A novel nitric oxide scavenger decreases liver injury and improves survival after hemorrhagic shock

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Menezes, John, Christian Hierholzer, Simon C. Watkins, Valerie Lyons, Andrew B. Peitzman, Timothy R. Billiar, David J. Tweardy, and Brian G. Harbrecht. A novel nitric oxide scavenger decreases liver injury and improves survival after hemorrhagic shock. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G144–G151, 1999.—We tested the ability of a nitric oxide (NO) scavenger to reduce tissue injury in a rodent model of hemorrhagic shock. Rats were hemorrhaged to a mean arterial blood pressure (MAP) of 40 mmHg and then resuscitated when either 30% of their shed blood had been returned (group 1) or after 100 min of continuous shock (group 2). Selected animals were treated with the NO scavenger NOX (30 mg·kg⁻¹·h⁻¹) infused over 4 h. Hemorrhaged rats had a lower MAP after resuscitation compared with sham-shock control rats. NOX treatment significantly increased MAP after resuscitation from hemorrhage. Hemorrhagic shock also increased liver injury as reflected by elevated ornithine carbamoyltransferase (OCT) plasma levels, and NOX treatment significantly reduced OCT release. In addition, NOX was associated with significantly decreased hepatic neutrophil infiltration and improved 24-h survival (n = 8 of 9) compared with saline-treated shock animals (n = 3 of 9). These data suggest that excess NO mediates shock-induced tissue injury and that suppression of NO availability with NO scavengers may reduce the pathophysiological sequelae of severe hemorrhage.

nitric oxide synthase; trauma; kidney; multiple organ failure

HEMORRHAGIC SHOCK contributes to both short-term and long-term morbidity and mortality after traumatic injury (17). The specific mechanisms involved in the pathophysiology of hemorrhage have been incompletely defined. Hemorrhagic shock results in an oxidative stress to cells and in the induction of the inflammatory response, with an increased expression of a number of proinflammatory mediators and cytokines (22). Hemorrhage thus results in a combined oxidative and inflammatory insult to tissues that can contribute to cellular dysfunction, produce tissue injury, and profoundly alter organ function.

Nitric oxide (NO) has been shown to combine with superoxide to form the highly toxic peroxynitrite radical and produce oxidative injury (26, 29). NO also plays a role in proinflammatory cell signaling, altering cellular gene expression, enzyme activity, and transcription factor activation (18, 21). In addition, NO regulates vasodilation, platelet and neutrophil aggregation, and local organ blood flow (18). Therefore, NO may influence cell and tissue function following hemorrhage in a variety of ways. Hemorrhagic shock decreases endothelial constitutive nitric oxide synthase (eNOS) activity (27, 34) but increases inducible nitric oxide synthase (iNOS) expression and activity (8, 30). Previous work has demonstrated that providing exogenous NO donors early in shock is beneficial (2, 25) and that inhibiting eNOS activity during shock is harmful (2, 3). Other authors have shown that inhibition of NO activity reduces organ injury and improves survival, suggesting that the role of NO in the pathophysiology of hemorrhagic shock is complex (35). We have shown that selective iNOS inhibition after hemorrhage reduces lung injury, transcription factor activation, and proinflammatory cytokine expression (4). This body of evidence supports the hypothesis that distinct NOS isoforms may regulate different metabolic and physiological aspects of the response to hemorrhage and that excess NO from iNOS promotes inflammation and tissue injury.

The above hypothesis suggests that removal of excess NO while basal beneficial levels of NO are preserved could improve outcome after resuscitation from hemorrhagic shock. Most studies investigating the use of NOS inhibitors in hemorrhagic shock, however, are limited by the use of inhibitors that are either more selective for eNOS or are nonselective, inhibiting both eNOS and iNOS isoforms (1, 36). Even highly selective iNOS inhibitors can potentially interfere with eNOS activity at high doses or high tissue concentrations. Problems with NOS isoform selectivity and dose-response relationships complicate the potential therapeutic utility of NOS inhibitors in shock states. Recently, a number of compounds have been developed that are designed to scavenge excess extracellular NO. Because NO scavengers bind NO that diffuses from the site of production (13), they may potentially reduce toxicity from excessive iNOS activity. Poor water solubility may limit the potential use of some scavengers in biological systems (16). The compound NOX, however, is a dithiocarbamate that is water soluble and effectively binds NO in vivo (11, 20, 31, 33). We therefore tested the ability of NOX to ameliorate tissue injury after hemorrhagic shock. Our data demonstrate that NOX infusion reduced liver injury, improved systemic blood pressure after hemorrhage, and improved survival following hemorrhagic shock.
MATERIALS AND METHODS

