NON-INVASIVE CHARACTERIZATION OF DYSTROPHIC SKELETAL AND CARDIAC MUSCLE

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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. While continuing work to establish which combination of adeno-associated virus (AAV) serotype, promoter and delivery route is the most advantageous for cardiac gene delivery, we have initiated studies to non-invasively characterize hearts in mouse models of the various forms of muscular dystrophy which we intend to treat. The following studies include murine models of Limb Girdle Muscular Dystrophy; 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.

Methods

Gene Delivery: Hindlimb muscles of 6 ASG/- mice were injected with 1x10^{11} particles of AAV1 expressing the human form of the missing sarcolemmal protein (ASG). Using MRI, legs were imaged every 3 weeks post injection in order to observe dystrophic lesion development. In Vivo measures of muscle damage: Mice were imaged on a 4.7T Oxford Magnet using a Bruker Advance console and Paravision software with a 3 cm quadrature birdcage coil for the 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=5x3cm^2, 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, 2048 pts, n=128). For delayed contrast enhancement, baseline SPGR images (TR=200ms, TE=5ms, FLIP=90, n=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; Berlex;NJ). 3D SPGR MR images were repeated every 5 min for a total of 30 min post contrast 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 hyperintense regions correlated with regions of myocyte damage. AAV1-ASG treated ASG/- legs display an 85% reduction in dystrophic lesion development. Passive stretch force measurements on isolated muscles 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 also demonstrated the 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.