MRI for Characterization and Cardiac and Skeletal Muscle Gene Therapy Evaluation in Murine Models of Muscular Dystrophy.

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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 genetic diseases affecting the heart. Whereas gene therapy shows great promise for the treatment of cardiomyopathies associated with muscular dystrophy, noninvasive methods need to be developed to monitor gene transfer efficacy. This is particularly important because the scope of gene correction is highly variable, depending on the virus serotype, promoter, and delivery route. In order to determine whether MRI can be used to monitor the effectiveness of cardiac gene therapy, we determined the natural history of disease progression in three different forms of muscular dystrophy and whether gene transfer alters these endpoints in a model of Limb Girdle Muscular Dystrophy (LGMD). We have studied a model of Limb Girdle Muscular Dystrophy Type 2D; alpha-sarcoglycan knockout (ASG-/-), a model for Myotonic Dystrophy Type 1 (MDNL1-/-) in which exon 3 of MBNL has been deleted and the MDX mouse model for Duchenne Muscular Dystrophy which lacks dystrophin. In addition, we addressed whether gene transfer was associated with changes in proton transverse relaxation time and delayed contrast enhancement by Gd-DTPA. LGMD-2D is the result of mutations in the alpha sarcoglycan (ASG) gene that encodes for a transmembrane protein located in the sarcolemma of muscle fibers. It is characterized by the progressive development of lesions in cardiac and ambulatory muscles due to sarcolemma deterioration and chronic cycles of damage and regeneration. Using MRI, functional measurements, and histology our studies have assessed the hypothesis that adeno-associated virus (AAV) is a highly efficient vehicle for delivery of the ASG gene and therapeutic treatment of muscle in the ASG-/- mice. In addition, we addressed whether gene transfer was associated with changes in proton transverse relaxation time and delayed contrast enhancement by Gd-DTPA.

Methods:

Gene Delivery: Hindlimb muscles of 6 ASG-/- mice were injected with $1x10^{11}$ particles of AAV1 expressing human ASG. Using MRI, legs were imaged every 3 weeks post injection in order to observe dystrophic lesion development. *In Vitro* measures of muscle damage: In all mice, Evans Blue Dye (EBD) was injected intravenously 24hr prior to tissue harvest to detect membrane permeability to albumin as previously described. EBD was dissolved in PBS (0.15 M NaCl, 10mM phosphate buffer, pH 7.4) and sterilized by passage through membrane filters with a 0.2-mm pore size. The EBD concentration was 1mg/0.1ml/10g body weight, and tissues were harvested 24 hours later. EBD positive fibers were determined under fluorescent microscopy on 10um cryosections. In order to recognize lesions in the later stages of development on cryosections we have utilized the trichrome stain. This stains for the presence of collagen that infiltrates more progressed dystrophic lesions as they undergo fibrosis. At harvest, force mechanics were performed on muscles in order to assess function. Histological stains and immunohistochemistry were performed on sectioned tissues to show morphology and distribution of ASG expression. *In Vivo* measures of muscle damage: Mice were imaged on a 4.7T Oxford Magnet using a Bruker Advance console and Paravision software with X cm quadrature birdcage coil for cardiac studies and a single tuned 1.6cm solenoid coil for the hindlimbs. 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. Dorsal and sagittal images were acquired using a cardiac gated cine-gradient echo sequence (FOV=50x30mm, matrix=256x128, TR=12msec, TE=2.2msec, NEX=4AVG, slice thickness=1.5mm, 14 frames with one frame per 12ms). Short axis images were prescribed from base to apex and collected with the Cine-GE sequence described above except with FOV=$3x2cm^2$, TR=12msec, TE=2.3msec, and 14 frames to capture the entire cardiac cycle. Both cardiac and skeletal muscle T2 was measured using a DWI-SE sequence in which the diffusion terms were minimized and held constant at a TE of 14 and 40ms (TR=2,000ms, FOV=1.2x1.2 cm^2, matrix=256x128, 1mm slice thickness). ^1H MRS was acquired from affected and unaffected regions within the mouse hindlimb muscle using STEAM localization (TR=2,000ms, TE=20ms, SW=3000Hz, 2048pts, nex=128). For delayed contrast enhancement, baseline SPGR images (TR=200ms, TE=5ms, FLIP=90, nex=8, matrix=256x128, FOV=1.2x1.2 cm^2,) were acquired prior to the i.p. injection of Gd-DTPA (0.1mL/10g body-weight; Gd-DTPA, Magnevist; Barlex;NJ). 3D SPGR MR images were repeated every 5 min for a total of 30 min post injection.

Results & Conclusions:

Characterization of the cardiac manifestations of these diseases demonstrates lesion development, hypertrophy, arrhythmias, localized contractility defects and irregular ECG readings, all of which progress with age. In older MDX mice (6-52wk), the heart shows focal lesions of inflammatory cell infiltration, myocyte damage and fibrosis generally located in the ventricle or septum and display regions of increased MR signal intensity. The hypertintense regions correlated with regions of myocyte damage. AAV1-ASG treated ASG-/- legs display an 85% reduction in dystrophic lesion development. Passive stretch force measurements demonstrate wild-type elasticity in treated muscles and a 3-fold decrease in elasticity of untreated muscles. ASG expression was present in 50-100% of fibers in treated muscles. Using a variety of functional and morphological measurements our studies have demonstrated our ability to non-invasively characterize the hearts of murine models of cardiomyopathy. Cardiac MR provides high-resolution images that offer structural as well as global and regional functional information. In addition to standard cardiac imaging measurements and techniques, we are currently establishing a cardiac tagging protocol to allow us to identify areas of localized contractility defects. We expect this to be particularly beneficial for our mouse models which may display regional dysfunction due to areas of necrotic tissue throughout the heart. Also, we have shown that AAV is a highly efficient vehicle for both delivery of the ASG gene and therapeutic treatment of dystrophic skeletal muscle in ASG-/- mice. Due to the success of our gene therapy in the skeletal muscle, we are currently developing cardiac gene therapy to prevent dystrophic lesion formation and provide functional correction to the heart and non-invasively demonstrate functional correction in murine models of cardiomyopathy.