Cardiac effects of burn injury complicated by aspiration pneumonia-induced sepsis

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White, Jean, James Thomas, David L. Maass, and Jureta W. Horton. Cardiac effects of burn injury complicated by aspiration pneumonia-induced sepsis. Am J Physiol Heart Circ Physiol 285: H47–H58, 2003. First published March 13, 2003; 10.1152/ajpheart.00833.2002.—Early fluid resuscitation, antimicrobials, early excision, and grafting have improved survival in the early postburn period; however, a significant incidence of pneumonia-related sepsis occurs after burn injury, often progressing to multiple organ failure. Recent studies have suggested that this initial injury (burn injury) primes the subject, producing an exaggerated response to a second insult, such as pneumonia-related sepsis. We developed an experimental animal model that included a third-degree burn over 40% of the total body surface area, followed by sepsis (intratracheal administration of Streptococcus pneumoniae, 4 × 106 colony-forming unit), which was produced either 48 or 72 h after burn injury in adult male rats. Hearts harvested after either burn alone, sepsis alone, or burn plus sepsis were used to assess either contractile function (Langendorff) or cardiomyocyte secretion of tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-10 (ELISA). Experimental groups included the following: 1) sham (sham burn and no sepsis); 2) burn injury alone studied either 24, 48, or 72 h postburn; 3) pneumonia-related sepsis in the absence of burn injury; and 4) pneumonia-induced sepsis studied either 48 or 72 h after an initial burn injury. Burn injury alone (24 h) or sepsis alone produced myocardial contractile defects and increases in pro- and anti-inflammatory cytokine secretion by cardiomyocytes. Sepsis that occurred 48 h postburn exacerbated the cardiac contractile defects seen with either burn alone or sepsis alone. Sepsis that occurred 72 h postburn produced contractile defects resembling those seen in either burn alone or sepsis alone. In conclusion, our data suggest that burn injury primes the subject such that a second insult early in the postburn period produces significantly greater cardiac abnormalities than those seen with either burn alone or sepsis alone.

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burn injury. A review of our burn patient population suggested that pneumonia-related sepsis occurs 2–10 days after admission to the burn unit, and the effects of delayed sepsis after burn injury on cardiac inflammatory cytokine response, cation redistribution, and cardiac contractile function have not been examined.

Therefore, the purpose of this present study was to examine cardiac contractile responses in a rodent model of burn injury complicated by sepsis. This model included intratracheal administration of Streptococcus pneumoniae in rats with a previous burn injury >40% total body surface area. We hypothesized that burn injury complicated by pneumonia-induced sepsis would produce greater cardiac dysfunction than either burn injury alone or sepsis alone.

METHODS AND MATERIALS

Experimental model. Adult Sprague-Dawley male rats (320–350 g) were used in the present study. The animals (Harlan Laboratories; Houston, TX) were conditioned in house for 5 or 6 days after arrival, and commercial rat chow and tap water were provided at will. All studies performed in this study were reviewed and approved by The University of Texas Southwestern 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 anesthetized lightly with methoxyflurane 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, and the wet carotid artery was exposed, and a polyethylene-50 (PE-50) catheter inserted into the artery was advanced retrogradely to the level of the aortic arch. In addition, a PE-50 catheter 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, the animals were deeply anesthetized with methoxyflurane and secured in a set-up to ensure that 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 (4 × 10^6 CFU/0.3 ml) or sterile endotoxin-free PBS was injected directly into the trachea using a 23-gauge needle; the wound was then closed with surgical staples. Animals were observed to move freely about the cage and consumed food and water within 20 min after recovering from anesthesia. In the sham burn group, 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. Body temperature was measured with a rectal temperature probe (model 44TA; YSL-Tele Thermometer) and respiratory rate was monitored by counting respiratory movement. Systemic blood pressure was measured with the use of a pressure transducer (model IDP23, Gould Instruments; Oxnard, CA) connected to a medical recorder (model 7D polygraph, Grass Instruments; Quincy, MA). A tachycardiograph (model 7P4F, Grass Instruments) was used to monitor heart rate. Data from the Grass recorder were input to a Dell Pentium computer, and a data-acquisition system (Poly VIEW, Grass) was used to detect heart sound and convert them to computer-readable data. A tachycardiograph (model 7P4F, Grass Instruments) was used to monitor heart rate.

