Caspase inhibition reduces cardiac myocyte dyshomeostasis and improves cardiac contractile function after major burn injury

Deborah L. Carlson, David L. Maass, Jean White, Patricia Sikes, and Jureta W. Horton

Departments of Surgery and Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas

Submitted 6 November 2006; accepted in final form 11 April 2007

Caspase inhibition reduces cardiac myocyte dyshomeostasis and improves cardiac contractile function after major burn injury. J Appl Physiol 103: 323–330, 2007. First published 12 April 2007; doi:10.1152/japplphysiol.01255.2006.—In the heart, thermal injury activates a group of intracellular cysteine proteases known as caspases, which have been suggested to contribute to myocyte inflammation and dyshomeostasis. In this study, Sprague-Dawley rats were given either a third-degree burn over 40% total body surface area plus conventional fluid resuscitation or sham burn injury. Experimental groups included 1) sham burn given vehicle, 400 μl DMSO; 2) sham burn given Q-VD-OPh (6 mg/kg), a highly specific and stable caspase inhibitor, 24 and 1 h prior to sham burn; 3) burn given vehicle, DMSO as above; and 4) burn given Q-VD-OPh (6 mg/kg) 24 and 1 h prior to burn. Twenty-four hours postburn, hearts were harvested and studied with regard to myocardial intracellular sodium concentration, intracellular pH, ATP, and phosphocreatine (31P nuclear magnetic resonance); myocardial caspase-1, -3, and -8 expression; myocyte Na+ (fluorescent indicator, sodium-binding benzofuran isophthalate) loading; cytokine secretion of TNF-α, IL-1β, IL-6, and IL-10; and myocardial performance (Langendorff). Burn injury treated with vehicle alone produced increased myocardial expression of caspase-1, -3, and -8, myocyte Na+ loading, cytokine secretion, and myocardial contractile depression; cellular pH, ATP, and phosphocreatine were stable. Q-VD-OPh treatment in burned rats attenuated myocardial caspase expression, prevented burn-related myocardial Na+ loading, attenuated myocyte cytokine responses, and improved myocardial contraction and relaxation. The present data suggest that signaling through myocardial caspases plays a pivotal role in burn-related myocardial dyshomeostasis and myocyte inflammation, perhaps contributing to burn-related contractile dysfunction.

A series of methods and materials were described in detail. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
TBSA. This burn technique produces complete destruction of the underlying neural tissue. After immersion, the rats were immediately dried, and each animal was placed in an individual cage. All burned animals received standard fluid resuscitation consisting of lactated Ringer solution (4 ml·kg⁻¹·% TBSA burned), with one-half of this calculated volume given over the first 8 h postburn and the remaining volume given over the next 16 h postburn. Animals immersed in room-temperature water served as appropriate controls. In addition, all rats were given analgesic (buprenorphine, 0.5 mg/kg, im) every 12 h. Animals were monitored closely for the first 8 h after burn to ensure adequate recovery from anesthesia, responsiveness to external stimuli, absence of pain, and ability to consume food and water.

Experimental groups. Rats were randomized to receive either burn injury or sham burn as described above. In addition, burned and sham-burned rats were further divided into subgroups and given either intravenous vehicle (400 μl of 15% DMSO) or the caspase inhibitor Q-VD-OPh [quinoline-Val-Asp(OME)-CH₂-PH; R&D Systems, Minneapolis, MN]. The total dose of Q-VD-OPh given in a 300-g rat was 6 mg, with the first dose (3 mg dissolved in 400 μl of DMSO) given 24 h preburn and the second dose (3 mg) given 1 h preburn (9). Experimental groups included: group 1, sham burn given vehicle (400 μl of DMSO, iv); group 2, sham burn given Q-VD-OPh; group 3, burns given vehicle (400 μl of DMSO, iv); and group 4, burns given Q-VD-OPh as described above.

