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Mechanistic aspects of inducible nitric oxide synthase-induced lung injury in burn trauma[☆]

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Abstract

Introduction—Although the beneficial effects of inducible nitric oxide synthase (iNOS) inhibition in acute lung injury secondary to cutaneous burn and smoke inhalation were previously demonstrated, the mechanistic aspects are not completely understood. The objective of the present study is to describe the mechanism(s) underlying these favourable effects. We hypothesised that iNOS inhibition prevents formation of excessive reactive nitrogen species and attenuates the activation of poly(ADP) (poly(adenosine diphosphate)) ribose polymerase, thus mitigating the severity of acute lung injury in sheep subjected to combined burn and smoke inhalation.

Methods—Adult ewes were chronically instrumented for a 24-h study and allocated to groups: sham: not injured, not treated, $n = 6$; control: injured, not treated, $n = 6$; and BBS-2: injured treated with iNOS dimerisation inhibitor BBS-2, $n = 6$. Control and BBS-2 groups received 40% total body surface area 3rd-degree cutaneous burn and cotton smoke insufflation into the lungs under isoflurane anaesthesia.

Results—Treatment with iNOS inhibitor BBS-2 significantly improved pulmonary gas exchange (partial pressure of oxygen in the blood/fraction of inspired oxygen (PaO_2/FiO_2) 409 ± 43 mmHg vs. 233 ± 50 mmHg in controls, $p < 0.05$) and reduced airway pressures (peak pressure 20 ± 1 cm H₂O vs. 28 ± 2 cm H₂O in controls, $p < 0.05$) and lung water content (lung wet-to-dry ratio 4.1 ± 0.3 vs. 5.2 ± 0.2 in controls, $p < 0.05$) 24 h after the burn and smoke injury. BBS-2 significantly reduced the increases in lung lymph nitrite/nitrate (10 ± 3 μ M vs. 26 ± 6 μ M in controls, $p < 0.05$) and 3-nitrotyrosine (109 ± 11 (densitometry value) vs. 151 ± 18 in controls, $p < 0.05$). Burn/smoke-induced increases in lung tissue nitrite/nitrate, poly(ADP)ribose polymerase, nuclear factor- κ B (NF- κ B) activity, myeloperoxidase activity and malondialdehyde formation and interleukin (IL)-8 expression were also attenuated with BBS-2.

Conclusions—The results provide strong evidence that BBS-2 ameliorated acute lung injury by inhibiting the inducible nitric oxide synthase/reactive nitrogen species/poly(ADP-ribose) polymerase (iNOS/RNS/PARP) pathway.

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Conflict of interest

The authors do not have any financial or personal relationships that could bias their work.

Keywords

Thermal injury; Reactive nitrogen species; Poly(ADP)ribose

1. Introduction

Acute respiratory distress syndrome (ARDS) is a life-threatening condition as a manifestation of many serious diseases including combined burn and smoke inhalation injury. It represents a clinical syndrome defined by acute pulmonary dysfunction with deteriorated gas exchange (partial pressure of oxygen in the blood/fraction of inspired oxygen (PaO_2/FiO_2) less than 200 mmHg) and bilateral pulmonary edema not related to myocardial contractility failure [1].

There have been a large number of reports dedicated to exploring the fundamental mechanisms that initiate and propagate this lethal injury. However, despite the long-term and intense investigation, the exact mechanisms underlying the pathophysiology of this syndrome are still not completely understood. The complexity of the pathomechanisms leading to the development of ARDS might be related to different pathogenic factors that may vary depending on the nature of the initial cause of damage.

For instance, ARDS could be caused by direct (pneumonia, gastric aspiration, drowning, embolism, contusion, haemorrhage and reperfusion) and indirect (sepsis, transfusions, shock, etc.) injuries and a broad spectrum of the pathogenic factors, such as coagulopathy, vasoactive compounds, angiotensin-converting enzyme, angiopoietins, angiogenic growth factors and other inflammatory mediators, which play a critical role in the manifestation of this serious condition. Combined cutaneous burn and smoke inhalation is one of the serious illnesses that eventually lead to the onset of ARDS. In the previous studies, we have described the pathophysiology of ovine ARDS secondary to combined burn and smoke inhalation injury and reported a crucial role of inducible nitric oxide synthase (iNOS)-derived excessive nitric oxide (NO) [2,3]. The exact mechanism of how excessive NO contributes to pulmonary dysfunction in these animals, however, remains to be determined. In the present study, we hypothesised that excessive iNOS-derived NO causes lung tissue injury via forming toxic reactive nitrogen species (RNS) with subsequent cellular dysfunction as a result of over-activation of the poly(ADP-ribose) polymerase (PARP). We tested this hypothesis using a potent and novel iNOS dimerisation inhibitor BBS-2 in our well-established and clinically relevant ovine model of ARDS.