Preparation of inoculum. Streptococcus pneumoniae type 3 was obtained from the American Type Culture Collection (ATCC 6303, Rockville, MD) 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 phosphate-buffered saline (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 six sterile tubing syringes in 0.5-ml aliquots. Bacterial colony-forming units (CFU) were determined by platting 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 group or in the burn plus pneumonia group was 3.0 ± 2.8 × 10^8 CFU (6, 26, 41). Preliminary studies showed that the tracheal injection of nonviable bacteria (N = 4) or PBS (N = 3) produced no ill effects and no cardiac dysfunction (unpublished data).

Induction of aspiration pneumonia. At the designated time after either burn or sham burn (described under Experimental Groups; see also Fig. 7), animals were again anesthetized with methoxyflurane and 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 (4 × 10^6 CFU/0.3 ml) or sterile endotoxin-free PBS was injected directly into the trachea using a 23-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.

Experimental groups. Twenty-four hours after catheter placement, rats were randomly divided to receive either burn or sham burn and injury >40% of the total body surface area (Fig. 7). All burns received standard fluid resuscitation (lactated Ringer solution) and analgesic, as described in Catheter placement and burn procedure. Sham burns and burns were then randomly assigned to either protocol 1 or protocol 2 (Fig. 7). Under protocol 1, sham burns were divided into subgroups...
to receive either intratracheal vehicle (0.3 ml PBS) to produce a sham burn/sham sepsis group (group 1) or were given intratracheal *S. pneumoniae* (4 × 10⁶ CFU/0.3 ml PBS) to produce sepsis in the absence of burn injury (group 2). Similarly, groups of burned rats under protocol 1 were given either intratracheal vehicle to produce burn injury in the absence of sepsis (group 3) or *S. pneumoniae* (4 × 10⁶ CFU/0.3 ml PBS) to produce burn injury complicated by early sepsis (sepsis produced 48 h postburn, group 4).

Under protocol 2, *S. pneumoniae* administration was delayed until 48 h after burn injury. Groups included sham burns given intratracheal PBS (group 5, sham burn, sham sepsis) and sham burns given intratracheal *S. pneumoniae* to produce sepsis in the absence of burn injury (group 6). Similarly, burns given intratracheal vehicle allowed us to examine burn in the absence of sepsis (group 7), whereas burned rats given intratracheal *S. pneumoniae* and studied 72 h postburn allowed us to examine the effects of burn injury complicated by delayed sepsis (group 8). Animals under both experimental protocols 1 and 2 were studied 24 h after intratracheal administration of *S. pneumoniae*, regardless of the time of a previous burn injury (Fig. 7). Additional rats (groups 9A and 9B, N = 8) were studied 48 and 72 h, respectively, after burn injury in the absence of sepsis to examine the resolution of cardiac deficits after major burn. The development of sepsis in our rat model was determined from histological evidence of pulmonary infection and inflammation, the presence of positive blood cultures, and a rise in body temperature ≥1.5°C.

Isolated coronary perfused hearts. Twenty-four hours after intratracheal challenge, rats from each experimental group (N = 7–9 rats/group) were heparinized, and a blood sample was collected to measure circulating cytokines and to determine the presence/absence of circulating bacteria. One lung was harvested and placed in ice-cold Ca²⁺-free Tyrode solution composed of (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/glucose. All solutions were prepared each day with demineralized, deionized water and bubbled with 95% O₂–5% CO₂/glucose.

Cardiomyocyte isolation. Similarly, 24 h after intratracheal challenge, additional animals from each experimental group (N = 4–5 rats/group) were heparinized, blood samples were collected, and rats were decapitated; the heart was removed through a medial sternotomy with the use of sterile techniques. The isolated heart was immediately placed in ice-cold Ca²⁺-free Tyrode solution composed of (in mM) 136 NaCl, 5 KCl, 0.57 MgCl₂, 0.33 NaH₂PO₄, 10 HEPES, and 10 glucose. The aorta was cannulated within 60 s, and the excised heart was perfused for 5 min in a Langendorff mode. Perfusion was then switched to a collagenase-containing solution composed of 80 ml of Ca²⁺-free Tyrode and 40 mg collagenase A (0.05%, Boehringer Mannheim, Indianapolis, IN) plus 4 mg of protease, Polysaccharide XIV (Sigma; St. Louis, MO) with continuous oxygenation (95% O₂–5% CO₂). After enzymatic digestion over an 8-min period, the heart was removed from the cannula. Ventricular tissue was separated from the base of the heart, placed in a petri dish containing 100 μM Ca²⁺ Tyrode, and gently minced to increase cell dispersion over 6 min. The myocyte suspension was filtered and the cells were allowed to settle. This rinsing and settling step was repeated three times with 10 min between each step and with gentle swirling between each step to allow myocyte separation. The [Ca²⁺] of the rinsing solution was gradually increased during these steps from 100 μM to 200 μM and finally to 1.8 mM. The cell viability was measured and cell suspensions with >85% viability were used for subsequent studies. Myocytes with a rodlike shape, clear defined edges, and sharp striations were prepared with a final cell count of 5 × 10⁴ cells/ml−1·well−1 (16, 18).

