Molecular and pharmacological approaches to inhibiting nitric oxide after burn trauma

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White, Jean, Deborah L. Carlson, Marita Thompson, David L. Maass, Billy Sanders, Brett Giroir, and Jureta W. Horton. Molecular and pharmacological approaches to inhibiting nitric oxide after burn trauma. Am J Physiol Heart Circ Physiol 285: H1616–H1625, 2003. First published May 8, 2003; 10.1152/ajpheart.00061.2002.—Whereas controversial, several studies have suggested that nitric oxide (NO) alters cardiac contractility via cGMP, peroxynitrite, or poly(ADP ribose) synthetase (PARS) activation. This study determined whether burn-related upregulation of myocardial inducible NO synthase (iNOS) and NO generation contributes to burn-mediated cardiac contractile dysfunction. Mice homozygous null for the iNOS gene (iNOS knockouts) as well as wild-type mice were given a cutaneous burn over 40% of the total body surface activity were evident 6–12 h after cytokine challenge iNOS mRNA within 4–6 h, whereas iNOS protein/activity were evident 6–12 h after cytokine challenge (5, 6, 23).

A role for inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in the myocardial depression that is characteristic of trauma and sepsis is well recognized (18, 21, 24, 28, 29). Specific mechanisms by which these cytokines induce cardiac contractile abnormalities remain unclear, but interest in NO as a final mediator of cytokine-mediated contractile dysfunction has developed from studies showing that a NOS inhibitor (Nω-nitro-l-arginine) blocked the negative inotropic effects of inflammatory cytokines (11). Numerous studies followed this initial observation, but the results have led to considerable controversy regarding the role of NO as a mediator of cardiac dysfunction. Several studies have suggested that cardiomyocytes per se secrete NO in response to an inflammatory stimulus such as cytokine or lipopolysaccharide (LPS) challenge.

CONSIDERABLE EVIDENCE has accumulated supporting the presence of nitric oxide (NO) signaling pathways in the myocardium. NO synthesis/secretion is regulated by a family of enzymes known as NO synthase (NOS). Finksky and associates (33) and Kelly and colleagues (23) reported that constitutive NOS plays a significant regulatory role in the heart under normal physiological conditions, adjusting cardiac performance on a beat-to-beat basis and modulating cardiac contraction and relaxation in responses to changes in preload. Other studies have shown that injury, disease, and inflammation promote iNOS activity in the myocardium (3–5, 35), but the complex mechanisms that regulate iNOS expression remain unclear. Increased mitogen-activated protein kinase (MAPK) activity and signal transducer and activator of transcription 1α (STAT-1α) phosphorylation/nuclear translocation have been shown to proceed iNOS induction in several tissues, and intracellular L-arginine content, activation of PKC isozymes, α-adrenergic stimulation, and cAMP analogs modulate iNOS transcription and activity in cardiomyocytes (23). More recent attention has focused on cytokine-related stimulation of NO signaling pathways, and IL-1β, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) challenge in primary cultures of adult ventricular myocytes. These were shown to increase iNOS mRNA within 4–6 h, whereas iNOS protein/activity were evident 6–12 h after cytokine challenge (5, 6, 23).


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and NO may produce negative inotropic effects by modulating myofilament responsiveness to calcium (13). Under normal physiological conditions, constitutive NO likely plays a beneficial role in modulating cardiac contraction and relaxation responses to cholinergic/adrenergic stimulation; however, it is likely that the iNOS-related surge in NO levels occurring in response to inflammation causes excessive vasodilation and significant negative inotropic effects. This hypothesis is consistent with our previous finding that high doses of NO donors, but not low doses, exacerbated TNF-α-mediated cardiac contractile dysfunction (19). In addition, the finding of iNOS mRNA and protein in myocardial specimens obtained from patients with ischemic heart disease and dilated cardiomyopathy have provided further evidence for the detrimental role of NO in myocardial dysfunction and failure (26, 39).

