Reducing susceptibility to bacteremia after experimental burn injury: a role for selective decontamination of the digestive tract

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Submitted 27 October 2005; accepted in final form 22 January 2007

Horton JW, Maass DL, White J, Minei JP. Reducing susceptibility to bacteremia after experimental burn injury: a role for selective decontamination of the digestive tract. J Appl Physiol 102: 2207–2216, 2007. First published February 1, 2007; doi:10.1152/japplphysiol.01365.2005.—We proposed that selective decontamination of the digestive tract (SDD) initiated after experimental burn injury would decrease myocardial inflammation and dysfunction after a second insult such as septic challenge. Rats were divided into eight experimental groups. Groups included sham burn plus sham sepsis, burn alone, sepsis alone, and burn plus sepsis given either water by oral gavage for 5 days after burn (or sham burn) or given oral antibiotics (polymyxin E, 15 mg; tobramycin, 6 mg; 5-flucytosin, 100 mg given by oral gavage, 2× daily for 5 days after burn or sham burn). Cardiac function and inflammation were studied 24 h after septic challenge. In the absence of SDD, burn alone, sepsis alone, or burn plus septic challenge promoted cardiac myocyte secretion of TNF-α (burn, 174 ± 11; sepsis, 269 ± 19; burn + sepsis, 453 ± 14 pg/ml), IL-1β (burn, 35 ± 2; sepsis, 29 ± 1; burn + sepsis, 48 ± 7 pg/ml), and IL-6 (burn, 143 ± 18; sepsis, 116 ± 3; burn + sepsis, 248 ± 12 pg/ml) compared with values measured in sham (TNF-α, 3 ± 1; IL-1β, 1 ± 0.4; IL-6, 6 ± 1.5 pg/ml) (P < 0.05). Impaired ventricular contraction and relaxation responses were evident in the absence of SDD [burn + sepsis: left ventricular pressure (LVP), 65 ± 4 mmHg; rate of LVP rise (+dP/dt), 1,320 ± 131 mmHg/s compared with values measured in sham: LVP, 96 ± 4 mmHg; +dP/dt, 2,095 ± 90 mmHg/s, P < 0.05]. SDD treatment of experimental burn attenuated septic challenge-related inflammatory responses and improved myocardial contractile responses, producing cardiac TNF-α, IL-1β, and IL-6 levels, LVP, +dP/dt, and rate of LVP fall (-dP/dt) values that were significantly better (P < 0.05) than values measured in burn plus sepsis in the absence of SDD. This work confirms that endogenous gut organisms contribute to susceptibility to subsequent infectious challenge.

oral antibiotics; postburn cardiac function; adult Sprague-Dawley rats; isolated cardiac myocytes; inflammatory cytokines; Streptococcus pneumoniae; intratracheal bacterial challenge

AN INFECTION-PREVENTION REGIMEN consisting of oral antibiotics to selectively decontaminate the digestive tract was introduced in 1984 (31). The use of selective decontamination of the digestive tract (SDD) is of particular interest for application in patients with major burn injury where the need for intubation and mechanical ventilation likely contributes to oropharyngeal and intestinal colonization with pathogenic microorganisms; in addition, nosocomial infection and pneumonia contribute significantly to morbidity and mortality in burn units (24, 29). Furthermore, burn injury produces a complex interaction of gut-derived inflammatory responses paralleled by systemic inflammatory responses that may render the burn patient more sensitive to a subsequent infectious challenge. In this study, we proposed that administration of SDD in rats during the early postburn period may decrease the chance of developing bacteremia and indirectly improve postburn outcome.

SDD, a strategy directed to eliminate aerobic microflora from the oropharynx and digestive tract, does not alter endogenous anaerobic flora (4, 9, 26). SDD has been used to prevent gut-derived infection in pancreatitis, liver transplantation, and trauma. Furthermore, SDD strategies have been shown to reduce mortality in critically ill patients, and patient survival was improved while the incidence of antibiotic resistance was lowered (4, 7, 9, 10, 16, 22, 26, 30).

Despite the abundance of evidence supporting routine use of SDD in critically ill patients, several investigators (6, 15) have argued against routine application of SDD, reasoning that this strategy may increase antimicrobial resistance and promote the emergence of antibiotic-resistant bacterial strains. In this regard, Lingnau and colleagues (17) examined bacterial resistance in a randomized, placebo-controlled trial of SDD in surgical intensive units; these investigators concluded that SDD could not be recommended as a prophylactic tool in critically ill patients.