Rats from each of the experimental groups were killed either 2 or 4 h after burn injury or sham burn (n = 5 rats per group per time period). Hearts were collected and freeze-clamped to assess myocardial caspase activity. Additional rats from each experimental group were killed 24 h postburn, and hearts were harvested and placed in a petri dish containing ice-cold heart medium [113 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 d-glucose; 0.5 × MEM amino acids (50×, Gibco/BRL 11310-051); 10 mM HEPES; 30 mM taunine; 2.0 mM carnitine; and 2.0 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. 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 (endotoxin free), Fraction V (Gibco/BRL 11018-025), 0.5 ml trypsin (2.5%, Gibco/BRL 15909-046), and 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 per 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 mg 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, 200, and 500 μM, to a final concentration of 1,000 μM). After the final pelleting step, the supernatant was removed, and the pellet was resuspended in MEM [prepared by adding 10.8 mg 1× MEM (Sigma M-1018), 11.9 mM NaHCO₃, 10 mM Hapes, and 10 ml penicillin/streptomycin (100×, Gibco/BRL 1540-122) with 950 ml MilliQ water]; total volume was adjusted to 1.1 l. At the time of MEM preparation, the medium was bubbled with 95% O₂-5% CO₂ for 15 min, and the pH was adjusted to 7.1 with 1 M NaOH. The solution was then filter sterilized and stored at 4°C until use. At the final concentration of calcium, the cardiomyocyte cell number was calculated and myocyte viability was determined.

Cytokine secretion by cardiomyocytes. Myocytes were pipetted into microtiter plates at 5 × 10⁴ cell/ml per well (12-well cell culture cluster, Corning,Coming, NY) for 18 h (CO₂ incubator at 37°C). Supernatants were collected to measure myocyte-secreted TNF-α,

Table 1. Hemodynamic and metabolic effects of Q-VD-OPH treatment in burned rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham + Vehicle</th>
<th>Group 2 Sham + Q-VD-OPH</th>
<th>Group 3 Burn + Vehicle</th>
<th>Group 4 Burn + Q-VD-OPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>151±5</td>
<td>160±8</td>
<td>130±3*</td>
<td>139±5*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>523±12</td>
<td>530±17</td>
<td>536±10</td>
<td>518±12.0</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>38.8±0.1</td>
<td>38.8±0.3</td>
<td>38.9±0.1</td>
<td>38.0±0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.46±0.1</td>
<td>7.49±0.02</td>
<td>7.54±0.03</td>
<td>7.50±0.01</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>23.2±1.8</td>
<td>26.7±1.3</td>
<td>27.1±1.9</td>
<td>27.2±0.6</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>110.6±5.6</td>
<td>105.9±3.4</td>
<td>118.6±4.4</td>
<td>117.2±3.7</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.6±4.3</td>
<td>42.2±1.0</td>
<td>36.1±1.4*</td>
<td>38.0±0.7*</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>21.4±0.9</td>
<td>21.8±1.1</td>
<td>21.3±0.8</td>
<td>20.9±0.3</td>
</tr>
<tr>
<td>O₂ saturation, % volume</td>
<td>99.9±0.3</td>
<td>99.2±0.5</td>
<td>96.8±0.7</td>
<td>100.3±0.2</td>
</tr>
<tr>
<td>Lactate, mmol</td>
<td>2.20±0.3</td>
<td>2.50±0.7</td>
<td>3.33±0.4*</td>
<td>2.57±0.1†</td>
</tr>
<tr>
<td>Base excess, mmol</td>
<td>1.6±0.5</td>
<td>1.4±0.3</td>
<td>-2.17±0.7*</td>
<td>-0.88±0.4†</td>
</tr>
<tr>
<td>Serum ionized calcium, mM</td>
<td>1.29±0.01</td>
<td>1.06±0.09</td>
<td>0.85±0.04*</td>
<td>0.72±0.02*</td>
</tr>
<tr>
<td>Serum ionized sodium, mM</td>
<td>141.2±0.6</td>
<td>141.7±0.9</td>
<td>137.8±1.4</td>
<td>142.3±0.4</td>
</tr>
</tbody>
</table>

All values are means ± SE. *P < 0.05 from respective sham groups. †P < 0.05 from vehicle-treated burn (group 4 vs. group 3).
IL-1β, IL-6, and IL-10 (rat ELISA, Endogen, Woburn, MA). We previously examined the contribution of contaminating cells (non-myocytes) in our cardiomyocyte preparations, using flow cytometry, cell staining (hematoxylin and eosin), and light microscopy. We confirmed that less than 2% of the total cell number in a myocyte preparation was noncardiomyocytes. Since our preparations are 98% cardiomyocytes, we concluded that a majority of the inflammatory cytokines measured in the cardiomyocyte supernatant was indeed cardiomyocyte derived.