Our previous studies have shown that traumatic injury such as burn trauma promotes cardiomyocyte secretion of several inflammatory cytokines, including TNF-α, IL-1β, and IL-6 (20). Whether burn trauma and the accompanying cytokine burst initiate iNOS activity in the myocardium remains unclear or has the effect of inhibiting iNOS activity on burn-mediated myocardial dysfunction been studied. Therefore, this present study used a molecular approach [iNOS knockout (KO) mice] as well as a pharmacological approach (the NO inhibitor aminoguanidine) to further examine the role of NO in the cardiac contraction and relaxation deficits that are characteristic of burn trauma.

MATERIALS AND METHODS

Animal Model

Adult mice (C57BL/6J) obtained from Jackson Laboratories (Bar Harbor, ME) were used in this study. All animals were housed in a temperature-controlled environment with a 12-h/12-h light/dark cycle, and food and water were available at will. All animals were used in compliance with the guidelines established by the Institutional Review Board for Animal Research at the University of Texas Southwestern Medical Center and were performed in accordance with National Institutes of Health guidelines for the use of laboratory animals.

Burn Protocol

Mice were weighed immediately before being included for the study. With the mice under anesthesia (methoxybarbital), the side and back of each mouse were closely clipped and then carefully shaved from the base of the tail to the base of the neck. Animals were randomly assigned to sham burn or burn groups. In those animals designated for burn trauma, a cutaneous burn injury was produced over 40% of the total body surface area (TBSA) by applying brass probes (2 × 3 cm with a 3-mm thickness) heated to 100°C in boiling water to the animals' side and back for 5 s as previously described (38). The TBSA was calculated using murine-specific data (14); this calculation was verified by removing the animal’s pelt and measuring the actual burned area. The percent burned area was then calculated based on the animal’s measured TBSA. After burn trauma was completed, the mice were given lactated Ringer fluid resuscitation (4 ml/kg per %burn) intraperitoneally. Half of the fluid was administered at the time of burn trauma, and the remaining fluid was given 8 h postburn. All animals received analgesic (buprenorphine, 0.05 mg/kg im) every 8 h after burn trauma. Animals designated for sham burn groups received identical regimens of anesthesia and handling but no burn injury was given. Animals were monitored closely for the first 8 h after burn trauma to determine adequate recovery from the anesthesia, animal responsiveness to external stimuli, the absence of pain, and the ability to consume food and water.

Experimental Groups

**Molecular approach.** The molecular approach to inhibiting NO included the use of genetically engineered mice in which the inducible NO gene had been deleted. iNOS knockout mice (C57BL/6J background), weighing 20–25 g, were obtained from Jackson Laboratories. Mice were bred in house, and the absence of iNOS mRNA and protein was confirmed by Northern and Western blots, respectively (37). The iNOS-deficient mice were randomly divided into sham and burn groups; experimental groups included: 1) wild-type shams, 2) wild-type mice given burn injury over 40% TBSA and fluid resuscitated with lactated Ringer solution as described above (wild-type burn), 3) iNOS KO mice given sham burn injury (iNOS KO/sham), and 4) iNOS KO mice given burn trauma and fluid resuscitation as described above (iNOS/KO burn). All mice were euthanized 24 h postburn, and the hearts were perfused to examine cardiac mechanical function (n = 7–8 mice/group).

**Pharmacological approach.** Pharmacological inhibition of NO was achieved by dividing C57BL/6J mice into four experimental groups: 1) sham burn mice given vehicle (VE) only (0.3 ml of saline given ip for 7 days preburn and on the morning of burn trauma, VE sham); 2) additional sham burn mice were given the iNOS inhibitor aminoguanidine (Sigma Chemical, St. Louis, MO, 200 mg/kg suspended in 0.3 ml of saline and given ip for 7 days preburn and on the day of burn trauma, AmG sham); 3) the third group of mice received burn injury and fluid resuscitation plus vehicle (0.3 ml saline given ip as described above, VE burn); and 4) in the fourth group, mice were given burn injury over 40% TBSA and fluid resuscitated with lactated Ringer as described above and were given aminoguanidine (200 mg/kg given ip as described for group 2, AmG burn).

