Novel Cardioprotective Role of a Small Heat-Shock Protein, Hsp20, Against Ischemia/Reperfusion Injury

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Background—Heat-shock proteins (Hsps) have been shown to render cardioprotection from stress-induced injury; however, little is known about the role of another small heat-shock protein, Hsp20, which regulates activities of vasodilation and platelet aggregation, in cardioprotection against ischemia injury. We recently reported that increased expression of Hsp20 in cardiomyocytes was associated with improved contraction and protection against β-agonist–induced apoptosis.

Methods and Results—To investigate whether overexpression of Hsp20 exerts protective effects in both ex vivo and in vivo ischemia/reperfusion (I/R) injury, we generated a transgenic (TG) mouse model with cardiac-specific overexpression of Hsp20 (10-fold). TG and wild-type (WT) hearts were then subjected to global no-flow I/R (45 minutes/120 minutes) using the Langendorff preparation. TG hearts exhibited improved recovery of contractile performance over the whole reperfusion period. This improvement was accompanied by a 2-fold decrease in lactate dehydrogenase released from the TG hearts. The extent of infarction and apoptotic cell death was also significantly decreased, which was associated with increased protein ratio of Bcl-2/Bax and reduced caspase-3 activity in TG hearts. Furthermore, in vivo experiments of 30-minute myocardial ischemia, via coronary artery occlusion, followed by 24-hour reperfusion, showed that the infarct region–to–risk region ratio was 8.1±1.1% in TG hearts (n=7), compared with 19.5±2.1% in WT hearts (n=11, P<0.001).

Conclusions—Our data demonstrate that increased Hsp20 expression in the heart protects against I/R injury, resulting in improved recovery of cardiac function and reduced infarction. Thus, Hsp20 may constitute a new therapeutic target for ischemic heart diseases. (Circulation. 2005;111:1792-1799.)

Key Words: ischemia ■ reperfusion ■ myocardial infarction ■ proteins

Ischemic heart disease causes approximately one third of all deaths in men and approximately one quarter of all deaths in women. This detriment reflects lack of effective therapies, targeted to the underlying biological processes within ischemic cardiomyocytes. In the heart, transient ischemia followed by reperfusion (ischemia/reperfusion, I/R) induces necrosis and apoptosis, leading to myocardial dysfunction. Preservation of myocardial function after I/R depends on critical adaptive responses, some of which are believed to involve the heat-shock proteins (Hsps). It is well known that Hsp synthesis arises transiently as a tool to protect cellular homeostasis after exposure to heat and a wide spectrum of stressful and potentially deleterious stimuli. Accumulating evidence has implicated Hsps as mediators of myocardial protection, particularly in experimental models of ischemia and reperfusion injury. The cardioprotective effects of Hsp 70 have been shown in isolated adult feline cardiomyocytes, rabbit hearts after adenovirus-mediated gene transfer, and transgenic (TG) mouse hearts after global or regional ischemia. Recently, protection during myocardial ischemia has also been shown for the small heat-shock proteins Hsp27 and αB-crystallin; however, little is known about the role of another small Hsp, Hsp20, which shares considerable sequence homology with Hsp27 and αB-crystallin, in cardioprotection against ischemic injury.

The small Hsp, Hsp20, was initially identified as a member of the crystallin Hsp family from skeletal muscle. It provides resistance to heat treatment in Chinese hamster ovary cells, and its expression levels are elevated on heat pretreatment of swine carotid artery, insulin exposure of skeletal muscle, and β-agonist stimulation of cardiomyocytes. Interestingly, Hsp20 has been shown to regulate vasodilation and suppress platelet aggregation. Moreover, our recent studies have indicated that adenoviral gene transfer of Hsp20 in isolated cardiomyocytes improved contractile function and protected against β-agonist–mediated apopto-
To further define the functional significance of Hsp20 in vivo and its potential protective mechanisms, we generated a TG mouse model with cardiac-specific overexpression of this protein. Our findings indicate that increased Hsp20 expression in the heart protects against IR injury, resulting in full functional recovery and reduced infarction, which implicates this protein as a potential therapeutic target for ischemic heart disease.

