A selective inducible NOS dimerization inhibitor prevents systemic, cardiac, and pulmonary hemodynamic dysfunction in endotoxemic mice

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Cardiovascular dysfunction in sepsis contributes to the high mortality rate for this syndrome (22). Nitric oxide (NO) is produced by NO synthases (NOS1, NOS2, and NOS3), and induction of NOS2 has been implicated in the pathogenesis of endotoxin-induced hypotension and vascular hyporeactivity (28). However, treatment of septic animals with inhibitors of all three NOS isoforms such as N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) and N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) significantly decreases cardiac output and oxygen delivery (21), further impairs renal (24) and liver (16) function, and increases mortality rate (1, 16, 24).

These observations may relate to the deleterious effects of inhibition of NOS1 and/or NOS3 by nonselective NOS inhibitors in the setting of systemic sepsis (21).

In contrast, selective NOS2 inhibitors may protect against sepsis-induced end-organ damage without inhibiting the ability of NOS1 and NOS3 to participate in cardiovascular homeostasis (3, 9). NOS2 inhibitors with variable selectivity have been examined in endotoxin-induced cardiovascular dysfunction. Although less selective NOS2 inhibitors (e.g., aminoguanidine) showed variable effects depending on species and model (20, 25), recent studies with more selective NOS2 inhibitors [e.g., L-N\textsuperscript{G}-(1-iminoethyl)lysine hydrochloride (27) and 1400W (32)] were more encouraging. Studying NOS2-deficient mice, we reported the key roles played by NOS2 in the endotoxin-induced impairment of myocardial function and hypoxic pulmonary vasoconstriction (HPV; Refs. 29, 30).

NOS isoforms are only active for NO formation as stable homodimers (18). Pyrimidylimidazole-based NOS dimerization inhibitors provide a novel approach to selective pharmacological NOS2 inhibition (4, 19).

Here we hypothesized that the NOS2 dimerization inhibitor BBS-2 would be highly selective for NOS2 in vivo and would prevent endotoxin-induced systemic, nitric oxide synthase; inflammation; inhibitors

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cardiac, and pulmonary hemodynamic dysfunction. Specifically, we investigated the effects of BBS-2 on baseline hemodynamics and endotoxin-induced systemic hypotension, myocardial dysfunction, and impairment of HPV in mice.

MATERIALS AND METHODS

After we received institutional approval by the Massachusetts General Hospital Subcommittee on Research Animal Care, we studied male C57BL/6J wild-type mice and B6.129P2-NOS2md−/− NOS2-deficient mice with an age range of 2–5 mo and body wt 18–29 g (Jackson Laboratory; Bar Harbor, ME) and Lewis rats with body wt 250–350 g.

NOS2 dimerization inhibitor. Stock solution of BBS-2 (compound 4 in Ref. 4) was prepared at 30 mg/ml in HCl-acidified sterile water (pH 3–4) and stored at room temperature. HCl-acidified sterile water was used as vehicle. BBS-2 and vehicle were injected (10 μg/g ip) after appropriate dilution of stock solutions.

Serial measurement of blood pressure by tail cuff. Blood pressure was measured in mice with a tail-cuff pressure-recording device (Kent Scientific; Torrington, CT) before and 4 and 7 h after challenge with endotoxin or saline. At time 0, 14 mice received an injection of Escherichia coli 0111:B4 endotoxin (10 mg/kg ip, Sigma; Detroit, MI), and 1 h later they were injected with BBS-2 or vehicle (n = 9 and 5, respectively). An additional 4 mice received an intraperitoneal injection of saline at time 0 and were injected with BBS-2 1 h later. After the measurement of blood pressure 7 h after challenge, whole blood was sampled to analyze plasma nitrate and nitrite (NOx) levels.

