Improved Myocardial Mechanics as Assessed by MRI Generated Pressure-Volume Loops Following AAV6-mediated βARKct Gene Delivery.

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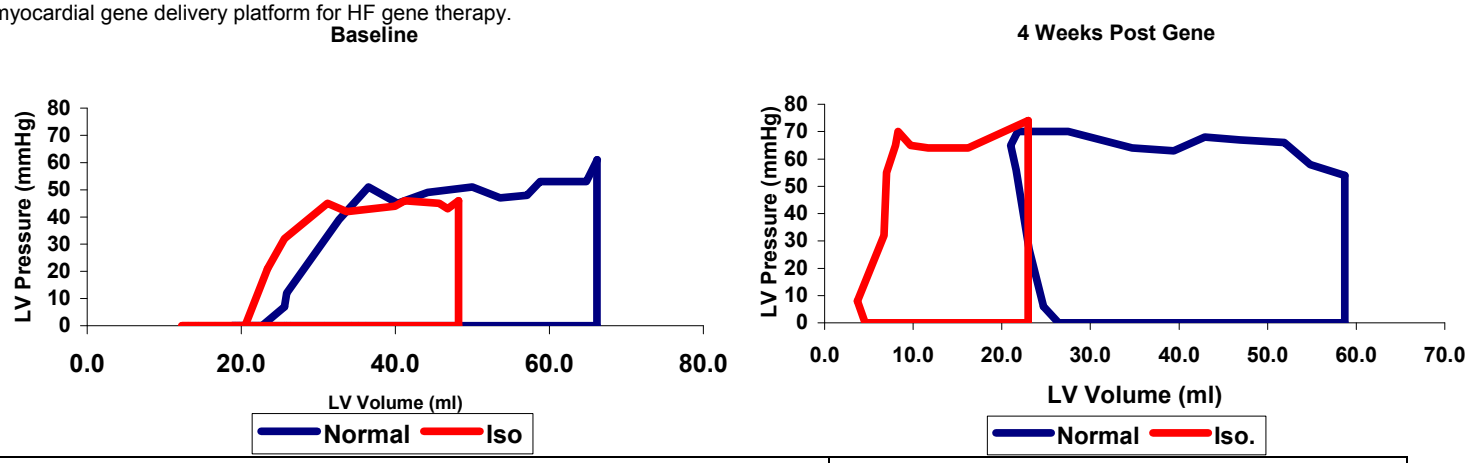
Introduction: Genetic modulation of ventricular function and remodeling may offer a novel therapeutic strategy for patients with acute ischemic left ventricular (LV) dysfunction and also chronic heart failure (HF). Manipulation of molecular targets has shown therapeutic potential in HF models. Among these is the G protein-coupled receptor kinase-2 (GRK2 or βARK1), which is up-regulated in failing human myocardium. The carboxyl-terminal portion of GRK2 (βARKct) has been shown to be an effective inhibitor of GRK2 activity in myocytes. This present study focuses on the effects of AAV6-mediated βARKct gene delivery on LV function using a novel gene delivery platform, MCARD™, unlike existing gene delivery platforms, allows for highly efficient, global and cardiac-specific gene expression in large animals. We hypothesize that βARKct gene therapy will amplify the cardiac response to a beta-adrenergic agonist resulting in improved function and efficiency as measured by MRI.

Material and Methods: Using a 3T scanner (Tim Trio, Siemens Medical Solutions, Malvern, PA, USA), MRI generated LV pressure-volume loops (PV) were obtained on a baseline sheep for normal and isoproterenol (Iso) challenge states. LV volume was measured from images acquired using a 3D SSFP sequence with the following parameters: TR=13-26ms, TE=1.65ms, slice thickness=4mm, FOV= 350x280, matrix=192x158, BW=930, FA=25 degrees. LV pressure was measured via a Millar catheter placed in the ventricle and along with the cardiac trigger were recorded with a custom designed acquisition and gating system. Following the baseline study, cardiac-specific delivery of 1014 vg of the selected AAV serotype/construct [scAAV6.CMV.βARKct] and VEGF commenced in the cardioplegic arrested heart of the animal. The infusion proceeded in retrograde fashion into the coronary sinus of an isolated cardiac circuit created through control of the vena cava, aorta, pulmonary artery, azygous and hemiazygous veins, while 100% of blood was collected from the left and right ventricular cavities and connected in sequence to the coronary sinus. Cardiac circuit flow was 50-120 cc/min and coronary sinus pressure, 80mm Hg. The vector was re-circulated for 20 minutes and then flushed from the isolated circuit. Cross clamp time was 64 min and CPB time was 109 min. A follow up MRI study was performed 4 weeks post gene delivery using the procedure described above.

Results: PV loops generated for the baseline and 4 week time points are shown in **Figure 1**. At baseline Iso challenge produces a slight leftward shift of the loops while at 4wks the shift is more significant. The changes in end-diastolic volume (EDV), stroke volume (SV), and ejection fraction (EF) with Iso are more significant post gene therapy compared to baseline (**Table**). External Work (EW) which is the work performed on the blood is greater at the 4 week point and the potential energy (PE) which is lost energy is decreased at 4 weeks with Iso (**Table**). This alteration in PE results in a more efficient LV post gene therapy (**Table**).

Discussion and Conclusion: MRI generated PV loops have demonstrated that βARKct expression improves global LV systolic performance and efficiency relative to controls 4 weeks after gene delivery. These results in normal ovine subject, using a novel, cardiac-specific gene delivery platform (MCARD™) are predictive of long term efficacy in a clinically relevant large animal HF model. Further, MCARD™ is a safe, clinically translatable global myocardial gene delivery platform for HF gene therapy.



Baseline			4 Weeks Post Bypass	
	Normal	Iso.	Normal	Iso.
EDV (ml)	66.2	48.2	58.7	23.0
SV (ml)	47.4	35.9	37.7	19.3
EF (%)	71	74	64	84
Pressure (mmHg)	61	46	70	74
EW	1839	1053	2333	1130
PE	583	452	924	161
Efficiency (%)	75	69	71	87

Figure 1. MRI generated pressure-volume loops for baseline and 4 weeks post gene therapy sheep pre and post isoproterenol (Iso) challenge. Gene therapy results in an increased response to the beta-adrenergic agonist indicted by the greater leftward shift.