Intracellular calcium and sodium concentration measurements with fluorescent probes. Separate aliquots of cells were loaded with either fura-2 AM (Molecular Probes, Carlsbad, CA) for 45 min or sodium-binding benzofurzan isophthalate (Molecular Probes) for 1 h at room temperature in the dark. Myocytes were then suspended in 1.0 mM calcium containing MEM, washed to remove extracellular dye, and placed on a glass slide on the stage of a Nikon inverted microscope. The microscope was interfaced with Grooney optics for epi-illumination, a triocular head, phase optics, and 30× phase contrast objective and mechanical stage. Excitation illumination source (300 nm)

Table 2. Plasma cytokines measured 24 h after burn or sham burn injury

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham + Vehicle</th>
<th>Sham + OPH</th>
<th>Burn + Vehicle</th>
<th>Burn + OPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/ml</td>
<td>3.6±0.3</td>
<td>0.6±0.3*</td>
<td>4.8±0.2*</td>
<td>1.7±0.5†</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>1±0.4</td>
<td>0.4±0.3</td>
<td>8.3±0.6*</td>
<td>0.4±0.1†</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>6.0±0.4</td>
<td>0.18±0.1*</td>
<td>19±0.2*</td>
<td>1.1±0.1†</td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>4±0.3</td>
<td>1.7±0.2*</td>
<td>10±0.3*</td>
<td>2±0.4†</td>
</tr>
</tbody>
</table>

All values are means ± SE. *P < 0.05 from respective sham group. †P < 0.05 from vehicle-treated burn (group 4 vs. group 3).
W compact Xenon arc illuminator was equipped with a power supply. In addition, this InCyt Im2 Fluorescence Imaging System (Intracellular Imaging, Cincinnati, Ohio) included an imaging workstation and Intel Pentium Pro 200 MHz-based PC. The computer-controlled filter changer allowed alternation between the 340- and 380-nm excitation wavelengths. Images were captured by monochrome charge-coupled device camera equipped with a TV relay lens. InCyt Im2 Image software allowed measurement of intracellular calcium and sodium concentrations from the ratio of the two fluorescent signals generated from the two excitation wavelengths (340 and 380 nm); background was removed by the InCyt IM2 software. The calibration procedure included measuring fluorescence ratio with buffers containing different concentrations of either calcium or sodium. At each wavelength, the fluorescence emissions were collected for 1-min intervals, and the time between data collection was 1–2 min. Since quiescent or noncontracting myocytes were used in these studies, the calcium levels measured reflect diastolic levels.

NMR spectroscopy. To determine if changes in either high-energy phosphates or pH contributed to Na\(^{+}\)/Ca\(^{2+}\) dyshomeostasis after burn injury, separate groups of hearts from sham-burned, burned, and burn plus Q-VD-OPH-treated rats (n = 6 rats/group) were harvested 24 h after burn or sham burn. Hearts were harvested through a midline thoracotomy, and a cannula placed in the aorta was used to perfuse the coronary arteries (Krebs-Henseleit bicarbonate buffer) with a Langendorff approach. The hearts were perfused in a water-jacketed reservoir located in the bore of the NMR magnet (37, 38, 42). The hearts were suspended in a 20-mm OD magnetic resonance spectroscopy tube with a microtube holding 50 μl of 1.3 mM NaCl and 3.3 mM Dy(DOTP)\(^{-5}\) as a sodium external standard. The shift reagent Tm(DOTP)\(^{5}\) was used to separate intra- and extracellular 23Na signal in the isolated heart. A Varian INOVA 300 spectrometer equipped with an Oxford 4.2 T vertical-bore superconducting magnet with a Bruker 20-mm BB probe was used, tunable to either 23Na or 31P. The resonance areas were determined using NUTS (NMR Utility Transfer Transform Software 2D version; Acorn, NMR, 1996) curve analysis program. Intracellular pH was determined from the relationship:

\[ p\text{H} = 6.70 + \log[(v - 3.148)/(5.696 - v)] \]

where v is the chemical shift difference between inorganic phosphate and the PCr resonance (37, 38, 42).