**Langendorff Perfused Hearts**

To examine cardiac contraction and relaxation, mice were anticoagulated 24 h after burn trauma with heparin sodium (200 units/100 g). All animals were anesthesia and were then decalciﬁed and surgically isolated. The heart was rapidly removed and placed in a petri dish containing ice-cold (4°C) Krebs-Henseleit bicarbonate-buffered solution (in mM: 118 NaCl, 4.7 KCl, 21 NaHCO₃, 1.25 CaCl₂, 1.2 MgSO₄, 1.2 K₂HPO₄, and 11 glucose). All solutions were prepared each day with demineralized, deionized water and bubbled with 95% O₂-5% CO₂ (pH 7.4; Po₂, 500 mmHg; Pco₂, 38 mmHg). A cannula placed in the ascending aorta was connected via glass tubing to a buffer-filled reservoir for perfusion of the coronary circulation at a constant flow rate. Hearts were suspended in a temperature-controlled chamber maintained at 35 ± 0.5°C, and a constant flow pump (Ismatec, model 7355-30, Cole-Palmer Instrument; Chicago, IL) was used to maintain perfusion of the coronary artery (ml/min) by retrograde perfusion of the aortic stump cannula as described previously (38). Coronary perfusion pressure was measured and effluent was collected to confirm coronary flow rate. Contractile function was assessed by measuring intraventricular pressure with a water-filled...
latex balloon attached to a polyethylene tube and threaded through the apex of the left ventricular chamber. Peak systolic left ventricular pressure (LVP) was measured with a Statham pressure transducer (model P23 ID, Gould Instruments; Oxnard, CA) attached to the balloon cannula, and the rate of LVP rise \((\dfrac{dP}{dt})\) and fall \((\dfrac{dP}{dt})\) were obtained using an electronic differentiator (model 7P20C, Grass Instruments; Quincy, MA) and recorded (model 7DWL8P; Grass Recording Instruments). Left ventricular developed pressure was calculated from peak systolic LVP and LV end-diastolic pressure. After stabilization of the hearts for 35–40 min at a constant level of coronary flow (3.0 ml/min) and a constant end-diastolic pressure of 10 mmHg, cardiodynamic function was assessed by measuring developed LVP, \(\pm \dfrac{dP}{dt}\), developed pressure at 40 mmHg, the times to peak pressure, as well as times to 90% relaxation and to \(\pm \dfrac{dP}{dt}\), coronary vascular resistance, and heart rate (data are summarized in Tables 2 and 3). After baseline cardiodynamic function was assessed, the hearts from all experimental groups were then perfused at a coronary flow rate of 1 ml/min and after 2–3 min stabilization, developed LVP and \(\pm \dfrac{dP}{dt}\) responses were recorded; coronary flow rate was then incrementally increased from 2 to 3 to 4 ml/min; and at each level of coronary flow rate, developed LVP and \(\pm \dfrac{dP}{dt}\) responses were measured. These data were then used to generate ventricular performance-coronary flow relationships. After these studies were completed, hearts were then divided into two subsets; hearts in the first subset \((n = 7–8\) hearts/group\) were perfused at a constant coronary flow rate (3.0 ml/min) and a constant left ventricular end-diastolic pressure (10 mmHg) and a constant heart rate; perfusate calcium was then incrementally increased from 1 to 8 mM, and developed LVP and \(\pm \dfrac{dP}{dt}\) responses were measured at each level of perfusate calcium. The other subset of hearts from each experimental group \((n = 5\) group\) were perfused at a constant coronary flow rate and constant end-diastolic pressure as described above; isoproterenol was then added to the perfusate in a recirculating manner for 2–3 min. Developed LVP and \(\pm \dfrac{dP}{dt}\) responses were measured at each perfusate concentration of isoproterenol, and the absolute values of developed LVP and \(\pm \dfrac{dP}{dt}\) (as well as the percent change in these parameters at each isoproterenol concentration compared with perfusion in the absence of isoproterenol) were determined. Data from the Grass recorder was input into a Dell Pentium computer, and a Grass Poly VIEW Data Acquisition system was used to convert acquired data into digital form.