**Methods**

**Generation of TG Mouse Model**

TG mice were generated by using mouse cardiac Hsp20 cDNA (mcHsp20) under control of α-myosin heavy chain promoter (α-MHCp) (Figure 1a). The TG lines used in this study have a 10-fold overexpression of Hsp20. Routine genotyping was performed by polymerase chain reaction (PCR) with the use of an upper primer from the α-MHC promoter (5′-CACATAGAAAGCCTAGCCCACAC-3′) and a lower primer from the mcHsp20 cDNA (5′-GCTTTGCTCTGTGGACA-GCTGGGAC-3′) to amplify a 600-bp fragment spanning the junction between the α-MHC promoter and mcHsp20 cDNA. The control PCR was set up to amplify a 350-bp fragment of TSH-β using an upstream primer: 5′-TCCTCAAAAGATGCTATTAG-3′ and a downstream primer: 5′-GTAACCTCCTCATTAGCAGT-3′ (Figure 1b).

**Immunoblotting**

Heart homogenates were analyzed by standard Western blotting to compare Hsp20, Hsp25, αB-crystallin, Bcl-2, Bax, actin, and α-actin levels. Binding of the primary antibody was detected by peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham), and bands were quantified with densitometry. Antibodies Hsp20 (Research Diagnostics Inc), Hsp25 (Affinity BioReagents Inc), αB-crystallin (Calbiochem), Bcl-2(C-2), Bax (B-9), actin, and α-actin (Santa Cruz Biotechnology Inc).

**Immunoprecipitation**

Association of Hsp20 with Bax and Bcl-2 was studied using Dynabeads Protein G (Dynal Biotech) according to the manufacturer’s instructions. Briefly, cardiac homogenate from wild-type (WT) heart was precleared by incubating with the beads for 1 hour at 4°C to minimize nonspecific binding. Fresh beads were washed with 0.1
mol/L sodium phosphate buffer and then coated with anti-Hsp20 or anti-Bax antibody. The bound antibody was crosslinked to the beads using 20 mmol/L dimethyl pimelidate in a 0.2 mol/L triethanolamine solution. The precleared homogenate was then added to the cross-linked beads, and binding was mediated at 4°C for 1 hour. Finally, the proteins were eluted off (using 0.1 mol/L citrate), and their identity was determined by immunoblotting.

**Global Ischemia Ex Vivo**

The cellular and functional responses to I/R were assessed in mice by using an isolated perfused heart model as previously described. Male adult mice (12 to 14 weeks old) were anesthetized intraperitoneally (IP) with pentobarbital sodium (50 mg/kg). Hearts were rapidly excised and mounted on a Langendorff apparatus, perfused with Krebs-Henseleit buffer (noncirculating), and stabilized for 30 minutes, and then the hearts were subjected to 45 minutes of no-flow global ischemia and 2 hours of reperfusion. A fluid-filled balloon made of plastic film was inserted into the left ventricle via the mitral valve and inflated to yield a left ventricular end-diastolic pressure of 10 mm Hg. The balloon was attached to polyethylene tubing (PE50) to a pressure transducer connected to a Heart Performing Analyzer (Micro-Med), and continuous left ventricular pressure was measured. A bipolar electrode (NuMed) was inserted into the right atrium, and atrial pacing was performed at 400 bpm with a Grass S-5 stimulator. Pacing was stopped during ischemia and restarted at reperfusion. After reperfusion, the hearts were weighed, frozen, and cut into 2-mm-thick slices parallel to the atrioventricular groove. The slices were thawed and stained by incubation in 1% triphenyl tetrazolium chloride (TTC) solution in phosphate buffer (Na2HPO4 88 mmol/L, NaH2PO4 1.8 mmol/L) at 37°C for 10 to 20 minutes as previously described. The area of infarction, risk zone, and nonrisk myocardium were determined by planimetry of each slice.

