

## Inducible nitric oxide synthase dimerization inhibitor prevents cardiovascular and renal morbidity in sheep with combined burn and smoke inhalation injury

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**Enkhbaatar, Perenlei, Kazunori Murakami, Katsumi Shimoda, Akio Mizutani, Lillian Traber, Gary Phillips, John Parkinson, John R. Salsbury, Nettie Biondo, Frank Schmalstieg, Ann Burke, Robert Cox, Hal Hawkins, David Herndon, and Daniel Traber.** Inducible nitric oxide synthase dimerization inhibitor prevents cardiovascular and renal morbidity in sheep with combined burn and smoke inhalation injury. *Am J Physiol Heart Circ Physiol* 285: H2430–H2436, 2003. First published August 14, 2003; 10.1152/ajpheart.00055.2003.—Inducible nitric oxide synthase (iNOS) is implicated in the pathogenesis of acute respiratory distress syndrome (ARDS). ARDS treatment is frequently complicated by significant extrapulmonary comorbidity. This study was designed to clarify the role of iNOS in mediating extrapulmonary comorbidity in sheep after combined burn and smoke inhalation injuries using a potent and highly selective iNOS dimerization inhibitor, BBS-2. Twenty-two female sheep were operatively prepared. After 5 days of recovery, tracheostomy was performed under ketamine-halothane anesthesia. Sheep were given a 40% total body surface third-degree burns and insufflated with cotton smoke (48 breaths, <40°C). Sheep were divided into four groups: noninjured and nontreated (sham group;  $n = 6$ ), noninjured but treated with BBS-2 (sham/BBS-2 group;  $n = 4$ ), injured but nontreated (control group,  $n = 6$ ), and injured but treated with  $100 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  BBS-2 (BBS-2 group;  $n = 6$ ). Evaluation was in a laboratory intensive care unit setting for 48 h. The sham group had stable cardiopulmonary and systemic hemodynamics. Control animals showed multiple signs of morbidity. Decreased left ventricular stroke work index and stroke volume index with elevated left atrial pressure indicated myocardial depression. Systemic vascular leak was evidenced by robust hemoconcentration, decreased plasma oncotic pressure, and increased transvascular fluid flux into the lymphatic system. Finally, severely impaired renal function (urinary output) was associated with adverse net fluid balance. Treatment with BBS-2 prevented all these morbidities without adversely affecting cardiovascular hemodynamics such as cardiac index and mean arterial pressure. The results identify a major role for iNOS in mediating extrapulmonary comorbidity in a clinically relevant and severe

trauma model and support the use of highly selective iNOS inhibitors as novel treatments in critical care medicine.

heart; kidney

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) with pulmonary edema occurs frequently in trauma, particularly in patients with >30% total body surface area (TBSA) cutaneous burns combined with smoke inhalation injury (5). ARDS contributes to, and is complicated by, the systemic inflammatory response syndrome (SIRS) (33), which promotes significant comorbidity in multiple organs (23). These may include myocardial depression (32), acute renal failure (ARF) (16), and systemic vascular leak. Capillary hyperpermeability occurs not only at injured sites but also in regions distant from the injury, including the lung (2). The hyperpermeability is more severe when burn injury is associated with smoke inhalation (6). This hyperpermeability leads to marked fluid loss from the circulation and may result in hypovolemia, requiring fluid resuscitation (21). Maintaining a good net fluid balance without exacerbating systemic vascular leak and pulmonary edema is thus a complex process in the setting of burn and smoke inhalation injury with ARDS and ARF. Novel treatment strategies for these patients should therefore ideally promote not only improved pulmonary status but also prevent extrapulmonary comorbidity.

Nitric oxide (NO) is synthesized from L-arginine by the enzyme NO synthase (NOS). Constitutive NOSs [endothelial NOS (eNOS) and neuronal NOS (nNOS)] play important roles in regulation of vascular tone and homeostasis (19). Cytokine-inducible NOS (iNOS) generates excessive amounts of NO and has been implicated in the pathogenesis of sepsis, ARDS, and multiple traumas. Recent publications report beneficial ef-

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fects of highly selective iNOS inhibitors on myocyte damage in acute cardiac allograft rejection (30), ischemia-reperfusion injury in skeletal muscle (35), and multiple organ dysfunction in hemorrhagic shock (17). Numerous studies with partially selective iNOS inhibitors support a role for iNOS and NO in thermal injury (26), but definitive data with highly selective iNOS inhibitors are lacking.