We recently applied selective decontamination in an experimental model of burn trauma in adult rats and confirmed that this strategy applied either preburn (13) or implemented within 4 h after burn injury (32) attenuated cardiac myocyte secretion of inflammatory cytokines and provided remarkable protection against the cardiac dysfunction that routinely occurs 24 h postburn. However, the long-term benefits of SDD, particularly with regard to the effects of SDD on susceptibility to infection during the late postburn course, have not been studied. These data led us to design a study that would address the hypothesis that a pharmacological intervention that eradicated colonization of pathogenic organisms from the digestive tract in the early postburn period would decrease the sensitivity to a second insult such as Streptococcus pneumoniae challenge. We further proposed that SDD treatment after burn injury would indirectly improve cardiac performance and survival after septic challenge. Our study examined septic challenge on postburn day 7, and all experimental groups were studied 24 h after septic challenge on postburn day 8.

METHODS AND MATERIALS

Experimental model. Adult Sprague-Dawley male rats (320–350 g) obtained from Harlan Laboratories (Houston, TX) were conditioned in-house, for 5–6 days after arrival, with commercial rat chow and tap water available at will. All studies performed in this study were reviewed and approved by The University of Texas Southwestern...
Table 1. Experimental groups

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn</td>
<td>−</td>
<td>40%</td>
<td>40%</td>
<td>−</td>
<td>−</td>
<td>40%</td>
<td>40%</td>
</tr>
<tr>
<td>SDD</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intratracheal <em>Streptococcus pneumoniae</em></td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intratracheal vehicle</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

−, Groups not receiving specific interventions; +, groups receiving specific interventions; SDD, selective decontamination of the digestive tract; 40%, 40% total body surface area.

Medical Center’s Institutional Review Board for the care and handling of laboratory animals and conformed to all guidelines for animal care as outlined by the American Physiological Society and the National Institutes of Health.

**Catheter placement and burn procedure.** Rats were lightly anesthetized with isoflurane 18–20 h before the burn experiment, and body hair on the side, back, and neck was closely clipped. The neck region was treated with a surgical scrub, the left carotid artery was exposed, and a polyethylene catheter (PE-50) was inserted into the artery and advanced retrogradely to the level of the aortic arch. In addition, a polyethylene catheter (PE-50) placed in the right external jugular vein was used to administer fluids and drugs. All catheters were filled with heparinized saline, exteriorized, and secured at the nape of the neck. Eighteen hours after catheter placement, animals were deeply anesthetized (isoflurane), secured in a constructed template device, and the surface of the skin exposed through the aperture in the template was immersed in 100°C water for 10 s on the back and upper sides. Use of the template produced well-circumscribed full-thickness dermal burns over 40% of the total body surface area (TBSA). Exposure to this water temperature in adult rats destroys all underlying nerves and avoids injury to underlying organs. Sham burn rats were subjected to identical preparation, except that they were immersed in room temperature water to serve as controls. Immediately after immersion, rats were dried, returned to individual cages, and each external jugular catheter was connected to a swivel device (BSP99 Syringe Pump, Braintree Scientific, Braintree, MA) for fluid administration. Body temperature was measured with a rectal temperature probe (YSL-Tele Thermometer, model 44TA, Yellow Springs Laboratory, Herefordshire, UK), and respiratory rate was monitored by counting respiratory movement. Systemic blood pressure was measured intermittently during the first 24 h postburn to determine adequacy of fluid resuscitation using a Gould-Statham pressure transducer (model IDP23, Gould Instruments, Oxnard, CA) connected to a Grass medical recorder (model 7D Polygraph, Grass Instruments, Quincy, MA). A Grass tachycardiograph (model 7P4F) was used to monitor heart rate. A Grass Poly VIEW Data Acquisition System was used to convert acquired data into digital form. Blood pressure and heart rate were also measured 24 h after septic challenge (postburn day 8) in all experimental groups.

**Lactated Ringer resuscitation after burn injury.** Fluid resuscitation consisted of lactated Ringer solution, 4 ml/kg per percent burn with one-half of the calculated volume given intravenously during the first 8 h postburn and the remaining volume given over the next 16 h postburn. The total volume of Ringer given over the first 24 h postburn was 50–56 ml. Buprenorphine (0.5 mg/kg) was given every 12 h during the postburn period. Burned rats did not display discomfort or pain, moved freely about the cage, and consumed food and water within 15 min after recovering from isoflurane anesthesia. In the sham burn animals, the external jugular vein was cannulated, and lactated Ringer solution was given to maintain catheter patency (0.2 ml·kg\(^{-1}\)·h\(^{-1}\)); sham burns also received identical regimens of analgesics (buprenorphine) throughout the study period.

