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Endothelin-1 and Blood Pressure After Inhibition of Nitric Oxide Synthesis in Human Septic Shock

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Background—The systemic hypotension during human sepsis has been ascribed to increased production of nitric oxide (NO). Therefore, inhibitors of NO synthesis have been used in the treatment of hypotension in patients with septic shock. In addition, NO production may inhibit the synthesis and vasoconstrictor effects of endothelin-1 (ET-1). In this study, we tested whether ET-1 contributed to the vasopressor action of the NO synthase inhibitor N^G -nitro-L-arginine methyl ester (L-NAME) in patients with severe septic shock.

Methods and Results—Compared with healthy volunteers, patients with septic shock had increased plasma levels of nitrite/nitrate (37 ± 5 [SEM] versus 12 ± 5 mmol/L, $P < 0.01$), the stable end products of NO metabolism, and ET-1 (45 ± 7 versus 3 ± 2 pg/mL, $P < 0.001$). Plasma ET-1 concentration was not related to plasma nitrite/nitrate concentration or blood pressure. Continuous infusion of L-NAME ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IV) for 12 hours increased mean arterial pressure by $43 \pm 5\%$ and systemic vascular resistance by $64 \pm 10\%$ (both $P < 0.01$). The increase in blood pressure and systemic vascular resistance correlated positively with the level of ET-1 (both $P < 0.005$) but not with plasma nitrite/nitrate level. L-NAME infusion did not result in significant changes in the plasma concentrations of ET-1 or nitrite/nitrate.

Conclusions—NO and ET-1 may both play a role in the cardiovascular derangements of human sepsis. Although L-NAME does not increase ET-1 concentration in patients with septic shock, the vasopressor response induced by L-NAME depends on the plasma level of ET-1. These findings may indicate that inhibitors of NO synthesis unmask a tonic pressor response of ET-1 in human septic shock. (*Circulation*. 1999;99:271-275.)

Key Words: endothelium-derived factors ■ shock ■ endothelin

Recent evidence suggests a complex interaction of endothelium-derived factors, including endothelin-1 (ET-1) and nitric oxide (NO), in the control of vascular smooth muscle tone in health and disease.¹ ET-1 is a 21-amino-acid peptide with potent vasoconstrictor actions in animals and humans.^{2,3} NO is a short-lived radical, derived from the amino acid L-arginine by the enzyme NO synthase, with direct vasodilatory action.^{4,5} In vitro experiments show that continuous NO production inhibits the synthesis⁶ and vasoconstrictor action of ET-1.⁷ Furthermore, endothelin receptor antagonists fail to affect blood pressure in normal animals.⁸ This apparent absence of vascular effects of ET-1 has been attributed to continuous NO production. This is further supported by the finding that inhibition of NO synthesis in rats can unmask a tonic pressor response of endothelin and results in increased plasma levels of ET-1.^{9,10} So far, the precise interaction of ET-1 and NO in humans remains to be determined.^{11,12}

Sepsis in humans is characterized by massive vasodilatation with low systemic vascular resistance, high cardiac output, and severe hypotension.¹³ Increased production of NO

by an inducible isotype of NO synthase has been held responsible for the cardiovascular derangements during sepsis.^{5,14} High levels of nitrite and nitrate, the stable end products of NO metabolism, are found in patients with severe sepsis, and these levels may correlate with vasodilation.¹⁵ Analogues of L-arginine competitively inhibit the production of NO from L-arginine¹⁶ and can reverse hypotension in endotoxin- and cytokine-induced shock in animals.^{5,17} More recently, these inhibitors of NO synthesis have been used to increase blood pressure in sepsis in humans.^{18–20} At present, it is unknown whether inhibition of NO synthesis modulates endothelin release in septic shock. Because high levels of endothelin-1 have been found in patients with septic shock,^{21,22} we hypothesized that part of the vasoconstrictive response after inhibition of NO synthesis may result from increased production of ET-1.

The purpose of the present study was to examine whether ET-1 plays a role in the increase in blood pressure after inhibition of NO synthesis in patients with severe sepsis. Plasma concentrations of ET-1 and nitrite/nitrate, as an indirect measure of NO production, were measured during

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continuous infusion of the L-arginine analogue N^G -nitro-L-arginine methyl ester (L-NAME) in patients with severe septic shock.