2. Materials and methods

2.1. Animal model

The ovine model of combined burn and smoke inhalation injury and the animal surgical preparation technique have previously been described in detail [3–5]. The following groups have been studied: sham, $n = 6$; control, $n = 6$; and BBS-2, $n = 6$. The control and BBS-2 groups were given a flame burn (40% total body surface area (TBSA), 3rd degree) and inhalation injury (48 breaths of cotton smoke, $<40^\circ\text{C}$) under anaesthesia. The BBS-2 group received a continuous intravenous infusion of a new potent iNOS dimerisation inhibitor BBS-2 (ZK-809984, Berlex, Richmond, CA, USA) [6]. BBS-2 was started 1 h post-injury, and infused for 23 h with a dose of $100\text{ }\mu\text{g kg}^{-1}\text{ h}^{-1}$. The experiment continued for 24 h. The IV infusion dose of BBS-2 used in the ovine model was selected based on pharmacokinetic and pharmacodynamic considerations. A pharmacodynamic assay for systemic iNOS inhibition in rats with acute lipopolysaccharide (LPS)-induced endotoxaemia using plasma nitrate/nitrite levels (plasma NO_x) at 6 h post-LPS dosing was used to establish an ED_{50} (the

median dose that produces the desired effect) for BBS-2 of $\sim 1 \text{ mg kg}^{-1}$ after bolus i.p. dosing, as described [7]. Constant IV infusion of BBS-2 for 6 h in this model gave an ED_{50} for plasma NO_x inhibition by BBS-2 of $100 \mu\text{g kg}^{-1} \text{ h}^{-1}$ at plasma concentrations of $\sim 40 \text{ nM}$ BBS-2. ED_{95} in this rat IV assay was achieved at 650 nM circulating BBS-2 plasma levels. Based on a longer IV half-life and better exposures for BBS-2 in dogs and pharmacokinetic scaling considerations to the larger body weight ovine species, a continuous IV infusion dose of $100 \mu\text{g kg}^{-1} \text{ h}^{-1}$ was selected (J.F. Parkinson, R. Vergona and C. Mallari unpublished observations). The effectiveness of this dose selection was demonstrated in our previous studies in ovine trauma, which confirmed a robust effect on injury-induced elevations in plasma NO_x levels over 48 h [3].

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 (NIH).

2.2. Measured variables

Haemodynamics was monitored continuously for 24 h. Arterial and mixed venous blood samples were taken at different time points for the measurement of blood gases (Blood Gas Analyser 1302 IL, Instrumental Laboratory, Lexington, MA, USA). The $\text{PaO}_2/\text{FiO}_2$ ratio was measured to assess pulmonary gas exchange. Pulmonary shunt fraction (Q_s/Q_t) was calculated using standard equations.

2.2.1. Measurement of lung tissue and lung lymph nitrite/nitrate (NO_x)—Lung tissue and lung lymph NO levels were evaluated by measuring the intermediate and end products, nitrate/nitrite (NO_x) as described previously [3,8,9].

2.2.2. Measurement of lung tissue 3-nitrotyrosine level—Lung tissue 3-nitrotyrosine, a stable metabolite of RNS such as peroxynitrite, was evaluated by Western blot analysis [10].

2.2.3. Lung myeloperoxidase (MPO) activity and malondialdehyde (MDA) formation—The activity of myeloperoxidase (MPO) [11] and malondialdehyde (MDA) [12] formation was evaluated in homogenised lung tissue with a commercially available assay, as previously described.

2.2.4. Immunohistochemistry of poly(ADP-ribose) polymerase (PARP) activity—For the immunohistochemical detection of poly(ADP-ribose), mouse monoclonal anti-poly(ADP-ribose) (PAR) antibody (Calbiochem, San Diego, CA, USA) was used. The intensity of PAR staining of individual sections was determined by a masked experimenter, according to a semiquantitative PAR-positivity score from 1 to 10 [13,14].