**Experimental groups.** Rats were randomly divided into sham and burn groups. In the rats designated for the burn groups, a full-thickness burn was accomplished over 40% TBSA, and lactated Ringer solution was initiated as described above. Four hours after lactated Ringer infusion was initiated, subgroups of sham burn and burn animals were divided to receive either no oral antibiotic therapy (vehicle, 1 ml water given by oral gavage, groups 1–4) or oral antibiotics (polymyxin E, 15 mg; tobramycin, 6 mg; 5-flucytosin, 100 mg given by oral gavage twice daily for 5 days after burn injury; groups 5–8) to achieve SDD. A total of eight experimental groups were produced (Table 1 and Fig. 1). These included group 1: sham burn, no antibiotics plus sham septic challenge on post-sham burn day 7; group 2: sham burn, no antibiotics plus intratracheal *S. pneumoniae* challenge on post-sham burn day 7 to produce septic challenge in the.
Table 2. Blood and BAL cultures and mortality in all experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood cultures</th>
<th>BAL cultures</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>9%</td>
</tr>
<tr>
<td>2</td>
<td>1.05x10^7±0.2x10^7</td>
<td>1x10^5±1x10^5</td>
<td>17%</td>
</tr>
<tr>
<td>3</td>
<td>1.72x10^7±0.1x10^7</td>
<td>1x10^5±1x10^5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3.0x10^7±1x10^7</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>5.3x10^7±1x10^7</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE of St. pneumoniae colony-forming units. BAL, bronchoalveolar lung aspirates used for culture. Blood and BAL cultures detected only S. pneumoniae; no other bacterial species were detected.

absence of previous burn injury; group 3: burn over 40% TBSA, no antibiotics, intratracheal vehicle on postburn day 7 to produce septic challenge complicated by previous burn injury; group 5: sham burn plus antibiotics for 5 days after sham burn, intratracheal vehicle on postburn day 7 to produce sham burn + SDD + sham septic challenge; group 6: sham burn given SDD as described for group 5 plus intratracheal S. pneumoniae on postburn day 7 (sham burn + SDD + septic challenge); group 7: burns given SDD plus intratracheal vehicle on postburn day 7 (burn + SDD + sham septic challenge); and group 8: burns given SDD plus intratracheal S. pneumoniae on postburn day 7 (burn + SDD + septic challenge). All animals were studied 24 h after intratracheal vehicle/S. pneumoniae challenge and on postburn (or post-sham burn) day 8 (Table 1 and Fig. 1). Rats were used to examine either myocardial function (Langendorff, n = 8–11 rats/group) or to prepare cardiomyocytes (n = 4–5 rats/group) to examine myocyte cytokine secretion and myocyte calcium and sodium handling (total of 12–16 rats/experimental group).

Preparation of bacterial inoculum. S. pneumoniae type 3 was obtained from the American Type Culture Collection (ATCC 6303, Manassas, VA) in lyophilized form. Bacteria were reconstituted and then passed through the cerebrospinal fluid of a rabbit to increase virulence; aliquots were prepared and stored at -80°C. Before each experiment, individual aliquots were thawed, inoculated into Muller Hinton broth with supplement C (Difco, Kansas City, MO), and incubated overnight at 37°C in the presence of 5% CO2. The broth was then centrifuged, and the resultant pellet was washed twice with sterile endotoxin-free PBS to remove any impurities adherent to the bacteria. The bacteria were then resuspended in sterile endotoxin-free PBS, agitated, and then drawn up into sterile tuberculin syringes in 0.4-ml aliquots. Bacterial colony-forming units (CFU) were determined by plating 100 µl of the bacterial suspension onto blood agar plates in serial dilutions and incubating the plates overnight at 37°C. The number of viable bacteria inoculated into animals in either the pneumonia alone or in the burn plus pneumonia groups was ~4x10^6 CFU (8, 21, 28, 33). With each batch of S. pneumoniae prepared on an experimental day, two to three animals from each group designated for septic challenge (groups 2, 4, 6, and 8) were inoculated as described above. This approach ensured that a single batch of bacteria was used for all experimental groups.

Induction of aspiration pneumonia. Seven days after either burn or sham burn, animals were again anesthetized with isoflurane, placed in a supine position, and the area over the trachea was prepped with a surgical scrub (povidine-iodine, Betadine). A midline incision was made over the trachea; the trachea was identified and isolated via blunt dissection. An aliquot of either bacterial suspension (4x10^6 CFU/0.4 ml) or sterile endotoxin-free PBS was injected directly into the trachea using a 27-gauge needle; the wound was then closed with surgical staples. Animals were placed on a 30° incline, with the head up, to ensure that the injected fluid entered the lungs. Anesthesia, intratracheal administration of bacteria or vehicle, and closure of the incision were completed within 4–5 min (8, 28, 33).

