

Establishing Cellular Context for Pre-Amyloid Oligomer Toxicity in Human Heart Failure
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Introduction: The presence of pre-amyloid oligomer (PAO) has been demonstrated in a number of cardiomyopathy etiologies. PAO, by itself, can cause cardiomyocytes to fail but the pathological processes remain largely undefined. One preliminary hypothesis is that PAO induces pore formation on membranes. Other mechanisms such as calcium dysregulation and oxidative stress have been demonstrated in other amyloid-related disorders such as Alzheimer's disease but have not been explored in heart failure. In this study, we utilized immunohistochemistry to identify potential toxicity pathways in mouse and human heart failure.

Methods: Tissue samples were harvested from non-transgenic mouse controls, crystallin- α B R120G mutant mice known to express PAO, failing human atria removed during aortic valve replacement surgery, and ventricular tissue from Alzheimer's patients. The samples were either fixed and embedded in paraffin using standard methodology or cryopreserved and embedded in OCT. Five micron sections were prepared and mounted on glass slides. Tissue samples were treated with the following primary antibodies and subsequently tagged with Alexa 488 fluorochrome (green): anti-ryanodine receptor 2 (anti-RyR2) as a surrogate for aberrant calcium flux, anti-connexin43 (anti-Cx43) to identify aberrant cell-cell signal conduction and anti-nitrotyrosine (anti-NTyr) to localize proteins that have undergone oxidative stress. Counter-stains consisted of phalloidin conjugated to Alexa 586 fluorochrome (red) in order to identify the cardiomyocytes, wheat germ agglutinin conjugated to tetramethylrhodamine isothiocyanate-dextran (TRITC) (red) to clearly outline cell boundaries, or to a second antibody subsequently tagged with Alexa 586. The tissue was then visualized using confocal microscopy with a 60x objective and the images were captured on a CCD camera.

Results: In failing human heart tissue, connexin43 localized within the sarcolemma along the long axis of the fiber and at the intercalated disk. Nitrotyrosine localization showed limited amounts of nitrotyrosine within diseased myocytes and intense heterogenous staining within Alzheimer's heart tissue. Ryanodine receptor 2 immunofluorescence localized only to the extracellular margins of the cardiomyocytes and did not appear within myocytes.

Conclusions: Localization of connexin43 away from intercalated disks in diseased human atria suggests a possible mechanism for impaired cell-to-cell conduction signaling and subsequent cardiomyocyte dysfunction. Nitration of tyrosine residues on intracellular proteins does not appear to have a major role in cardiomyocyte toxicity.

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