Effects of nitric oxide on blood flow distribution and $O_2$ extraction capabilities during endotoxic shock

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Zhang, Haibo, Peter Rogiers, Nadia Smail, Ana Cabral, Jean-Charles Preiser, Marie-Odile Peny, and Jean-Louis Vincent. Effects of nitric oxide on blood flow distribution and $O_2$ extraction capabilities during endotoxic shock. J. Appl. Physiol. 83(4): 1164–1173, 1997.—The effects of the nitric oxide (NO) synthase inhibitor $N^\text{G}$-monomethyl-$l$-arginine ($l$-NMMA) and the NO donor 3-morpholinosydnonimine ($l$-SNAP) were tested in 18 endotoxic dogs. $l$-NMMA infusion (10 mg·kg$^{-1}$·h$^{-1}$) increased arterial and pulmonary artery pressures and systemic and pulmonary vascular resistances but decreased cardiac index, left ventricular stroke work index, and blood flow to the hepatic, portal, mesenteric, and renal beds. $l$-SNAP infusion (2 µg·kg$^{-1}$·min$^{-1}$) increased cardiac index; left ventricular stroke work index; and hepatic, portal, and mesenteric blood flow. It did not significantly influence arterial and pulmonary artery pressures but decreased renal blood flow. The critical $O_2$ delivery was similar in the $l$-NMMA group and in the control group (13.3 ± 1.6 vs. 12.8 ± 3.3 ml·kg$^{-1}$·min$^{-1}$) but lower in the $l$-SNAP group (9.1 ± 1.8 ml·kg$^{-1}$·min$^{-1}$, both $P < 0.05$). The critical $O_2$ extraction ratio was also higher in the $l$-SNAP group than in the other groups (58.7 ± 10.6 vs. 42.2 ± 7.6%) in controls, $P < 0.05$; 43.0 ± 15.5% in $l$-NMMA group, $P = $ not significant). We conclude that NO is not implicated in the alterations in $O_2$ extraction capabilities observed early after endotoxin administration.

cardiac output; hypotension; organ perfusion; oxygen delivery; sepsis; endothelium-derived relaxing factor; nitrite; tumor necrosis factor-$\alpha$.
Hence, the present study investigated the effects of l-NMMA and SIN-1 in a dog model of endotoxic shock. In addition to global hemodynamics, we studied regional blood flow in hepatic, portal, mesenteric, and renal vasculatures. We also studied the effects of these interventions on tissue O_2 extraction capabilities by progressively decreasing blood flow induced by cardiac tamponade.

**MATERIALS AND METHODS**

Surgical preparation. Eighteen mongrel dogs weighing 24.5 ± 6.5 kg were anesthetized with pentobarbital sodium, with an initial loading dose of 30 mg/kg followed by a constant intravenous infusion of 4 mg·kg^{-1}·h^{-1} (Infusomat II; B. Braun, Melsungen, Germany) through the right forepaw vein. After being endotracheally intubated with a cuffed endotracheal tube, the dog was mechanically ventilated with room air by using a servo ventilator (model 900B; Siemens-Elema, Solna, Sweden). Controlled ventilation was facilitated with pancuronium bromide given as an initial bolus of 0.15 mg·kg^{-1} followed by an infusion of 0.075 mg·kg^{-1}·h^{-1}. Respiratory rate was set at 12 breaths/min and tidal volume was adjusted to obtain an end-tidal PCO_2 (PETCO_2) between 28 and 34 Torr. These ventilatory conditions were not changed thereafter. A right femoral arterial catheter was inserted and connected to a pressure transducer for arterial pressure monitoring. The left forepaw vein was cannulated for normal saline and drug infusion. A balloon-tipped pulmonary artery catheter (model 93A-131–7-Fr; Baxter, Irvine, CA) was inserted through the right external jugular vein under guidewire (model 93A-131–7-Fr; Baxter, Irvine, CA) by using three to five 5-ml injections of cold 5% dextrose in ice water. Each injection was started at end inspiration. A temperature probe was placed on the chest, COM-2, Baxter) by using three to five 5-ml injections of cold 5% dextrose in ice water. Each injection was started at end inspiration. A temperature probe was used on-line to control for variations in injectate temperature. Regional blood flow was estimated simultaneously in the common hepatic artery, portal vein, mesenteric artery, and left renal artery by a previously calibrated blood flowmeter (model T208; Transonic Systems, Ithaca, NY). Because 90% of common hepatic blood flow goes to the liver, and the other 10% goes to the gastroduodenal artery in control conditions (30), one should consider that the hepatic blood flow we obtained was an estimation rather than an exact measurement.