2.2.5. Measurement of lung tissue IL-8 mRNA and lung lymph IL-8 protein levels—IL-8 messenger RNA (mRNA) was determined real-time polymerase chain reaction (RT-PCR) in lung tissue 24 h post-injury, as previously described [15]. All results were expressed as copy numbers per microgram of total RNA. Lung lymph IL-8 protein levels were determined by Western blot analysis at different time points after the injury [10].

2.2.6. Evaluation of lung tissue nuclear factor- κB (NF- κB) expression—Western blot analysis for p65 was performed to evaluate nuclear factor- κB (NF- κB) expression [16]. For the determination of the heterodimeric transcription factor p65, the lung tissue samples were homogenised, preserving the nuclear extract that contains p65. Thereafter, the nuclear extract was processed for Western blot analysis for p65 using an established protocol [16].

Blots were quantified by NIH IMAGE J scanning densitometry, and normalised to total actin expression.

2.2.7. Lung wet-to-dry ratio and airway obstruction—Sheep were sacrificed under deep anaesthesia 24 h after injury. The right lung was then removed and the lower half of the lower lobe was used for determination of bloodless wet-to-dry weight ratio. A 1-cm thick section was taken from the middle of the lower lobe, injected with 10% formalin, and immersed in formalin. Four tissue samples were taken at predetermined sites for histological examination. Fixed samples were embedded in paraffin, sectioned at 4 μ m and stained with haematoxylin and eosin. The pathologist evaluated all bronchioles in each section of the four tissue samples taken from each individual animal. The percentage (0–100%) of the surface area of the lumen of each bronchiole obstructed by cast material was estimated and the mean obstruction score was averaged for each animal. Then, the mean obstruction score per group was determined averaging the means from each animal [3].

2.2.8. Statistical analysis—Data were analysed using one-way analysis of variance (ANOVA). Fisher's least significant difference procedure was used for multiple comparisons. For immunohistochemistry study, a non-parametric Kruskal–Wallis test was performed, and Mann–Whitney's *U*-test was used to compare data within the groups. All statistics were performed with a commercially available statistical software (GraphPad Software, Inc., San Diego, CA, USA). A *p* value of <0.05 was considered significant.

3. Results

All animals in the three groups survived 24 h. Control animals showed severe signs of lung injury evidenced by deteriorated PaO_2/FiO_2 ratio (<300 at 24 h), increased airway pressures and airway obstruction scores and elevated lung water content. All these changes were significantly attenuated by iNOS inhibition with BBS-2 (Table 1).

3.1. Effect of iNOS inhibition on lung tissue and lung lymph NO_x levels and lung tissue formation of RNS

Lung tissue NO_x and 3-nitrotyrosine levels measured at 24 h post-injury were significantly increased in the control animals compared with the sham animals (Fig. 1(A) and (C)). Lung lymph NO_x started to increase 6 h following the combined injury peaking at 12 h (Fig. 1(B)). Statistically significant differences were found at 12, 18 and 24 h vs. the sham group. Post-treatment with iNOS inhibitor BBS-2 completely reversed these pathological changes with most robust effects observed on lung lymph NO_x .

3.2. Effect of iNOS inhibition on lung tissue MPO and MDA formation

Lung tissue MPO activity (Fig. 2(A)) and MDA formation (Fig. 2(B)) were elevated ~2-fold compared with the sham animals. However, these changes were extensively reversed (~75–100%) by iNOS inhibition with BBS-2 (Fig. 2).

3.3. Effects of iNOS inhibition on lung tissue poly(ADP)-ribose expression

Poly(ADP)-ribose was significantly increased by the combined injury in lung tissue (Fig. 3): airway epithelium (A), alveoli (B), airway glands (C) and microvasculature (vessels) (D) compared with those seen in sham animals. However, inhibition of iNOS with BBS-2 significantly reduced these increased values to the sham levels.

3.4. Effect of iNOS inhibition on the mRNA for IL-8 in lung tissue and its protein level in lung lymph

Lung lymph protein levels of IL-8 started to increase in control animals at 3 h after the injury, and remained elevated throughout the 24-h study compared with sham levels that were unchanged. BBS-2 significantly inhibited this increase at 3, 6 and 12 h post-injury (Fig. 4(A)). Lung tissue IL-8 mRNA was increased ~2.5-fold by the combined injury compared with those seen in sham animals at 24 h. iNOS inhibition with BBS-2 largely prevented this increase (Fig. 4(B)). However, statistical significance could not be found between the control and treated groups ($p = 0.0725$).