RT-PCR Studies

All reagents and primers were purchased from Gibco-BRL (Grand Island, NY). RNA (1.5 µg) was reverse transcribed with the use of 6 units of SuperScript RTII, 12.5 ng/µl of oligo(dT)12–18, and 500 µM of each dNTP in a 1-unit RNase solution, 5 µM 1,4-dithiothreitol, and 1X buffer (50 mM Tris·HCl (pH 8.3), 75 mM KCl, and 3 mM MgCl2 in a total volume of 20 µl. The reaction was carried out at 37°C for 60 min, followed by 95°C for 5 min to heat inactivate the reverse transcriptase. The primers for murine iNOS were 5'-ATGTCGAGGAAAAATCACA-3' and 5'-TAACTGCCCAGGAAGTTAAGTGG-3'. The primers for murine GAPDH were 5'-CGGGACTGACGGATTTGGCTGAT-3' and 5'-AGCCCTTCTCCATGGGTGGAAGAC-3'.

Reverse-transcribed cDNA \((-0.5\) µg in 2 µl) was then mixed with 1X PCR buffer (20 mM Tris·HCl (pH 8.4), 50 mM KCl), 1 mM MgCl2, 20 µM of each deoxynucleotide triphosphate, 20 ng/µl of each primer set, and 1 unit platinum Taq in a 20-µl reaction. The following conditions were used for amplification: one cycle of 94°C for 2 min followed by 27 cycles (94°C for 45 s, 63°C for 45 s, and 72°C for 45 s) and then one cycle of 72°C for 5 min.

Protein Isolation

Frozen mouse hearts were homogenized in ice-cold lysis buffer at a concentration of 0.5 g tissue/ml buffer. The composition of the lysis buffer was 20 mM HEPES (pH 7.4), 2 mM EDTA, 20 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 0.2 mM Na3VO4, 20 mM sodium fluoride, 10 mM benzamidine, 1 mM DTT, 20 ng/ml leupeptin, 0.4 mM Pefabloc SC, and 0.05% Triton X. The homogenized samples were allowed to sit on ice for 15 min and were then centrifuged at 10,000 g for 20 min at 4°C. The protein concentration was determined by the Bradford assay using bovine serum albumin for the standard curve (Bio-Rad Protein Assay Reagents; Hercules, CA).

Western Immunoblots

Total heart protein (50 µg) was added to an equal volume of gel-loading buffer [0.2 M Tris (pH 6.7), 5% sodium dodecyl sulfate (SDS), 12.2 M β-mercaptoethanol (Sigma), 0.1% bromophenol blue, and 20% glycerol], boiled for 5 min at 100°C, resolved on a 12.5% polyacrylamide gel, and then transferred to a polyvinylidene fluoride microporous membrane. Membranes were blocked for 2 h in 5% powdered milk dissolved in Tris-buffered saline-Tween 20 (TBS-T) [20 mM Tris (pH 7.6), 137 mM NaCl, and 0.1% Tween 20]. Membranes were then incubated for 12 h at 4°C with a polyclonal rabbit iNOS antibody (Santa Cruz Biotechnology; Santa Cruz, CA) used at a 1:200 dilution. After incubation, the membranes were washed one time in TBS-T for 15 min, followed by five 5-min washes. The membranes were then incubated for 1 h at room temperature with a 1:5,000 dilution of anti-rabbit immunoglobulin G horseradish peroxidase-linked antibody (Amer sham; Piscataway, NJ). After incubation, the membranes were washed two more times in TBS-T for 15 min, followed by five 5-min washes. Membranes were then developed using SuperSignal West Pico Luminol/Enhancer Solution (Bio-Rad), and the intensity of the resulting chemiluminescent reaction was registered by autoradiography. Equal loading of the samples was confirmed by both Bradford assay and by Ponceau S staining of the membranes (Sigma Chemical).

Statistical Analysis

All values are expressed as means ± SE. ANOVA was used to assess an overall difference among the groups for each of the variables. Levene’s test for equality of variance was used to suggest the multiple-comparison procedure to be used. If equality of variance among the four groups was suggested, multiple comparison procedures were performed (Bonferroni); if inequality of variance was suggested by Levene’s test, Tamhane multiple comparison (which do not assume equal variance in each group) was performed. Probability values <0.05 were considered significant (analysis was performed using SPSS for Windows, version 7.5.1).