Exhaled gases were directed through a mixing chamber for sampling of expired gases to measure expired O_2 fraction (FE_O2) (P. K. Morgan, Chatham, UK). PE_TCO_2 was monitored simultaneously (47210A capnometer; Hewlett-Packard, Waltham, MA). The gas analyzers were calibrated before the experiment. Expired minute volume (VE) was measured with a spirometer (Haloscale Wright Respirimeter; Edmonton, London, UK).

Arterial and mixed venous blood samples were simultaneously withdrawn for immediate determination of blood gases and lactate concentration (ABL 500; Radiometer, Copenhagen, Denmark; lactate/glucose analyzer 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). In each sample, hemoglobin and O_2 saturation were measured (OSM 3 Hemoximeter, calibrated for dog blood; Radiometer). DO_2 was calculated as the product of arterial O_2 content and CI. VO_2 was determined by the formula

\[
\text{VO}_2 (\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{\text{VE}}{\text{wt (kg)}} \times \left[\frac{(1 - \text{FEO}_2 - \text{FE}_{\text{CO}_2})}{(1 - \text{FIO}_2)}\right] \times \left(\text{Fi}_O_2 - \text{FEO}_2\right)
\]

where FE CO_2 and Fi O_2 represent expired CO_2 fraction and inspired O_2 fraction, respectively. ERO_2 was derived from the ratio of VO_2/DO_2.

TNF-α levels were measured by using murine immunoglobulin G1 antibody to TNF-α (W. Buurman). This antibody to human TNF-α sees canine TNF-α in Western blot (unpublished data) and inhibits the biological activity of canine TNF-α to a lesser degree than it inhibits human TNF-α.

NO production was determined spectrophotometrically by measuring the accumulation of both nitrite and nitrate (the latter is reduced to nitrite) in plasma and was reported by the percentage of changes in nitrite from the baseline. Nitrate was stoichiometrically reduced to nitrite by incubation of sample (100 µl plasma) for 2 h at 37°C, in the presence of 0.1 U/ml nitrate reductase [NAD(P)H, nitrate oxidoreductase (EC 1.6.6.2; Aspergillus species) Sigma Chemical, St. Louis, MO], 120 µM NADPH, and 5 µM flavinadenine dinucleotide (Sigma Chemical) in a final volume of 103 µl. After nitrate had been reduced to nitrite, NADPH that interfered with the subsequent nitrite determination was oxidized with 10 U/ml l-lactic dehydrogenase (EC 1.1.1.27, type XI, from rabbit muscle; Sigma Chemical) and 10 mM sodium pyruvate for 30 min at 37°C in a final volume of 114 µl. Sodium nitrate was used as a standard. Nitrite concentration in plasma was assayed by a standard Griess reaction. Briefly, 100 µl plasma were incubated with an equal volume of Griess reagent (1% sulphanilamide-0.1% naphthylenediamine dihydrochloride-2.5% H_3PO_4) at room temperature for 10 min. The absorbance of the chromophore formed was determined at 540 nm by using a microtiter plate reader (Molecular Devices, Menlo Park, CA). Sodium nitrite was used as a standard, with control baseline plasma as a blank control reference.

Experimental protocol. After surgical preparation, the dog was placed in supine position and allowed to stabilize for...
the endotoxin 
intrapericardial pressure (IPP) in 3 groups of animals. B1, baseline; B2, 30 min after endotoxin; B3, 30 min after fluid resuscitation; B4, 30 min after administration of either N\textsubscript{G}-monomethyl-L-arginine (L-NMMA) or 3-morpholinosydnonimine (SIN-1) in treated groups. ○, Endotoxin; △, endotoxin + L-NMMA; □, endotoxin + SIN-1. *P < 0.05 vs. endotoxin; †P < 0.05 vs. endotoxin + SIN-1.