Cardiomyocyte isolation. Twenty-four hours after septic or sham septic challenge, animals from each experimental group (n = 4–5 rats/group) were heparinized, blood samples were collected, and rats were decapitated; hearts were harvested and placed in a petri dish containing room temperature heart medium [115 mM NaCl, 4.7 mM KCl, 0.6 mM KH2PO4, 0.6 mM Na2HPO4, 1.2 mM MgSO4, 12 mM NaHCO3, 10 mM KHCO3, 20 mM t-glucose, 0.5X minimum essential medium (MEM) amino acids (50x, Gibco/BRL, 11130–051), 10 mM HEPES, 30 mM taurine, 20 mM creatine]. Hearts were cannulated via the aorta and perfused with heart medium at a rate of 12 ml/min for a total of 5 min in a nonrecirculating mode.

Table 3. Hemodynamic and cardiodynamic responses to burn alone, septic challenge alone, or burn complicated by septic challenge measured on postburn day 8

<table>
<thead>
<tr>
<th>Oral Vehicle</th>
<th>Oral Antibiotics for SDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn + Sham</td>
<td>Burn + SDD + Sham Septic Challenge</td>
</tr>
<tr>
<td>In vivo</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>150.6±4.9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>523±12</td>
</tr>
<tr>
<td>pH</td>
<td>7.47±0.04</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>2.20±0.3</td>
</tr>
<tr>
<td>Base excess, mM</td>
<td>1.64±0.4</td>
</tr>
<tr>
<td>Ca²⁺, mM</td>
<td>1.29±0.01</td>
</tr>
<tr>
<td>In vitro</td>
<td></td>
</tr>
<tr>
<td>LVP, mmHg</td>
<td>96±4</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2,095±99</td>
</tr>
<tr>
<td>-dP/dt, mmHg/s</td>
<td>1,800±138</td>
</tr>
</tbody>
</table>

All values are means ± SE. MAP, mean arterial pressure; HR, heart rate; Ca²⁺, serum calcium; LVP, left ventricular pressure; +dP/dt and -dP/dt, rate of LVP rise and fall, respectively. ☆Difference from respective control group (group 1 or 5) at P < 0.05. †SDD-treated burns given septic challenge (group 8) different from burns given septic challenge but no SDD (group 4) (P < 0.05).
Enzymatic digestion was initiated by perfusing the heart with digestion solution that contained 34.5 ml of heart medium described above plus 50 mg of collagenase II (Worthington 4177, lot MOB3771), 50 mg BSA, fraction V (Gibco/BRL 11018–025), 0.5 ml trypsin (2.5%, 10×). Gibco/BRL 15090–046), 15 μM CaCl₂. Enzymatic digestion was accomplished by recirculating this solution through the heart at a flow rate of 12 ml/min for 20 min. All solutions perfusing the heart were maintained at a constant temperature of 37°C. At the end of the enzymatic digestion, the ventricles were removed and mechanically disassociated in 6 ml of enzymatic digestion solution containing a 6-ml aliquot of 2× BSA solution (2 mg BSA, fraction V to 100 ml of heart media). After mechanical disassociation with fine forceps, the tissue homogenate was filtered through a mesh filter into a conical tube. The cells adhering to the filter were collected by washing with an additional 10-ml aliquot of 1× BSA solution (100 ml of heart medium described above, and 1 g of BSA, fraction V). Cells were then allowed to pellet in the conical tube for 10 min. The supernatant was removed, and the pellet was resuspended in 10 ml of 1× BSA. The cells were washed and pelleted further in BSA buffer with increasing increments of calcium (100 μM, 200 μM, 500 μM, to a final concentration of 10×) for sham septic challenge controls or Streptococcus pneumoniae for septic challenge. The sham burn + sham septic challenge groups (groups 1 and 5) had n = 4 rats/group. All other groups had 5 rats/group. All values are means ± SE. *Significant difference from sham burns given sham septic challenge within each treatment group (i.e., groups 2–4 compared with group 1, and groups 6–8 compared with group 5). †Significant SDD-related effect (for example, group 1 vs. group 5, group 2 vs. group 6, etc.). ‡Significant difference in group 4 vs. either group 2 or group 3, indicating that burn + septic challenge had a greater effect than either septic challenge alone (group 2) or burn alone (group 3) at P < 0.05 (ANOVA, Student-Neuman-Keuls).
Table 4. Plasma cytokine levels in all experimental groups