Cytokine secretion by cardiomyocytes. Myocytes were pipetted into microtiter plates at 5 × 10⁴ cells·ml⁻¹·well⁻¹ (12-well cell culture cluster, Corning; Corning, NY) for 18 h (CO₂ incubator at 37°C). Supernatants were collected to measure myocyte-secreted tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 (rat ELISA, Endogen; Woburn, MA). We previously examined the contribution of contami-

<table>
<thead>
<tr>
<th>Groups 1 and 5</th>
<th>Groups 3 and 7</th>
<th>Groups 9A 48-h Burn Alone</th>
<th>Groups 9B 72-h Burn Alone</th>
<th>Groups 2 and 6</th>
<th>Groups 4 Sepsis 48-h Postburn</th>
<th>Group 8 Sepsis 72-h Postburn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>4.8 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>8.4 ± 0.9†‡</td>
<td>70.0 ± 8.9†‡</td>
<td>123.5 ± 13.3*‡</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.6 ± 0.3</td>
<td>2.3 ± 1</td>
<td>17.3 ± 0.9*</td>
<td>29.2 ± 3*</td>
<td>290.5 ± 64.8*†‡</td>
<td>793.2 ± 62.9*‡</td>
</tr>
<tr>
<td>IL-1β</td>
<td>8.3 ± 0.6*</td>
<td>12.8 ± 0.6*</td>
<td>11.3 ± 0.6</td>
<td>89.4 ± 2.8*</td>
<td>225.6 ± 60†</td>
<td>579 ± 12.3†‡</td>
</tr>
<tr>
<td>IL-6</td>
<td>9.0 ± 4.4</td>
<td>485.5 ± 31.4</td>
<td>524.6 ± 20.6*</td>
<td>796.6 ± 33.3*</td>
<td>2483.1 ± 534*‡</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>5.9 ± 0.3</td>
<td>13.6 ± 1.4</td>
<td>54.5 ± 4.5*</td>
<td>89.4 ± 2.8*</td>
<td>225.6 ± 60†</td>
<td>579 ± 12.3†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. TNF-α, tumor necrosis factor-α; IL, interleukin. *P < 0.05, significant difference among groups; †P < 0.05, significant difference between sepsis alone (groups 2 and 6) and sepsis complicated by burn (groups 4 and 8); ‡P < 0.05, significant difference between groups 4 and 8 (t-test).
Table 2. *In vivo* hemodynamic and metabolic responses to burn sepsis

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>Groups 3 and 7</th>
<th>Group 9A 48h Burn Alone</th>
<th>Group 9B 72h Burn Alone</th>
<th>Groups 2 and 6 Sepsis Alone</th>
<th>Group 4 Sepsis 48h Postburn</th>
<th>Group 8 Sepsis 72h Postburn</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>139 ± 4</td>
<td>111 ± 8*</td>
<td>121 ± 3*</td>
<td>130 ± 4</td>
<td>130 ± 5</td>
<td>91 ± 17†</td>
<td>137 ± 15‡</td>
</tr>
<tr>
<td>BT, °C</td>
<td>38.7 ± 0.1</td>
<td>38.8 ± 0.1</td>
<td>39.0 ± 0.1</td>
<td>38.9 ± 0.1</td>
<td>38.6 ± 0.1</td>
<td>38.4 ± 0.2</td>
<td>38.6 ± 0.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>506 ± 7</td>
<td>500 ± 5</td>
<td>505 ± 5</td>
<td>497 ± 12</td>
<td>468 ± 12</td>
<td>508 ± 10</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.48 ± 0.01</td>
<td>7.49 ± 0.02</td>
<td>7.49 ± 0.01</td>
<td>7.49 ± 0.01</td>
<td>7.49 ± 0.03</td>
<td>7.41 ± 0.03†</td>
<td>7.51 ± 0.03‡</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>30 ± 0.5</td>
<td>26 ± 1*</td>
<td>28 ± 0.05*</td>
<td>29 ± 3</td>
<td>25 ± 1*</td>
<td>27 ± 3*</td>
<td>26 ± 2*</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>106 ± 4</td>
<td>113 ± 4</td>
<td>102 ± 4</td>
<td>96 ± 5</td>
<td>105 ± 5</td>
<td>103 ± 5</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>Hct, %</td>
<td>45.7 ± 0.4</td>
<td>31.7 ± 2.4*</td>
<td>30.3 ± 1.9*</td>
<td>30.9 ± 3.5*</td>
<td>41.0 ± 3.3</td>
<td>34.2 ± 3.5†</td>
<td>36.7 ± 1.1†</td>
</tr>
<tr>
<td>Ca2⁺, mmol/l</td>
<td>1.21 ± 0.02</td>
<td>0.87 ± 0.04*</td>
<td>0.81 ± 0.04*</td>
<td>1.18 ± 0.02</td>
<td>1.0 ± 0.04*</td>
<td>0.66 ± 0.05†</td>
<td>1.0 ± 0.02*</td>
</tr>
<tr>
<td>Base excess, mmol/l</td>
<td>3.2 ± 0.5</td>
<td>-2.1 ± 0.7*</td>
<td>1.5 ± 0.8*</td>
<td>2.5 ± 0.3</td>
<td>-1.0 ± 0.7*</td>
<td>-8.1 ± 3.0*</td>
<td>-4.2 ± 2.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; BT, body temperature; HR, heart rate; PaO2, arterial PO2; Hct, hematocrit. *P < 0.05, significant difference from respective control; †P < 0.05, significant difference from sepsis alone; ‡P < 0.05, significant difference between groups 4 and 8 (t-test).