Hemorrhagic shock model. The model of hemorrhagic shock used in these studies has been previously described (2, 3). Briefly, male Sprague-Dawley rats (250–310 g) were fasted overnight but allowed free access to water before the experiment. After being anesthetized with pentobarbital (30 mg/kg ip), the rats were mechanically ventilated with room air, and catheters were placed in the right jugular vein and left carotid artery for infusion therapy and monitoring of mean arterial blood pressure (MAP), respectively. After a stabilization period of 20 min, baseline hemodynamic data were recorded and a blood sample was obtained. The rats were then hemorrhaged to a MAP of 40 mmHg and were maintained at that level by the withdrawal or reinfusion of shed blood as needed. Once the endpoint for resuscitation was reached, the rats were resuscitated with all the remaining shed blood plus 2× the maximum shed-blood volume as lactated Ringer solution. Sham-shock animals were subjected to anesthesia and instrumentation for a period of time identical to that of shock rats but were not hemorrhaged. When not receiving test solutions, all animals received a maintenance infusion of 0.9% NaCl at 1.5 ml/h. Animal care was in accord with the guidelines of the University of Pittsburgh Animal Care and Use Committee and followed guidelines prescribed by the National Institutes of Health.

The animals were studied in two separate experimental protocols. In the first (group 1; Fig. 1A), the rats were hemorrhaged, and the point of vascular decompensation (end of compensated shock, CE) was recorded. Vascular decompensation is defined as the point at which shed blood must be reinfused to maintain the MAP at 40 mmHg. Once 30% of the total shed-blood volume had been reinfused, the rats were resuscitated and observed during the 4 h after resuscitation, at which point blood was collected for analysis. Randomly selected animals received either saline or NOX (30 mg·kg⁻¹·h⁻¹; provided by Dr. Ching-San Lai, Medinox, San Diego, CA) by intravenous infusion, beginning at CE and continuing for 4 h.

In the second experimental protocol (group 2; Fig. 1B), the rats were maintained at a MAP of 40 mmHg for a total of 100 min and then were resuscitated as described above. Randomly selected animals received either saline or NOX (30 mg·kg⁻¹·h⁻¹) by continuous intravenous infusion beginning at 60 min of shock and continuing until 4 h after resuscitation. At this time, a blood sample was obtained, the vascular catheters were removed, and the animals were returned to their cages and allowed to recover. Survival was recorded 24 h after shock, and blood and tissues were collected from surviving animals for analysis. Sham-shock and sham-shock plus NOX (sham + NOX) animals for each separate experimental protocol were used as controls.

Tissue injury. Blood samples were collected at the indicated time points, the plasma was separated by centrifugation, and samples were stored at −70°C until analyzed. Plasma samples were analyzed for the liver-specific urea-cycle enzyme ornithine carbamoyltransferase (OCT) as a marker of hepatic injury (2, 3). Plasma OCT levels were determined by the method of Oshita et al. (19). As an index of renal damage, plasma creatinine levels were measured with the use of an automated analyzer. Excised tissue samples were placed in 10% buffered Formalin and stored at −4°C. Tissue injury was assessed histologically by staining with hematoxylin and eosin (H + E). Tissue polymorphonuclear cell (PMN) infiltration was quantitated by staining tissues for myeloperoxidase (MPO) (5). Tissue nitrotyrosine immunoreactivity was measured as an index of peroxynitrite formation by immunohistochemistry and fluorescence microscopy (28). To perform these microscopic analyses, 5-µm cryosections were cut and prepared for H + E and MPO staining by standard methods. For immunocytochemical detection of nitrotyrosine-nonspecific antibody, binding to sections was blocked with 10% BSA in TBS (50 mM Tris, pH 7.0; 150 mM NaCl) for 30 min, and cells were then washed with 0.25% BSA in TBS. Sections were incubated with monoclonal mouse anti-nitrotyrosine antibody (1:200) (Transduction Laboratories, Lexington, KY) in 0.25% BSA in PBS for 1 h, the sections were washed, and a secondary antibody, Alexa 488-conjugated anti-mouse (1:100) (Molecular Probes, Eugene, OR) antibody, was added for 1 h. After additional washing, the nuclei of cells were labeled with a specific DNA dye (Hoechst 24232) (Sigma), mounted with Gelvatol (Monsanto, St. Louis, MO), and viewed with an Olympus Provis microscope with the use of a ×100 objective and epifluorescence optics. Images in perfect registration were collected with the use of appropriate cubes for each color. Individual color images were then added together with Adobe Photoshop 5.0 without further filtration or processing. To perform the quantitative analyses, 10 randomly chosen fields were examined blindly and scored for the number of positive cells in each section.