Preventing the dimerization of inactive NOS monomers into active homodimers has emerged as a novel pharmacological strategy to obtain isoform-selective NOS inhibitors (1, 18). The selective iNOS dimerization inhibitors BBS-1 and BBS-2 are efficacious in allograft rejection (30). Both BBS-1 and BBS-2 are highly selective for inhibiting iNOS versus eNOS dimerization, but the fivefold selectivity of BBS-1 versus nNOS dimerization is not optimal (12). BBS-2 has a >100-fold selectivity for iNOS versus nNOS dimerization (Ref. 1 and G. Phillips and J. Parkinson, unpublished observations). We (8) have previously shown that BBS-2 prevents intrapulmonary pathology and ARDS in sheep with combined burn and smoke inhalation injury. In the present report, we focus on the effects of BBS-2 treatment on the extrapulmonary comorbidity. BBS-2 was provided by constant intravenous infusion, beginning 1 h after injury. BBS-2 prevented myocardial depression, renal failure, and systemic vascular leak. In contrast to nonselective NOS inhibitors, these beneficial results for BBS-2 were achieved without signs of adverse effects on cardiopulmonary and systemic hemodynamics.

## METHODS

**Animal model.** The model of burn/smoke injury has been described in detail (25). Briefly, 22 adult female sheep (30–40 kg) were surgically prepared for the study under halothane anesthesia. The right femoral artery was cannulated with a polyvinyl chloride catheter (18 gauge, 36 in., Parke-Davis; Sandy, UT) for continuous measurement of systemic arterial blood pressure and for intermittent sampling of arterial blood. A Swan-Ganz thermal dilution catheter (model 93A-131-7F, Edwards Laboratories; Irvine, CA) was introduced through the right external jugular vein and advanced into the pulmonary artery for measurement of pulmonary arterial pressures (PAPs). The catheter was also used for intermittent sampling of mixed venous blood, measurement of core body temperature, and determination of cardiac outputs. A Silastic catheter [0.062 in. inner diameter (ID) and 0.125 in. outer diameter (OD), Dow Corning; Midland, MI] was positioned in the left atrium through a left thoracotomy in the fifth intercostal space. This catheter was used for continuous measurement of left atrial pressure (LAP). To evaluate the changes in systemic lymph flow, an efferent lymphatic from the prefemoral lymph node was cannulated (Silastic catheter, 0.025 in. ID and 0.047 in. OD, Dow Corning). After a 5-day recovery period, the sheep were anesthetized with halothane and given a burn (40% TBSA, third degree) and inhalation injury. After burn/smoke injury, all sheep were placed on ventilator with the positive end-expiratory pressure (PEEP) set to 5 cmH<sub>2</sub>O and tidal volume at 15 ml/kg. The animals were provided fluid resuscitation with Ringer solution strictly according to the Parkland formula (4 ml·kg<sup>-1</sup>·%TBSA burned<sup>-1</sup>·24 h<sup>-1</sup>). Urine volume was mea-

sured every 6 h via a urinary tract catheter. Sheep had no access to water. The experiment was continued for 48 h. The sheep were randomly divided into four groups: noninjured and nontreated (sham group; *n* = 6), noninjured but treated with BBS-2 (sham/BBS-2 group; *n* = 4), injured but nontreated (control group; *n* = 6), and injured but treated with BBS-2 (BBS-2 group; *n* = 6). For treatment, we used a new potent and selective iNOS dimerization inhibitor, BBS-2 (ZK-809984). The pyrimidylimidazole-based iNOS dimerization inhibitor BBS-2 (Medicinal Chemistry, Berlex Biosciences; Richmond, CA) was prepared as described previously (18). This compound is highly selective for the iNOS isoform and inhibits its dimerization (1). BBS-2 was dissolved at 30 mg/ml in sterile water by the addition of a 1 mol equivalent of HCl with gentle heating and stored at room temperature. BBS-2 was diluted in normal saline before use and administered by continuous intravenous infusion beginning 1 h after injury, at a dose of 100 μg·kg<sup>-1</sup>·h<sup>-1</sup> for 48 h. Sham animals received no injury but were surgically prepared as control and treated animals and placed on a ventilator. The Animal Care and Use Committee of the University Texas Medical Branch approved the experimental protocol, and all the animals were handled according to guidelines established by the American Physiology Society and the National Institutes of Health.