Table 3. *In vitro* stabilization data from perfused hearts

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham (N = 12)</th>
<th>Groups 3 and 7 24 h Burn Alone (N = 8)</th>
<th>Group 9A 48 h Burn Alone (N = 9)</th>
<th>Group 9B 72 h Burn Alone (N = 7)</th>
<th>Groups 2 and 6 Sepsis Alone (N = 9)</th>
<th>Group 4 Sepsis 48 h Postburn (N = 8)</th>
<th>Group 8 Sepsis 72 h Postburn (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP, mmHg</td>
<td>97 ± 2</td>
<td>65 ± 2*</td>
<td>87 ± 3</td>
<td>88 ± 5</td>
<td>66 ± 3*</td>
<td>54 ± 2*</td>
<td>63 ± 2‡*</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2,074 ± 54</td>
<td>1,321 ± 90*</td>
<td>1,772 ± 96</td>
<td>1,889 ± 84</td>
<td>1,356 ± 53*</td>
<td>1,270 ± 55†</td>
<td>1,356 ± 72‡</td>
</tr>
<tr>
<td>-dP/dt, mmHg/s</td>
<td>1,774 ± 49</td>
<td>999 ± 96*</td>
<td>1,591 ± 83</td>
<td>1,683 ± 78</td>
<td>1,166 ± 59*</td>
<td>957 ± 92†</td>
<td>1,054 ± 78‡</td>
</tr>
<tr>
<td>DP, mmHg/s</td>
<td>1,802 ± 45</td>
<td>1,260 ± 108*</td>
<td>1,584 ± 96</td>
<td>1,478 ± 78</td>
<td>1,184 ± 53*</td>
<td>1,150 ± 66*</td>
<td>1,163 ± 62‡</td>
</tr>
<tr>
<td>Time to +dP/dt, ms</td>
<td>55 ± 4</td>
<td>50 ± 1</td>
<td>51 ± 1</td>
<td>55 ± 3</td>
<td>53 ± 1</td>
<td>50 ± 1</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>Time to -dP/dt, ms</td>
<td>53 ± 2</td>
<td>50 ± 1</td>
<td>50 ± 1</td>
<td>54 ± 1</td>
<td>51 ± 1</td>
<td>50 ± 1</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>51 ± 5</td>
<td>56 ± 6</td>
<td>52 ± 3</td>
<td>54 ± 2</td>
<td>47 ± 3</td>
<td>44 ± 3</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>CVR, mmHg</td>
<td>102 ± 11</td>
<td>96.2 ± 0.91</td>
<td>10.4 ± 0.6</td>
<td>10.8 ± 1.3</td>
<td>9.5 ± 0.5</td>
<td>8.5 ± 0.6</td>
<td>10.3 ± 0.6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>250 ± 13</td>
<td>250 ± 8</td>
<td>249 ± 4</td>
<td>261 ± 13</td>
<td>261 ± 7</td>
<td>240 ± 2</td>
<td>278 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of rats per experimental group. LVP, left ventricular pressure; +dP/dt, maximal rate of LVP rise; -dP/dt, maximal rate of LVP fall; DP, developed pressure at 40 mmHg; CPP, coronary perfusion pressure; CVR, coronary vascular resistance. *P < 0.05, significant difference from the control group; †P < 0.05, significant difference from sepsis in the absence of burn injury; ‡P < 0.05, significant difference between groups 4 and 8 (t-test).
No evidence of burn-related pain and no mortality with burn injury in the absence of sepsis over the 72-h postburn. Intratracheal *S. pneumoniae* challenge produced consistent evidence of pulmonary infection and inflammation ranging from moderate to severe. Blood and BAL cultures from rats with either sepsis in the absence of burn (groups 2 and 6) or in burn complicated by sepsis (groups 4 and 8) had positive blood and BAL cultures 24 h after intratracheal *S. pneumoniae* challenge. There was no significant difference in the CFU measured in the BAL from experimental groups; similarly, there was no significant difference in the number of CFU measured in blood of rats given intratracheal *S. pneumoniae*, regardless of the presence/absence of a previous burn injury. In all rats given intratracheal *S. pneumoniae*, histological confirmation of pneumonia was provided by examining lung tissue harvested from each experimental animal. The lungs of rats in the sham burn/sham sepsis groups (groups 1 and 5) and in rats with burn injury in the absence of sepsis (groups 3 and 7) were histologically normal, and blood and BAL cultures from these groups were negative for *S. pneumoniae*. There were no deaths with burn alone (groups 3 and 7); mortality in sepsis alone (groups 2 and 6) was 22%, whereas mortality in burn complicated by sepsis (groups 4 and 8) was 29%.

