Subcellular Localization of Calpastatin in Cardiomyocytes Subjected to Simulated Ischemia and Reperfusion

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Background: Cardiopulmonary bypass (CPB) is often required for the surgical repair of complex congenital heart malformations. Ischemia and reperfusion (I/R) injury to the myocardium during these repair procedures may be mediated by the proteins calpain (a calcium dependent cysteine protease) and calpastatin (the endogenous inhibitor of calpain).
Aims/Hypothesis: We aimed to localize calpastatin within cardiomyocytes undergoing simulated I/R and within cardiomyocytes treated with calpain inhibitor with or without simulated I/R. The hypothesis is that calpain and calpastatin interaction are mediated by changes in subcellular localization of calpastatin from a location around the nucleus to the cytosol.
Methods: Cardiomyocytes isolated from neonatal rats and plated on laminin-coated slides were subjected to simulated ischemia in 0.5% oxygen media without glucose for 18 hours followed by reperfusion in media with glucose at 21% oxygen for 15, 30, 60, and 120 minutes. Calpain inhibitor (2, 10, and 50 μM Z-LLY-FMK) was added 30 minutes prior to ischemia. The cells were fixed on the slide and incubated in primary antibody conjugates (Zenon Antibody Labeling, Molecular Probes) against calpain I (Alexa Fluor 594 conjugated antibody) and calpastatin (Alexa Fluor 488 conjugated antibody). Images of cardiomyocytes were captured with sequential scans on a Leica Microsystems SP5 confocal microscope using Leica Application Suite-Advanced Fluorescence confocal software (1.7.0 build 1240).
Results: In cardiomyocytes that underwent simulated I/R, calpastatin was localized around the nucleus while the cells were still ischemic (i.e. before reperfusion). After reperfusion, calpastatin was observed in localized regions in the cytosol. For untreated cells, this location change occurred by 15 minutes after reperfusion. For cells treated with 10 μM calpain inhibitor, this change occurred in some cells by 15 minutes after reperfusion and was observed in most cells by 60 minutes after reperfusion. For cells treated with 50 μM of calpain inhibitor, calpastatin was observed in the perinuclear position until 120 minutes after reperfusion. Control cells maintained in normoxic conditions did not demonstrate this change in calpastatin localization. When calpain I and calpastatin channels were overlaid, it was observed that calpain and calpastatin were colocalized after calpastatin moved to the cytosol.
Conclusions: During reperfusion, calpastatin moved to localized regions in the cytosol (possibly in association with mitochondria). In cells treated with calpain inhibitor, the time for calpastatin to move to localized regions in the cytosol was extended with increasing calpain inhibitor dose. After I/R, calpastatin and calpain I were colocalized within the cardiomyocyte. No changes in calpastatin location or colocalization with calpain were observed in control cells maintained in normoxic conditions. Future studies will aim to determine if phosphorylation or dephosphorylation of calpastatin mediates the location change with I/R. Determining how calpain and calpastatin interact during I/R can lead to deciphering the pathways of cell injury in cardiomyocytes.