**Measured variables.** Mean arterial pressure (MAP) and LAP were measured using pressure transducers (model PX-1800, Baxter, Edwards Critical Care Division; Irvine, CA), which were adapted with a continuous flushing device. The transducers were connected to a hemodynamic monitor (model 78304A, Hewlett-Packard; Santa Clara, CA). Cardiac output was measured by the thermomodulation technique using a cardiac output computer (COM-1, Baxter, Edwards Critical-Care Division). For the evaluation of cardiac function, the cardiac index (CI), stroke volume index (SVI), and left ventricular stroke work index (LVSWI) were calculated using standard equations. Hematocrit (Hct) was measured in heparinized micro-Hct capillary tubes (Fisherbrand; Pittsburg, PA). Blood gases were measured using a blood gas analyzer (model IL 1600, Instrumental Laboratory; Lexington, MA). Plasma colloid osmotic pressure was measured by colloid osmometer (model 4420, Wescor; Logan, UT). Net fluid balance was calculated by a standard equation. The cardiac tissue NO levels were evaluated by measuring the intermediate and end products, nitrate/nitrite (NO<sub>x</sub>). Cardiac tissue was taken 48 h after injury. The tissue homogenate was centrifuged, and the supernatant was taken for the measurement of NO<sub>x</sub>. For the conversion of NO<sub>x</sub> to NO, the plasma samples were mixed with vanadium (III) and hydrochloric acid at 90°C in a NO<sub>x</sub> reduction assembly (model 745, Antek Instruments; Houston, TX). Thereafter, the NO reacted with ozone in the reaction chamber of the chemiluminescent NO detector (model 7020, Antek), and the emitted light signal was recorded by dedicated software as the NO content.

**Statistical analysis.** Data are presented as means ± SD. Results were compared through ANOVA and Scheffé's post hoc test or unpaired *t*-test. A value of *P* < 0.05 was accepted as statistically significant.

## RESULTS

All animals survived after the combined injury with 40% (TBSA) burn and smoke inhalation during the 48-h experimental period. Fluid resuscitation was strictly by following the Parkland formula (4 ml·kg<sup>-1</sup>·%TBSA<sup>-1</sup>·24 h<sup>-1</sup>). The arterial carboxyhemoglobin levels immediately after the smoke exposure were

Table 1. Cardiovascular variables

	Time, h						
	Baseline	6	12	18	24	30	48
CI, l·min <sup>-1</sup> ·m <sup>-2</sup>							
Sham	5.8±0.5	6.0±0.4	6.2±0.3	6.1±0.5	5.8±0.2	5.9±0.3	5.9±0.3
Sham/BBS-2	6.2±0.8	6.3±0.6	6.0±0.6	6.4±0.5	6.1±0.5	6.3±0.9	5.7±0.4
Control	5.9±0.1	5.6±0.3	5.9±0.4	6.1±0.6	5.9±0.3	5.2±0.2	5.6±0.3
BBS-2	6.3±0.3	5.6±0.3	5.7±0.2	6.0±0.4	6.2±0.4	6.7±0.4*	6.4±0.4
MAP, mmHg							
Sham	99±2.9	106±3.8	104±4.7	104±4.1	105±4.0	100±4.4	101±3.4
Sham/BBS-2	100±4.6	100±2.3	101±3.4	98±3.6	96±1.5	99±3.3	97±1.8
Control	98±2.6	106±4.0	106±3.9	104±2.8	99±2.3	103±2.8	110±4.9
BBS-2	102±2.7	114±4.4	106±3.2	104±2.8	109±5.2	109±3.2	110±3.4
LAP, mmHg							
Sham	6.8±0.6	8.8±1.6	9.4±1.0	10.1±0.9	8.4±0.4	8.7±0.8	9.0±1.1
Sham/BBS-2	6.8±0.3	6.8±0.8	6.3±0.6	6.5±1.0	7.0±0.4	6.8±0.8	7.0±1.1
Control	6.5±0.6	9.0±0.9	7.5±0.9	7.0±0.7†	7.0±0.8	8.0±1.2	6.3±0.9
BBS-2	6.8±0.4	10.2±0.7	10.8±0.6*	9.3±0.6*	9.7±0.9	9.8±0.8	8.6±0.5

Values are means ± SD. CI, cardiac index; MAP, mean arterial pressure; LAP, left atrial pressure; sham, noninjured, nontreated animals; sham/BBS-2, noninjured animals treated with BBS-2; control, injured but nontreated animals; BBS-2, injured animals treated with BBS-2. Vascular pressures were measured constantly using fluid-filled pressure transducers. \**P* < 0.05 vs. sham; †*P* < 0.05 vs. sham/BBS-2.

