CHARACTERIZATION OF AAV2/9 MEDIATED GENE THERAPY FOR THE CARDIAC PHENOTYPE IN A MOUSE MODEL OF POMPE DISEASE

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Introduction

The long term goal of this project is to develop a clinically relevant gene therapy approach for the treatment of Pompe Disease. Pompe Disease is a form of muscular dystrophy and metabolic myopathy caused by mutations in the acid alpha glucosidase (GAA) gene. An insufficient amount of GAA leads to the accumulation of glycogen in lysosomes and consequent cellular dysfunction. In human patients there is a direct correlation between the amount of GAA produced and severity of disease. Without treatment, cardio-respiratory failure typically occurs in the early onset patients within the first year of life. Here we present an MRI-based characterization study of the cardiac phenotype in our GAA knockout mouse model (GAA-/-) at various ages and subsequent correction of the phenotype following gene therapy treatment.

Experimental

Using a 4.7 T Bruker Avance spectrometer and Paravision software and a quadrature transmit receive surface coil designed for acquiring data from a mouse chest cavity (AMRIS Facility, University of Florida, Gainesville, FL) we have characterized the progressive development of the cardiac phenotype in our GAA-/- mice at 3, 6, 12, 18, and 24 months of age. Mice were anesthetized with 1.5-2% isoflurane and 1L/min oxygen and monitored using the Small Animal Instrument (SAI) monitoring and gating system for respiration rate and cardiac triggering. Four chamber and two chamber images were acquired using a cardiac gated Fast Low Angle Shot (FLASH) sequence (FOV=50x30mm, matrix=256x128, TR=12 msec, TE=2.2 msec, NEX=4 AVG, slice thickness=1.5mm, 14 frames with one frame per 12 ms). Short axis images were prescribed from base to apex and collected with the Cine-GE sequence described above except with FOV=3x2cm², TR=12msec, TE=2.3msec, and 12 frames to capture the entire cardiac cycle. All MRI images were processed using PIE analysis software (Netherlands).

We have combined the most optimal recombinant Adeno-Associated Virus (rAAV) serotype for 
\textit{in vivo} cardiomocyte transduction with the clinically relevant intra-venous delivery route in order to administer the human GAA (hGAA) gene to GAA-/- mice. Experiments are currently underway in which GAA-/- mice have been injected with various doses of rAAV2/9-CMV-hGAA and MRI is being used to non-invasively assess the ability of our gene delivery method to provide disease prevention and/or correction in the heart.

Results and Discussion

Following MRI post-processing we have found that at the age points analyzed thus far (3, 6 and 12 months) the cardiac mass is significantly higher in the GAA-/- mice than in age-matched wild-type controls (B6/129). This matches the extreme cardiac hypertrophy that is observed in the most severe human form of the disease. In addition, we have found that both the stroke volume (SV) and the cardiac output (CO) are significantly lower in the GAA-/- mice than in age-matched mice from the B6/129 control strain. For our purposes, these three measurements: cardiac mass, SV and CO are the parameters by which we will assess the success of our cardiac gene therapy treatments. At every age analyzed thus far, we see no significant difference in the ejection fraction % (EF%). This is most likely due to a large amount of cardiac reserve present in the GAA-/- mouse model and explains why the mice are able to survive far longer than the most severe cases of the human form (infants born making no GAA) of the disease. In the preliminary mouse treatment experiments, we have determined that at a low dose our gene delivery strategy results in higher CA and SV at 3 months of age than in age-matched untreated control GAA-/- mice. At this low dose treatment, these numbers are not as high as we observe in healthy B6/129 control mice. Our preliminary results are therefore promising in this ongoing study.

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