague-Dawley rats (Dépré, Saint
Doulchard, 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 in-
duced 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 Insti-
tutes of Health Guidelines for the Care and Use of Labora-
tory 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 moni-
tored using an electrochemical sensing system (Draeger,
Lubeck, Germany) that was calibrated at the start of each
experiment using known standard gas concentrations.

Intravitral observation of leukocyte adhesion in the mesen-
teric venules. Rats were anesthetized with 50 mg/kg ketamine
xylazine intramuscularly, and the carotid artery was cannu-
lated with a PE-50 tube connected to a pressure transducer
(Kontron, Basel, Switzerland) to monitor mean arterial pres-
sure. The abdomen was then opened via a midline lapa-
rotomy, 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 main-
tained at 37°C. After a 30-min equilibration period, the
mesenteric microcirculation was observed with the use of an
intravitral 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 acrid-
eine orange (excitation, 470 nm FWHM 40; emission, 540 nm
FWHM 40). A Hamamatsu C2400-08 videocamera (Hama-
matsu 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. Conse-
quently, the final resolution was 0.5 µm per pixel.

As previously described (8, 24), single unbranched mesen-
teric 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-
rat IgG1 was used as a negative control (mouse anti-rat IgG1
negative control: FITC, Serotec). Fluorescence intensity (exci-
tation 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).

Isolated and perfused heart preparation. Myocardial con-
tractile function was studied using a modified Langendorff
isolated heart preparation (28, 35). Briefly, after hepariniza-
tion and ether anesthesia, the heart was rapidly excised, and
the atria were cut away. The heart was then implanted into a
modified Langendorff apparatus and perfused with Krebs-
Henseleit solution (in mM: 120 NaCl, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.2 CaCl2, 25 NaHCO3, and 11 glucose) saturated with a 95% O2-5% CO2
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 –dP/dt), and
coronary perfusion pressure (CPP) were monitored and re-
corded on a chart recorder (recorder and modules, Kontron
Supermon, Basel, Switzerland). After baseline measure-
ments were taken, LVDP- and LV volume-preload relationships
(LVDP 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 de-
scribed (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 cyto-
chrome c by superoxide radicals was used as an indirect
measurement of SOD activity. SOD activity measured in this
assay is expressed as total tissue SOD activity and one unit
of SOD activity is defined as 1 µmol of NADPH converted to NADP
by superoxide dismutase per minute per milligram of protein. GPX activity was
measured according to the method described by Brown et al.
(1). GPX activity was 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 resuspended 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). Endotoxin NO-treated animals received an intravenous infusion of endotoxin (10 mg/kg) and received inhaled NO at 10 ppm. In each group, NO therapy was started immediately after intravenous injection of either saline or endotoxin and continued for 4 h. After the 4-h period, animals were prepared for either heart preparation or mesenteric venule measurement protocol.

To further evaluate the role of inhaled NO as a molecule that could affect the peripheral vasculature, we devised some experiments in which rats inhaled either 0 or 10 ppm in air for 4 h. The mesentery was then exposed for intravital microscopy while being locally superfused with the NO inhibitor N^6-nitro-L-arginine methyl ester (L-NAME) hydrochloride (100 μM for 60 min) (Sigma), which has been shown to increase leukocyte rolling and adhesion.

Statistical analysis. Data were presented as means ± SE. Data were analyzed using an ANOVA with Scheffé's post hoc test. We tested for differences in LVDP- and LV volume-preload relationship of the isolated hearts over LVEDP using a two-way ANOVA. Statistical significance was set at P < 0.05.

RESULTS

Physiological variables. The mean arterial blood pressure after a 4-h period of NO inhalation (10 ppm) was not different between animals breathing room air and those breathing air plus NO. After 4 h, endotoxin-treated rats had similar blood pressure compared with saline-treated rats. In endotoxin-treated rats, inhaled NO (10 ppm) did not contribute to a change in mean arterial blood pressure (Table 1). Circulating leukocyte counts were not different between animals breathing room air and those breathing air plus NO. Endotoxin rats developed profound leukopenia (Table 1). In endotoxin NO-treated rats, inhaled NO neither affected total circulating leukocyte counts (Table 1) nor neutrophil differential counts (0.9 ± 0.2 10^8 per liter in endotoxin animals vs. 1.1 ± 0.3 10^8 per liter in inhaled NO-treated endotoxin 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 increase leukocyte rolling and adhesion.

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

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<tr>
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<th>CTR</th>
<th>EDTX</th>
<th>CTR/NO</th>
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<tr>
<td>Mean arterial pressure, mmHg</td>
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<td></td>
<td>80 ± 2</td>
<td>72 ± 8</td>
<td>83 ± 4</td>
<td>77 ± 6</td>
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<td>Circulating leukocytes, 10^9/liter</td>
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<td></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>
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<tr>
<td>Shear rate, s^-1</td>
<td>623 ± 80</td>
<td>157 ± 22*</td>
<td>678 ± 52</td>
<td>143 ± 29*</td>
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<td>Leukocyte rolling flux, cells/min</td>
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<tr>
<td></td>
<td>15 ± 7</td>
<td>78 ± 9*</td>
<td>20 ± 6</td>
<td>27 ± 8</td>
</tr>
<tr>
<td>Adherent leukocyte, cells/100 μm</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>
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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 (CTR) animals received 1 ml iv saline. Endotoxemic (EDTX) animals received 10 mg/kg iv endotoxin. CTR/nitric oxide (NO) and EDTX/NO (CTR) animals received 1 ml iv saline.
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 Cat activity was increased, whereas 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.

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

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

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<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>
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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.
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 postischemic 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 endotoxemic 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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