3.5. Effect of iNOS inhibition on the lung tissue NF- κ B activity

Lung tissue p65, a subunit of NF- κ B, was increased ~3.5-fold in control animals vs. sham animals. BBS-2 largely attenuated this increase; however, statistical significance was not found ($p = 0.0608$) between the control and treated groups (Fig. 5).

4. Discussion

Previously, we have reported beneficial effects of iNOS inhibition on the pathophysiological responses to combined burn and smoke inhalation injury in sheep for the 48-h time period [3]. In that study, we described that a potent iNOS inhibitor BBS-2 significantly improved pulmonary function by reducing augmented airway blood flow, pulmonary transvascular fluid flux, airway obstruction, lung water content and pulmonary shunting in burn-and-smoke-inhalation-injured sheep. The BBS-2-treated sheep also displayed near normal values in plasma NO levels evaluated by measuring its stable metabolite nitrite/nitrate [3]. However, the molecular mechanisms behind these beneficial effects of BBS-2 remained not fully understood. In the present study, we aimed to elucidate the mechanistic aspects underlying these beneficial effects of iNOS inhibition, performing a short-term 24-h study and testing effects of the same iNOS inhibitor BBS-2 in the same ovine burn-and-smoke-inhalation-injury model. The altered pathophysiological responses (acute lung injury indices) of injured non-treated control animals and the effectiveness of iNOS inhibition in ameliorating these alterations were reproducible at 24 h post-injury.

NO, a ubiquitous intracellular messenger, regulates physiological functions, such as neural and cardiovascular activities. However, during pathologic conditions, it can become deleterious because of its high reactivity with other free radicals, such as the superoxide anion. Although NO is reactive itself, it forms the more potent RNS—peroxynitrite by interacting with superoxide [17]. Peroxynitrite is a strong oxidant that mainly nitrates tyrosine residues in non-enzymatic reactions. Further, peroxynitrite reacts with CO₂ in biological systems, resulting in the formation of an unstable nitrosoperoxycarbonate anion, which interacts with guanine in DNA to form a variety of oxidation and nitration products, such as 8-nitro-2' deoxyguanosine [18]. The DNA strand breakage by peroxynitrite triggers the activation of poly(-ADP-ribose) polymerase (PARP), a DNA repair enzyme [18]. However, repairing the DNA lesions induced by peroxynitrite requires a great amount of energy (adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD⁺)), leading to necrotic cell death [19,20]. Thus, RNS can cause cellular dysfunction leading to tissue injury by direct oxidative and nitrosative stress or via overactivating PARP. More importantly, excessive NO contributes to tissue injury acting as RNS itself or forming more potent reactive agents, such as peroxynitrite. Excessive NO also contributes to lung injury by abolishing pulmonary vasoconstriction reflex to hypoxia [21,22].

In the present study, as mentioned, iNOS inhibition with the potent iNOS inhibitor BBS-2 ameliorated pulmonary lesions induced by combined burn and smoke inhalation. The

associated increases in lung tissue and lung lymph nitrite/nitrate, 3-nitrotyrosine, a stable metabolite of RNS such as peroxynitrite, and PARP activity were significantly inhibited by BBS-2. Previously, we have reported that the PARP inhibitor, INO1001, and different NOS inhibitors significantly attenuated ARDS in ovine burn and smoke inhalation and smoke inhalation/pneumonia models [3,12,23–25]. Chen et al. demonstrated the beneficial effects of iNOS inhibition in a rat model of burn injury [26]. We have also shown that inhibition of peroxynitrite using a peroxynitrite decomposition catalyst ameliorated interleukin (IL)-2-induced pulmonary dysfunction [27] and burn/smoke injury in sheep (unpublished data). Taken together, it is obvious that the NO/RNS/PARP pathway plays a central role in burn and inhalation injury-related pulmonary function deterioration. Therefore, it is essential to disrupt this consequent chain reaction in treating fire victims. As NO is an initiator of the pathway, prevention of its excess production at the earliest possible time point could benefit fire victims. In the present study, we report a dominating role of iNOS-derived excessive NO in these pathological responses to cutaneous burn and smoke inhalation, using a highly selective and potent iNOS dimerisation inhibitor BBS-2 with 620- and 1500-fold selectivity towards neuronal NOS (nNOS) and eNOS [3,6], respectively.