Methods

Subjects

Our hospital Medical and Ethics Committee approved the study. First-degree relatives were informed of the nature of the study and gave informed consent. Eleven adult, critically ill patients in the intensive care unit of our hospital were included in the study. All patients met the criteria of sepsis as described by Bone et al.²³ The source for sepsis was peritonitis (n=5), pneumonia (n=2), pancreatitis (n=2), mediastinitis (n=1), or peritonitis with pneumonia (n=1). Patients were in shock (systolic blood pressure <90 mm Hg or a decrease >40 mm Hg from baseline unresponsive to fluid challenge) on admission, requiring therapy with pressor agents. At the time of the study, all patients were receiving dopamine >15 mg · kg⁻¹ · min⁻¹ and/or norepinephrine >0.1 mg · kg⁻¹ · min⁻¹. All patients required mechanical ventilation because of respiratory failure. Four patients required continuous hemodialysis because of renal failure. Only patients with cardiac index >3.0 L · min⁻¹ · m⁻² were included, because earlier reports have shown reductions of cardiac output during inhibition of NO synthesis. Exclusion criteria for the study were severe coronary artery stenosis (angina pectoris grade III according to NYHA classification), pregnancy, and cardiac index <3.0 L · min⁻¹ · m⁻².

Study Protocol

All patients underwent continuous ECG monitoring and had indwelling radial artery and pulmonary artery catheters (Criticath, Ohmeda). Measurements of systemic arterial blood pressure, central venous pressure, pulmonary artery pressure, and cardiac output (thermodilution method) were made at baseline and at 0.5-, 1-, 3-, and 6-hour intervals, for a total period of 24 hours. Systemic and pulmonary vascular resistance were calculated according to standard formulas. After baseline measurements, infusion of L-NAME 1 mg · kg⁻¹ · h⁻¹ was started and continued for 12 hours. To minimize the potential of developing toxicity from high serum levels of L-NAME, infusion was not continued for longer periods. Maximum changes in blood pressure and systemic vascular resistance were noted after 30 minutes of L-NAME administration,²⁰ and hemodynamic variables at this time were used to compare with the baseline value. Concomitant therapy was at the discretion of the clinician managing the patient.

Blood Samples

For later determination of plasma ET-1, nitrite/nitrate, and cortisol, arterial blood samples (10 mL) were obtained in heparin-coated blood collection tubes (Vacutainer) at baseline (t=0), during L-NAME infusion (t=0.5, 1, 3, 6, and 12 hours), and 3, 6, and 12 hours after L-NAME infusion had stopped (t=15, 18, and 24 hours). Control values were established in 10 healthy volunteers. Blood samples were immediately centrifuged (1500g, 10 minutes, 4°C), and plasma was collected and stored at -80°C until tested. Creatinine concentration was determined by a standard method.

Determination of ET-1

ET-1 was determined, after Sep-Pak extraction, with a commercially available radioimmunoassay kit (Nichols Institute) as previously described.²⁴ Normal values in our laboratory were 1 to 5 pg/mL. The limit of detection was 1 pg/mL.

Determination of Nitrite/Nitrate Concentration

Total nitrite plus nitrate concentration was assayed as described by Phizackerley and Al-Dabbagh.²⁵ To reduce nitrate to nitrite, supernatant or standards were incubated at room temperature in the presence of *Klebsiella pneumoniae* under anaerobic conditions. Total nitrite in the supernatant was subjected to the Griess reaction and assayed spectrophotometrically. Data are reported as the sum of

Patient Characteristics, Plasma Endothelin, Nitrite/Nitrate, and Cortisol Concentration in Severe Septic Shock

	Septic Patients	Control
Age, y	51.7±3.9	
Sex, M/F, n	10/1	
MAP, mm Hg	65±3	[70–110]
SVR, dyne · s · cm ⁻⁵ · m ⁻²	962±121	[1900–2400]
CO, L · min ⁻¹ · m ⁻²	4.8±0.4	[2.5–3.6]
Creatinine, μmol/L	283±56	[60–110]
ET-1, pg/mL	45±7*	3±2
NO _x , μmol/L	37±5†	12±5
Cortisol, nmol/L	753±87‡	521±62
Mortality at day 28	6/11	

MAP indicates mean arterial pressure; SVR, systemic vascular resistance; CO, cardiac output; and NO_x, nitrite plus nitrate. Values are displayed as mean±SEM. Values in brackets are normal ranges.

* $P<0.001$, † $P<0.01$, and ‡ $P<0.05$ vs healthy volunteers.

nitrite plus nitrate. Normal values in our laboratory were 5 to 15 mmol/L. The limit of detection was 0.1 mmol/L.

