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REMODELING OF MICROSTRUCTURES ASSOCIATED WITH CARDIAC EXCITATION-CONTRACTION COUPLING AND ETIOLOGY BASED EFFECT ON HEART FUNCTION IN PATIENTS WITH END-STAGE HEART FAILURE

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Introduction

Cardiovascular diseases including heart failure (HF) are the leading cause of death worldwide.[1] HF currently afflicts 64.34 million people worldwide and accounts for nearly $350 billion in medical costs each year in the United States alone.[2] HF leads to cardiac structural remodeling and impairs heart function. HF is associated with loss of the transverse tubular system (t-system) and couplons in ventricular myocytes that leads to defective excitation-contraction (EC) coupling.[3] T-system-associated couplons comprise ryanodine receptor (RyR) and L-type calcium channels (LTCC).[3] Junctophilin 2 (JPH2) maintains the assembly of junctional membrane complexes (JMC) underlying EC coupling.[4] Improvement in heart function after left ventricular assist device (LVAD) implant are observed in patients with intact t-system. Heart function improvements vary in ischemic (ICM) and non-ischemic (ICM) patients. However, the effect of improved heart function by LVAD implant on the remodeling of t-system, RyRs, and JPH2 in HF patients needs to be investigated. Here, we studied etiology-based differences in improved heart function after LVAD implant and their effect on microstructures associated with EC coupling.

Methods

Left ventricular tissue biopsies were collected from end-stage HF patients and control donor hearts. Tissue samples were also collected from healthy donor hearts that were not suitable for transplant. Biopsies were immediately fixed in 2% paraformaldehyde. 100µm thickness tissue sections were prepared using a Leica Vibratome. The tissue samples were washed with PBS three times for 10 minutes each after every step. Tissue slices were permeabilized using 0.5% Triton-X 100 for 2 hours on a shaker followed by incubation with signal enhancer for 30 minutes. Tissue slices were blocked in 10% Donkey Serum for 90 minutes followed by overnight incubation with primary antibodies for JPH2 and RyR. Secondary antibodies conjugated with Alexa-Fluor 488 and Alexa-Fluor 633 for 3 hours the next day. Primary and secondary antibodies were diluted in the ratio of 1:100 in 1XPBS. The samples were stained with DAPI for 15 minutes followed by a wheat germ agglutinin (WGA) Alexa Fluor 555 conjugate, applied for 4 hours to stain the t-tubules. Upon

completion of the tissue preparation, they were placed on a glass slide and covered with Fluoromount-G mounting medium, secured by a coverslip, and left to set overnight.

The prepared slides were imaged using a Leica TCS SP8 confocal microscope. Three-dimensional stacks of size 100μm square were imaged. The JPH2, RyR, and t-tubule systems were imaged in each of these cases. The image stacks were processed using methods of digital image processing by MATLAB. The sarcolemma, including t-tubules, RyR clusters, and JPH2 clusters, was segmented following digital image processing in the form of noise reduction, background removal, attenuation correction, and deconvolution. As part of the image analysis, the distances of JPH2(ΔJPH2-SL) and RyR(ΔRyR-SL) clusters to the sarcolemma (SL) were measured. Additionally, the distances between intracellular sites RyR (ΔRyR), JPH2 (ΔJPH2) and t-system (Δ (μm)) were calculated as a means of determining cluster density in the tissue samples. The density of clusters was also measured. To evaluate the relationships between the collected measures, linear regression analysis was performed using MATLAB. The correlation coefficient (R^2) was used to determine the strength of said relationships. An R^2 value of close to 1 demonstrates a close relation, while an R^2 value close to 0 demonstrates little to no association between selected measures. A one-way ANOVA test was also performed to determine the correlation between different groups. In the ANOVA test, a p-value < 0.05 demonstrates significance.

Results

In this study, confocal microscopy images of cardiomyocytes demonstrated the clustering and remodeling patterns of microstructures associated with the EC coupling. First, we compared left ventricular ejection fraction (LVEF) before and after LVAD unloading. Overall cardiac function improved in HF patients significantly. The percent change in LVEF for both NCIM and ICM HF tissues was shown to be significantly different from control tissue. Additionally, it was shown that NCIM tissue recovers significantly more in LVEF after LVAD unloading when compared to the ICM tissue ($p=0.046$). The image data was also utilized to analyze the remodeling of microstructures in the cardiomyocyte. The distances between microstructures such as t-systems, RyR, and JPH2 were calculated. However, no significant differences were found between RyR (ΔRyR) or JPH2 (ΔJPH2) cluster density when comparing HF to control tissue. Linear regression analysis was performed, revealing a statistically significant difference between the distance among t-systems (Δ (μm)) and the change in LVEF (ΔLVEF (%)) in NICM tissue ($p=0.002$).

Conclusions

Our study revealed that cardiac structural remodeling in HF patients incorporates t-system defects as well as a reduction in clusters of RyR and JPH2 proteins with increased cluster distances. The remodeling of these microstructures was more prominent in ICM patients as compared to NICM patients. Clinical data demonstrated that the cardiac functional improvement in HF patients after LVAD implantation was significant. However, the improvement was greater in NICM patients in comparison to ICM. While not statistically significant, a trend was observed that with an increase in heart function, t-system distances were reduced. Similarly, with an increase in heart

function, RyR distances were reduced. Our study indicates that the level of cardiac functional recovery differs between the NICM and ICM groups that leads to a difference in remodeling of microstructures associated with EC coupling in cardiomyocytes. Our study suggests that there should be different clinical therapeutic approaches to treat ICM and NICM patients.

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