Measurements of left ventricular function. Echocardiographic measurements were obtained before and 7 h after challenge with endotoxin (100 mg/kg) or saline. Invasive measurements of systemic and left ventricular (LV) pressure were obtained 7 h after endotoxin challenge. Of the 16 mice challenged with endotoxin, 6 received BBS-2 (30 mg/kg), 5 received l-NAME (100 mg/kg), and 5 received vehicle 1 h later. Of the 9 mice challenged with saline, 4 received BBS-2 (30 mg/kg) and 5 received vehicle 1 h later. Pilot experiments established that a higher dose of endotoxin (100 mg/kg) was required to evoke consistent myocardial depression within 7 h than was required to cause hypotension or impair HPV (10 mg/kg). The dose of BBS-2 was adjusted to 30 mg/kg ip for these experiments to ensure complete inhibition of NOS2 dimerization. The dose of l-NAME was chosen based on a previous study (26).

Echocardiographic measurements. Echocardiography was performed using a 13-MHz ultrasound probe (Sequoia, Acuson; Mountain View, CA) in sedated mice (ketamine, 50 μg/g ip) as previously described (30). Fractional shortening (FS) was calculated from M-mode echocardiogram as described previously (23).

Invasive hemodynamics. Invasive measurements of LV pressure were performed with a 1.4-Fr high-fidelity pressure catheter (Millar Instruments; Houston, TX) in anesthetized mice (fentanyl, 250 ng/g ip, and ketamine, 50 μg/g ip) as previously described (30). The maximum and minimum first derivative of the developed LV pressure (dP/dtmax and dP/ dtdmin, respectively) were calculated by differentiation of the digitized analog LV pressure tracing. The time constant of LV isovolumic relaxation (τ) was calculated using the method of Weiss et al. (31).

Measurement of HPV in mice. Measurements of HPV were performed on anesthetized mice 22 h after challenge with endotoxin (10 mg/kg) or saline as previously described (12, 29). Of the 10 mice challenged with endotoxin, 5 received BBS-2 (10 mg/kg ip) and 5 received vehicle 1 and 8 h later. Of the 9 mice challenged with saline, 4 received BBS-2 (10 mg/kg) and 5 received vehicle 1 and 8 h later. Two doses of BBS-2 were given due to a relatively short elimination half-life in rodents (~1 to 1.5 h; J. F. Parkinson and B. Subramanyam, unpublished observations). Changes in left lung pulmonary vascular resistance (LPVR) in response to left mainstem bronchus occlusion (LMBO) were estimated by changing cardiac output via inferior vena cava occlusion while continuously measuring left pulmonary artery blood flow and pulmonary artery pressure as previously described (12).

Pulmonary NOS2 mRNA. Lung was harvested from BBS-2-treated (10 mg/kg, n = 3) and vehicle-treated (n = 3) mice 7 h after endotoxin challenge (10 mg/kg). Lung tissue mRNA was extracted by the guanidine isothiocyanate-cesium chloride method. RNA (10 μg) was fractionated in formaldehydeagarose gels and transferred to nylon membranes. Membranes were hybridized with a 32P-labeled 0.3-kb mouse NOS2 cDNA probe, washed, and exposed to X-ray films as described previously (30).

Low-temperature partially denaturing SDS-PAGE and immunoblot analysis. Lung and heart were harvested from BBS-2-treated mice (30 mg/kg, n = 3) and vehicle-treated mice (n = 3) 7 h after endotoxin challenge (100 mg/kg). NOS2 dimerization was analyzed by low-temperature PAGE as described previously (6, 33). Briefly, homogenates of lung and heart were mixed with sample buffer (250 mM Tris, pH 6.8, 8% SDS, 4% 2-mercaptoethanol, 0.004% bromphenol blue, 40% glycerol) and loaded on gels without boiling. After SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose membranes and incubated with a polyclonal anti-NOS2 antibody (Biomol Research Laboratories; Plymouth Meeting, PA) at a 1:500 dilution. Bound antibodies were detected using horseradish peroxidase-labeled protein A and visualized using chemiluminescence.