It is noteworthy that iNOS inhibition was accompanied with markedly attenuated inflammatory responses witnessed by reduced expression in lung tissue IL-8 mRNA and its concentration in lymph, lung tissue MPO activity and MDA formation. Although the exact mechanism of how iNOS inhibition modulates the inflammation is not fully understood, we speculate that BBS-2 may suppress the expression of IL-8 through inhibiting NF- κ B activation by attenuating RNS/PARP. It has been reported that PARP enhances the NF- κ B activity at promoter sites by facilitating the binding of NF- κ B subunits to DNA and by promoting transcription complex formation [28,29]. The latter leads to the activation of specific subsets of genes, such as those that encode cytokines, including tumour necrosis factor (TNF)- α , IL-1, IL-6 and IL-8 [30]. The production of IL-8 induces an influx of neutrophils towards the site of production, for example, to the lung in patients with ARDS [31]. Bhatia et al. reported that major trauma primes circulating neutrophils for increased migration in response to IL-8 [32]. Thus, it is possible that the iNOS inhibitor ameliorated acute lung injury by inhibiting neutrophil activation via suppressing inflammatory cytokines, such as IL-8. Previously, we have demonstrated a potential role of activated neutrophils in the pathogenesis of acute lung injury secondary to combined burn and smoke inhalation [23].

NF- κ B is responsible for the production of large amounts of iNOS-derived NO in both human and other species [33,34]. In the present study, we do not provide direct evidences that RNS/ROS or PARP activation is critically involved in the pathogenesis of burn/smoke-induced ARDS. However, we have documented central roles of these factors using peroxynitrite decomposition catalyst (unpublished data) and PARP inhibitor in the same model of injury [12]. In the present study, we show that the iNOS inhibitor BBS-2 significantly inhibits both RNS formation and PARP activation. Thus, it is apparent that iNOS/RNS/PARP is predominantly responsible for the burn-and-smoke-inhalation-induced ovine ARDS. As mentioned, we have previously demonstrated that iNOS inhibitors [3,35] ameliorated ARDS in burn-and-smoke-injured sheep. It appears that interruption of the above pathway at any level leads to improved pulmonary function. Nonetheless, in the present study, we describe the pathophysiological mechanism(s) underlying the beneficial effects of iNOS inhibition with the potent and selective iNOS dimerisation inhibitor BBS-2 in the pathogenesis of burn/smoke inhalation-related ARDS. The results of our previous and present studies strongly suggest that inhibition of excessive iNOS-derived NO could be a novel and beneficial treatment strategy in the management of burn patients with concomitant smoke inhalation injury.

In the previous studies, we have also demonstrated the beneficial effects of nNOS inhibitors 7-nitroindazole [36,37] and ZK234238 [23] in the same model of injury. Taken together, the results of the previous and present studies suggest that both nNOS and iNOS-derived NO are critically involved in the pathophysiology of burn/smoke-induced acute lung injury. These nNOS inhibitors inhibited plasma nitrite/nitrate and iNOS mRNA and protein expression. Although the precise mechanisms are not completely understood, the results of our previous studies suggest that early expression of nNOS may cause later induction of iNOS expression. Future studies are warranted to investigate the possible time-dependent expression of these enzymes to target a dominant isoform expression.

The limitation of the study is that we were not able to demonstrate other potentially important downstream pathways of iNOS inhibition, such as the interlink between iNOS and cyclooxygenase-2 (COX-2). Previous studies reported a critical role of COX-2 in burn injury [38–40]. There are also a number of studies showing that NOS and COX are co-localised in a variety of inflammation disorders [41] and activate each other [42,43]. Thus, it is possible that the beneficial effect of iNOS inhibition may, at least in part, be due to suppression of COX-2 expressions or vice versa. This hypothesis should be tested in future studies.