<table>
<thead>
<tr>
<th>Group 1: Sham Burn + Septic Challenge</th>
<th>Group 2: Sham Burn + Septic Challenge</th>
<th>Group 3: Burn + Septic Challenge</th>
<th>Group 4: Burn + SDD + Septic Challenge</th>
<th>Group 5: Sham Burn + SDD + Septic Challenge</th>
<th>Group 6: Sham Burn + SDD + Septic Challenge</th>
<th>Group 7: Septic Challenge</th>
<th>Group 8: Burn + SDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α pg/ml</td>
<td>4.7±0.3</td>
<td>8.4±0.8*</td>
<td>5.0±0.9</td>
<td>19±0.2*</td>
<td>2.7±0.3†</td>
<td>3.6±0.3*</td>
<td>3.7±1.2†</td>
</tr>
<tr>
<td>IL-1β pg/ml</td>
<td>2.3±0.3</td>
<td>14.6±0.9*</td>
<td>4.0±0.2*</td>
<td>29±3*</td>
<td>1.3±0.1†</td>
<td>2.3±1.1†</td>
<td>3.1±0.4†</td>
</tr>
<tr>
<td>IL-6 pg/ml</td>
<td>59.6±4.4</td>
<td>326±28*</td>
<td>61±0.6†</td>
<td>337±10.2*</td>
<td>18±3.3†</td>
<td>26.1±4.4†</td>
<td>19±0.2†</td>
</tr>
<tr>
<td>IL-10 pg/ml</td>
<td>3.9±0.2</td>
<td>21.7±6.6*</td>
<td>11±0.5*</td>
<td>89±2.8*</td>
<td>2±0.1†</td>
<td>9.6±0.8*†</td>
<td>5.1±1.2†</td>
</tr>
</tbody>
</table>

All values are means ± SE. *Significant difference between groups and appropriate control, P < 0.05. †Significant difference between SDD-treated groups and groups given no SDD, P < 0.05.

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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 KH₂PO₄, and 11 glucose). All solutions were prepared each day with demineralized, deionized water and bubbled with 95% O₂-5% CO₂ (pH 7.4; PO₂, 550 Torr; PCO₂, 38 Torr). 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 38°C, and a constant-flow pump (Ismatec, model 7335–30, Cole-Parmer Instrument, Chicago, IL) was used to maintain perfusion of the coronary artery (ml/min) by retrograde perfusion of the aortic stump cannula. 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 P23ID, Gould Instruments, Oxnard, CA) attached to the balloon cannula, and the rate of LVP rise (+dP/dt) and fall (−dP/dt) were obtained using an electronic differentiator (model 7P20C, Grass Instruments, Quincy, MA) and recorded (Grass, model 7DWL8P). Left ventricular developed pressure was calculated from peak systolic LVP and left ventricular end-diastolic pressure. Data from the Grass recorder were input to a computer, and a Grass PolYVIEW Data Acquisition System was used to convert acquired data into digital form.

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. Cardiac function determined by the Langendorff preparation (including stabilization data) is expressed as the mean ± SE, and separate analyses were performed for each LVP, maximal +dP/dt (+dP/dt_max), and maximal −dP/dt (−dP/dt_max) as a function of treatment group and coronary flow rate using a repeated-measures ANOVA. Levene’s test for equality of variance was used to confirm that differences in the degree of lung inflammation related to the administration of SDD therapy, and there was no correlation between the degree of pulmonary inflammation and the presence or absence of previous burn injury. Pulmonary congestion and inflammation were evident from lung infiltrates and increased appearance of neutrophils in bronchoalveolar lavage. As shown in Table 1, all blood and bronchoalveolar lavage cultures from animals receiving intratracheal vehicle (no bacterial challenge in groups 1, 3, and 7) were negative for microorganisms. Blood cultures and bronchoalveolar lavage cultures were positive in the groups given no oral antibiotics plus bacterial challenge after sham burn injury (group 2) or S. pneumoniae challenge 7 days after a previous burn injury (group 4). SDD administration in group 6 (animals given intratracheal S. pneumoniae challenge after sham burn) and group 8 (burns given intratracheal S. pneumoniae challenge 7 days after a previous burn injury) was associated with negative blood cultures 24 h after intratracheal bacterial challenge despite the persistence of S. pneumoniae CFU in bronchoalveolar lavage (Table 2). Mortality in group 4 (burns given bacterial challenge in the absence of SDD therapy) was 17% 24 h after septic challenge compared with the 9% mortality observed after septic challenge in the absence of previous burn injury (group 2) (χ² = 0.2904, P = 0.589). In addition, mortality in burns given bacterial challenge in the absence of SDD therapy (group 4, 17% mortality) was not significantly higher than mortality measured in burns plus septic challenge in the presence of SDD therapy (group 8, 0% mortality) (χ² = 2.182, P = 0.139).