**Lactate Dehydrogenase Release and TUNEL Assays**

In addition to cardiac function, cardiac injury was assessed by measuring lactate dehydrogenase (LDH) release. Perfusion effluent was collected every 15 minutes of preconditioning and also during reperfusion. Total LDH released from the heart was determined using a CytoTox 96 assay (Promega) and expressed as units per gram of wet heart weight. For terminal dUTP nick end-labeling (TUNEL) assays, hearts were removed from the apparatus after I/R, and the atrial tissue was dissected away. The ventricles were fixed in 10% buffered formalin and later embedded in paraffin according to standard procedures, and sections 3 μm thick were obtained to perform TUNEL assays using the ApoTag Plus Peroxidase In situ Apoptosis Detection Kit (Chemicon) according to the manufacturer’s instructions. TUNEL-positive myocytes were determined by randomly counting 10 fields of the midventricular section and were expressed as a percentage of the total cardiomyocyte population.

**DNA Fragmentation**

Heart samples were Dounce-homogenized in 2.5 vol of cell lysis buffer (RIPA, in μmol/L: 1 DTT and 50 PMSF) and centrifuged at 13,000g for 10 minutes, and supernatant (100 μg) from each heart was used in a photometric enzyme immunoassay for the quantitative determination of cytoplasmic histone-associated DNA fragments (mononucleosomes and oligonucleosomes) of programmed cell death using the commercial assay kit Cell Death Detection ELISA (Roche). Results were normalized to the standard provided in the kit and expressed as folds of increase over control.

**Caspase-3 Activity**

To detect caspase-3 activity, 200 μg of lysate from each heart was combined with fluorogenic caspase-3 substrate, diluted to 300 mg/L in caspase assay buffer (250 mmol/L PIPES, 50 mmol/L EDTA, 2.5% CHAPS, and 125 mmol/L DTT), and measured immediately in a fluorometer. Measurements were repeated every 10 minutes for 1 hour, the slope of fluorescent units per hour was calculated, and values were compared with known standards to determine enzymatic activity.

**Regional Ischemia In Vivo**

Mice weighing 25 to 30 g were anesthetized with sodium pentobarbital (90 mg/kg IP), intubated with PE 90 tubing, and ventilated by use of a mouse miniventilator (Harvard Apparatus) with room air supplemented with oxygen. The respiratory rate was 100 to 105 breaths per minute, and P02, P02, blood pH, and body temperature were maintained within normal limits throughout the procedure as previously described. ECG electrodes were placed subcutaneously, and data were recorded with a Digi-Med Sinus Rhythm Analyzer (Micro-Med). A lateral thoracotomy (1.5-cm incision between the second and third ribs) was performed to provide exposure of the left anterior descending coronary artery (LAD), while avoiding rib and sternal resection, retraction, and rotation of the heart. Vascular bundles in the vicinity were coagulated by use of a microcoagulator (Medical Industries). An 8-0 nylon suture was placed around the LAD at 2 to 3 mm from the tip of the left auricle, and a piece of soft silicon tubing (0.64 mm ID, 1.19 mm OD) was placed over the artery. All mice were subjected to a 30-minute coronary occlusion by tightening and tying the suture. Ischemia was confirmed by visual observation (cyanosis) and continuous ECG monitoring. After 24 hours of reperfusion, the aorta was cannulated, and the heart was perfused with 1% TTC (37°C, 60 mm Hg) as previously described. The occluder, which had been left in place, was retied, and the heart was perfused with 5% pthalgo blue. Hearts were transversely cut into 5 to 6 sections, with 1 section made at the site of the ligature. Infarct sizes were determined and expressed as a percentage of the region at risk.

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical analysis was performed using a 2-tailed Student t test for unpaired observations and ANOVA for multiple comparisons. Values of P<0.05 were considered statistically significant.