Plasma NOx concentration. Plasma NOx concentrations were determined by Greiss reagent detection at 540 nm using nitrite standards in nine endotoxin-challenged BBS-2-treated, five endotoxin-challenged vehicle-treated, and four saline-challenged wild-type mice. In addition to samples obtained from wild-type mice, plasma was sampled from NOS2-deficient mice 7 h after endotoxin challenge (n = 5).

Ex vivo NOS3 selectivity assay. Lewis rats received BBS-2 at 30 mg/kg sc or vehicle twice daily for 10 days (8 animals/group). On the day of ex vivo evaluation, the aorta was harvested and cut into eight rings while care was taken not to damage the endothelium. Aortic rings were mounted on a tension transducer device and immersed in organ baths of physiological salt solution that contained (in mM) 138.3 NaCl, 4.7 KCl, 0.6 MgSO4, 1.2 K2HPO4, 2.5 CaCl2, 25 NaHCO3, 0.26 CaNa2EDTA, and 11.1 glucose. Phenylephrine (PE)-induced contraction was allowed to reach steady state before addition of ACh. Concentration-response curves for ACh were constructed by sequential addition of ACh in increasing concentrations.

Statistical analysis. Differences between groups were determined by two-way ANOVA. When significance was detected by ANOVA, a post hoc Student-Newman-Keuls test was employed (Statistica for Windows, StatSoft; Tulsa, OK). A P value <0.05 indicated a significant difference. All data are expressed as means ± SD.
RESULTS

*BBS-2 does not improve mortality rate in endotoxicemic mice.* After endotoxin challenge, animals manifested weakness, lethargy, piloerection, and diarrhea. No mice died within 7 h after challenge with 10 mg/kg endotoxin whether or not they were treated with BBS-2. At 22 h after administration of 10 mg/kg endotoxin, mortality rate was similar between mice treated with BBS-2 (4 of 20) and vehicle (5 of 18). At 7 h after challenge with 100 mg/kg endotoxin, mortality rate was similar between mice treated with BBS-2. At 22 h after administration of 10 mg/kg endotoxin whether or not they were treated with BBS-2 completely prevented systemic hypotension (*P < 0.001 vs. endotoxin plus vehicle at 7 h*).

**BBS-2 prevents endotoxin-induced myocardial dysfunction.** BBS-2 (30 mg/kg) had no adverse effects on hemodynamics or LV function in saline-challenged mice (Table 1). Seven hours after administration of 100 mg/kg endotoxin, FS, \(dP/dt_{\text{max}}\), and \(dP/dt_{\text{min}}\) were markedly impaired in endotoxin-challenged vehicle-treated mice compared with saline-challenged mice (all *P < 0.05*; Table 1). Similarly, mean systemic arterial pressure (SAP) and LV end-systolic pressure (LVESP) were lower and \(\tau\) was greater in endotoxin-challenged vehicle-treated mice than in saline-challenged mice 7 h after challenge (all *P < 0.05*; Table 1). BBS-2 completely prevented the endotoxin-induced decreases in \(dP/dt_{\text{max}}\), LVESP, and SAP and attenuated the endotoxin-induced reduction in FS (*P < 0.05*; Fig. 1B). BBS-2 also prevented the endotoxin-induced prolongation of \(\tau\) 7 h after endotoxin-challenge (*P < 0.05*; Table 1). Treatment with \(L\)-NAME prevented the endotoxin-induced decrease in SAP (*P < 0.05* vs. endotoxin-challenged vehicle-treated mice; Table 1), whereas \(dP/dt_{\text{max}}\) and FS were further impaired by \(L\)-NAME (*P < 0.05 for both vs. endotoxin-challenged vehicle-treated mice; Table 1 and Fig. 1B). Measures of diastolic function, LVESP, \(dP/dt_{\text{min}}\), and \(\tau\) were markedly impaired by treatment with \(L\)-NAME after endotoxin challenge (for all three measurements, *P < 0.01 vs. saline-challenged mice and endotoxin-challenged BBS-2-treated mice; *P < 0.05 vs. endotoxin-challenged vehicle-treated mice; Table 1).