References

1. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000; 342:1334–49. [PubMed: 10793167]
2. Enkhbaatar P, Traber DL. Pathophysiology of acute lung injury in combined burn and smoke inhalation injury. *Clin Sci (Lond)*. 2004; 107:137–43. [PubMed: 15151496]
3. Enkhbaatar P, Murakami K, Shimoda K, Mizutani A, Traber L, Phillips GB, et al. The inducible nitric oxide synthase inhibitor BBS-2 prevents acute lung injury in sheep after burn and smoke inhalation injury. *Am J Respir Crit Care Med*. 2003; 167:1021–6. [PubMed: 12663341]
4. Enkhbaatar P, Murakami K, Cox R, Westphal M, Morita N, Brantley K, et al. Aerosolized tissue plasminogen activator improves pulmonary function in sheep with burn and smoke inhalation. *Shock*. 2004; 22:70–5. [PubMed: 15201705]
5. Morita N, Traber MG, Enkhbaatar P, Westphal M, Murakami K, Leonard SW, et al. Aerosolized alpha-tocopherol ameliorates acute lung injury following combined burn and smoke inhalation injury in sheep. *Shock*. 2006; 25:277–82. [PubMed: 16552360]
6. Blasko E, Glaser CB, Devlin JJ, Xia W, Feldman RI, Polokoff MA, et al. Mechanistic studies with potent and selective inducible nitric-oxide synthase dimerization inhibitors. *J Biol Chem*. 2002; 277:295–302. [PubMed: 11689556]
7. McMillan K, Adler M, Auld DS, Baldwin JJ, Blasko E, Browne LJ, et al. Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. *Proc Natl Acad Sci USA*. 2000; 97:1506–11. [PubMed: 10677491]
8. Enkhbaatar P, Lange M, Nakano Y, Hamahata A, Jonkam C, Wang J, et al. Role of neuronal nitric oxide synthase in ovine sepsis model. *Shock*. 2009; 32:253–7. [PubMed: 19008779]
9. Enkhbaatar P, Murakami K, Traber LD, Cox R, Parkinson JF, Westphal M, et al. The inhibition of inducible nitric oxide synthase in ovine sepsis model. *Shock*. 2006; 25:522–7. [PubMed: 16680018]
10. Gillett AM, Wallace MJ, Gillespie MT, Hooper SB. Increased expansion of the lung stimulates calmodulin 2 expression in fetal sheep. *Am J Physiol Lung Cell Mol Physiol*. 2002; 282:L440–7. [PubMed: 11839537]
11. Hollingsworth JW, Chen BJ, Brass DM, Berman K, Gunn MD, Cook DN, et al. The critical role of hematopoietic cells in lipopolysaccharide-induced airway inflammation. *Am J Respir Crit Care Med*. 2005; 171:806–13. [PubMed: 15618460]
12. Shimoda K, Murakami K, Enkhbaatar P, Traber LD, Cox RA, Hawkins HK, et al. Effect of poly(ADP ribose) synthetase inhibition on burn and smoke inhalation injury in sheep. *Am J Physiol Lung Cell Mol Physiol*. 2003; 285:L240–9. [PubMed: 12626333]