**Results**

**Generation of Hsp20 TG Mice**

We generated TG mice that carry the mouse cardiac Hsp20 cDNA under the control of the α-MHC mouse promoter (Figure 1a, a and b). All Hsp20 TG mice were healthy and showed no apparent cardiac morphological or pathological abnormalities (Figure 1c). Western blot analysis (Figure 1d) of tissue homogenates revealed that Hsp20 was expressed predominantly in the cardiac and in much lower abundance in skeletal and smooth muscles of WT mice. Transgenesis resulted in a 10-fold increase of the Hsp20 protein level in the

**Baseline Functional Parameters in Langendorff-Perfused Isovolumically Contracting Mouse Hearts**

<table>
<thead>
<tr>
<th></th>
<th>HR, bpm</th>
<th>BW, g</th>
<th>HW, mg</th>
<th>LVDP, mm Hg</th>
<th>+dP/dt, mm Hg/s</th>
<th>−dP/dt, mm Hg/s</th>
<th>Outflow, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, n=8</td>
<td>354±15</td>
<td>27.4±1.2</td>
<td>174±14</td>
<td>83±6*</td>
<td>4429±149*</td>
<td>2370±128*</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>WT, n=9</td>
<td>363±20</td>
<td>27.8±0.9</td>
<td>171±12</td>
<td>69±4</td>
<td>3311±185</td>
<td>1891±100</td>
<td>1.4±0.3</td>
</tr>
</tbody>
</table>

HR indicates heart rate; BW, body weight; HW, heart weight; LVDP, left ventricular developed pressure; +dP/dt, rate of contraction; and −dP/dt, rate of relaxation.

*P<0.05 vs corresponding WT group.
TG hearts (Figure 1e). Cardiac overexpression of Hsp20 did not alter the expression of other small Hsps, such as Hsp25 and αB-crystallin, in the heart (Figure 1f).

Improvement in the Postischemic Recovery of Function

Because acute expression of Hsp20 in rat cardiomyocytes is protective against apoptosis,23 we examined whether increased in vivo expression of Hsp20 protects against postischemic injury. To exclude the involvement of inflammatory components on reperfusion, we used an isolated perfused-heart preparation. Body weights and heart weights of the mice used in these studies were similar between the TG and WT groups (Table). Hearts were stabilized for 30 minutes, and baseline function was measured. Hsp20 overexpression resulted in an increased contractile function under basal conditions (Table), consistent with our previous report using adenovirus-mediated Hsp20 gene transfer in cardiomyocytes.20 Hearts were then subjected to 45 minutes of global ischemia and 2 hours of reperfusion. During reperfusion, the TG hearts exhibited significantly better functional recovery than the WT hearts (Figure 2). It is noteworthy that recovery of left ventricular developed pressure and the rates of contraction (+dP/dt) and relaxation (−dP/dt) were 54±5% and 59±4%, respectively, in the TG hearts and only 20±3% and 21±3%, respectively, in WT hearts after 10 minutes of reperfusion. Most importantly, these parameters were completely recovered after 1 hour of reperfusion in TG hearts, whereas WT hearts recovered to 69±6% and 71±4%, respectively, of preischemic values.

Attenuation of IR-Induced Necrosis and Apoptosis

To determine the degree of necrosis in these IR hearts, we assessed the level of LDH released during the first hour of reperfusion after global ischemia. The total LDH was twice as high in WT hearts compared with TG hearts (Figure 3a), which indicated increased necrosis in Hsp20-overexpressing hearts. Therefore, we examined whether the functional pro-
Detection of the Hsp20 TG hearts was related to the antiapoptotic property of small Hsps. Heart lysates from a subset of experimental WT and TG animals were assayed for DNA fragmentation by use of a quantitative nucleosome assay. WT hearts exhibited a 3-fold increase over Hsp20 TG hearts (Figure 3b). To substantiate the protective effect of Hsp20 via its antiapoptotic action, we also performed TUNEL assays of the hearts. TUNEL-positive cardiomyocytes from the left ventricles of WT animals were 4-fold higher than those in TG animals (Figure 3c). Thus, both methods of detecting apoptosis (DNA fragmentation and TUNEL assay) demonstrated significant attenuation in TG hearts.