Isolated coronary perfused hearts. Twenty-four hours after burn injury, rats from each experimental group (n = 7–8 rats/group) were heparinized, and a blood sample was collected to measure circulating cytokines. Hearts were removed and placed on ice in ice-cold (4°C) Krebs-Henseleit bicarbonate buffered solution (in mM: 118 NaCl, 4.7 KCl, 1.25 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 11 glucose; 95% O\(_2\)-5% CO\(_2\); pH 7.4; PO\(_2\), 550 mmHg; PaCO\(_2\), 38 mmHg). The ascending aorta was cannulated, and the coronary circulation was perfused (Krebs-Henseleit bicarbonate, flow rate 6 ml/min; ISMATEC Model 7335-30, Cole Palmer, Chicago, IL). A pressure transducer connected to the pressure tubing between the heart and the heating coil was used to measure coronary perfusion pressure; effluent was collected and measured to confirm coronary flow rate. In vitro contractile function was monitored by placing a latex balloon attached to a polyethylene tube into the left ventricular chamber through an apical stab wound. Left ventricular pressure was measured with a Statham P23ID pressure transducer attached to the balloon cannula. Left ventricular ±dP/dt\(_{max}\) values were obtained using an electronic differentiator (model 7P20C, Grass Instruments, Quincy, MA). All variables were recorded on an ink-writing Grass polygraph (model 7DWL8P); a Grass tachycardiograph (Model 7P4F) was used to monitor heart rate; and a Grass Poly VIEW Data Acquisition System was used to convert acquired data into digital form.

Statistical analysis. All values are expressed as mean ± 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 or Neuman Keuls); if inequality of variance was suggested by Levene’s test, Tamhane multiple comparisons (which do not assume equal variance in each group) were performed. Probability

### Table 3. Cardiac stabilization data

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Sham + Vehicle</th>
<th>Group 2 Sham + Q-VD-OPH</th>
<th>Group 3 Burn + Vehicle</th>
<th>Group 4 Burn + Q-VD-OPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular pressure, mmHg</td>
<td>96±4</td>
<td>96±3</td>
<td>58±3</td>
<td>80±3*†</td>
</tr>
<tr>
<td>+dP/dt(_{max}), mmHg/s</td>
<td>2,095±99</td>
<td>2,008±85</td>
<td>1,086±51*</td>
<td>1,600±158*‡</td>
</tr>
<tr>
<td>−dP/dt(_{max}), mmHg/s</td>
<td>1,800±138</td>
<td>1,775±101</td>
<td>814±67*</td>
<td>1,350±96‡</td>
</tr>
<tr>
<td>dP40, mmHg/s</td>
<td>1,758±105</td>
<td>1,763±55</td>
<td>843±61*</td>
<td>1,388±133†</td>
</tr>
<tr>
<td>Time to peak pressure, ms</td>
<td>88.3±2.8</td>
<td>96.5±5.9</td>
<td>101.2±3.6*</td>
<td>91.8±4.4</td>
</tr>
<tr>
<td>Time to 90% relaxation, ms</td>
<td>83.0±4.5</td>
<td>88.0±4.4</td>
<td>91.6±4.5</td>
<td>89.8±6.3</td>
</tr>
<tr>
<td>Time to max +dP/dt, ms</td>
<td>58.8±2.7</td>
<td>57.5±2.5</td>
<td>56.4±1.4</td>
<td>54.5±2.5</td>
</tr>
<tr>
<td>Time to max −dP/dt, ms</td>
<td>53.8±1.8</td>
<td>54.0±1.7</td>
<td>56.4±2.6</td>
<td>55.7±0.8</td>
</tr>
<tr>
<td>Coronary perfusion pressure, mmHg</td>
<td>43.0±2.1</td>
<td>48.0±3.9</td>
<td>47.7±3.1</td>
<td>52.0±9.7</td>
</tr>
<tr>
<td>Coronary vascular resistance, mmHg</td>
<td>8.6±0.4</td>
<td>9.6±0.8</td>
<td>9.54±0.6</td>
<td>10.4±1.9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>251±5</td>
<td>250.3</td>
<td>256±10</td>
<td>248.3</td>
</tr>
</tbody>
</table>