Hemodynamic and metabolic responses in the absence of antibiotic therapy. In the groups of animals given no antibiotics to selectively decontaminate the digestive tract (groups 1–4), intratracheal S. pneumoniae challenge in the absence of previous burn (group 2) or septic challenge after burn injury (group 4) were associated with a significant fall in mean arterial blood pressure (MAP) (group 2, 137 ± 5 mmHg; group 4, 101 ± 5 mmHg) measured 24 h after septic challenge and compared with values measured in sham-burn animals given intratracheal vehicle (group 1: 151 ± 4 mmHg, P < 0.05). MAP response to septic challenge was significantly lower in group 4 (burn + septic challenge) compared with group 2 (septic challenge in the absence of burn, P < 0.05) (Table 3).

In the absence of oral antibiotics to decontaminate the digestive tract, septic challenge in the absence of burn injury (group 2) produced a modest acidosis as indicated by the change in arterial lactate and change in base excess (Table 3). Burn complicated by septic challenge on postburn day 7 (group 4) was associated with a significant metabolic acidosis as indicated by the rise in arterial lactate and change in base excess, and these metabolic responses in burn + septic challenge were greater than responses observed after septic challenge alone (P < 0.05). Septic challenge in the absence of a previous burn injury (group 2) or as a complication of burn injury (group 4) was associated with a significant fall in serum ionized calcium levels (Table 3). There was no significant difference in arterial PO₂, PCO₂, or bicarbonate levels among experimental groups (P > 0.05, data not shown).

**RESULTS**

All animals survived for 7 days after either sham burn or burn injury alone. On day 7, groups of both sham burn and burn animals were given intratracheal S. pneumoniae, 4 × 10⁶ CFU, a bacterial challenge that has been shown previously to produce positive blood cultures within 24 h after intratracheal challenge (8, 28). Blood samples and lung lavage samples were cultured for S. pneumoniae 24 h after intratracheal bacterial challenge. All animals given S. pneumoniae challenge had evidence of inflammation as evidenced by pulmonary inflammation that was moderate to severe. There was no difference in the degree of lung inflammation related to the administration of SDD therapy, and there was no correlation between the degree of pulmonary inflammation and the presence or absence of previous burn injury. Pulmonary congestion and inflammation

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**Fig. 4.** Left ventricular function assessed in several experimental groups as either preload (A) or perfuse calcium (B) was incrementally increased. Septic challenge after previous burn injury in the absence of SDD (group 4) produced significant contractile depression, and this contractile dysfunction was significantly attenuated by post-burn SDD treatment (group 8, P < 0.05). SDD treatment in sham burns (group 5) had no significant effect on ventricular function compared with that measured in sham-burned animals given oral vehicle (group 1). Both sham groups (groups 1 and 5) had n = 8 rats/group. All other groups had n = 10–11 rats/group. All values are means ± SE. LVP, left ventricular pressure; +dP/dt_max, maximal rate of LVP rise; −dP/dt_max, maximal rate of LVP fall. *Significant difference from group 1 (sham burn + sham septic challenge + no SDD) and group 5 (sham burn + sham septic challenge + SDD) at P < 0.05. + Difference between groups 4 and 8 at P < 0.05 (ANOVA, Student-Neuman-Keuls).
Effects of SDD on hemodynamic and metabolic response to burn and/or septic challenge. SDD administration (groups 5–8) ablated the fall in MAP associated with either septic challenge alone (group 6) or with septic challenge + previous burn injury (group 8). SDD therapy after burn injury also prevented the septic challenge-related bradycardia. SDD therapy in group 6 (septic challenge alone) or in group 8 (burn complicated by S. pneumoniae challenge) attenuated the rise in arterial lactate and change in base excess but failed to alter serum ionized calcium responses to burn and or septic challenge.

Cardiomyocyte secretion of inflammatory cytokines. As shown in Fig. 2, septic challenge in the absence of previous burn (group 2) produced significant TNF-α (Fig. 2A), IL-1β (Fig. 2B), IL-6 (Fig. 2C), and IL-10 (Fig. 2D) secretion by cardiomyocytes compared with values measured in cardiomyocytes prepared from sham burns given intratracheal vehicle (group 1). Animals in group 3 (burn injury + sham septic challenge) had increased cardiomyocyte secretion of inflammatory cytokines, an effect that was likely related to the burn injury 8 days earlier. Intratracheal S. pneumoniae challenge on day 7 after a previous burn injury (group 4) produced significantly greater cardiomyocyte secretion of TNF-α, IL-1β, IL-6, and IL-10 compared with cytokine secretion by cardiomyocytes prepared from animals given a septic challenge in the absence of a previous burn injury (group 2, P < 0.05). Systemic inflammation occurred after septic challenge alone (group 2) as well as after burn plus septic challenge (group 4) as indicated by the rise in plasma TNF-α, IL-1β, and IL-6 levels (Table 4); this inflammatory response was paralleled by an anti-inflammatory response (rise in plasma IL-10, P < 0.05).