Decreased Myocardial Infarct Size Ex Vivo and In Vivo

After 45 minutes of no-flow, global ischemia, followed by 120 minutes of reperfusion ex vivo, we determined myocardial infarct size by histochemical and TTC staining. Histochemical examination of post–ischemic/reperfused WT hearts revealed contraction bands (hypercontracted myofibers) and vacuolizations (Figure 4, a and c), which are both indicative of cardiomyocyte damage; however, the myofibers were well preserved in Hsp20 TG hearts (Figure 4, b and d), suggesting that priming the heart with Hsp20 maintains the integrity of the muscle during IR injury. Myocardial infarct size as a percentage of the area at risk was reduced by 6-fold in TG compared with WT hearts (Figure 4e).

These studies were extended to an in vivo model of 30-minute myocardial ischemia, via coronary artery occlusion, followed by 24-hour reperfusion. Importantly, the infarct region–to–risk region ratio was 8.1 ± 1.1% in TG hearts (n = 7), compared with 19.5 ± 2.1% in WT hearts (n = 11, P < 0.001), under in vivo conditions (Figure 4f).

Mechanism(s) of Hsp20 Cardioprotective Effects in IR

To elucidate the potential mechanism(s) of Hsp20 cardioprotection, we first assessed Bcl-2 and Bax protein expression levels in hearts before or after IR, because alterations of Bcl-2 and Bax protein levels have been shown in isolated cardiomyocytes after hypoxia/reoxygenation and in hearts during IR. Overexpression of Hsp20 did not alter the expression levels of either Bcl-2 or Bax (Figure 5, a and b); however, on IR, the levels of Bcl-2 were significantly reduced, whereas the levels of Bax were greatly increased, in WT hearts, compared with their preischemic samples (Figure 5, a and b). Consequently, the relative ratio of Bcl-2 to Bax expression in the TG hearts was more than 2-fold higher, compared with WT hearts after IR (Figure 5c). Moreover, immunoprecipitation of homogenates from WT hearts using the Hsp20 antibody revealed that Hsp20 interacted with Bax but not Bcl-2 (Figure 5d). A reciprocal immunoprecipitation approach using the Bax antibody also demonstrated an association of Bax with Hsp20 (Figure 5e). Furthermore, the activity of caspase-3 was significantly reduced in TG compared with WT hearts on IR (Figure 5f).