**BBS-2 treatment prevents endotoxin-induced impairment of murine HPV.** In saline-challenged mice, LMBO increased LPVR (101 ± 38%) without affecting SAP or pulmonary artery pressure (Table 2 and Fig. 1C).

![Graph A: effects of treatment with BBS-2 (10 mg/kg) or vehicle on tail-cuff systolic blood pressure measurements at baseline and 4 and 7 h after mice were challenged with saline or endotoxin (10 mg/kg). Endotoxin-challenged mice were subsequently treated with BBS-2 (●, *n* = 9) or vehicle (○, *n* = 5). Saline-challenged mice were treated with BBS-2 (●, *n* = 4). *P < 0.05 vs. corresponding baseline blood pressure values; *#P < 0.01 vs. blood pressure measurements of endotoxin-challenged BBS-2-treated mice at 7 h after endotoxin challenge. B: left ventricular (LV) fractional shortening before and 7 h after mice were challenged with endotoxin (100 mg/kg) followed by treatment with BBS-2 (30 mg/kg, ●, *n* = 6), vehicle (○, *n* = 5), or \(N\)^\(\text{G}\)-nitro-\(L\)-arginine methyl ester (\(L\)-NAME, 100 mg/kg, ■, *n* = 5). Additional saline-challenged mice were treated with BBS-2 (●, *n* = 4). *P < 0.05 vs. corresponding baseline and saline-challenged BBS-2-treated mice at 7 h after endotoxin challenge; *#P < 0.05 vs. endotoxin-challenged BBS-2-treated mice; *†P < 0.01 vs. endotoxin-challenged vehicle-treated mice. C: percent increase of lung pulmonary vascular resistance (LPVR) in response to left mainstem bronchus occlusion in mice challenged with saline (endotoxin −) or endotoxin (endotoxin +) 22 h earlier. Some mice received BBS-2 or vehicle at 1 and 8 h after challenge. *P < 0.05 vs. saline-challenged mice; *#P < 0.01 vs. endotoxin-challenged BBS-2-treated mice."

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BBS-2 had no adverse effects on baseline pulmonary hemodynamics or their response to LMB0 in saline-challenged mice. In contrast, LMB0 did not increase the LPVR in mice 22 h after endotoxin challenge and treatment with vehicle (23 ± 22%; P < 0.01 vs. saline-challenged mice). In endotoxin-challenged BBS-2-treated mice, the LMB0-induced increase in LPVR was preserved (85 ± 16%; P < 0.01 vs. endotoxin-challenged vehicle-treated mice; Fig. 1C).

Effects of BBS-2 on NOS2 gene expression, NOS2 dimerization, and plasma NOx concentration. Lungs of saline-challenged mice had undetectable levels of NOS2 mRNA. In contrast, 7 h after endotoxin challenge, pulmonary NOS2 mRNA levels were markedly increased (Fig. 2A). Similarly, the plasma NOx level in saline-challenged mice was 41 ± 6 μM and increased >10-fold 7 h after endotoxin challenge (461 ± 240 μM; P < 0.001; Fig. 2C). Administration of BBS-2 did not affect the endotoxin-induced increase in pulmonary NOS2 gene expression. However, BBS-2 prevented cardiac and pulmonary NOS2 protein dimerization (Fig. 2B) and the increase of plasma NOx concentration at 7 h after endotoxin challenge (91 ± 33 μM; P < 0.001 vs. endotoxin-challenged mice). Despite only partially denaturing conditions, we were able to readily detect NOS2 homodimers in tissues from endotoxin-challenged mice but not in tissues from endotoxin-challenged mice treated with BBS-2 (Fig. 2B). Endotoxin did not increase plasma NOx levels in NOS2-deficient mice at 7 h (56 ± 17 μM; P < 0.001 vs. endotoxin-challenged wild-type mice).