Effects of SDD therapy on cardiomyocyte and systemic cytokine levels. As shown in Fig. 2, SDD treatment after sham burn followed by S. pneumoniae challenge on day 7 after sham burn (group 6) blunted TNF-α, IL-1β, and IL-6 secretion by cardiomyocytes compared with values measured in group 2 animals given sham burn plus intratracheal S. pneumoniae challenge in the absence of SDD (P < 0.05). SDD treatment after burn injury also blunted inflammatory cytokine responses to a subsequent S. pneumoniae challenge (group 8) compared with responses seen after S. pneumoniae challenge in the absence of SDD therapy (group 4, P < 0.05). Similarly, SDD therapy attenuated the systemic pro- and anti-inflammatory responses (Table 4), producing plasma cytokine levels that were similar to levels measured in animals given sham burn + sham sepsis (group 1).

Cardiomyocyte calcium and sodium responses to burn injury and SDD therapy. In the absence of SDD treatment, S. pneumoniae challenge on postburn day 7 (group 4) produced significantly greater cardiomyocyte accumulation of calcium (Fig. 3, top) and sodium (Fig. 3, bottom) compared with cation derangements in animals given septic challenge in the absence of a previous burn injury (group 2, P < 0.05). Oral antibiotic therapy significantly attenuated the sodium and calcium accumulation by cardiomyocytes in either sham burns given septic challenge (group 6) or in burns complicated by subsequent bacterial challenge (group 8) compared with calcium and sodium levels measured after septic challenge in the absence of SDD (groups 2 and 4, P < 0.05).

Cardiac contractile function after burn and/or septic challenge. In the absence of SDD therapy, septic challenge alone (group 2) and septic challenge after a previous burn injury (group 4) produced a significant fall in left ventricular developed pressure (LVP) as well as decreases in ±dP/dt max responses (P < 0.05). As shown in Table 3, there was significant cardiac contractile dysfunction in group 2 (sepsis alone) and in group 4 (burn + sepsis) compared with that seen after sham burn + sham septic challenge (group 1) as indicated by the significantly lower LVP and ±dP/dt max during stabilization of the hearts at a constant preload, constant heart rate, and constant coronary flow rate (P < 0.05). Cardiac contraction and relaxation defects were greater (P < 0.05) in burn + sepsis (group 4) compared with sepsis alone (group 2).

The greater cardiac contractile dysfunction (P < 0.05) in group 4 compared with group 2 was also evident from the examination of left ventricular function curves that describe LVP and ±dP/dt responses to incremental increases in left ventricular volume or increases in perfusate calcium (data not shown).

Effects of SDD therapy on cardiac responses to burn and/or septic challenge. SDD therapy attenuated the fall in LVP and ±dP/dt max seen with septic challenge, producing values in the group given S. pneumoniae challenge alone (group 6) and in the group given S. pneumoniae challenge after a previous burn injury (group 8) that were significantly improved compared with values measured in the respective septic-challenged groups given no SDD (groups 2 and 4, respectively, P < 0.05) (Table 3). That SDD improved myocardial contraction and relaxation was also evident from the LVP and ±dP/dt responses to incremental increases in left ventricular volume, as well as LVP and ±dP/dt responses to increases in perfusate calcium. SDD therapy improved myocardial contraction and relaxation responses in group 6 compared with values measured in the absence of SDD (group 2, P < 0.05). A significant SDD-related improvement in all indexes of myocardial contraction and relaxation was evident in group 8 compared with function measured in animals given identical burn injury plus septic challenge but no SDD therapy (group 4, P < 0.05) (Fig. 4, A and B).

DISCUSSION

In this present study, administration of oral antibiotics to selectively decontaminate the digestive tract after burn over 40% of TBSA in adult rats significantly attenuated the systemic as well as myocardial pro- and anti-inflammatory responses to a second hit (S. pneumoniae challenge) on postburn day 7 as indicated by the decreases in TNF-α, IL-1β, IL-6, and IL-10 secretion. The SDD-related decrease in cardiac inflammation was associated with attenuation of cardiac myocyte loading of calcium and sodium and a significant improvement in myocardial contraction and relaxation. These data suggest that oral antibiotic therapy after the initial injury decreased sensitivity to a second infectious challenge and indirectly decreased the cardiac inflammation and dysfunction associated with a septic challenge.

In this present study, septic challenge in the absence of SDD produced positive blood cultures (groups 2 and 4), while administration of antibiotics to selectively eliminate gram-negative aerobes from the digestive tract prevented bacteremia...
as indicated by the negative blood cultures (groups 6 and 8). The absence of systemic bacteremia after S. pneumoniae challenge in SDD-treated rats was attributed to decreased sensitivity to the infectious challenge. Thus SDD after burn injury decreased the chances of developing bacteremia and indirectly improved cardiac function.