Hsp20 has been shown previously to be phosphorylated under β-adrenergic stimulation of isolated cardiomyocytes. To determine whether the protective effects of Hsp20 overexpression alleviates IR injury. a–d, Representative trichrome-stained myocardial sections were from ex vivo postischemic hearts. Contraction bands (long arrows) and vacuoles (short arrows), all signs of cellular damage, were present in WT (a and c) but not TG (b and d) hearts. e, Cardiac-specific overexpression of Hsp20 significantly reduced ex vivo myocardial infarct size, expressed as a percentage of area at risk, after 45 minutes of no-flow ischemia and 120 minutes of reperfusion (5 to 6 slices per heart; n = 6, TG; n = 8, WT; *P < 0.01 vs postischemic WT hearts). f, Hsp20 overexpression greatly reduced in vivo myocardial infarct size after 30 minutes of myocardial ischemia, via LAD occlusion (top left), followed by 24 hours of reperfusion. Hearts were infused through aorta with 1% TTC, followed by 5% phthalo blue, and then cut transversely into slices 2 to 3 mm thick. Area of necrosis and area at risk were quantified as previously described (n = 7, TG; n = 11, WT; *P < 0.001 vs postischemic WT hearts). Region at risk was not significantly different between groups (TG, 52.9 ± 1.5; WT, 45.6 ± 3.2; P > 0.05). Infarct sizes before normalization were TG, 4.3 ± 0.5; WT, 8.8 ± 1.2; P < 0.05.
Effects of Hsp20 overexpression on apoptosis-related proteins. In ex vivo WT hearts, protein levels of Bcl-2 (a) were significantly decreased, whereas Bax (b) levels were significantly increased, after 45 minutes of no-flow ischemia, followed by 120 minutes of reperfusion. These proteins showed no alterations in TG hearts. c, Ratio of Bcl-2/Bax protein levels in post–ischemic/reperfused TG hearts was significantly higher than in WT hearts.
d and e, Hsp20 coimmunoprecipitated with Bax but not with Bcl-2. Mouse heart homogenates were immunoprecipitated with anti-Hsp20 (d) or anti-Bax antibodies (e). Proteins were separated on SDS-PAGE gels and probed with anti-Bax (d1) or anti-Hsp20 antibodies (e1). Then, membrane (d1) was stripped and rebotted with anti-Bcl-2(d2); subsequently, this membrane (d2) was stripped and rebotted with anti-Hsp20 (d3). Similarly, membrane (e1) was stripped and rebotted with anti-Bax antibodies (e2). Preimmunoprecipitated heart homogenate was used as positive control (H11001), and immunoprecipitate without Hsp20 or Bax antibodies was used as negative control (H11002).
f, Caspase-3 activity was significantly lower in TG hearts than in WT hearts after IR. *P<0.05 vs post–ischemic/reperfused WT hearts; n=6 in each group.
g–j, Hsp20 phosphorylation was analyzed by use of 2D gel electrophoresis. Homogenates were obtained from pre–ischemic/reperfused (g and i) and post–ischemic/reperfused (h and j) WT and TG hearts that were subjected to occlusion of LAD artery for 30 minutes and reperfusion for 24 hours. Homogenates were extracted by use of a solubilization solution of 7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 20 mmol/L DTT, 20 mmol/L spermine, and 1 mmol/L PMSF. Extract was centrifuged (100,000g, 1 hour, at 10°C), and 500 μg from each preparation was separated by 2D electrophoresis, using isoelectric focusing strips (Immobiline Drystrips, 18 cm, pH 4 to 7). 2D gels were Sypro Ruby–stained, and protein spots corresponding to phosphorylated (pI of 5.5) or nonphosphorylated (pI of 5.7) Hsp20 were further confirmed by Western blotting and LC-MS/MS analysis (data not shown).
Hsp20 against IR injury in vivo may be associated with increases in its phosphorylation levels, we applied 2D gel electrophoresis. Our findings indicated that 16±1% of total Hsp20 was phosphorylated in TG hearts (Figure 5i). This phosphorylation was significantly increased to 37±2% after 24 hours of reperfusion (Figure 5j), suggesting that Hsp20 phosphorylation plays an important role in cardioprotection against IR injury. Similar assessment of altered Hsp20 phosphorylation in WT hearts was not feasible because of the low levels of endogenous Hsp20 expression (Figure 5, g and h).

Discussion

In experimental IR models, although apoptosis may be initiated during ischemia, the number of apoptotic cells is increased during reperfusion.3 These observations suggest that the apoptotic component contributes to the death of the myocardium particularly during reperfusion. Thus, unraveling these apoptotic mechanisms becomes very important in developing new therapeutic approaches to attenuate cell death resulting from IR injury.

Increasing evidence has shown that several Hsps have antiapoptotic roles, and overexpression of Hsp27, αB-crystallin, Hsp32 (HO-1), and Hsp70 in the heart can attenuate the IR injury and improve cardiac function9–15,31; however, the Hsp20 studied here is different from the other Hsps in its unique protein kinase A/protein kinase G phosphorylation site HRRAS20,21,23 (phosphorylation of the Ser-16 site significantly increases the contractility in cardiomyocytes; unpublished data), regulatory activities of vasorelaxation,21 and platelet aggregation.22 Furthermore, previous studies have shown that Hsp20 is translocated to actin filaments on stress, suggesting its cytoskeletal stabilizing function in cardiomyocytes.23,32 Taken together, these properties of Hsp20 suggest that it may benefit the ischemic heart at multiple levels.