Fig. 1. Changes in mean arterial pressure (MAP) and mean pulmonary arterial pressure (MPAP) in relation to incremental changes in intrapericardial pressure (IPP) in 3 groups of animals. B1, baseline; B2, 30 min after endotoxin; B3, 30 min after fluid resuscitation; B4, 30 min after administration of either N\textsubscript{G}-monomethyl-L-arginine (L-NMMA) or 3-morpholinosydnonimine (SIN-1) in treated groups. ○, Endotoxin; △, endotoxin + L-NMMA; □, endotoxin + SIN-1. *P < 0.05 vs. endotoxin; †P < 0.05 vs. endotoxin + SIN-1.
blood flow to the hepatosplanchnic vasculature but decreased blood flow to the renal vasculature (Fig. 4). During tamponade, SIN-1 delayed the occurrence of supply dependency, so that the $D\dot{O}_2\text{crit}$ was significantly lower in the SIN-1-treated group ($9.1 \pm 1.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) than in the other groups ($13.3 \pm 1.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the L-NMMA-treated group and $12.8 \pm 3.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the control group, both $P < 0.05$), and the $E\dot{O}_2\text{crit}$ was higher in the SIN-1-treated group (58.7 ± 10.6%) than in the other groups [43.0 ± 15.5% in the L-NMMA, $P$ = not significant (NS); 42.2 ± 7.6% in the control group, $P < 0.05$]. The slopes were identical in all three groups during the supply-independent phase, whereas the slope was significantly steeper in the SIN-1-treated group than in the L-NMMA-treated group during the supply-dependent phase ($P < 0.05$).

There were no significant differences in heart rate, cardiac filling pressures, and hematocrit level between any paired groups. Plasma lactate concentrations were identical in all three groups (data not shown).

The amount of normal saline infusion was identical in the three groups of animals ($4.2 \pm 0.8$ vs. $3.5 \pm 0.5$ liters in endotoxin alone vs. endotoxin + L-NMMA and vs. $3.7 \pm 0.5$ liters in endotoxin + SIN-1 group; $P = NS$ for both comparisons). In the three groups, hematocrit decreased from 39–40 to 29–33%.

Plasma nitrite and nitrate levels were somewhat lower in the L-NMMA-treated than in the control animals at 240 min. Nitrite and nitrate levels were higher in the SIN-1-treated animals than in the other groups at 180 min and higher than the L-NMMA group at 240 min. The L-NMMA-treated group had higher TNF-$\alpha$ levels than the other groups at 150, 180, and 240 min (Fig. 8).

Pathological examination of the liver biopsies showed a decreased number of polymorphonuclear cells, located either in the portal spaces or in the sinusoids, in both L-NMMA-treated and SIN-1-treated animals compared with the control group. The sinusoidal or centrolobular stasis and steatosis were less expressed in the SIN-1-treated group than the other groups. These
observations may suggest less inflammatory response in the SIN-1-treated animals than in the other groups of animals. Pathological examination of the small intestine and the kidney revealed only minor alterations, characterized by a vascular congestion, but there were no significant differences between the control and either L-NMMA-treated or SIN-1-treated animals.

**DISCUSSION**

Although an increased production of NO in sepsis has been well demonstrated both in animals and humans (8, 28, 38), the beneficial effects of L-arginine analogs such as L-NMMA in septic shock have been much debated. These NOS inhibitors consistently increase
arterial pressure and SVR, but may reduce tissue blood flow and alter organ function (20). The present study investigated the effects of a NOS inhibitor and a NO donor on regional blood flow and O₂ extraction capabilities in experimental endotoxic shock. We used a large dog model to have easy access to global and regional blood flow measurements. Also, the administration of endotoxin followed by generous fluid infusion in this model results in an initial hyperdynamic form of endotoxic shock. We applied cardiac tamponade in the second phase of the experiments to study the O₂-extraction capabilities of the animals.