13. Tóth-Zsámboki E, Horváth E, Vargova K, Pankotai E, Murthy K, Zsengellér Z, et al. Activation of poly(ADP-ribose) polymerase by myocardial ischemia and coronary reperfusion in human circulating leukocytes. *Mol Med*. 2006; 12:221–8. [PubMed: 17225870]
14. Beller CJ, Radovits T, Kosse J, Gerö D, Szabó C, Szabó G. Activation of the peroxynitrite-poly (adenosine diphosphate-ribose) polymerase pathway during neointima proliferation: a new target to prevent restenosis after endarterectomy. *J Vasc Surg*. 2006; 43:824–30. [PubMed: 16616243]
15. Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular cloning. A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
16. Shi XZ, Pazdrak K, Saada N, Dai B, Palade P, Sarna SK. Negative transcriptional regulation of human colonic smooth muscle *cav1.2* channels by p50 and p65 subunits of nuclear factor-kappaB. *Gastroenterology*. 2005; 129:1518–32. [PubMed: 16285952]
17. Hughes MN. Relationships between nitric oxide, nitroxyl ion, nitrosonium cation and peroxynitrite. *Biochim Biophys Acta*. 1999; 1411:263–72. [PubMed: 10320662]
18. Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Disc*. 2007; 6:662–80.
19. Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science*. 1994; 263:687–9. [PubMed: 8080500]
20. Szabó C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in a rat model of endotoxic shock. Importance of the activation of poly (ADP-ribose) synthetase by peroxynitrite. *J Clin Invest*. 1997; 100:723–35. [PubMed: 9239421]
21. Fischer SR, Deyo DJ, Bone HG, McGuire R, Traber LD, Traber DL. Nitric oxide synthase inhibition restores hypoxic pulmonary vasoconstriction in sepsis. *Am J Respir Crit Care Med*. 1997; 156:833–9. [PubMed: 9310001]
22. Light RB. Effect of sodium nitroprusside and diethylcarbamazine on hypoxic pulmonary vasoconstriction and regional distribution of pulmonary blood flow in experimental pneumonia. *Am J Respir Crit Care Med*. 1996; 153:325–30. [PubMed: 8542138]
23. Enkhbaatar P, Connelly R, Wang J, Nakano Y, Lange M, Hamahata A, et al. Inhibition of neuronal nitric oxide synthase in ovine model of acute lung injury. *Crit Care Med*. 2009; 37:208–14. [PubMed: 19050603]
24. Lange M, Connelly R, Traber DL, Hamahata A, Cox RA, Nakano Y, et al. Combined neuronal and inducible nitric oxide synthase inhibition in ovine acute lung injury. *Crit Care Med*. 2009; 37:223–9. [PubMed: 19050630]
25. Murakami K, Enkhbaatar P, Shimoda K, Cox RA, Burke AS, Hawkins HK, et al. Inhibition of poly (ADP-ribose) polymerase attenuates acute lung injury in an ovine model of sepsis. *Shock*. 2004; 21:126–33. [PubMed: 14752285]
26. Chen LW, Hwang B, Chang WJ, Wang JS, Chen JS, Hsu CM. Inducible nitric oxide synthase inhibitor reverses exacerbating effects of hypertonic saline on lung injury in shock. *Shock*. 2004; 22:472–7. [PubMed: 15489641]
27. Maybauer DM, Maybauer MO, Szabó C, Westphal M, Traber LD, Enkhbaatar P, et al. Lung-protective effects of the metalloporphyrinic peroxynitrite decomposition catalyst WW-85 in interleukin-2 induced toxicity. *Biochem Biophys Res Commun*. 2008; 377:786–91. [PubMed: 18951875]
28. Hassa PO, Hottiger MO. The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-kappaB in inflammatory disorders. *Cell Mol Life Sci*. 2002; 59:1534–53. [PubMed: 12440774]
29. Kraus WL, Lis JT. PARP goes transcription. *Cell*. 2003; 113:677–83. [PubMed: 12809599]
30. Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev*. 2004; 18:2195–224. [PubMed: 15371334]
31. Hietbrink F, Koenderman L, Rijkers G, Leenen L. Trauma: the role of the innate immune system. *World J Emerg Surg*. 2006; 1:15. [PubMed: 16759367]
32. Bhatia RK, Pallister I, Dent C, Jones SA, Topley N. Enhanced neutrophil migratory activity following major blunt trauma. *Injury*. 2005; 36:956–62. [PubMed: 15998513]

33. Mendes AF, Caramona MM, Carvalho AP, Lopes MC. Role of mitogen activated protein kinases and tyrosine kinases on IL-1-induced NF- κ B activation and iNOS expression in bovine articular chondrocytes. *Nitric Oxide*. 2002; 6:35–44. [PubMed: 11829533]
34. Palmer RM, Hickery MS, Charles IG, Moncada S, Bayliss MT. Induction of nitric oxide synthase in human chondrocytes. *Biochem Biophys Res Commun*. 1993; 193:398–405. [PubMed: 7684906]
35. Soejima K, Traber LD, Schmalstieg FC, Hawkins H, Jodoin JM, Szabo C, et al. Role of nitric oxide in vascular permeability after combined burns and smoke inhalation injury. *Am J Respir Crit Care Med*. 2001; 163:745–52. [PubMed: 11254534]
36. Saunders FD, Westphal M, Enkhbaatar P, Wang J, Pazdrak K, Nakano Y, et al. Molecular biological effects of selective neuronal nitric oxide synthase inhibition in ovine lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2010; 298:L427–36. [PubMed: 19965980]
37. Traber DL, Hawkins HK, Enkhbaatar P, Cox RA, Schmalstieg FC, Zwischenberger JB, et al. The role of the bronchial circulation in the acute lung injury resulting from burn and smoke inhalation. *Pulm Pharmacol Ther*. 2007; 20:163–6. [PubMed: 16798035]
38. He LK, Liu LH, Hahn E, Gamelli RL. The expression of cyclooxygenase and the production of prostaglandin E2 in neutrophils after burn injury and infection. *J Burn Care Rehabil*. 2001; 22:58–64. [PubMed: 11227686]
39. Grbic JT, Mannick JA, Gough DB, Rodrick ML. The role of prostaglandin E2 in immune suppression following injury. *Ann Surg*. 1991; 214:253–62. [PubMed: 1929607]
40. Shoup M, He LK, Liu H, Shankar R, Gamelli R. Cyclooxygenase-2 inhibitor NS-398 improves survival and restores leukocyte counts in burn infection. *J Trauma*. 1998; 45:215–20. [PubMed: 9715175]
41. Salvemini D, Masferrer JL. Interactions of nitric oxide with cyclooxygenase: in vitro, ex vivo, and in vivo studies. *Methods Enzymol*. 1996; 269:12–25. [PubMed: 8791633]
42. Chen L, Salafranca MN, Mehta JL. Cyclooxygenase inhibition decreases nitric oxide synthase activity in human platelets. *Am J Physiol*. 1997; 273:H1854–9. [PubMed: 9362253]
43. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA*. 1993; 90:7240–4. [PubMed: 7688473]