NOX decreases tissue injury after shock. To establish that NOX scavenged excess NO, the quantity of NO bound to hemoglobin (NO-Hb) was measured in 4-h blood samples from group 2 animals. Red blood cells (RBCs) were separated from plasma, washed twice, and resuspended in 10 mM Tris·HCl buffer (pH 7.4) containing 0.15 M NaCl. After sonication and centrifugation, the supernatant containing Hb was passed through a Sephadex G-25 column to remove nitrite and nitrate, and the eluate was analyzed by diazotization (23).
RT-PCR amplification. Liver samples were obtained immediately after killing, frozen in liquid nitrogen, and stored at −80°C until analyzed. Total cellular RNA was isolated, and RT-PCR was performed as described (4). Briefly, total RNA (2.5 μg) was subjected to first-strand cDNA synthesis with the use of oligo(dT) primer and Moloney murine leukemia virus (MMLV) RT. PCR primers and conditions were as previously described (4). RAW 264.7 murine macrophages stimulated with endotoxin were used as positive controls, with water as a negative control. PCR products were separated on a 10% polyacrylamide gel and were exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA), and radioactivity was determined by scanning densitometry (4).

Statistical analysis. Data are presented as means ± SE. For the analysis of survival, statistical significance was determined by χ². For all other analyses, significance was determined by ANOVA followed by Fisher’s least significant difference test. A P value of < 0.05 was considered statistically significant.

RESULTS

In the first experimental protocol, rats were maintained at a MAP of 40 mmHg until 30% of their shed-blood volume was returned, and then they were resuscitated. NOX treatment was begun at CE. As shown in Table 1, there were no differences in baseline MAP between groups. All rats responded to hemorrhage in a similar manner with no differences in the time to reach the point of vascular decompensation or in the total volume of blood removed to maintain the MAP of 40 mmHg (Table 1). However, once the NOX infusion was instituted, NOX-treated rats required a less-rapid rate of return of shed blood to maintain the MAP at 40 mmHg compared with saline-treated animals (P < 0.05, Table 1). There were no differences in MAP between sham and sham + NOX animals in this experiment (data not shown). The MAP for hemorrhaged rats was significantly lower than the MAP for sham-shock rats at 120, 180, 210, and 240 min after resuscitation (data not shown). After hemorrhage, NOX-treated rats had a significantly higher MAP 120 min after resuscitation than saline-treated rats (shock alone, 94 ± 9 mmHg; shock + NOX, 115 ± 5 mmHg, P < 0.05). NOX-treated rats had a higher MAP at later time points after resuscitation that approached but did not reach statistical significance (at 180 min: shock alone, 83 ± 10 mmHg, shock + NOX 101 ± 5 mmHg, P = 0.070; at 210 min: shock alone 76 ± 11 mmHg, shock + NOX 98 ± 7 mmHg, P = 0.077). NOX treatment alone had no effect on plasma OCT levels in sham-shock rats. Despite having a longer total time in shock (Table 1), NOX-treated rats that were hemorrhaged had lower OCT plasma levels than saline-treated rats when measured 4 h after resuscitation (Fig. 2).