The timing of interventions may be of great importance. The rapid (5–30 min) development of hypotension in response to endotoxin in vitro and in vivo may be mediated by an enhanced release of mediators such as kinins, prostacyclin, or atrial natriuretic factor (13, 28), whereas the iNOS release contributing to the hypotension may take several hours to occur (27). It is, therefore, reasonable to hypothesize that early inhibition of NOS may produce greater vasoconstriction than needed, resulting in tissue hypoperfusion. On the other hand, the activity of the cNOS may be depressed during the early phase of endotoxemia, resulting in impaired endothelium-dependent vasodilation (24), suggesting that early administration of a NO donor may be beneficial to preserve tissue perfusion. We started L-NMMA or SIN-1 infusion 1 h after endotoxin challenge to study the effects of L-NMMA preventing excessive iNOS and the effects of SIN-1 maintaining cNOS during early endotoxic shock. The increase in plasma nitrite/nitrate levels 180 min after endotoxin administration suggests that iNOS was significantly expressed. We recently observed in the same model that tissue iNOS was expressed in the heart, lung, liver, and small intestine 3 h after endotoxemia (unpublished data).

As a NOS inhibitor, we used L-NMMA, which has been widely used in experimental studies and has been tested in humans (12). Because only one dose of the compound could be administered, we selected a moderate dose of 10 mg·kg⁻¹·h⁻¹ to avoid excessive vasoconstricting effects. Higher doses, especially those exceeding 30 mg/kg may have harmful hemodynamic effects and may even increase mortality rates (38). The dose of 10 mg/kg has been shown to be effective and well tolerated in humans with hyperdynamic septic shock (12).

L-NMMA increased arterial pressure and SVR but reduced CI and SI. Similar observations have been reported previously (6, 20, 22), although some of them involved hypodynamic models in which the fluid status might have been inadequate for endotoxic shock. In the second phase of the study, when tamponade was induced, the effects of L-NMMA on arterial pressure were not sustained. This observation was at least in part related to a more profound myocardial depression, as shown by a lower LVSWI for a similar degree of ventricular filling as in the other animals. Although an...
excessive release of NO has been implicated in the development of sepsis-related myocardial depression (5), the effects of NOS inhibition on the endotoxin-induced myocardial depression are still controversial. The studies reporting that NOS inhibitors may improve myocardial contractility usually have involved isolated myocardiums (5), but other studies involving entire organisms failed to observe such effects (16, 35). \textsuperscript{\textsuperscript{NG}}-nitro-L-arginine could even alter myocardial function in endotoxic shock in rats (16). Treatment with aminoguanidine, a selective iNOS inhibitor, reduced plasma nitrite and nitrate but did not affect cardiac depression (14). Results of a preliminary clinical trial showed that L-NMMA treatment failed to increase ventricular stroke work despite the frequent addition of dobutamine to L-NMMA administration (35). Taken together, these observations bring into question any beneficial effect of NOS inhibition on myocardial function in septic shock.

The vasoconstricting effects of L-NMMA on the pulmonary circulation were more dramatic than on the systemic circulation. In the present model, with only moderate pulmonary hypertension, L-NMMA dramatically increased Ppa and PVR. Other studies have also emphasized that NOS inhibitors can potentiate endotoxin-induced pulmonary hypertension in endotoxic shock (20). The increase in Ppa induced by NOS inhibition may represent a major limitation to clinical use of NOS inhibitors.

The administration of L-NMMA decreased blood flow to the hepatosplanchnic circulation. Our observations are consistent with a number of recent studies underscoring that NOS inhibitors can significantly exacerbate regional vasoconstriction and ischemia (1, 22, 23, 25, 38). In endotoxic rats, Mulder et al. (22) demonstrated that NOS inhibition increased organ vascular resistance in the splanchnic vasculature during the first hour of endotoxic shock. In endotoxic pigs, Ayuse et al. (1) showed that NOS inhibition could alter local control of liver blood flow and markedly increase resistance to venous return across the liver. In endotoxic rabbits, Pastor and Payen (26) reported that \textsuperscript{\textsuperscript{N}}-nitro-L-arginine significantly reduced portal vein and hepatic artery blood flows. Walker et al. (36) showed that L-NAME reduced gut blood flow by increasing gut vascular resistance in endotoxic dogs. In most of these studies, NOS inhibitors were administered during early endotoxemia or as a pretreatment, suggesting that NO plays an important role in regional hemodynamic effects and that NOS inhibition may be deleterious in acute endotoxemia. Other studies (3, 22) showed that NOS inhibition enhanced both macroscopic and histological intestinal and liver damage, but these effects were not always attributed to a reduced blood flow in this region.