Fig. 1.

Effects of iNOS inhibitor BBS-2 on burn and smoke-induced lung tissue (A) and lung lymph (B) nitrite and nitrate (NO_x), a stable metabolite of nitric oxide and lung tissue 3-nitrotyrosine (C), a stable metabolite of reactive nitrogen species such as peroxynitrite. Data are expressed as means ± SEM. **p* < 0.05 vs. sham; †*p* < 0.05 vs. control.

Fig. 2. Effects of iNOS inhibitor BBS-2 on burn and smoke-induced lung tissue myeloperoxidase activity (A) and malondialdehyde formation (B). Data are expressed as means $\pm$ SEM. * $p < 0.05$ vs. sham; † $p < 0.05$ vs. control.

Fig. 3.

Effects of iNOS inhibitor BBS-2 on burn and smoke-induced lung tissue PARP activity expressed as scores. (A) Airway, (B) alveoli, (C) airway gland and (D) vessels. Data are expressed as means $\pm$ SEM. * $p < 0.05$ vs. sham; † $p < 0.05$ vs. control.

Fig. 4. Effects of iNOS inhibitor BBS-2 on burn and smoke-induced lung lymph IL-8 expression (Western blot) (A) and lung tissue IL-8 mRNA (B). Data are expressed as means $\pm$ SEM. * p < 0.05 vs. sham; † p < 0.05 vs. control.

Fig. 5.
Effects of iNOS inhibitor BBS-2 on burn and smoke-induced lung tissue NF-κB activity.
Data are expressed as means $\pm$ SEM.

Table 1

Pulmonary variables.

	0 h	3 h	6 h	12 h	18 h	24 h
$\text{PaO}_2/\text{FiO}_2$ (mmHg)						
Sham	508 ± 13	483 ± 26	577 ± 21	553 ± 15	571 ± 12	590 ± 8
Control	495 ± 11	477 ± 28	490 ± 51	466 ± 58	339 ± 72*	233 ± 50*
BBS-2	494 ± 17	488 ± 21	534 ± 38	507 ± 47	468 ± 41	409 ± 43 [†]
$\dot{Q}_s/\dot{Q}_t$ (%)						
Sham	0.17 ± 0.01	0.2 ± 0.01	0.14 ± 0.01	0.15 ± 0.0	0.15 ± 0.0	0.15 ± 0.0
Control	0.15 ± 0.00	0.18 ± 0.01	0.15 ± 0.0	0.16 ± 0.02	0.28 ± 0.03*	0.31 ± 0.04*
BBS-2	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.02 [†]	0.19 ± 0.02 [†]
Peak (cm H ₂ O)						
Sham	18 ± 1.1	16 ± 0.9	17 ± 0.9	17 ± 0.6	17 ± 1.0	16 ± 0.6
Control	21 ± 0.4	19 ± 1.3	21 ± 1.0	23 ± 0.7	26 ± 2*	28 ± 2*
BBS-2	21 ± 0.4	20 ± 0.5	21 ± 0.8	20 ± 0.6	21 ± 1.0	20 ± 1.1 [†]
W/D ratio						
Sham						3.8 ± 0.1
Control						5.2 ± 0.2*
BBS-2						4.1 ± 0.3 [†]
Br. obstr						
Sham						1.7 ± 0.1
Control						4.4 ± 0.4*
BBS-2						2.8 ± 0.3 [†]

$\dot{Q}_s/\dot{Q}_t$: pulmonary shunt fraction; Ppeak: peak airway pressure; W/D: lung bloodless wet-to-dry weight ratio; Br. obstr: bronchiole obstruction.

Date presented as means ± SEM. Each group includes 6 sheep.

* $p < 0.05$ vs. sham.

[†] $p < 0.05$ vs. control.