58.8 ± 4.9% in the BBS-2-treated group and 61.2 ± 5.0% in the control group. There was no statistical difference (*P* = 0.79) between these values, indicating that both control and treated animals received similar injuries. There were also no statistical differences between the baseline values of hemodynamic data obtained in both the treated and untreated groups of animals (Table 1). The sham animals treated with BBS-2 (sham/BBS-2 group) showed no significant changes in cardiovascular parameters during the 48-h experimental period.

In Table 1, cardiovascular parameters are summarized. The CI and MAP were stable in the sham group. The CI was slightly decreased in control animals at 30 h after injury, but this decline was not statistically different versus the sham group. There was a transient increase in LAP in control animals at 3 h after insult, followed by a rapid decline in this variable. LAP was increased in the sham and BBS-2-treated groups in the same manner and remained slightly elevated throughout the experimental period. There was a significant difference between the control and BBS-2-treated groups at 12 and 18 h after injury.

In the noninjured sham and sham/BBS-2 groups, the LVSWI and SVI were stable over 48 h (Fig. 1, A and B). However, control animals with combined injury exhibited a progressive decline in both LVSWI (Fig. 1A) and SVI (Fig. 1B), which reached a nadir at ~30 h with evidence of a partial recovery by 48 h. These modest signs of cardiac dysfunction were largely prevented by BBS-2 (Fig. 1). Figure 2 shows the relationship between LVSWI and LAP, as an index of myocardial contractile function. In the control group, the index was shifted downward, indicating that the cardiac dysfunction was due to myocardial contractile depression. The cardiac tissue NO<sub>x</sub> levels were significantly increased in control animals 48 h after insult compared with the sham group, but this increase was attenuated by BBS-2 treatment (Fig. 3).

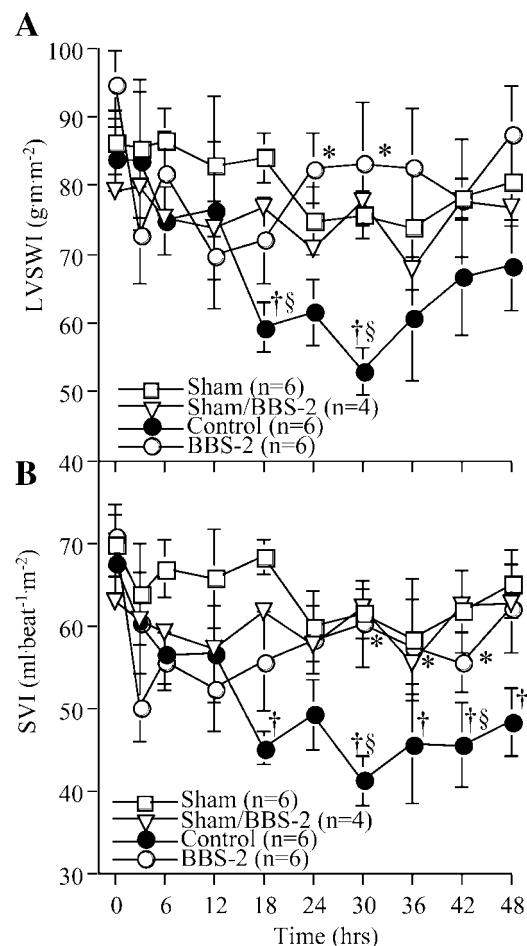


Fig. 1. Effect of BBS-2 on left ventricular (LV) stroke work index (LVSWI; A) and stroke volume index (SVI; B). LVSWI and SVI were calculated by standard equation. Data are expressed as means ± SD. \**P* < 0.05, BBS-2 vs. control; †*P* < 0.05, control vs. sham; §*P* < 0.05, control vs. sham/BBS-2.