RESULTS

All mice survived their respective experimental protocol regardless of the presence or absence of iNOS. After anesthesia and burn trauma, all mice moved freely about the cage and consumed food and water at will. There was no significant change in body weight
during the 24 h of the experimental protocol. In addition, the percent TBSA burn was calculated for each mouse at euthanasia; there were no significant differences in the percent TBSA burn among experimental groups (Table 1). RT-PCR studies confirmed that burn injury increased myocardial iNOS RNA in C57BL/6J wild-type mice 2 and 4 h postburn (Fig. 1A). There was no burn-related increase in iNOS RNA in iNOS/KO mice. Western blot analysis of hearts harvested from burned mice 2 h or 4 h postburn (n = 4–5 mice/group) and compared with hearts harvested from wild-type sham burns confirmed that burn trauma produced significant upregulation of myocardial iNOS in wild-type mice (Fig. 1B). In contrast, burn-related upregulation of myocardial iNOS was absent in iNOS KO mice (Fig. 1C). Burn trauma had no significant effect on myocardial constitutive NOS (data not shown).

Molecular Approach to Inhibiting iNOS

Neither the absence of iNOS (Table 2) nor inhibiting iNOS with aminoguanidine (Table 3) in sham burn mice altered any aspect of cardiac stabilization data nor responsiveness to increase in either coronary flow, isoproterenol challenge, or increases in perfusate calcium (Figs. 2–4). The effects of burn trauma, in the presence and absence of iNOS inhibition, are shown in Fig. 2.

Left ventricular function was examined in all experimental groups as coronary flow was increased from 1 to 4 ml/min; all hearts retained the ability to respond to incremental increases in coronary flow rate with significant increases in left ventricular developed pressure (Fig. 2, left) as well as increases in the rate of LVP rise (Fig. 2, middle) and increases in the rate of LVP fall (Fig. 2, right). However, burn trauma in the wild-type mice (Fig. 2A) and in the vehicle-treated mice (Fig. 2B) produced considerable cardiac contraction and relaxation defects as indicated by the significantly lower LVP and ±dP/dt responses at each level of coronary flow rate compared with values measured in either the wild-type littermate shams (Fig. 2A) or the vehicle-treated shams (Fig. 2B). Inhibiting iNOS by either a molecular approach or a pharmacological approach significantly attenuated the negative inotropic effects of burn trauma (Fig. 2, A and B). Ventricular function curves generated by iNOS/KO burn mice and by AmG burn mice were not significantly different from those generated by either iNOS KO shams or by the wild-type shams.

Figure 3 examined ventricular responsiveness to isoproterenol challenge and confirmed that burn trauma impaired LVP and ±dP/dt max responses to β-adrenergic challenge. LVP and ±dP/dt max were significantly lower (P < 0.05) in the wild-type burn group at each level of isoproterenol challenge; this burn-mediated defect was ablated by the absence of the iNOS gene (iNOS/KO burn) or by AmG burn to inhibit NO synthesis (Fig. 3B).

All hearts responded to increases in perfusate Ca2+ with significant increases in LVP and ±dP/dt max (Fig. 4). However, wild-type burn (Fig. 4A) and VE burn mice (Fig. 4B) showed significantly impaired left ventricular responsiveness to increases in perfusate Ca2+. LVP and ±dP/dt max were significantly lower at each perfusate Ca2+ concentration in burns compared with values measured in the respective shams. In contrast, burn-related contraction and relaxation defects were attenuated by either a molecular approach to inhibiting iNOS (iNOS/KO burn, Fig. 4A) or the pharmacological approach to inhibit iNOS (AmG burn, Fig. 4B).

