Phosphorylation and translocation of heat shock protein 27 and αB-crystallin in human myocardium after cardioplegia and cardiopulmonary bypass

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Supplemental material is available online.

Objectives: Cardiac surgery using cardioplegia and cardiopulmonary bypass subjects myocardium to hypothermic reversible ischemic injury that can impair cardiac function. Research in animal and cell models demonstrates that acute myocardial ischemia/reperfusion injury causes phosphorylation of heat shock protein 27 and αB-crystallin. Phosphorylation of heat shock protein 27 and αB-crystallin is implicated in the regulation of both beneficial and detrimental responses to ischemic injury. The phosphorylation status of these proteins in human myocardium after ischemic insults associated with cardioplegia and cardiopulmonary bypass is unknown.

Methods: Right atrial appendage and chest wall skeletal muscle samples were collected from patients before and after cardioplegia and cardiopulmonary bypass. Cardioplegia and cardiopulmonary bypass-induced changes in phosphorylation and localization of heat shock protein 27 and αB-crystallin were determined using immunoblot and confocal microscopy with total and phospho-specific antibodies.

Results: Cardioplegia and cardiopulmonary bypass increased the phosphorylation of heat shock protein 27 on serine 15, 78, and 82, and αB-crystallin on serine 59 and 45, but not serine 19. The majority of heat shock protein 27 and αB-crystallin localized to I-bands of cardiac myofilaments and shifted to a detergent insoluble fraction after cardioplegia and cardiopulmonary bypass. Cardioplegia and cardiopulmonary bypass–induced phosphorylation of specific heat shock protein 27 and αB-crystallin residues were associated with additional subcellular locations. Increases in phosphorylation of heat shock protein 27 and αB-crystallin were negatively correlated with cardiac function after surgery.

Conclusion: Cardiac surgery using cardioplegia and cardiopulmonary bypass is associated with phosphorylation and myofilament translocation of heat shock protein 27 and αB-crystallin in human myocardium. Phosphorylation of specific heat shock protein 27 and αB-crystallin serine residues is associated with distinct localization. Understanding the human myocardial small heat shock protein response may have significant implications for surgical myocardial protection.

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The majority of coronary artery bypass graft (CABG) and valve repair surgeries use cardioplegia and cardiopulmonary bypass (CP/CPB) to respectively arrest the heart and systemically circulate oxygenated blood. Hypothermic CP solutions provide myocardial protection during prolonged surgically induced global ischemia that would otherwise prove lethal. However, cardioplegic arrest of the heart during surgery results in reversible ischemic injury that manifests as impaired contractility of viable myocardium and reductions as cardiac function (myocardial stunning).[1,2] Ischemic insults associated with CP/CPB-induced contractile deficits include myocyte hypoxia, intracellular acidosis, increased generation

Abbreviations and Acronyms
CABG = coronary artery bypass graft
CP = cardioplegia
CPB = cardiopulmonary bypass
cryAB = αB-crystallin
HSP = heat shock protein
ICU = intensive care unit
Ser = serine
sHSP = small heat shock protein

of reactive oxygen species, and metabolic alterations.[3] The small heat shock proteins (sHSP) heat shock protein (HSP)-27 and αB-crystallin (cryAB) are regulated in response to all of these ischemic insults.[4-6] Furthermore, growing evidence indicates that HSP27 and cryAB may modulate contractile function in response to ischemic insults.[7-9]