Fig. 1. A: left ventricular (LV) performance measured in hearts from rats given an initial sham burn injury, followed either 24 or 48 h by sham sepsis. Sham sepsis was produced by intratracheal (IT) injection of phosphate-buffered saline (PBS). There were no significant differences in ventricular function measured in these two experimental groups (*N* = 7–8 hearts/group). B: sepsis produced significant cardiac contraction and relaxation defects regardless of the time that sepsis was induced after a burn injury. LVP, LV developed pressure; +dP/dt max, maximal rate of LVP rise; −dP/dt max, the maximal rate of LVP fall. All values are means ± SE. *P* < 0.05, significant difference among groups (ANOVA and multiple-comparison procedure). There were 7–9 animals per experimental group.
Circulating TNF-α, IL-1β, IL-6, and IL-10 levels. Burn injury in the absence of sepsis produced a modest increase in circulating IL-1, whereas IL-6 and IL-10 progressively rose over the postburn period (Table 1). Similarly, sepsis in the absence of burn injury produced a rise in plasma cytokine levels and this cytokine response was exacerbated when sepsis was preceded by a major burn injury (Table 1). Because all rats given intratracheal S. pneumoniae had similar blood levels of bacteria, there was no correlation between circulating cytokine levels and blood levels of bacteria.

Hemodynamic-metabolic responses to burn sepsis. Mean arterial blood pressure fell 24 h after burn injury despite aggressive fluid resuscitation (Table 2); blood pressure remained below sham values 48 h postburn but recovered 72 h after burn injury in the absence of sepsis. After burn injury alone, hematocrit and serum ionized Ca$^{2+}$ levels fell significantly ($P < 0.05$). The fall in PaCO$_2$ was paralleled by an increase in respiratory rate (from 13 ± 2 to 19 ± 1 breaths/min, $P < 0.05$). Hemodynamic and metabolic function gradually returned to sham values by 72 h postburn. Sepsis in the absence of burn injury was associated with significantly lower arterial PaCO$_2$, and lower serum-ionized Ca$^{2+}$ levels compared with values measured in sham burns. Sepsis produced 48 h after burn injury produced a significantly lower mean arterial blood pressure, heart rate, PaCO$_2$, hematocrit, and serum-ionized Ca$^{2+}$ levels compared with time-matched values measured in sham burns (groups 1 and 5). Similarly, sepsis produced 72 h after a previous burn injury was characterized by a lower PaCO$_2$, hematocrit, and serum-ionized Ca$^{2+}$ levels compared with values measured in sham burns ($P < 0.05$, Table 2).

Cardiac contractile function. Table 3 summarizes the cardiodynamic data measured in perfused hearts after 30 min of stabilization. Figure 1A compares ventricular contraction and relaxation responses to increases in preload in sham burn/sham sepsis rats (groups 1 and 5); these data showed that ventricular responsiveness to increases in ventricular volume were nearly identical in all shams. Thus all data from these sham groups were combined and are hereafter described as sham. Similarly, sepsis in the absence of burn injury (groups 2 and 6) produced significant and similar cardiac contraction and relaxation defects regardless of the time of intratracheal S. pneumoniae challenge after sham burn injury. Therefore, the data from groups 2 and 6 were combined and are described as sepsis in the absence of burn injury hereafter (Fig. 1B).