DISCUSSION

Numerous studies have shown that both clinical and experimental burn trauma are characterized by profound myocardial depression; however, burn-related myocardial contraction and relaxation deficits are...
transient and gradually resolve over the first 48 to 72 h after burn trauma (17, 19, 31). Cardiac dysfunction in the early postburn period is not corrected by either aggressive fluid resuscitation to optimize ventricular filling (2) or by β-adrenergic drugs to improve cardiac contractility (22). The precise mechanisms by which burn trauma alters the ability of the myocardial contractile proteins to respond effectively to either inotropic agonists or antagonists remain unclear. However, studies using models of ischemia-reperfusion and sepsis have implicated the negative inotropic effects of NO. Similarly, data from this present study suggest that NO contributes, in part, to the cardiac contractation and relaxation deficits that are characteristic of major burn trauma. In this present study, our RT-PCR and Western blot data confirm that burn trauma in wild-type mice produced significant upregulation of myocardial iNOS, whereas constitutive NOS was unchanged. In this study, we used both a molecular as well as a pharmacological approach to inhibit NO. Our molecular studies showed that the absence of the iNOS gene was associated with improved left ventricular contractile performance, despite a major burn injury over 40% of the TBSA and aggressive fluid resuscitation regimen that was identical to that used in the wild-type mice. Our pharmacological approach included administration of aminoguanidine, a known inhibitor of NOS enzyme activity (1). The dose of aminoguanidine used in this study was identical to that applied in a rodent model by Alden and colleagues (1). Because the half-life of aminoguanidine is 4 h and burn trauma alters drug absorption and metabolism, we elected to administer aminoguanidine daily for 7 days before burn trauma to ensure adequate blood as well as tissue levels of inhibitor.

Our finding of burn-related upregulation of myocardial iNOS is consistent with numerous studies that have described increased myocardial iNOS expression in injury and disease states. deBelder and colleagues (8) described increased iNOS activity in the myocardium of patients with dilated cardiomyopathy; and LPS and inflammatory cytokine challenge have been shown to upregulate iNOS expression in the myocardium (23, 27).

Our interest in NO as a potential mediator of burn-related cardiac contractile dysfunction was based on our previous finding that cardiomyocytes secrete a number of inflammatory cytokines, including TNF-α, IL-1β, and IL-6. We recently showed that burn trauma

Table 2. Ventricular responses to burn trauma in AmG-treated and VE-treated mice

<table>
<thead>
<tr>
<th>LVP, mmHg</th>
<th>VE</th>
<th>AmG</th>
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<tbody>
<tr>
<td>Sham</td>
<td>94±1</td>
<td>94±2</td>
</tr>
<tr>
<td>Burn</td>
<td>72±4*</td>
<td>84±4†</td>
</tr>
<tr>
<td>-dP/dtmax, mmHg/s</td>
<td>2,188±59</td>
<td>2,222±50</td>
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<tr>
<td>-dP/dtmax, mmHg/s</td>
<td>1,889±83</td>
<td>1,867±67</td>
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<tr>
<td>dP40, mmHg/s</td>
<td>1,800±122</td>
<td>1,916±64</td>
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<tr>
<td>TPP, ms</td>
<td>72.5±2.5</td>
<td>72.2±2.2</td>
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<tr>
<td>Time to 90% relax, ms</td>
<td>78.8±5.9</td>
<td>73.8±2.4</td>
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<tr>
<td>Time to max, +dP/dt, ms</td>
<td>49.0±0.6</td>
<td>48.2±0.4</td>
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<tr>
<td>Time to max, -dP/dt, ms</td>
<td>42.0±1.8</td>
<td>50.1±1.2</td>
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<tr>
<td>CPP, mmHg</td>
<td>83.0±9.3</td>
<td>72.7±7.8</td>
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<tr>
<td>CVR, mmHg/ml</td>
<td>55.3±6.2</td>
<td>48.5±5.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>332±7</td>
<td>355±12</td>
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Values are means ± SE. LVP, left ventricular pressure; -dP/dt, relaxation; +dP/dt, contraction; TPP, time to peak pressure at 10% ventricular relaxation; Time to 90% relaxation; Time to max, +dP/dt; Time to max, -dP/dt. *Significant difference among groups at *0.05 (ANOVA, Student-Newman-Keuls). †Significant difference between VE-treated burn and AmG Burn at *0.05 (Student’s t-test).

Table 3. Ventricular responses to burn trauma in iNOS-deficient and wild-type littermates