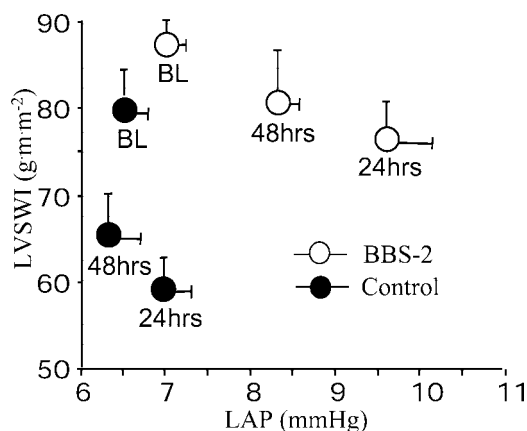


Fig. 2. Relationship between preload and stroke work, an index of myocardial contractility: left atrial pressure (LAP) vs. LVSWI. The index is shifted downward in the control group. BL, baseline.

Despite fluid resuscitation strictly followed according to the Parkland formula, the control group showed a marked and progressively worsening hemoconcentration with significant increases in both Hct and hemoglobin (Fig. 4). Relative to the sham group, hemoglobin (Fig. 4A) and Hct (Fig. 4B) began to increase at 3 h after insult and remained increased throughout the experimental period. Posttreatment with BBS-2 significantly inhibited this increase in both parameters. In control animals, urine output was markedly decreased by 12 h compared with the sham group and almost stopped on the second experimental day (Fig. 5A). Moreover, net fluid balance was markedly increased in these animals (Fig. 5B). Taken together, the increase in Hct, hemoglobin, and net fluid balance and the decrease in urine output are evidence of a severe systemic vascular leakage and ARF in control animals. Treatment with BBS-2 significantly attenuated all these pathological changes. The plasma colloid oncotic pressure was significantly decreased in control animals (Fig. 6A). Treatment with BBS-2 significantly attenuated this decrease. The prefemoral lymph flow

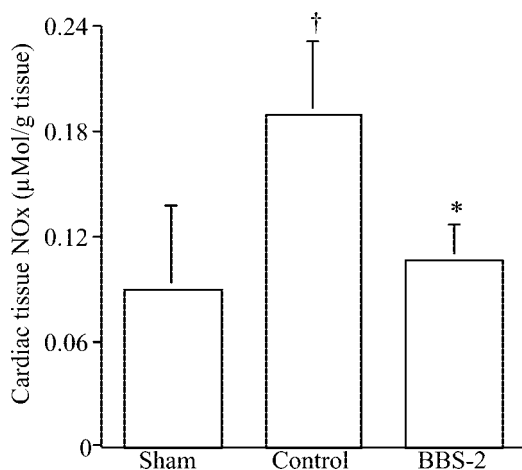


Fig. 3. Effect of BBS-2 on cardiac tissue nitrite/nitrate ( $\text{NO}_x$ ) levels. Data are expressed as means  $\pm$  SD. \* $P < 0.05$ , BBS-2 vs. control; † $P < 0.05$ , control vs. sham.