The transient nature of burn-mediated myocardial contractile deficits is evident from the data shown in Table 3 and Fig. 2. Twenty-four hours after burn injury in the absence of sepsis, cardiac contraction and relaxation deficits were significant, as indicated by the downward shift of LV function curves compared with those calculated for shams. The transient nature of burn-mediated contractile dysfunction was evident from the upward shift of function curves calculated from hearts harvested 48 and 72 h after thermal injury in the absence of sepsis ($N = 7–8$ hearts per experimental group).

Figure 3 compares LV function calculated for shams and all sepsis groups: (sepsis alone, groups 2 and 6; sepsis plus 48 h postburn, group 4; and sepsis plus 72 h postburn, group 8). LVP and maximal rate of LVP rise and fall (±dP/dt$_{max}$) responses were plotted versus incremental increases in preload (LV volume). All hearts retained the ability to respond to increases in preload; however, cardiac contractile dysfunction was greatest when sepsis occurred 48 h postburn (group 4) compared with ventricular dysfunction observed with the development of sepsis 72 h postburn (group 8), $P < 0.05$ (t-test comparing the two burn plus sepsis groups).
Figure 4 describes the cardiac responsiveness to increases in perfusate Ca\textsuperscript{2+} examined in all sepsis groups. All experimental groups retained the ability to respond to incremental increases in perfusate Ca\textsuperscript{2+} with increases in LVP and dP/dt\textsubscript{max}. Similar to the data described in Fig. 3, S. pneumoniae-related sepsis, which occurred 48 h postburn (group 4), was associated with greater cardiac contraction and relaxation deficits compared with those seen when sepsis developed 72 h postburn (group 8, \(P < 0.05\), t-test).

Cardiomyocyte cytokine responses to burn injury and/or sepsis. Twenty-four hours after burn injury, there was a significant increase in proinflammatory cytokines TNF-\(\alpha\) (Fig. 5A), IL-1\(\beta\) (Fig. 5B), anti-inflammatory cytokines IL-6 (Fig. 5C), and IL-10 (Fig. 5D); proinflammatory cytokine levels (TNF-\(\alpha\) and IL-1\(\beta\)) returned toward baseline levels 48 and 72 h after burn injury alone, but remained significantly above sham values (\(P < 0.05\)). Nitric oxide (NO) secretion by cardiomyocytes was significantly increased 24 h after burn injury compared with NO secreted by sham myocytes; however, NO secretion by cardiomyocytes fell progressively after burn alone, achieving levels measured 72 h postburn that were significantly less than values measured 24 h postburn (\(P < 0.05\)) and achieving values that were nearly identical to values measured in myocytes from control rats (Fig. 5E). Sepsis in the absence of burn injury produced a significant rise in pro- and anti-inflammatory cytokine secretion by cardiomyocytes (\(P < 0.05\)). In contrast, sepsis that occurred after a previous burn injury was associated with significantly...
lower TNF-α, IL-1β, and NO levels compared with those measured in sepsis alone. Furthermore, sepsis complicated by previous burn injury produced cardiomyocyte secretion of IL-6 and IL-10 that resembled that measured after either burn injury alone (24 h) or sepsis alone.

Cardiomyocyte Ca$^{2+}$ and Na$^{+}$ levels in burn sepsis. Either burn injury alone or sepsis alone promoted a significant rise in cardiomyocyte [Ca$^{2+}$]; (Fig. 6A) and cardiomyocyte [Na$^{+}$]; (Fig. 6B) compared with values measured in cardiomyocytes prepared from sham burned hearts. Myocyte Na$^{+}$ and Ca$^{2+}$ levels gradually returned to basal values 48 and 72 h after burn injury. Sepsis, which occurred either 48 or 72 h postburn, promoted cardiomyocyte Ca$^{2+}$ and Na$^{+}$ accumulation that resembled that seen in either burn injury alone or in sepsis alone.