Data are collected from hearts perfused at constant left ventricular end-diastolic volume, constant heart rate, and constant coronary flow rate. All values are means ± SE. *P < 0.05 from appropriate sham groups. †P < 0.05 from vehicle-treated burn (group 4 vs. group 3).
values <0.05 were considered statistically significant (analysis was performed using SPSS for Windows, Version 7.5.1).

RESULTS

All animals survived for 24 h after burn injury (or sham burn injury). As shown in Table 1, mean arterial blood pressure was lower 24 h after burn injury compared with values measured in sham burns regardless of Q-VD-OPh administration. Acidosis occurred in vehicle-treated burns as indicated by arterial lactate and base excess changes; Q-VD-OPh treatment of burn injury attenuated metabolic acidosis. Serum ionized calcium levels were lower 24 h after burn injury compared with values measured in sham burns, regardless of Q-VD-OPh administration.

Caspase-1, caspase-3 and caspase-8 activation. Since caspase-1, caspase-3, and caspase-8 have been shown to be

Fig. 4. Left ventricular responses to increases in either preload (left ventricular volume, \( A \)) or increases in perfusate calcium concentrations (\( B \)). Administration of the caspase inhibitor Q-VD-OPh significantly improved measures of left ventricular contraction and relaxation after major burn injury. All values are mean \( \pm \) SE. LVP, left ventricular pressure. *Significant difference from sham at \( P < 0.05 \) (ANOVA/Student Neuman Keuls). +Significant difference from vehicle treated burn at \( P < 0.05 \) (ANOVA and Student Neuman Keuls).
activated by a number of injuries and insults, we first examined whether burn injury over 40% TBSA altered this signaling cascade. As shown in Fig. 1, significant increases in caspase-1 (A), caspase-3 (B), and caspase-8 (C) activity in the myocardium were evident as early as 2 and 4 h postburn, times consistent with caspase activation in the heart in a model of sepsis (8). We next examined whether Q-VD-OPh inhibited this burn-related caspase activation. Rats pretreated with Q-VD-OPh and subsequently subjected to burn injury over 40% TBSA had significantly lower myocardial levels of caspase-1, caspase-3, and caspase-8 activity 2 and 4 h postburn. Taken together, these findings indicate unequivocally that several caspases are activated in the heart by burn injury; furthermore, this burn-related increase in caspase activation was attenuated by the administration of the cell-permeable, broad spectrum caspase inhibitor Q-VD-OPh.

Inflammatory responses to burn injury in the presence or absence of caspase inhibition. To evaluate the effects of caspase inhibition on systemic and compartmental (myocardial) inflammation, we measured pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 and the anti-inflammatory cytokine IL-10 in plasma 24 h after burn injury as well as pro- and anti-inflammatory cytokine secretion by cardiomyocytes. Vehicle-treated burn injury produced a robust systemic inflammatory response, as indicated by the burn-related increase in plasma TNF-α, IL-1β, IL-6, and IL-10 secretion in vehicle-treated burn compared with values measured in shams (Table 2). This systemic inflammatory response was paralleled by a burn-related increase in cardiomyocyte secretion of TNF-α (Fig. 2A), IL-1β (Fig. 2B), and IL-6 (Fig. 2C), as well as a compensatory increase in anti-inflammatory cytokine IL-10 (Fig. 2D). Administration of the broad-spectrum caspase inhibitor ablated the burn-related increase in systemic inflammation (Table 2) and ablated the myocardial inflammatory response, as indicated by the significantly lower TNF-α, IL-1β, and IL-6 levels measured in plasma 24 h after Q-VD-OPh-treated burn injury. Similarly, caspase inhibition was associated with a significant attenuation of the anti-inflammatory response, as indicated by decreased systemic (plasma) and cardiomyocyte IL-10 concentrations (Table 2 and Fig. 2).