Determination of Cortisol

Cortisol concentrations were determined with a commercially available radioimmunoassay kit (DPC) as described previously.²⁶ Normal values were 400 to 800 nmol/L (15 to 30 mg/dL). The limit of detection was 28 nmol/L.

Drugs

L-NAME was obtained from Sigma Chemical Co. The hospital pharmacy prepared a sterile and pyrogen-free solution of L-NAME 10 mg/mL, ready for intravenous infusion.

Data Analysis

All results are expressed as mean±SEM. Changes over time were compared with baseline values by repeated-measures ANOVA. Differences between groups were compared by Student's *t* test. Pearson correlation and scatterplots were used for analysis of correlation between variables. A value of $P<0.05$ was considered statistically significant.

Results

Patient characteristics are shown in the Table. All patients had hypotension, low systemic vascular resistance, high cardiac output, and increased serum creatinine due to septic shock (Table). Compared with healthy volunteers, plasma levels of ET-1, nitrate/nitrate, and cortisol were significantly increased (all $P<0.05$, Table). Mortality at 28 days was 55%.

No correlation was found between plasma ET-1 concentration and plasma nitrite/nitrate concentration ($r=0.07$, $P>0.80$), blood pressure ($r=-0.34$, $P=0.30$), systemic vascular resistance ($r=-0.41$, $P=0.22$), or creatinine concentration ($r=-0.13$, $P=0.69$). ET-1 concentration was $46±5$ pg/mL in survivors and $43±12$ pg/mL in nonsurvivors ($P>0.05$). Plasma nitrite/nitrate concentration was negatively correlated with systemic vascular resistance ($r=-0.78$, $P=0.012$) (Figure 1) but not with creatinine concentration ($r=-0.03$, $P=0.93$).

Continuous infusion of L-NAME 1 mg · kg⁻¹ · h⁻¹ IV resulted in a maximum increase of mean arterial pressure by $43±5\%$ and systemic vascular resistance by $64±10\%$ (Figure 2). There was a concomitant reduction in cardiac output by

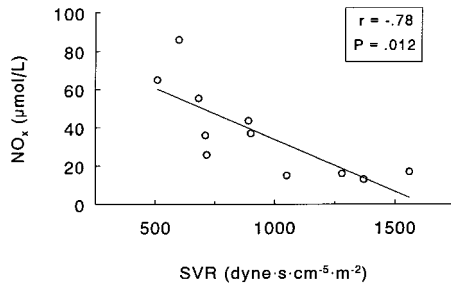


Figure 1. Relation between plasma nitrite/nitrate (NO_x) concentration and systemic vascular resistance (SVR) in patients with severe septic shock.

20±4%. The increase in blood pressure ($r=0.84$, $P=0.001$) and systemic vascular resistance ($r=0.81$, $P=0.002$) during L-NAME infusion correlated positively with the baseline plasma concentration of ET-1 (Figure 3). The increase in blood pressure with L-NAME did not correlate with plasma nitrite/nitrate level ($r=0.12$, $P=0.89$). L-NAME infusion for a period of 12 hours did not result in changes in the plasma concentration levels of ET-1 (from 45 ± 7 pg/mL at baseline to 43 ± 6 pg/mL after 12 hours of L-NAME, $P>0.05$) and/or cortisol (from 753 ± 86 nmol/L at baseline to 682 ± 73 nmol/L after 12 hours of L-NAME, $P>0.05$; Figure 4). Plasma nitrite/nitrate did not change during L-NAME (from 37 ± 5 mmol/L at baseline to 36 ± 5 mmol/L after 12 hours of L-NAME, $P>0.05$).

Discussion

Inhibitors of NO synthase have recently been presented as new therapeutic tools in the management of hypotension in septic shock in humans.¹⁸⁻²⁰ The present study demonstrates that the increases in blood pressure and the magnitude of the vasopressor response induced by the NO synthase inhibitor L-NAME are related to the plasma level of ET-1. These findings may suggest that inhibitors of NO synthesis unmask a tonic pressor response of ET-1 in human septic shock. In addition, this study shows that prolonged inhibition of NO synthesis with L-NAME does not further increase the already elevated plasma ET-1 concentration.