**BBS-2 does not affect NOS3-dependent aortic relaxation.** Contractions to 10−5 M PE were 2.2 ± 0.4 and 2.2 ± 0.5 g for aortic rings obtained from vehicle- and BBS-2-treated animals, respectively. ACh dose-response curves in aortic rings precontracted with 10−5 M PE did not differ between BBS-2- and vehicle-treated rats (Fig. 3). These results confirm that BBS-2 did not affect agonist-stimulated NOS3-dependent aortic relaxation ex vivo.

**DISCUSSION**

The present study demonstrates that systemic administration of a highly selective NOS2 dimerization inhibitor protected mice from endotoxin-induced systemic hypotension and impairment of HPV. BBS-2 also largely prevented endotoxin-induced myocardial dysfunction. These protective effects of BBS-2 were associated with inhibition of the endotoxin-induced increase in plasma NOx levels. Endotoxin-induced formation of NOS2 homodimers was blocked by BBS-2, whereas the increase in NOS2 gene expression was not altered. These observations are consistent with the pharmacological action of BBS-2, which prevents NOS2 dimerization (4).

The observation that BBS-2 treatment did not improve survival in endotoxemia confirms previous studies in NOS2-deficient mice (15, 30). Although the lack

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**Table 1. Invasive hemodynamic measurements at 7 h after saline or 100 mg/kg endotoxin challenge**

<table>
<thead>
<tr>
<th>Challenge (time 0):</th>
<th>Saline Vehicle</th>
<th>Saline BBS-2</th>
<th>Endotoxin Vehicle</th>
<th>Endotoxin BBS-2</th>
<th>Endotoxin L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (time = 1 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>624 ± 90</td>
<td>556 ± 132</td>
<td>561 ± 66</td>
<td>587 ± 35</td>
<td>546 ± 52</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>113 ± 18</td>
<td>109 ± 24</td>
<td>84 ± 12</td>
<td>118 ± 21</td>
<td>111 ± 18*</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>128 ± 19</td>
<td>123 ± 20</td>
<td>92 ± 12</td>
<td>138 ± 14</td>
<td>108 ± 18</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>3 ± 2</td>
<td>3 ± 4</td>
<td>5 ± 4</td>
<td>1 ± 3</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>15,062 ± 3,404</td>
<td>15,293 ± 1,846</td>
<td>9,479 ± 1,843b</td>
<td>14,676 ± 1,341</td>
<td>6,724 ± 1,74b,d</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>11,825 ± 1,193</td>
<td>11,854 ± 2,120</td>
<td>7,300 ± 2,575e</td>
<td>9,152 ± 1,377</td>
<td>4,363 ± 1,075f,d</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; SAP, mean systemic arterial pressure; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt max and dP/dt min, maximum and minimum first derivative of the developed left ventricular (LV) pressure, respectively; τ, time constant of LV isovolumic relaxation; L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 vs. saline-challenged mice and endotoxin-challenged BBS-2-treated mice; †P < 0.01 vs. saline-challenged mice and endotoxin-challenged BBS-2 treated mice; ‡P < 0.05 vs. saline-challenged mice; ‡P < 0.05, endotoxin-challenged vehicle-treated mice; ‡P < 0.001 vs. all other groups.

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**Table 2. Hemodynamic data during hypoxic pulmonary vasoconstriction studies at 22 h after 10 mg/kg endotoxin challenge**