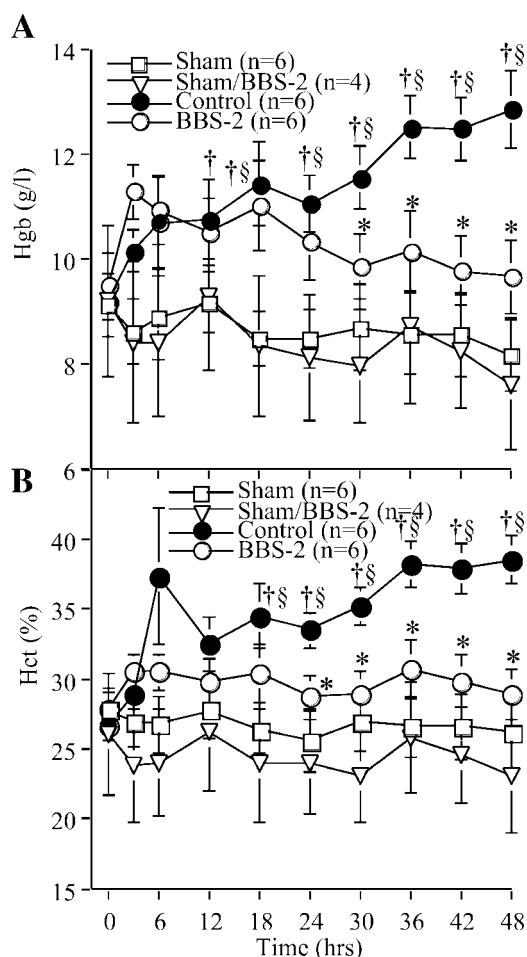


Fig. 4. Effect of BBS-2 on changes in hemoglobin concentration (Hgb; A) and hematocrit (Hct; B). Data are expressed as means  $\pm$  SD. \* $P < 0.05$ , BBS-2 vs. control; † $P < 0.05$ , control vs. sham; § $P < 0.05$ , control vs. sham/BBS-2.

(flank lymph) began to increase 3 h after the insult. The peak point was observed at 24 h after injury, and the increase was  $\sim 12$ -fold higher compared with the baseline value. This increase in transvascular fluid flux was significantly inhibited by iNOS inhibition (Fig. 6B).

## DISCUSSION

In this study, the role of iNOS in the pathogenesis of combined burn and smoke inhalation injury was explored using BBS-2, a potent and highly selective iNOS dimerization inhibitor. The combined injury resulted in a range of morbidities consistent with a SIRS and multiple organ failure (MOF). These included reversible myocardial depression, pronounced microvascular dysfunction (as evidenced by multiple signs of systemic vascular leakage), and ARF with markedly decreased urine output, resulting in an adverse net fluid balance. The marked beneficial effects of BBS-2 treatment on these morbidities strongly support a role for iNOS in their pathogenesis. Assessment of the cardiovascular profile of BBS-2 in sham (noninjured) animals indicated no remarkable effects, attesting to the high phar-

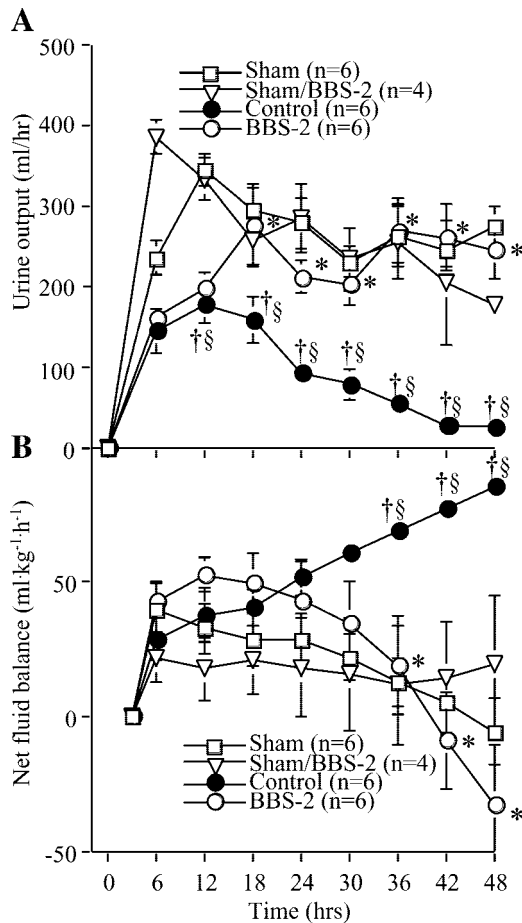


Fig. 5. Effect of BBS-2 on urine output (A) and net fluid balance (B). All animals received identical amount of fluid according to the Parkland formula. Data are expressed as means  $\pm$  SD. \* $P$  < 0.05, BBS-2 vs. control; † $P$  < 0.05, control vs. sham; § $P$  < 0.05, control vs. sham/BBS-2.