In the present study, elevated plasma levels of ET-1 were found in patients with severe sepsis, as has been reported by others.^{21,22} The mechanism involved in the increase of ET-1

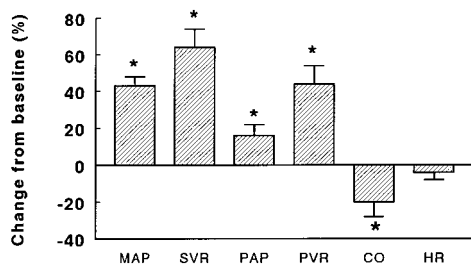


Figure 2. Effect of continuous infusion of L-NAME $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IV on hemodynamic variables in patients with severe septic shock. MAP indicates mean arterial pressure; SVR, systemic vascular resistance; PAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; CO, cardiac output; and HR, heart rate. Values are maximum changes vs baseline and are displayed as mean±SEM. * $P<0.05$.

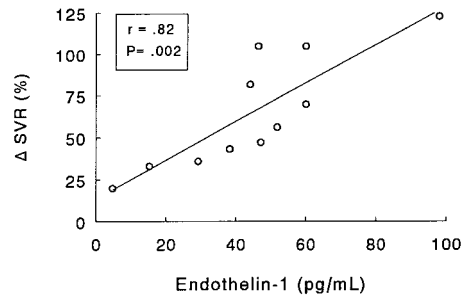
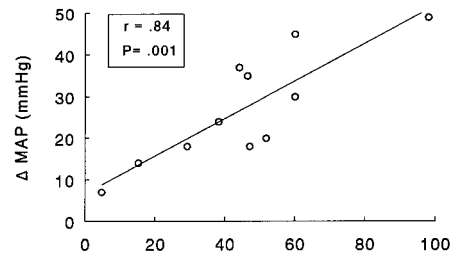


Figure 3. Relation between plasma ET-1 concentration and increase in blood pressure (top) and systemic vascular resistance (bottom) during continuous infusion of L-NAME $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IV in patients with severe septic shock.

concentration during sepsis is largely unknown. High ET-1 levels could have resulted from increased synthesis and/or diminished clearance of ET-1. Levels of circulating cytokines, such as tumor necrosis factor and interleukins, are increased in septic shock and may stimulate ET-1 production in endothelial cells and macrophages.^{27,28} Furthermore, intravascular leakage of ET-1 from damaged endothelial cells may have occurred.¹³ Because most patients with septic shock had impaired renal function, diminished renal clearance could have played a role.^{29,30} However, no correlation was found between plasma ET-1 and creatinine in the present study.

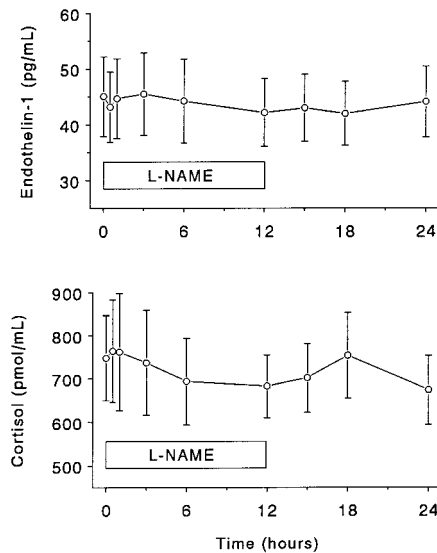


Figure 4. Effect of continuous infusion of L-NAME $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IV on plasma concentrations of ET-1 (top) and cortisol (bottom) in patients with severe septic shock. Time of L-NAME infusion is from $t=0$ to $t=12$ hours. No significant changes over time vs baseline. Values are mean±SEM.

This supports the idea that increased generation of endothelin occurs in sepsis in humans, although other vascular beds, including liver and skeletal muscles, may also contribute to the clearance of ET-1.

The finding that high ET-1 levels coincided with high nitrite/nitrate levels as an indirect measure of increased NO production is not in line with previous data that indicate an inhibitory action of NO on ET-1 release.^{1,6} This suggests that the normal interaction between NO and ET-1 is disturbed during sepsis. Abnormalities in the balance between vasoconstricting and vasodilating factors have been reported during sepsis.^{13,31} At present, however, the exact interaction of ET-1 and NO in septic shock remains to be determined. We hypothesize that the high circulating ET-1 concentration helps to maintain vascular tone during sepsis and opposes the vascular smooth muscle relaxation induced by increased NO production. Indeed, the high circulating ET-1 levels in patients with septic shock, in the present study 45 pg/mL (\approx 18 pmol/L), are sufficient to induce relevant vasoconstriction.^{32,33} The fact that the increase in blood pressure with L-NAME correlated with the level of ET-1 further supports the idea of a continuous vasopressor tone by ET-1 during sepsis in humans and that this vasopressor tone is unmasked by an inhibitor of NO synthesis. Our findings are consistent with previous reports in normal rats in which the increase in blood pressure with L-NAME was reduced with the specific endothelin receptor antagonist bosentan.^{9,10} In healthy humans³⁴ and humans with chronic heart failure,³⁵ endothelin receptor antagonists can reduce blood pressure. To demonstrate a direct relation between ET-1 and blood pressure, however, the use of a receptor blocker specific for ET-1 would be necessary. In the present study, we did not test the effects of an endothelin receptor antagonist. We hypothesize that endothelin receptor antagonism during septic shock in humans, in which plasma levels of ET-1 are high, would be detrimental, because it may result in severe hypotension. Whether endothelin receptor antagonists can prevent the increase in blood pressure of NO synthase inhibitors in humans remains to be determined.