The effects of NOS inhibition on renal blood flow have been less well studied. Our observation of a reduced renal blood flow is in keeping with those of Spain et al.
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Fig. 8. Changes in TNF-α and nitrate/nitrite concentrations in 3 groups over time. *P < 0.05 vs. endotoxin; §P < 0.05 vs. endotoxin + L-NMMA; † P < 0.05 vs. endotoxin + SIN-1.

(32), indicating that NOS inhibition can further constrain renal vasculature and decrease interlobular artery flow during sepsis. However, another recent study (2) reported that L-NMMA did not reduce renal blood flow in a sheep model of hyperdynamic sepsis.

We chose SIN-1, the vasoactive metabolite of molsidomide, as a NO donor. SIN-1 spontaneously decomposes into NO and the stable metabolite N-3-morpholinonoacetonitrile (SIN-1C). Although SIN-1 at high doses may simultaneously generate NO and superoxide anion (17), it is an effective agent in the treatment of coronary artery disease and has effects at least as powerful as nitroglycerin. Also, Pastor et al. (25) recently demonstrated that SIN-1 administration in the early phase of endotoxic shock in rabbits can preserve systemic and hepatic perfusion while preventing lactate acidosis. We chose a dose of 2 µg·kg⁻¹·min⁻¹ of SIN-1, because the improvement in myocardial function and splanchnic blood flow were most evident with this dose in previous experiments on a similar canine model of fluid-resuscitated endotoxic shock (41).

Although the model used was characterized by a low vascular resistance, SIN-1 had no deleterious effect on arterial pressure. The adequate fluid loading of the animal was probably a prerequisite of such good cardiovascular tolerance, as SIN-1 may otherwise reduce venous return to the heart by its effect of venous dilation. The lack of hypotension was also related to a significant increase in Cl associated with an improvement in cardiac function, as reflected by a greater LVSWI despite identical cardiac filling pressures. Such an improvement in cardiac performance after SIN-1 administration in endotoxic shock has been previously observed (41), but the exact mechanism is unclear. It may be related to a protective effect of SIN-1 on endothelial cell function or to NO-independent mechanisms related to preserved cytosolic Ca²⁺ levels. A reduced Ppa could also be involved.

SIN-1 administration selectively increased hepatic artery blood flow, compared with other groups, and maintained portal and mesenteric blood flow. Mulder et al. (22) showed that splanchnic blood flow is critically dependent on NO during the first hour of endotoxemia in rats. Boughton-Smith et al. (4) reported that exogenous supplementation of NO by S-nitroso-N-acetylpenicillamine administration could preserve gut blood flow and attenuate endotoxin-induced macroscopic jejunal damage in the rat. In a rabbit endotoxic shock model, Pastor et al. (25) recently demonstrated that pretreatment with SIN-1 could maintain portal vein blood flow at control level and significantly increase hepatic artery blood flow without any further effect on MAP. These studies suggest that NO release is essential to maintain splanchnic blood flow in the early phase of endotoxic shock. Whether these beneficial effects of SIN-1 are also present late in the course of endotoxic shock requires further study. On the contrary, SIN-1 administration decreased blood flow to the renal bed at moderate IPP compared with controls, an effect which was unexpected.