A second experimental protocol was undertaken to standardize the total duration of shock. Here, rats were subjected to 100 min of continuous shock at MAP of 40 mmHg with either saline or NOX given beginning at 60 min. As shown in Table 2, there were no differences in baseline MAP between groups. Shock and shock + NOX rats were also comparable in the amount of time required to reach CE and in the total volume of shed blood removed to maintain the MAP at 40 mmHg (Table 2). Once the NOX infusion was begun at 60 min, the NOX-treated animals again appeared to tolerate the hypotensive state better than saline-treated animals, requiring only 12% of their shed-blood volume to maintain their MAP at 40 mmHg over the next 40 min, compared with 29% for saline-treated animals (Table 2). Both sham-shock rats and sham + NOX rats had a stable MAP throughout the experimental period (Fig. 3). Shock rats had a significantly lower MAP compared with sham animals after resuscitation at all time points. The MAP following resuscitation was significantly higher in NOX-treated rats compared with saline-treated rats (Fig. 3).

To confirm the ability of NOX to scavenge excess NO after hemorrhage, we measured NO·Hb as an index of extracellular NO release. RBCs from sham and sham + NOX animals liberated little NO·Hb (Fig. 4). RBCs from shocked rats liberated increased quantities of NO, consistent with previous findings demonstrating increased NO activity after shock (8, 27). RBCs from shock + NOX rats had significantly less NO·Hb than

Table 1. Hemodynamic parameters in group 1 animals

<table>
<thead>
<tr>
<th></th>
<th>Baseline MAP, mmHg</th>
<th>CE, min</th>
<th>SBV, ml/100 g</th>
<th>30% SBR, min</th>
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<tbody>
<tr>
<td>Sham</td>
<td>128 ± 8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sham + NOX</td>
<td>137 ± 7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Shock</td>
<td>135 ± 7</td>
<td>57.7 ± 4.1</td>
<td>2.79 ± 0.10</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>Shock + NOX</td>
<td>122 ± 3</td>
<td>56.4 ± 2.9</td>
<td>2.66 ± 0.09</td>
<td>85 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; CE, end of compensated shock; SBV, shed-blood volume; SBR, shed blood returned; NA, not applicable. *P < 0.05 vs. shocked animals.

Table 2. Hemodynamic parameters in group 2 animals

<table>
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<tr>
<th></th>
<th>Baseline MAP, mmHg</th>
<th>CE, min</th>
<th>SBV, ml/100 g</th>
<th>SBR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>141 ± 5</td>
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<td>NA</td>
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<tr>
<td>Sham + NOX</td>
<td>137 ± 4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Shock</td>
<td>136 ± 6</td>
<td>53.1 ± 1.9</td>
<td>2.68 ± 0.05</td>
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</tr>
<tr>
<td>Shock + NOX</td>
<td>138 ± 3</td>
<td>54.4 ± 1.0</td>
<td>2.73 ± 0.06</td>
<td>11.6 ± 1.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. shocked animals.
saline-treated rats, consistent with the NO-scavenging effect of NOX after hemorrhage.

When the effect of NOX on liver injury was examined, sham-shock rats given either saline or NOX had low plasma levels of OCT throughout the experiment. Hemorrhaged rats given saline alone had elevated plasma OCT levels at both 4 and 24 h that were significantly decreased by NOX (Fig. 5A). All sham-shock and sham + NOX animals survived 24 h. The number of rats that survived for 24 h was significantly greater in hemorrhaged rats treated with NOX than in saline-treated rats (n = 8 of 9 vs. 3 of 9 24-h survivors, NOX vs. saline, respectively; P < 0.05 by χ²).

Plasma creatinine levels were measured in group 2 animals as an index of renal injury (Fig. 5B). Sham and sham + NOX rats had low plasma creatinine at all time points measured. Shocked rats treated with saline had low baseline creatinine levels that were increased at 4 h and elevated further at 24 h (Fig. 5B). However, shocked rats treated with NOX had plasma creatinine levels that were similar to the low baseline values at each time point.