Susceptibility to gram-negative and gram-positive infection, multiple organ dysfunction, and increased mortality continue to be major problems in burn units. Recent evidence has accumulated that bacteria produce immune signaling cascades that culminate in multiple organ failure by binding to a specific set of membrane receptors described as the Toll-like family of receptors (TLR). Gram-negative bacteria and the bacterial cell membrane component lipopolysaccharide (LPS) bind to LPS binding protein and CD14, which, in turn, transmit a signal through the TLR4 receptor to evoke a number of intracellular signaling mechanisms, while gram-positive bacteria bind the TLR2 class of receptors. Recent experimental and clinical data have suggested that gut-derived bacteria and/or endotoxin play a significant role in the pathogenesis of postburn sepsis and multiple organ dysfunction (5, 11, 13, 14). That gut-derived bacteria play a role in down syndrome inflammatory signaling and multiple organ failure has been supported by studies by us and others confirming that interventions such as mesenteric duct ligation or therapeutic strategies that eradicate aerobic microorganisms from the digestive tract provide significant protection after major burns (1, 2, 13, 25, 32). SDD was first introduced in 1984 as a strategy to decrease morbidity and mortality in intensive care units (31). Nonabsorbed antibiotics can be administered orally or applied through a nasogastric tube (7, 9, 22, 27), and several investigators have described that SDD treatment in intensive care patients decreased ICU stay and mortality (9, 10, 16, 22, 26, 30), reduced wound colonization, prevented postburn infection (18, 19, 20), and decreased the incidence of respiratory tract infection (30). In contrast, Barret et al. (3) described that SDD did not effectively decrease either bacterial colonization or infectious episodes in severely burned pediatric patients, while Kollef (15) described that SDD was associated with the emergence of antibiotic-resistant bacterial strains, limiting its overall utility.

That the intestinal tract is one source of gram-negative bacilli, as well as Enterococcus, Clostridium, and Candida species, is not a new concept. It is also well accepted that either clinical or experimental burn injury disrupts gut barrier function (5, 11, 14), allowing dissemination of bacteria from the intestinal tract and colonization of downstream organs (20). Strategies such as SDD would be expected to interrupt the spread of these pathogens and may be of particular relevance in reducing sensitivity to a subsequent infectious challenge or reducing the chance of developing bacteremia (13, 23, 34, 35).

In our animal model, SDD therapy prevented systemic bacteremia after S. pneumoniae challenge, producing negative blood cultures 24 h after septic challenge in SDD-treated rats (groups 6 and 8) despite intratracheal administration of a nearly identical number of S. pneumoniae CFU on postburn day 7. Furthermore, the number of CFU of S. pneumoniae measured in bronchoalveolar lavage fluid 24 h after septic challenge was not significantly different among groups given intratracheal bacterial challenge (P < 0.05), regardless of SDD treatment. While we considered that SDD may have reduced sensitivity to a subsequent infectious challenge, we also considered that bacterial killing was increased as a direct antibiotic effect of SDD therapy or by an indirect mechanism that preserved innate killing mechanisms. In our study, SDD therapy decreased both plasma and cardiac myocyte-secreted inflammatory cytokine levels; this reduced inflammatory response was paralleled by improved cardiac performance in SDD-treated animals, and these data are consistent with previous reports that inflammatory cytokines are primary mediators of postburn myocardial contraction and relaxation defects (12, 13). In our study, the SDD-related decrease in IL-10 response was attributed to the SDD-mediated attenuation of inflammatory responses and a decrease in compensatory anti-inflammatory responses.

Since infection is a common source of morbidity and mortality in critically burned patients, we developed an experimental model that would allow us to examine the role of SDD in burn complicated by septic challenge. The limits of extrapolating data from a rat model to the clinical scenario must be considered. Rats are particularly resistant to septic challenge and required far greater bacterial load to produce infectious-related complications. However, burn injury in rats alters gut barrier function, promoting bacterial translocation to downstream organs (2, 5, 11, 14), a finding consistent with bacterial dissemination from the intestinal tract after clinical burns (20).

In summary, the data from this present study suggest that the intestinal tract serves as an important source of potential pathogens, and elimination of these pathogens decreases susceptibility to subsequent infection and reduces the exaggerated inflammatory responses that occur with septic challenge after an initial burn injury. Our data further suggest that the application of standard antibiotic therapy to decontaminate the digestive tract after major injury may be a useful strategy in patient populations at increased risk for infectious complications after an initial injury.

GRANTS
This study was supported by National Institutes of Health Grant 5P50-GM-21681–40.

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