**DISCUSSION**

The data from this study show that either burn injury alone or aspiration pneumonia-induced-sepsis alone produced profound cardiac contractile deficits. In addition, sepsis complicated by a previous burn injury (48 h) exacerbated the cardiac contraction and relaxation deficits associated with either burn or sepsis alone. It was surprising that sepsis, which occurred 72 h after burn injury, produced less ventricular dysfunction than observed when sepsis developed 48 h postburn. During the first 24 h after burn injury, contraction and relaxation deficits are significant, and numerous compensatory mechanisms are upregulated to maintain circulatory function. Therefore, a second insult during this time of limited cardiac reserves likely overwhelms compensatory mechanisms, limiting
the ability of the organism to maintain cardiac function. In this regard, we (17) have shown that burn injury over 40% total body surface area produces cardiac contractile deficits that are evident as early as 8 h after injury, reaching a nadir 20–24 h postburn. In this present study, cardiac contractile depression was evident 24 h postburn, followed by a gradual recovery of myocardial function 48 and 72 h postburn. These data are consistent with our previous report of a progressive recovery in myocardial contractile function over the first 3–5 days after major burn injury (17–19, 41). The transient nature of the burn-related cardiac injury and contractile depression resembles myocardial stunning that has been described after ischemia reperfusion (30, 33). It was perhaps the transient nature of burn-related contractile depression that contributed to the lesser ventricular dysfunction observed when sepsis occurred 72 h after burn injury. Aggressive fluid resuscitation and maintenance of cardiac filling pressure after burn injury promotes recovery of cardiac compensatory mechanisms and likely contributes to the ability of the organism to respond to a later insult.

In our study, the marked cardiac dysfunction that was evident 24 h after burn injury was accompanied by inflammatory responses similar to those described with a variety of physiological insults, including ischemia-reperfusion, hemorrhage, congestive heart failure, and lipopolysaccharide challenge (23, 31, 32). This burn-mediated inflammatory cascade included a rise in circulating TNF-α, IL-1β, IL-6, and IL-10 as well as significant cytokine secretion by cardiomyocytes. We and others (3, 13, 22) have accumulated compelling evidence indicating that TNF-α and IL-1β are the primary mediators of the myocardial depression which occurs after ischemic heart disease, sepsis, burn injury, and congestive heart failure. The critical role of TNF-α as the mediator of myocardial depression has been further implicated by the finding that inhibition of TNF-α prevents cardiac dysfunction after experimental burn injury or septic challenge, and blockade of TNF-α activity attenuates cardiac contractile depression in human sepsis (13, 22). In this study, sepsis, which occurred after a previous burn injury, was associated with reduced TNF-α, IL-1β, and NO secretion by cardiomyocytes compared with proinflammatory cytokine levels measured after either burn injury alone or sepsis alone; in contrast, plasma TNF-α and IL-1β levels were elevated to a greater extent in sepsis complicated by previous burn injury compared with values measured in either sepsis alone or burn alone. The increased systemic cytokine levels were likely related to lung and liver cytokine synthesis and confirm previous reports that a previous injury (first hit) exacerbates the inflammatory response to a second injury (1, 7, 10, 12, 15, 21, 27, 35, 37, 43, 44, 47).

While it is well recognized that burn injury activates the immune system, triggering the release of numerous immunoactive mediators (18, 41), burn injury also alters several aspects of the anti-inflammatory cascade. In this regard, Napolitano and colleagues (31, 32) showed a significant decrease in IL-10 production after burn injury and altered T-cell activation. Previous studies (2, 3) have described that the initial hyperimmune and hypermetabolic responses to traumatic in-

![Intracellular Ca²⁺ levels](http://ajpheart.physiology.org/)
jury are followed by a state of generalized immune suppression. In our study, sepsis after a previous burn injury produced less cardiomyocyte TNF-α and IL-1β secretion than that observed after either burn injury alone or sepsis alone, whereas circulating levels of proinflammatory cytokines (TNF-α/IL-1β) were higher when sepsis was preceded by a previous burn injury. Furthermore, cardiomyocyte IL-6 and IL-10 responses to a second injury were nearly identical to those observed after either injury alone, but again sepsis complicated by a previous burn injury was characterized by an exaggerated anti-inflammatory response. Thus sequential injuries, which occurred in a close temporal order such as sepsis produced 48 or 72 h after a previous burn injury, were characterized by enhanced myocardial anti-inflammatory cytokine response. It is likely that this greater myocardial anti-inflammatory response, which was particularly evident in group 8 from the significantly greater IL-6 and IL-10 levels, provided a measure of cardioprotection, contributing to the improved LVP and ±dP/dt responses measured in group 8.