Increased PMN infiltration into the liver contributes to the hepatic injury produced by hemorrhagic shock and hepatic ischemia-reperfusion (6, 15, 32). Selective iNOS inhibition after hemorrhage decreased PMN infiltration into the lung (4). We therefore assessed the degree of PMN infiltration into liver and kidney utilizing MPO staining. Sham-shock rats had low numbers of MPO-positive cells in both liver and kidney sections, and this low number was unaffected by NOX treatment (data not shown). Liver sections from shocked rats had significantly increased numbers of MPO-positive cells compared with sham-shock controls. The number of MPO-positive cells was significantly reduced by NOX treatment after shock (Fig. 6). Hemorrhagic shock induced increased PMN infiltration into kidney sections compared with sham-shock animals, but this influx was not as marked as that seen in liver sections. Increased PMN infiltration was chiefly localized to the glomeruli. In addition, glomerular erythrocyte casts and altered mesangial cell morphology were seen in saline-treated hemorrhaged rats with H + E staining. These changes were difficult to quantitate because of
the lower number of infiltrating cells in kidney compared with liver sections, but qualitatively the shock-induced changes in renal histology were diminished with NOX treatment.

Increased tissue injury after shock has been proposed to be due, in part, to NO-mediated oxidative injury through the formation of peroxynitrite (26, 29). To determine if a reduction in peroxynitrite formation with NOX contributed to the changes in tissue injury seen in these experiments, we measured hepatic nitrotyrosine immunoreactivity in liver samples collected at 24 h. Sham-shock rats had little detectable nitrotyrosine immunoreactivity and no changes were seen in sham + NOX rats (data not shown). Increased nitrotyrosine staining was present in hemorrhaged rats, and NOX infusion reduced immunoreactivity in hemorrhaged rats to the levels present in sham animals (Fig. 7).

We have previously demonstrated that inhibition of iNOS with a selective inhibitor reduced transcription factor activation and proinflammatory cytokine production in the lung (4). To assess whether NOX decreased proinflammatory cytokine production in these experiments, we measured tumor necrosis factor (TNF-α) and interleukin (IL)-1β mRNA expression in the liver by RT-PCR. Sham-shock rats had low levels of TNF-α and IL-1β mRNA present in the liver, and these low levels were unaffected by NOX treatment. Rats subjected to hemorrhagic shock had increased hepatic TNF-α and IL-1β mRNA expression, and the expression of both proinflammatory cytokines was reduced by NOX treatment (Figs. 8 and 9).

**DISCUSSION**

NO plays an important role in a vast array of cellular functions by mediating intracellular signaling pathways, modulating cellular oxidative stress, regulating nuclear transcription factor activation, and mediating gene expression (14, 18, 21, 24). NO can also profoundly alter organ function by regulating regional blood flow and organ perfusion (18). NO was initially thought to mediate hypotension and vascular decompensation following hemorrhage through its effects on vascular tone (10). However, as our knowledge of the numerous effects of NO has increased, the complexity of the role of NO during hemorrhagic shock has become more evident. Most studies examining the role of NO in hemorrhagic shock have utilized NOS inhibitors that either act predominantly against ecNOS or nonselectively inhibit both ecNOS and iNOS. The activity of ecNOS is decreased following hemorrhage (27, 34), and delivering NO with NO donors early after hemorrhage improves blood pressure and short-term survival (25). Inhibition of NOS with compounds that are nonselective...
tive or principally inhibit ecNOS increases tissue injury, suggesting that ecNOS activity is essential in maintaining organ and tissue perfusion during hypovolemia (2, 3, 35). Use of NOS inhibitors with greater selectivity toward iNOS, however, is beneficial against shock-induced tissue injury (4). Therefore, maintaining the protective effects of NOS activity (ecNOS) while preventing the potentially harmful effects of NOS activity (excessive iNOS) is a significant obstacle in attempts to therapeutically manipulate NOS activity after hemorrhagic shock. Even the most selective iNOS inhibitors can potentially inhibit ecNOS if a sufficient dose is used, because the perfectly selective NOS inhibitor has yet to be developed. Potentially narrow ranges of safe and effective doses of these compounds, as well as altered tissue perfusion and bioavailability in shock, further complicate this issue.