Cardiomycyte Ca2+ and Na+ responses to burn injury in the presence or absence of caspase inhibitor. Since calcium is a pivotal effector of myocardial contractile function, we used fluorescent indicators to quantitate cardiac myocyte calcium and sodium levels in all experimental groups. Burned animals given vehicle had significant cardiac myocyte accumulation of calcium (Fig. 3, top) and sodium (Fig. 3, bottom). Cardiac myocyte calcium and sodium accumulation was significantly attenuated by the administration of caspase inhibitor prior to burn injury (P < 0.05).

Cardiac responses to burn injury in presence or absence of caspase inhibition. Despite aggressive fluid resuscitation to maintain cardiac filling and preload, there was a significant decrease in cardiac contractile performance 24 h after vehicle-treated burn injury, as indicated by the significantly lower left ventricular developed pressure (LVP) and ±dP/dt responses measured during stabilization of the heart at a constant preload, constant coronary flow rate, and constant heart rate. Administration of the caspase inhibitor in burns significantly improved left ventricular contraction and relaxation compared with values measured in vehicle-treated burns (Table 3). To further explore the effects of caspase inhibition on myocardial contractile function, hearts from all experimental groups were perfused in a Langendorff style with incremental increases in either left ventricular preload (left ventricular volume; Fig. 4A) or incremental increases in perfusate calcium (Fig. 4B). As shown in this figure, hearts from vehicle-treated burned rats generated significantly lower levels of LVP and ±dP/dt as either preload or perfusate calcium were incrementally increased compared with values measured in hearts from sham-burned rats. Administration of the caspase inhibitor significantly improved all aspects of myocardial contraction and relaxation, producing left ventricular function curves that were significantly improved in Q-VD-OPh-treated burned rats compared with those calculated for vehicle-treated burned rats (P < 0.05).

NMR spectroscopy studies. As shown in Table 4, ATP and PCR values measured in hearts from vehicle-treated burned rats were not significantly different from the values measured in sham-burned rats. In addition, pHi was similar in burned and sham-burned rats. Administration of Q-VD-OPh to an additional group of burned rats was associated with myocardial ATP, PCr, and pH values that were nearly identical to those measured in sham-burned rats. NMR measures of myocardial Na+ content in sham-burned, burned, and Q-VD-OPh-treated burned rats are summarized in Table 4. The ratio of Na+ signal from the intracellular space (i.e., [Na+]i) compared with external Na+ standard increased (P < 0.05) 24 h after vehicle-treated burns. Since cardiac mass was nearly identical in all experimental subjects, this change indicates an increase in [Na+]i level. Q-VD-OPh administration attenuated burn-related myocardial sodium loading (Table 4). These NMR measures of intracellular sodium were consistent with fluorescent indicator measures of myocyte sodium shown in Fig. 3, bottom.

DISCUSSION

In the present study, vehicle-treated burns produced a typical hemodynamic and metabolic response pattern that included a fall in mean arterial blood pressure (despite aggressive fluid resuscitation), moderate acidosis, and a fall in whole blood hematocrit that was attributed to fluid administration and hemodilution. In addition, burn injury produced significant myocardial caspase activation as indicated by the increased activity of caspase-1, caspase-3, and caspase-8 in the heart. Caspase activation was paralleled by a robust systemic and myocardial inflammatory response and myocardial contraction and relaxation defects. To determine whether the caspase enzymes play a role in the myocardial inflammation and dysfunction that we
have shown to be characteristic of burn injury (16, 40), we administered the cell-permeable caspase inhibitor Q-VD-OPh. This inhibitor has been shown to attenuate ischemia reperfusion-related injury (10, 11, 32, 34, 39). In our study, Q-VD-OPh inhibited the caspase-1, caspase-3, and caspase-8 cleavage activity, providing biochemical evidence of caspase inhibition. Of particular interest was our finding that a caspase inhibitor profoundly reduced both systemic and myocardial inflammation after burn injury, as indicated by the decrease in TNF-α, IL-1β, and IL-6 levels measured in plasma as well as cytokines secreted by cardiomyocytes (15, 27).