<table>
<thead>
<tr>
<th>Challenge (time 0):</th>
<th>Saline Vehicle</th>
<th>Saline BBS-2</th>
<th>Endotoxin Vehicle</th>
<th>Endotoxin BBS-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (time = 1 and 8 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>499 ± 41</td>
<td>458 ± 76</td>
<td>489 ± 88</td>
<td>487 ± 35</td>
</tr>
<tr>
<td>LMB0</td>
<td>492 ± 28</td>
<td>434 ± 56</td>
<td>447 ± 73</td>
<td>457 ± 46</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>73 ± 22</td>
<td>77 ± 4</td>
<td>63 ± 29</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>LMB0</td>
<td>71 ± 19</td>
<td>79 ± 3</td>
<td>51 ± 15</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>17 ± 4</td>
<td>15 ± 2</td>
<td>20 ± 4</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>QLPA, ml.min−1.g−1 body wt</td>
<td>17 ± 5</td>
<td>16 ± 1</td>
<td>19 ± 5</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>QLPA, ml.min−1.g−1 body wt</td>
<td>93 ± 25</td>
<td>86 ± 8</td>
<td>100 ± 41</td>
<td>104 ± 56</td>
</tr>
<tr>
<td>LPVR, mmHg·ml−1·min−1·g−1 body wt</td>
<td>68 ± 28</td>
<td>49 ± 18*</td>
<td>77 ± 26</td>
<td>60 ± 30</td>
</tr>
<tr>
<td>Baseline</td>
<td>81 ± 8</td>
<td>90 ± 17</td>
<td>96 ± 32</td>
<td>78 ± 23</td>
</tr>
<tr>
<td>LMB0</td>
<td>164 ± 40*</td>
<td>179 ± 86*</td>
<td>114 ± 25†</td>
<td>143 ± 42*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Hemodynamic parameters before (baseline) and 5 min after left mainstem bronchus occlusion (LMB0). Mice were challenged with intraperitoneal saline or endotoxin 22 h before hemodynamic measurements. Some mice received BBS-2 or vehicle 1 and 8 h after saline or endotoxin challenge. PAP, mean pulmonary artery pressure; QLPA, flow through left pulmonary artery; LPVR, left pulmonary vascular resistance. Effects of LMB0 on each parameter were analyzed in each group. *P < 0.05 vs. baseline; †P < 0.05 vs. saline-challenged vehicle-treated mice.
of a survival advantage for BBS-2 treatment in our model is not unexpected, it has been shown that NOS2 deficiency does promote survival in models of sepsis associated with sustained bacteremia (1, 11). It is possible that BBS-2 treatment might improve survival rates in different models of sepsis.

BBS-2 inhibits NOS2 enzyme activity in cytokine-stimulated A-172 cells (IC$_{50}$ = 0.49 nM) and has a high affinity for NOS2 monomers (K$_d$ = 0.1 nM; Ref. 4). In cell-based NOS dimerization assays, the IC$_{50}$ values of BBS-2 for NOS3 and NOS1 are >1,500- and 600-fold greater than that for NOS2 (J. F. Parkinson and G. B. Phillips, unpublished observations). The present results demonstrate that the high selectivity of BBS-2 in vitro translates into selective effects in vivo by showing the lack of effects of BBS-2 on baseline pulmonary and systemic hemodynamics (see Fig. 1 and Tables 1 and 2). To further examine possible inhibitory effects of BBS-2 on NOS3 dimerization, we administered BBS-2 to rats for 10 days and assessed NOS3-dependent vascular reactivity ex vivo. Agonist-stimulated (PE and ACh) vascular reactivity was indistinguishable in aortic rings from BBS-2- and vehicle-treated rats (see Fig. 3). Because NOS3 turnover has been reported to occur with a half-life of 43 h (7), a 10-day treatment period with BBS-2 should have afforded sufficient time for the inhibition of NOS3 dimerization by BBS-2. Taken together, these findings suggest that BBS-2 has no measurable effects on NOS3 activity in vivo or ex vivo at doses that are highly inhibitory for NOS2 activity.

Excessive production of NO by sepsis-induced NOS2 has been implicated in the refractory systemic hypotension of sepsis. A variety of selective (32) and nonselective (27) NOS2 inhibitors and congenital NOS2 deficiency (17) have been shown to attenuate endotoxin-induced hypotension. Our observation that BBS-2 maintains the SAP in conscious mice 4 and 7 h after endotoxin challenge suggests that augmented NOS2 activity contributes to the evolution of sepsis-induced systemic hypotension.

