Proteomic profile of differentially regulated proteins in human myocardium before and after cardiac surgery utilizing cardioplegia and cardiopulmonary bypass.

Richard T. Clements¹, Neel R. Sohda¹, Gary Smejkal¹, Jun Feng¹, Sirisha Emani¹, Venkatachalam Senthilnathan¹, Alexander Lazarev¹, Kamal R. Khabbaz¹, Cesario Bianchi¹, and Frank W. Sellke¹

1) Cardiothoracic Research Lab, Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; and 2)Proteomics and Small Molecules Applications Laboratory, Pressure BioSciences Inc., West Bridgewater MA

Abstract

Introduction: Although highly protective, cardiac surgery utilizing cardioplegia and cardiopulmonary bypass (CP/CPB) subjects myocardium in hypothermic reversible myocardial ischemia. This procedure is associated with a subset of patients that result in a greatly enhanced risk of mortality. Changes in metabolic, inflammatory, and proteolytic proteins potentially involved in myocardial contractile deficits. Specifically, assessment of the role of PEBP may have significant implications for CP/CPB-induced myocardial stunning.

Methods

Diced samples of right atrial appendage and chest wall skeletal muscle were harvested from patients before and after undergoing CP/CPB for CABG or valve surgery. Tissue was harvested by tightening of double purse string sutures placed on the right atrial appendage and immediately frozen in liquid nitrogen or placed in formalin. Pre-CP/CPB - harvested before CP/CPB at venous cannulation. Post-CP/CPB - harvested after a short period of reperfusion following removal of the aortic cross clamp (~5-10 min).

Proteins were extracted in RIPA buffer with protease and phosphatase inhibitors using RecPrime Plant and Animal Protein Extraction Kit (Thermo Scientific, Waltham, MA). The samples were subjected to 48 cycles at a maximum pressure of 35,000 psi as previously described (Smejkal et al, Analytical Biochemistry, 2007). The concentration of the supernatant was determined by the BCA protein assay (Pierce). A 100 μg of total protein per sample was used to hydrate immobilized pH gradients (IPG) (pH 3-10), pH 4-7, and pH 5-8 for 10 hours, isoelectric focusing and two-dimensional electrophoresis were performed as previously described (Shepherd and Robinson, Electrophoresis, 2007).

2D gels were silver-stained for phosphoproteins and total proteins using Ponceau S and SYPRO Ruby dye (Novix) according to manufacturer's instructions. Gels were imaged using a BioRad Imaging DigiDoc. Additional 2 gels were stained with colloidal Coomassie Blue and imaged with an infrared gel scanner (Licor).

Protein spot detection was performed on all gels using Phoretix 2-D gel analysis software. Densitometry values for spots matched between gels were determined following background subtraction and normalization to total spot intensity of each gel in every group.

At least 20 spots consistently up-regulated or present only in pre or post samples were excised and submitted to the proteomics core facility at BIDMC for identification by Mass Spectrometry. Identified sequences were entered into blast searches to determine protein identification.

Results: CP/CPB: 15 unique or preferentially expressed (as post-CP/CPB) protein spots were detected. Post-CP/CPB, 65 differential proteins (as pre-CP/CPB) were detected. We compared changes in the remaining 280 consistently detected proteins. Identified proteins that changed other expression parameters are: 31 proteins with MLC2a and MLC1, 4 proteins with ATP synthase F1 complex, 30 with S100 CaBP, 24 with S100 CaBP, 28 with ATP synthase F1 complex, 3 with actin. Of particular interest was phosphorylation of protein (S100CaBP), a secreted protein with recently identified potent negative regulatory effects.

Conclusions: Cardiac surgery results in dramatic changes in the human myocardial protein profile. CP/CPB modifies specific cytoskeletal, metabolic, inflammatory, and proteolytic proteins potentially involved in myocardial contractile deficits. Specifically, assessment of the role of PEBP may have significant implications for CP/CPB-induced myocardial stunning.

Proteomic profile of patients undergoing cardiac surgery utilizing cardioplegia and cardiopulmonary bypass reveals numerous consistent changes in the human myocardial protein profile.

CP/CPB modifies specific cytoskeletal, metabolic, inflammatory, and proteolytic proteins potentially involved in ischemic insults associated with cardiac surgery.

These results and subsequent analysis of additional proteins provide valuable insights to changes in the myocardial proteome during CP/CPB. Modulation of these specific proteins may lead to enhanced strategies for cardiac protection.

Table 1: Proteins regulated during CP/CPB identified by Mass Spectrometry.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Fold Change</th>
<th>P-value</th>
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<tbody>
<tr>
<td>MLC2a</td>
<td>2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>MLC1</td>
<td>1.8</td>
<td>0.005</td>
</tr>
<tr>
<td>ATP synthase</td>
<td>2.0</td>
<td>0.01</td>
</tr>
<tr>
<td>S100 CaBP</td>
<td>1.5</td>
<td>0.006</td>
</tr>
<tr>
<td>S100 CaBP</td>
<td>1.4</td>
<td>0.008</td>
</tr>
</tbody>
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Table 4: Quantification of CP/CPB regulated proteins identified in Table 1. Data indicates the fold change of the mean maximal response of each protein. Statistical significance (p<.05) determined using paired t-test.

* indicates p<.05