<table>
<thead>
<tr>
<th>LVP, mmHg</th>
<th>Wildtype</th>
<th>iNOS/KO</th>
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<tbody>
<tr>
<td>Sham</td>
<td>94±2</td>
<td>92±6</td>
</tr>
<tr>
<td>Burn</td>
<td>67±3*</td>
<td>1,620±94*</td>
</tr>
<tr>
<td>-dP/dtmax, mmHg/s</td>
<td>2,240±58</td>
<td>1,200±84*</td>
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<tr>
<td>-dP/dtmax, mmHg/s</td>
<td>1,800±42</td>
<td>1,200±84*</td>
</tr>
<tr>
<td>dP40, mmHg/s</td>
<td>1,840±67</td>
<td>1,367±111*</td>
</tr>
<tr>
<td>TPP, ms</td>
<td>76.6±1.0</td>
<td>75.1±2.2</td>
</tr>
<tr>
<td>Time to 90% Relax, ms</td>
<td>81.2±2.2</td>
<td>76.3±2.4</td>
</tr>
<tr>
<td>Time to max, +dP/dt, ms</td>
<td>47.2±1.8</td>
<td>49.3±0.4</td>
</tr>
<tr>
<td>Time to max, -dP/dt, ms</td>
<td>52.6±2.1</td>
<td>49.3±1.4</td>
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<tr>
<td>CPP, mmHg</td>
<td>87±4</td>
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<tr>
<td>CVR, mmHg/ml</td>
<td>58±3</td>
<td>48±5</td>
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<tr>
<td>HR, beats/min</td>
<td>342±4</td>
<td>359±5</td>
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Values are means ± SE. *Significant difference among groups at *0.05 (ANOVA, Student-Newman-Keuls). †Significant difference between wild-type burn and iNOS/KO burn at *0.05 (Student’s t-test). iNOS/KO indicates iNOS-deficient (knockout) mice.
in wild-type mice increased NO secretion by primary cardiomyocytes prepared 24 h after burn injury over 40% TBSA (14.1 ± 1.4 μM) and compared that with NO levels secreted by cardiomyocytes prepared from sham burn mice (4.8 ± 0.8 μM, P < 0.05) (unpublished data). Whereas inflammatory cytokines are produced in response to burn trauma by a variety of cells including fibroblasts, macrophages, and leukocytes, cardiomyocyte synthesis and secretion of inflammatory cytokines likely produce myocardial or compartmental levels of mediators that far exceed those measured in the systemic circulation. Thus the myocardium is exposed to cytokines of liver and lung origin, which enter the systemic circulation as well as cytokines released by several cell types within the myocardium, including cardiomyocytes, vascular endothelial cells, and emigrated leukocytes. Because inflammatory cytokines have been shown to promote the inducible form of NO in a variety of cell types, we recognized that myocardial-derived cytokines likely promote myocardial NO synthesis, increasing NO concentration which, in turn, may alter cardiac function. However, in our study, the molecular approach of inhibiting NO (deleting the iNOS gene) as well as the pharmacological approach (systemic administration of aminoguanidine) were not cardiосpecific and inhibited NO synthesis by all tissues. Thus our molecular and pharmacological approaches to inhibiting NO may have improved cardiac contractile function by several mechanisms, including downregulation of the overall inflammatory response.

Fig. 2. Left ventricular pressure (LVP) and rate of LVP rise (+dP/dt) and fall (-dP/dt) responses to increases in coronary flow rate in iNOS-deficient mice (A) and in aminoguanidine-treated mice (AmG, B). Neither the absence of the iNOS gene (iNOS/KO, A) nor pharmacological inhibition of iNOS (with AmG, B) altered ventricular responses in sham burn mice to increases in coronary flow. Whereas all hearts retained the ability to respond to increases in coronary perfusion, hearts from WT burns (Wild-type Burn, A) and from vehicle-treated burns (VE Burn, B) had considerable cardiac contraction and relaxation defects. Absence of the iNOS gene (iNOS/KO Burn) or AmG treatment in burns (AmG Burn) attenuated these burn-related defects. All values are means ± SE. *Significant difference among groups at P < 0.05 (ANOVA and multiple-comparison procedure).
to burn trauma. Alternatively, inhibiting systemic NO synthesis may have prevented burn-related disruption of gut barrier function, preventing bacterial/endotoxin translocation and subsequent downstream myocardial contractile abnormalities (16, 34).