In the present study, no increase was found in the plasma levels of ET-1 during 12 hours of inhibition of NO synthesis with L-NAME. These findings may suggest that ET-1 release itself is not modulated by L-NAME in patients with septic shock. In normal rats, acute inhibition of NO synthesis results in modest increases in ET-1 levels, but these ET-1 levels were measured only 15 and 35 minutes after L-NAME infusion.^{9,10} In contrast to these findings, chronic inhibition of NO synthesis with L-NAME (3 weeks) in normal rats does not increase ET-1 levels or gene expression.³⁶ Only a few reports are available on the NO-endothelin interaction in humans. Although nitrovasodilators can reduce ET-1 production by human endothelial cells in culture,³⁷ infusion of nitroglycerin, an NO-donating drug, does not change plasma ET-1 levels in healthy subjects.¹¹ In a recent study in healthy men, the NO synthase inhibitor *N*^G-monomethyl-L-arginine temporarily (only after 20 minutes) increased ET-1 levels from 7.6 to 9.6 pmol/L.¹² In the present study, however, we did not find a temporary increase in already elevated ET-1 concentration. One possible explanation is that the continuous high release

of ET-1 during sepsis resulted in a depletion of storage vesicles with ET-1 (or its precursor).³⁸ The results from the present study and previous reports suggest that increased production of ET-1 does not play a major role in the rise in blood pressure during prolonged inhibition of NO synthesis.

However, some reservations must be made regarding interpretation of plasma ET-1 levels. Plasma levels of ET-1 may not correctly reflect production rate, and the local concentration of the peptide at the vascular smooth muscle binding sites is probably more essential. For instance, big endothelin, which needs conversion to endothelin by tissue endothelin-converting enzyme to gain activity, is able to cause vasoconstriction without notable increases in plasma endothelin.³⁹ Because the main ET-1 release seems to occur abluminally, local concentrations of ET-1 are probably higher than in the plasma.⁴⁰ In the present study, therefore, increased local production of ET-1 may have occurred with L-NAME without detectable changes in plasma concentration. Similar reservations must be made regarding nitrite/nitrate levels. NO is but one of the ways that nitrite and nitrate are formed, and L-NAME infusion did not result in reduced serum levels of nitrite and nitrate, despite increased vasoconstriction. Possibly, plasma levels of nitrite and nitrate do not directly reflect the local amount of NO released, and active excretion of nitrite/nitrate through the kidneys and gastrointestinal tract may have influenced the plasma levels currently measured.⁴¹

Cortisol has a vital role in the maintenance of vascular tone and endothelial function.⁴² Furthermore, cortisol potentiates the vasoconstrictor actions of catecholamines.⁴³ Thus, increases in plasma cortisol may indirectly increase vasopressor tone. Animal studies have shown that inhibition of NO synthesis with L-NAME stimulates adrenal steroidogenesis.^{44,45} We hypothesized that increased cortisol production might be an additional mechanism by which L-NAME increases blood pressure. However, we found no evidence of changes in cortisol levels during inhibition of NO synthesis in patients with septic shock. We conclude that changes in cortisol levels do not contribute to the increase in blood pressure with L-NAME.

In conclusion, inhibitors of NO synthesis may unmask a tonic pressor response of ET-1 in humans with septic shock. Prolonged inhibition of NO synthesis does not influence the plasma levels of ET-1. These results suggest that ET-1 maintains blood pressure in human sepsis and that plasma ET-1 concentration may determine the vasopressor response of NO synthase inhibitors in septic shock. These findings may provide further evidence of interaction between NO and ET-1 in pathological conditions of the cardiovascular system in which an imbalance between endothelium-derived vasodilator and vasoconstrictor substances disturbs the normal regulation of vascular tone.

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