macological selectivity of the compound (Table 1). With regard to myocardial depression, there was a marked decrease in both LVSWI and SVI in control animals with combined injury. This reached a nadir at  $\sim$ 30 h, but appeared reversible, with partial recovery by 48 h. Examining the relationship between LVSWI and LAP indicated a depressed myocardial contractility index in injured animals. Treatment with BBS-2 prevented these changes. LAP was increased in both sham (non-injured) and BBS-2-treated groups. This might be explained by the large amounts of fluid resuscitation in these cohorts, with less injury-induced loss of plasma volume. The effect of BBS-2 on myocardial depression was correlated to inhibition of a small, but significant, injury-induced increase in cardiac NO metabolite ( $\text{NO}_x$ ) content as measured at 48 h. The small increase in  $\text{NO}_x$  content in the heart tissue of injured animals at 48 h may underestimate the amount of NO generated from iNOS in the heart at the point of maximal dysfunction i.e., 30 h. Given the high pharmacological selectivity of BBS-2 for iNOS inhibition, the results are consistent with an induction of iNOS in the heart as a result of combined injury and a direct effect of iNOS on

myocardial dysfunction. A limitation of the present study is that the time course for iNOS induction (mRNA and protein) and calcium-independent iNOS enzyme activity in the heart were not examined. Nevertheless, the findings are consistent with prior research on the functions of iNOS in cardiac pathophysiology and the known effects of BBS-2 in vivo. Constitutive NOS and iNOS isoforms can be expressed in isolated cardiomyocytes (24). A positive correlation between NO production and heart failure has been reported (34), and a role for iNOS in myocardial depression in SIRS has been proposed (32). The pathogenesis of burn and smoke inhalation-related myocardial dysfunction is still controversial but is likely to be mediated by cytokines such as IL-1, which is present in lung tissue from sheep with burn/smoke inhalation injury (31). Cytokines such as TNF or IL-1 are cardiac depressant substances (3, 10) and are potent stimuli for iNOS induction in vitro and in vivo. In addition, iNOS-derived NO acts as a myocardial depressant factor (15). Mungro et al. (20) reported that cardiac-specific overexpression of iNOS in transgenic mice caused acute cardiac death. Feng et al. (9) showed that increased NO production from iNOS expression contributes to myo-

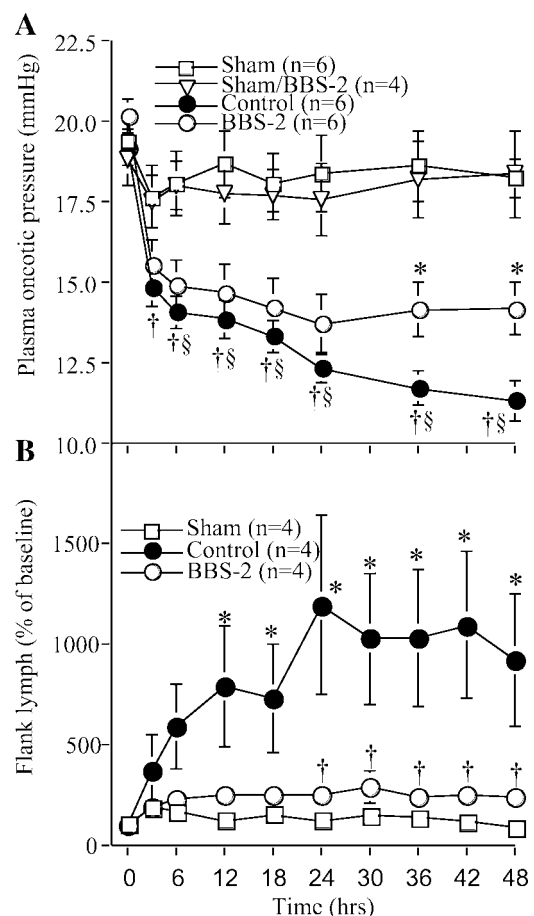


Fig. 6. Effect of BBS-2 on plasma oncotic pressure (A) and systemic transvascular fluid flux (flank lymph; B). Data are expressed as means  $\pm$  SD. \* $P$  < 0.05, BBS-2 vs. control; † $P$  < 0.05, control vs. sham; § $P$  < 0.05, control vs. sham/BBS-2.

cardial dysfunction and mortality after myocardial infarction in mice. Finally, the iNOS dimerization inhibitors BBS-1 and BBS-2 prolong survival and diminish myocardial inflammation as well as cardiomyocyte damage and apoptosis in acute cardiac allograft rejection (30). In these studies, BBS-1 and BBS-2 were shown to inhibit iNOS enzyme activity in cardiac allograft tissue lysates.