In the present study, we investigated the role of Hsp20 on cardioprotection during myocardial ischemia. Interestingly, cardiac-selective overexpression of Hsp20 was associated with full functional recovery and decreased infarct size both ex vivo and in vivo on IR injury. There are at least 2 mechanisms that underlie cardioprotection in Hsp20 TG mice from IR injury. The first involves stabilization of the apoptosis-related proteins Bcl-2 and Bax by Hsp20 overexpression (Figure 5, a–c), whereas WT hearts exhibited decreases in Bcl-2 and increases in Bax levels after IR, similar to recent findings in vivo.30 It has been shown previously that overexpression of Bcl-224 or ablation of Bax25 was associated with improved cardiac function, which correlated with a reduction of cardiomyocyte apoptosis, after IR. In addition, delayed ischemic preconditioning, on phenylephrine treatment of rabbit hearts, resulted in an increased Bcl-2/Bax ratio and a reduction of apoptosis.33 Thus, preserved Bcl-2 and Bax protein levels may be an important mechanism of inhibiting IR–induced apoptosis by overexpression of Hsp20. Importantly, we showed that Hsp20 complexed with the protein Bax (Figure 5, d and e), which possibly prevented the translocation of Bax from the cytosol into the mitochondria during IR. As a result, Hsp20 may preserve the integrity of mitochondria, restrict release of cytochrome c, and repress activation of caspase-3. In fact, our data demonstrated decreased caspase-3 activity in post–ischemic/reperfused TG hearts (Figure 5f), suggesting that cardioprotection from IR–induced apoptosis may involve inhibition of the conversion of procaspase-3 (p24) to active caspase-3 because of inhibition of Bax translocation (data not shown). It has been shown that caspase-3 expression is increased in association with heart failure and apoptosis in experimental animals.34 Several groups have reported that direct blockade of caspase activation with a peptide inhibitor protects the myocardium against lethal reperfusion injury.34,35 In contrast, heart-targeted overexpression of caspase-3 in mice has been shown to increase infarct size and depress cardiac function.36 Therefore, the decreased activation of caspase-3 might also be one of the cardioprotective mechanisms against IR-induced injury.

Recently, Hsp60 and αB-crystallin have been also shown to be complexed with the proapoptotic protein Bax.29,37–39 Under stress conditions, these Hsps and Bax dissociate, whereupon Bax translocates to the mitochondria to participate in apoptosis, suggesting a role for Hsps upstream of caspase activation; however, Hsp70 was found to protect cells from death induced by enforced expression of caspase-3, suggesting that protection by Hsp70 may occur downstream of caspase activation.40 In addition, Hsp70 has been reported to interfere with apoptosis by a direct interaction with Apaf-1.41,42 Moreover, Hsp27 has been reported to directly bind to cytosolic cytochrome c and sequester it from Apaf-1.43,44 Thus, different Hsps may act via different mechanisms to prevent cell death. The data presented here demonstrate that Hsp20 prevents IR-induced apoptosis, possibly through the Bax-caspase pathway.

Another cardioprotective mechanism of Hsp20 may involve the maintenance of muscle integrity during IR injury, as evidenced by the presence of less damaged myofibril in the TG hearts after IR. These cardioprotective effects may be associated with increased Hsp20 phosphorylation in the TG hearts after IR, consistent with previous reports on enhanced interaction of phosphorylated Hsp20 with actin and actinin, which further stabilizes the microfilaments.23,45 Because there is strong evidence that cytoskeletal injury plays a crucial role in the pathogenesis of myocardial ischemic injury,46 it is plausible that Hsp20 translocates from the soluble fraction of cardiomyocytes to the insoluble fraction after IR, leading to protection of the collapsed intermediate filament network or cytoskeletal protein damage.

In summary, our findings demonstrate that increased expression of Hsp20 protects the heart from IR-induced injury, leading to restoration of cardiac function and reduced infarction. Thus, Hsp20 may provide a new potential therapeutic target for heart disease. Future studies using adenoviral or adenovirus-associated viral gene transfer in vivo after myocardial infarction may further elucidate the potential clinical benefits of Hsp20.

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References

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