In the present study, either burn injury alone (24 h) or sepsis in the absence of burn injury increased cardiomyocyte [Ca^{2+}] and [Na^{+}], while serum Ca^{2+} levels fell; the changes in serum Ca^{2+} profiles could be related to the volume of lactated Ringer solution administered, producing a dilutional effect. However, the rise in cardiomyocyte Na^{+} and Ca^{2+} levels suggest that fluid and ion shifts that have been described previously as characteristic of burn injury also occurred in this present study. Furthermore, ion shifts gradually resolved 48 and 72 h after burn injury alone. It was of interest that burn injury complicated by sepsis produced myocyte Ca^{2+} accumulation that resembled that seen in either sepsis alone or burn injury alone. Thus burn injury complicated by sepsis did not exacerbate either the Ca^{2+} or Na^{+} dyshomeostasis seen with either injury alone.

We also considered that the failure of sepsis 72 h after burn injury to exacerbate the cardiac dysfunction seen with sepsis alone was related to the phenomenon of preconditioning. In this scenario, the initial burn injury likely evoked numerous compensatory mechanisms, which would allow the organism to survive the second insult. Previous studies have shown that a nonlethal lipopolysaccharide challenge (33), IL-1β pretreatment (5), transient moderate ischemia (30), or induction of heat shock proteins, such as heat shock protein 70 (11, 48) provide cardioprotection in the face of a second major insult. Indeed, we have shown previously that burn injury upregulates heat shock proteins within the myocardium, and persistent increases in heat shock protein 70 expression for several hours postburn may protect against subsequent injury (45). Upregulation of these acute phase proteins may con-
ttribute to the transient nature of cardiac dysfunction after burn injury in the absence of sepsis, producing the recovery of cardiac contraction and relaxation 72 h postburn (17). We also considered that our finding, that sepsis complicated by burn injury 72 h earlier produced measures of myocardial contraction/relaxation that were significantly better than those measured when sepsis occurred 48 h after burn, may have been related to upregulation of myocardial anti-inflammatory cytokine responses, which negated the negative inotropic effects of myocardial-derived proinflammatory mediators and improved cardiac mechanical function.

While our data confirm that the cardiodepressive consequences of sepsis after burn trauma are related to the time that sepsis develops after the initial injury, the limitations of a rodent animal model are clearly recognized. In addition, it is frequently difficult to mimic the multitude of complications that are encountered in the critically ill patient. However, our study included the presence of devitalized tissue, fluid, and electrolyte shifts, and aggressive fluid resuscitation that are present in clinical burn injury. We have shown previously that hematocrit rises significantly after burn injury in the absence of fluid resuscitation (authors’ unpublished data), suggesting that fluid shifts in our rodent model of burn injury contribute to decreased venous return, hypotension, and decreased peripheral perfusion (17, 18). The fluid resuscitation regimen used in this present study has been shown to improve ventricular filling and to improve postburn cardiac performance via a Frank-Starling mechanism (17). Our model of pneumonia-induced sepsis was designed to mimic clinical burn patients who develop pneumonia-related sepsis, the most frequent cause of sepsis in our burn unit. Because infection continues to be a major cause of morbidity and mortality in burn units, we deemed it imperative to develop an animal model that adequately explores burn injury complicated by clinically relevant sepsis. In our model, burn injury complicated by sepsis tended to increase mortality over that seen with either burn alone or sepsis alone, but this increase in mortality did not achieve statistical significance. This contrasts with previous studies describing that burn injury increases mortality with a subsequent septic challenge (cecal ligation and puncture) (29, 34). These differences in burn sepsis-related mortality may be related to differences in the experimental model (sepsis related to intratracheal S. pneumoniae challenge in our model compared with cecal ligation and puncture used to produce polymicrobial sepsis in studies by others). Alternatively, differences in mortality may be related to the time period selected for study; we examined myocardial inflammatory cytokine as well as myocardial contractile responses to sepsis produced within 72 h after burn injury, whereas others have examined sepsis induced 7–10 days after burn trauma (29, 34). A better understanding of the time course of organ dysfunction as well as the complex proinflammatory and anti-inflammatory responses that occur after burn injury may allow the development of appropriate therapeutic modalities aimed at providing significant cardioprotection and improving outcome.

In summary, our studies confirmed that pneumonia-related sepsis or major burn injury independently altered cardiac contractile performance and increased pro- and anti-inflammatory cytokine secretion by cardiomyocytes. Sepsis that occurred 48 h after burn injury produced significantly greater cardiac contractile depression than that observed with either burn alone or sepsis alone. In contrast, delaying sepsis until 72 h postburn produced cardiac abnormalities similar to those seen with either sepsis alone or burn injury alone. The data suggest that therapeutic interventions, which provide cardioprotection during the early post-burn period, may decrease morbidity and improve outcome in patients at risk for developing sepsis.

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