Hypovolemia, systemic vascular leak, and ARF are considered major causes of morbidity in burn patients. ARF is a significant contributing factor to mortality (14). Despite large amounts of fluid resuscitation, control injured animals showed severe hemoconcentration characterized by an increase in Hct and hemoglobin. Injured animals also showed severe decreases in plasma oncotic pressure and a marked increase in prefemoral lymph flow (flank lymph), indicating an increase in systemic transvascular fluid flux. BBS-2-treated animals showed a marked improvement in all these clinically relevant parameters. In addition to these severe signs of systemic vascular leak, injured animals showed marked signs of ARF, with severe and sustained decreases in urinary output to critically low levels by 48 h. BBS-2 treatment prevented ARF and maintained urinary output at acceptable levels. BBS-2-treated animals also required less fluid resuscitation and exhibited a marked improvement in net fluid balance. It is known that microvascular hyperpermeability occurs not only at the site of injury but also in regions distant from injury in burned patients (2). Microvascular permeability in the pulmonary vasculature as well as unburned tissue increases after major cutaneous burn (7). The resulting vascular leakage leads to a large amount of fluid flux from circulating blood passing into interstitial spaces, contributing to tissue edema and MOF. Although the mechanism of microvascular permeability increase in distant organs is not completely defined, there are recent reports showing an important role of NOS/NO. Both  $N^{\omega}$ -nitro-L-arginine methyl ester (L-NAME), a nonselective NOS inhibitor, and aminoguanidine, a partially selective iNOS inhibitor, suppress increased vascular permeability of second-degree burned skin in mice (27). The partially selective iNOS inhibitor mercaptoethylguanidine (MEG) decreases pulmonary microvascular permeability changes in sheep after combined burn and smoke inhalation injury (26).  $N^G$ -(1-iminoethyl)-L-lysine, a selective iNOS inhibitor, improves renal function in rats subjected to renal artery cross-clamping (22). However, some reports show negative or no effects of NOS inhibition by L-NAME on systemic microvascular permeability in canine (4) and ovine (13) sepsis models. The controversy regarding the role of NO in vascular permeability changes might be due to a lack of truly selective iNOS inhibitors. The NOS inhibitors mentioned above (L-NAME, aminoguanidine, and MEG) inhibit constitutive NOS to varying degrees. In addition, MEG is not only a weak NOS inhibitor but also a free radical scavenger (29). Thus it remains unclear which NOS isoform is specifically involved in the pathogenesis of microvascular alterations in sheep

after combined burn and smoke inhalation injury. BBS-2 is not known to significantly inhibit the constitutive NOS isoforms in vivo at the low infusion dose ( $100 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) used in this study (J. Parkinson, unpublished observations). The present results on hemoconcentration, plasma oncotic pressure, and transvascular fluid flux strongly support a pathogenic role for iNOS in systemic microvascular dysfunction in sheep with combined burn and smoke inhalation injury.

The adverse effects of nonselective NOS inhibitors on cardiac, pulmonary, and microvascular function have been documented extensively. These include systemic and pulmonary hypertension (MAP and PAP), adverse effects on heart rate, and decreased cardiac output. Elevated MAP and particularly PAP are negative prognostic indicators for patients with multiple traumas (11, 28). In the present study, BBS-2 did not adversely affect cardiac or systemic hemodynamic parameters in sham or injured animals. BBS-2 also shows no adverse increases in PAP in either sham or injured sheep (8). These findings indicate that BBS-2 in particular, and perhaps selective iNOS inhibitors in general, are likely to have a safer cardiovascular and pulmonary profile than first-generation NOS inhibitors such as L-NAME and  $N^{\omega}$ -monomethyl-L-arginine.

In summary, the present study using a potent and highly selective iNOS inhibitor firmly establishes an important role for iNOS in the pathogenesis of severe systemic morbidity and MOF in a clinically relevant sheep model of combined burn and smoke inhalation injury. Beneficial effects of BBS-2 were established on myocardial depression, systemic vascular leak, net fluid balance, and ARF. Taken together with a previous study showing marked benefits with BBS-2 on multiple aspects of ARDS in this model (8), it can be concluded that iNOS contributes significantly to both intra- and extrapulmonary pathology. Highly selective iNOS inhibitors should thus be considered at the forefront of emerging therapies for severe burn patients at high risk for developing ARDS. In addition to efficacy, significant cardiovascular and hemodynamic safety advantages compared with partially selective NOS inhibitors can be anticipated.

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