HSP27 and cryAB are abundant in heart and skeletal muscle.[10] These proteins are involved in the beneficial regulation of diverse cellular processes.[4,11] The function and localization of HSP27 and cryAB are closely linked to its phosphorylation status. In their nonphosphorylated state, HSP27 and cryAB are believed to provide heat tolerance for specific proteins, chaperone and cell signal scaffolding function, and protection against oxidant damage.[12-14] In their phosphorylated state, HSP27 and cryAB provide enhanced protection to apoptotic stimuli and stabilization of structural elements of the cytoskeleton, including actin, microtubules, titin, and intermediate filaments.[11,15-17] Nonphosphorylated HSP27 and cryAB exist as cytoplasmic proteins in large hetero-oligomeric complexes. Numerous isolated myocyte and animal models demonstrate that in response to ischemia, HSP27 and cryAB become phosphorylated and move from a diffuse cytoplasmic localization to a striated sarcomeric localization at or near z-lines and intercalated discs.[5,18] HSP27 and cryAB are each phosphorylated on multiple residues: serine (ser)82, ser78, and ser15 of HSP27 and ser59, ser45, and ser19 of cryAB. The p38–mitogen-activated protein kinase (MAPK)/MAPK activated protein-2 pathway can phosphorylate all 3 residues of HSP27 and ser59 of cryAB.[19,20] ERK can phosphorylate cryAB on ser45. The kinase responsible for phosphorylation of cryAB on ser19 is unknown.[20] Both p38-MAPK and ERK are activated in patients after CP/CPB.[21] To our knowledge, no studies have compared the ischemia-induced myocyte localization of HSP27 and cryAB phosphorylated on each residue. Previous studies in animal and isolated myocytes assessed ischemia-induced changes in localization of total protein or limited analysis to single phosphorylated residues. The purpose of the following study was to analyze the regulation of HSP27 and cryAB in human-reversible ischemic injury after CP/CPB. This is the first report to determine CP/CPB-induced changes in the phosphorylation and phospho-specific localization of HSP27 and cryAB.

Materials and Methods

Patient Tissue and Data Collection

Samples were obtained from patients undergoing cardiac surgery with cardioplegic arrest (CP) and moderately hypothermic (32°C-34°C) CPB for CABG or valve repair/replacement as described previously.[22] Briefly, samples of right atrium were isolated using a double purse-string suture method. During the placement of the venous cannula, the first sample of atrial appendage was harvested (pre-CP/CPB). The superior suture was tightened to secure the venous cannula. The inferior suture remained loose to allow this portion of the atrium to be perfused with blood, exposed to CPB and blood CP, and reperfused after removal of the aortic cross-clamp. The second suture was tightened after weaning from bypass and a brief period of reperfusion (~5-15 minutes), and the remaining distal tissue was harvested as the post-CP/CPB sample. Cold-blood CP (4°C) consisted of a 4:1 mixture of oxygenated blood with a hyperkalemic (K+ = 25 mmol/L) crystalloid solution. Skeletal muscle samples were discarded tissue from the internal thoracic artery harvest site. Myocardial and skeletal muscle tissue were immediately frozen in liquid nitrogen (n = 15 patients) or fixed in 10% formalin in phosphate-buffered saline (n = 5 patients) for molecular biology or microscopy studies, respectively. There were no detectable differences in any of the parameters studied between patients undergoing valve repair or CABG. For correlation analysis, ejection fraction (EF) was estimated by the anesthesiologist using intraoperative transesophageal echocardiography before and immediately after CP/CPB. The cardiac index was recorded from patients with a Swan-Ganz catheter in place at the time of surgical intensive care unit (ICU) admittance and 4 hours later. The Clinical Research Committee of the Beth Israel Deaconess Medical Center approved this study.

Sodium Dodecylsulfate–Polyacrylamide Gel Electrophoresis and Immunoblot Analyses

Sodium dodecylsulfate–polyacrylamide gel electrophoresis and immunoblot analyses were performed as previously described.[22] Briefly, tissue (50-150 mg) was homogenized in radioimmunoprecipitation assay buffer with 50 mmol/L NaF, protease inhibitors (Complete; Boehringer, Mannheim, Germany), and phosphatase inhibitor cocktail (I + II, 1:100, Sigma, St Louis, Mo). After homogenization, lysates were centrifuged at 10,000*g* for 10 minutes, and a bicinchroninic acid protein assay was performed to allow equal gel loading; 20 to 40 μg of lysates were loaded on tris-glycine 8% to 16% gradient gels and electrophoresed for approximately 1 hour at 150 volts. Gels were transferred to polyvinylidene difluoride membranes for 1 hour at 100 volts. Gels were blocked in 3% nonfat dry milk in tris-buffered saline (TBS) for 1 hour, followed by incubation in primary antibodies in 3% TBS milk or 3% bovine serum albumin according to the manufacturer's recommendation. Blots were washed 3 times in TBS and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody for 1 hour, washed 3 times in TBS, and detected using chemiluminescent detection (Pierce, Rockford, Ill). Antibodies for immunoblot were as follows: phospho-specific and total HSP27