The potential utility of reducing excess NO levels after hemorrhagic shock and the limitations of the NOS inhibitors developed to date combine to enhance the potential usefulness of compounds designed to scavenge or bind excess NO. A NO scavenger must be given in a manner that does not interfere with essential ecNOS function and yet neutralizes excess iNOS-produced NO that may mediate tissue damage. In these experiments, we have used a continuous infusion of the NO scavenger NOX in two separate shock protocols. In both protocols, NOX improved systemic MAP after resuscitation from hemorrhage and reduced shock-induced liver injury compared with saline-treated controls. NOX infusion also reduced renal dysfunction and improved 24-h survival compared with saline-treated animals, although it had no apparent noxious effect on either shock or sham animals. The reduction in injury with NOX was associated with a reduction in hepatic PMN infiltration, suggesting that NOX may reduce tissue injury, in part, by reducing the shock-induced inflammatory cell infiltrate. We cannot exclude the possibility, however, that the reduced PMN infiltrate is a result of the decrease in tissue injury produced by NOX, as opposed to a direct effect on PMN by NOX itself.

The ability of NOX to reduce shock-induced hepatic injury is similar to our previous findings of reduced shock-induced tissue injury in rats treated with the iNOS-selective inhibitor L-N³-(1-iminoethyl)lysine and

![Fig. 7. Nitrotyrosine immunoreactivity after hemorrhage. Liver sections were analyzed for nitrotyrosine immunoreactivity as described in MATERIALS AND METHODS. Representative fields (magnification, ×20) are shown for shock (A), shock + NOX (C), sham (D), and negative control (E). B: shock field (magnification, ×100).](http://ajpgi.physiology.org/)

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in iNOS-knockout mice (4). In these animals, inhibiting iNOS after shock was associated with reduced lung PMN infiltration and with a downregulation in the inflammatory response, with decreased IL-6 and granulocyte colony-stimulating factor expression and decreased nuclear factor-κB and STAT3 activation (4). NOX treatment likewise leads to a decreased expression of the proinflammatory cytokines TNF-α and IL-1β after hemorrhage in these experiments. This finding suggests that a reduction in the proinflammatory response by NOX may contribute to its beneficial effect after hemorrhage. The interaction of NO with superoxide to produce peroxynitrite has been hypothesized to contribute to tissue injury after ischemia and hemorrhage (26, 29). We detected reduced hepatic nitrotyrosine immunoreactivity, an index of peroxynitrite formation, in hemorrhaged rats treated with NOX. This finding suggests that the beneficial effects of NOX on hepatic injury could also be due, in part, to reduced peroxynitrite formation. Therefore, an infusion of NOX after hemorrhage is associated with a number of potentially beneficial effects, including a reduction in proinflammatory cytokine production, peroxynitrite formation, tissue neutrophil infiltration, and shock-induced hepatic and renal injury.

Other authors have hypothesized that NO mediates the vascular decompensation associated with prolonged hemorrhagic shock and that inhibiting NO may improve vascular tone and increase blood flow to vital tissues (30). Although it remains to be proven whether NO is responsible for vascular decompensation after hemorrhage, by experimental design the NOX infusion was begun after vascular decompensation had occurred. NOX-treated rats had a greater systemic MAP than saline-treated rats in both experimental protocols. Therefore, if NO contributes to hemorrhage-induced vascular decompensation, it is possible that NOX infusion may be improving vascular tone or tissue perfusion. Determining whether NOX can prevent or delay vascular decompensation after hemorrhage will require further study. NOX is a dithiocarbamate and readily binds NO with high affinity (20, 31, 33). Although some dithiocarbamates are poorly soluble in water, NOX is quite hydrophilic and binds NO readily in vivo (20, 31, 33). NOX therefore represents a promising compound for studying NO production and the effect of NO synthesis in in vivo systems.

In conclusion, we have demonstrated that infusion of the NO-scavenging compound NOX after hemorrhagic shock improves the hemodynamic response to hemorrhage, reduces tissue injury, proinflammatory cytokine expression, and peroxynitrite formation, and improves 24-h survival compared with saline-treated control animals. These data support the hypothesis that excessive NO contributes to hemorrhage-induced tissue injury and that reducing excess NO production may be beneficial after hemorrhage. The finding that trauma patients who have experienced hypotension express iNOS in liver tissue (7) suggests that additional work on the role of NO in hemorrhagic shock is merited.
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