HPV is markedly impaired in sepsis and may be associated with marked systemic hypoxemia. Although the mechanisms responsible for sepsis-induced attenuation of HPV remain incompletely elucidated, NOS2-deficient mice were protected from endotoxin-induced hypotension.

Fig. 2. A: pulmonary nitric oxide synthase 2 (NOS2) mRNA levels in wild-type mice 7 h after challenge with saline (endotoxin −) or endotoxin (endotoxin +) and 6 h after treatment with BBS-2 or vehicle. NOS2 mRNA was detected in RNA extracted from mouse lungs using RNA blot hybridization and a murine NOS2 cDNA probe. A photograph of ethidium bromide-stained 28S ribosomal RNA is shown to confirm equal loading of RNA on gels. Treatment with BBS-2 did not affect endotoxin-induced increases in pulmonary NOS2 mRNA levels. B: effects of BBS-2 (30 mg/kg) on cardiac and pulmonary NOS2 homodimerization in mice 7 h after endotoxin (100 mg/kg) challenge. An immunoblot for NOS2 protein in heart and lung homogenates after low-temperature gel electrophoresis is shown. SDS-stable NOS2 homodimers (Di-NOS2) are seen in heart and lung homogenates from endotoxemic animals that received vehicle but not in those from mice treated with BBS-2. NOS2 monomers are observed in all samples as expected under partially denaturing conditions. A representative blot from three independent experiments is shown. C: plasma nitrate and nitrite (NO$_x$) levels in wild-type mice (NOS2 +/+ ) 7 h after challenge with saline (n = 4, endotoxin −) or endotoxin (n = 14, endotoxin +). One hour after endotoxin challenge, 9 mice received BBS-2 (BBS-2 +) while 5 mice received vehicle (BBS-2 −). Additional samples were obtained from 5 NOS2-deficient mice (NOS2 −/− ) 7 h after endotoxin challenge. *P < 0.001 vs. saline-challenged wild-type mice, endotoxin-challenged BBS-2-treated wild-type mice, and endotoxin-challenged NOS2-deficient mice.
impairment of HPV (29). The present study using BBS-2 supports the hypothesis that NOS2-derived NO plays an important role in septic pulmonary vascular dysfunction and represents a potential therapeutic target for preventing the impairment of HPV associated with sepsis.

NOS1 and NOS3 are important regulators of myocardial, systemic, and pulmonary vascular homeostasis (3, 26). Although activation of NOS3 has been implicated in the myocardial depression that is observed early after exposure to inflammatory cytokines (13, 14), treatment of sepsis with agents that can inhibit all three NOS isoforms has been observed to further aggravate systemic and pulmonary hemodynamic alterations. For example, administration of L-NMMA causes systemic vasoconstriction and hypertension (10), decreases cardiac output (8) and dP/dt max (5), and promotes pulmonary hypertension (2). In the present study, L-NAME, which is another inhibitor of all three NOS isoforms, further impaired LV systolic function (as reflected by LVEF and dP/dt max) and diastolic function (as reflected by LVEDP, dP/dt min, and $\tau$) in endotoxin-challenged mice (see Table 1). We have previously reported that a single bolus administration of L-NAME (100 mg/kg iv) increased SAP without altering cardiac output in healthy mice (26). It is of note that BBS-2 and L-NAME similarly prevented endothin-derived hypotension while exerting markedly different effects on LV function. These results confirm the deleterious effects of nonselective NOS inhibition in endotoxemia and suggest the possibility that NOS1 and/or NOS3 activity protects against the LV dysfunction of sepsis.

In summary, we have demonstrated that the highly selective NOS2 dimerization inhibitor BBS-2 protects mice from the cardiovascular dysfunction of sepsis: endothin-induced hypotension, myocardial dysfunction, and impairment of HPV. Early treatment by selective inhibition of NOS2 may prove to be a valuable treatment strategy to prevent septic shock.

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DISCLOSURE

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