The precise mechanisms by which NO impairs cardiac contractile performance remains unclear, but Luss and colleagues (27) suggested that NO impairs cardiomyocyte oxidative metabolism, whereas others (7, 10) have shown that NO interaction with superoxide radicals produces the highly toxic peroxynitrite radical and hydroxyl radical. Whereas our study did not measure burn-related alterations in myocardial superoxide or peroxynitrite generation, we have shown previously that burn trauma in rodents promotes free radical-related lipid peroxidative injury in the myocardium (32). Thus it is likely that a burst of NO generation during the postburn period promoted NO-superoxide interaction, and peroxynitrite generated as a result of this NO burst likely contributed to burn-related myocardial depression. Alternatively, NO may mediate formation of metmyoglobin, alter myocyte mitochondrial respiration, or activate guanylate cyclase-cGMP production, which, in turn, would alter Ca^{2+} availability to the contractile proteins (9, 15, 30).

Whereas the studies described herein suggest a deleterious role for NO in major trauma, our study did not examine the long-term consequences of NO depletion. In this regard, NO inhibition may alter some aspect of the immune function, increasing susceptibility to infection after major burn trauma. Moreover, the beneficial effects of NO are clearly recognized; low levels of NO

Fig. 3. Ventricular responsiveness to isoproterenol challenge in all experimental groups. WT burn mice (A) or vehicle-treated burns (VE burn, B) had significantly impaired LVP and -dP/dt responses to isoproterenol challenge. Either absence of the iNOS gene (iNOS/KO burn, A) or pharmacological inhibition of iNOS with AmG (AmG Burn, B) attenuated this burn-mediated contractile defect. All values are means ± SE. *Significant difference among groups at P < 0.05 (ANOVA and multiple-comparison procedure).
interact with the superoxide radical, thus serving as a free radical scavenger; NO also prevents adherence and activation of platelets and leukocytes, preventing the release of deleterious mediators (12). The beneficial effects of NO have been further emphasized by studies that showed that the addition of NO donors to cardioplegic solution during ischemia-reperfusion afforded considerable cardioprotection (25, 36). Finally, NO is inhibitory to tumor cells as well as bacteria, thus serving a protective role in the heart by eliminating invading bacteria; the ability of NO to inhibit viral replication may also provide myocardial antiviral defense. Thus, whereas our short-term studies confirmed the cardioprotective effects of NO inhibition in burn trauma, these studies cannot be extrapolated to the clinical setting where inhibiting iNOS may alter susceptibility to secondary infection in patients with major burn trauma.

In this study, burn-related myocardial abnormalities were unmasked by several interventions including examining coronary flow-ventricular performance relationships as well as ventricular responsiveness to increases in perfusate Ca^{2+}. All values are means ± SE. *Significant difference among groups at \( P < 0.05 \) (ANOVA and multiple-comparison procedure).

Fig. 4. LVP and ±dP/dt responses to incremental increases in perfusate Ca^{2+} from 1 to 8 mM. Absence of the iNOS gene (iNOS/KO, A) and aminoguanidine treatment in shams tended to impair ventricular responsiveness to increases in perfusate Ca^{2+} in sham burns, but these changes did not achieve statistical significance. WT burn (A) and VE burn (B) hearts produced measures of LVP and ±dP/dt that were significantly less than those values measured in wild-type shams or in vehicle-treated shams (VE Sham) at each level of perfusate Ca^{2+} concentration. Absence of the iNOS gene (iNOS/KO Burn) or AmG treatment significantly improved ventricular responses to increases in perfusate Ca^{2+}. All values are means ± SE.
measured in burned hearts could not be overcome by maximally effective increases in coronary flow rate suggested that the mechanism of postburn contractile dysfunction in these hearts was not related to burn-related alterations in coronary vascular autoregulation. Finally, examining LVP and ±dP/dt responses to increases in coronary flow also provides another approach to examining postburn myocardial abnormalities and provides a means of unmasking burn-related disparity in ventricular performance. Similarly, our finding that all hearts retain the ability to respond to increases in perfusate calcium or isoproterenol challenge with increases in LVP and ±dP/dt suggest that burn trauma did not produce irreparable damage to the actin-myosin filaments of the myocardium.

In summary, this present study used both molecular and pharmacological approaches to inhibit iNOS in a model of murine burn trauma. Our data suggest that NO contributes to the early myocardial contraction and relaxation deficits that are characteristic of major burn trauma. However, further studies to determine the specific mechanisms by which NO alters myocardial function are warranted.

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REFERENCES


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