When O₂ supply becomes limited, the release of NO may contribute to the recruitment of unperfused capillaries and the increase in capillary density (7). However, previous studies, including those of healthy animals, demonstrated that NO inhibitors did not influence O₂ extraction capabilities when blood flow was acutely reduced (15, 33, 37). During hypoxic hypoxia or ischemia in dogs, L-NAME did not affect ER O₂ (31, 33). NG-nitro-L-arginine influenced neither D˙O₂crit nor maximum ER O₂ of the diaphragm during reductions in D₂O by hemorrhage in dogs (37). Recently, we observed (39) that the infusion of the NO donor sodium nitroprusside did not influence D₂Ocrit nor ER O₂crit, when blood flow was acutely reduced by cardiac tamponade in dogs. Thus it appears that, in basal conditions, neither increasing exogenous NO nor blocking its release can significantly influence the O₂ extraction capabilities.

We investigated the effects of NO on O₂ extraction capabilities during endotoxic shock. L-NMMA at a dose of 10 mg·kg⁻¹·h⁻¹ did not influence tissue O₂ extraction capabilities, because neither D₂Ocrit nor ER O₂crit was significantly altered. These observations are consistent with those reported by Schumacker et al. (31), who demonstrated that L-NAME administration did not reverse the O₂ extraction impairment seen during endotoxemia, either in whole body or in isolated intestines. Signals other than NO, such as hydrogen ion, calcium ion, and ATP products are largely related to capillary recruitment, vascular tone, and endothelial
function. Although NOS inhibitors may restore tissue perfusion pressure, they may also decrease capillary density, induce capillary leak (24), activate platelet aggregation, and promote leukocyte adhesion in post-capillary venules (18).

On the other hand, the NO donor SIN-1 increased the tissue O$_2$ extraction capabilities during endotoxin shock, as reflected by a decrease in D$_{O2crit}$ and an increase in ERO2crit. NO donors may promote the metabolic vasodilation of terminal arterioles governing flow through nutritive capillaries. However, if this were the only mechanism, L-NMMA might be expected to alter O$_2$ extraction, and this was not the case. SIN-1, like NO, can exert important anti-inflammatory effects mediated by the inhibition of TNF-α (17), oxygen free radicals (21), platelet-activating factor (3), or thromboxane A$_2$ (3), and these effects may have played a role in the improved O$_2$ extraction capabilities.

To study the interaction between NO and TNF-α, we determined TNF-α levels and found they increased after L-NMMA and SIN-1 administration. Other studies have shown that NOS inhibitors can enhance endotoxin- or bacteria-induced TNF-α production in experimental sepsis, both in vitro and in vivo (10), suggesting a regulatory feedback mechanism by which NO inhibits its own TNF-α-mediated production. Another recent study, however, showed that NO can increase TNF-α production from human neutrophils independently of guanosine 3′,5′-cyclic monophosphate stimulated by endotoxin in vitro (34). Kumsins et al. (17) more recently showed that the NO donor molsidomine, which acts only after it is converted by the liver to SIN-1, attenuated TNF-α, interleukin-1, and interleukin-6 production in endotoxic mice. The higher TNF-α release in the L-NMMA group may contribute to the detrimental global and regional hemodynamics. A sustained release of TNF-α caused by NOS inhibition was associated with an increased toxicity of bacteria in mice (10). Although SIN-1-treated animals had higher nitrite and nitrate concentrations than the other groups, (10). Although SIN-1-treated animals had higher nitrite and nitrate concentrations than the other groups, (10). Although SIN-1-treated animals had higher nitrite and nitrate concentrations than the other groups, (10). Although SIN-1-treated animals had higher nitrite and nitrate concentrations than the other groups, (10). Although SIN-1-treated animals had higher nitrite and nitrate concentrations than the other groups, (10).

In conclusion, the present study shows that although the NOS inhibitor L-NMMA could transiently reverse endotoxin-induced hypotension, it decreased hepatosplanchnic blood flow. These observations, made when L-NMMA was given before iNOS was expressed, suggest that L-NMMA should not be administered before iNOS is induced. L-NMMA did not influence O$_2$ extraction capabilities, suggesting that the loss in O$_2$ extraction capabilities observed early after endotoxemia is not caused by a NO-induced loss of vascular reactivity. On the other hand, the administration of the NO donor SIN-1 could significantly increase global and hepatosplanchnic blood flow without deleterious effects on arterial pressure. SIN-1 could also improve myocardial function and increase tissue O$_2$ extraction capabilities early during endotoxic shock.

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