Considerable evidence has accumulated in recent years that inflammatory cytokines contribute to myocardial dysfunction in models of ischemia and reperfusion, cardiac transplantation, hemorrhage, sepsis, and burn injury. Giroir and colleagues provided unequivocal evidence that TNF-α overproduction in the heart produced profound myocardial dysfunction. In their study, transgenic mice with increased TNF-α levels within the myocardium had ventricular dysfunction, cardiomyopathy, and ventricular dilatation (4). The late negative inotropic effects of inflammatory cytokines have been attributed to altered intracellular calcium homeostasis (44), while sphingosine or sphingosine metabolites have been hypothesized to mediate the early negative inotropic effects of inflammatory cytokines (31). Other studies have suggested that inflammatory mediators such as TNF-α, IL-1β, and IL-6 incite an inflammatory cascade that includes upregulation of iNOS and synthesis of nitric oxide, which in turn exerts negative chronotropic and inotropic effects (47). Meldrum provided an overview of the negative effects of TNF-α in ischemia/reperfusion injury, sepsis, chronic heart failure, viral myocarditis, and cardiac allograft rejection (28). This overview summarized considerable evidence implicating the myocardium itself as a source of TNF-α and defined several mechanisms of inflammatory cytokine-induced cardiac dysfunction (sphingosine/nitric oxide and cytokine-related myocardial apoptosis). Studies by several other investigators have suggested that the cardiac contractile depression that is characteristic of injuries such as ischemia/reperfusion, sepsis, or burn injury are not related to the effects of a single inflammatory cytokine, but instead are related to the synergistic and overlapping activities of multiple inflammatory mediators (5, 20, 26).

In our study, Q-VD-OPh attenuated burn-related caspase-1 activation in the heart, and this effect was paralleled by attenuation of compartmental (myocardial) inflammatory cytokine secretion. This finding is consistent with previous reports that the primary role of caspase-1 is regulation of inflammatory responses (2, 3); specifically, activation of caspase-1 has been shown to impair IL-1β processing (27). While the stimuli that activate inflammatory caspases are poorly understood, LPS, either through interaction with TLR-4 or through cell entry and LPS-direct interaction with a multiprotein complex termed the inflammasome, has been proposed as an activator of caspase-1 (27). Thus in our study, it is likely that Q-VD-OPh-related inhibition of burn-related caspase-1 activation prevented inflammatory cytokine responses to burn injury, contributing to improved ventricular performance.

However, other mechanisms by which caspase inhibition may have improved cardiac contraction and relaxation include inhibition of burn-related apoptosis of cardiomyocytes (7, 23). Studies from our laboratory have shown that burn injury over 40% TBSA promotes apoptosis of less than 3% of the total cardiomyocyte population, suggesting that programmed cell death of cardiomyocytes is not the sole mechanism of burn-related cardiac contractile dysfunction (7, 23). Recent studies from our laboratory have shown that burn injury promotes release of cytochrome c from mitochondria (45), which has been shown to produce caspase activation (21, 25). In our previous study, release of mitochondrial cytochrome C into the cytosol was paralleled by a burn-related loss of mitochondrial antioxidant capacity in the heart, a loss of mitochondrial membrane integrity, and lipid and protein oxidation (45). In this present study, Q-VD-OPh-related inhibition of postburn caspase activation may have prevented loss of mitochondrial membrane integrity, improved myocardial antioxidant capacity, and prevented burn-related lipid and protein oxidation. These data suggest that further studies examining myocardial caspases are warranted.

In summary, vehicle-treated burn injury was associated with increased myocardial expression of caspase-1, caspase-3, and caspase-8, myocyte sodium and calcium loading, cardiac myocyte cytokine secretion, and myocardial contractile dysfunction, while intracellular cardiac myocyte pH, ATP, and PCr were stable. Q-VD-OPh treatment of burns attenuated myocardial caspase expression, prevented burn-related myocardial sodium and calcium loading, attenuated cardiac myocyte cytokine responses, and improved myocardial contractile performance. Our data suggest that signaling through myocardial caspases plays a significant role in cardiac myocyte ion dyshomeostasis and inflammation, contributing to burn-related contractile dysfunction.

GRANTS

Supported by National Institute of General Medical Sciences Grant 57054-05.

REFERENCES

CASPASES IN BURN INJURY


