MRI Assessment of Magnetically-labeled Mesenchymal Stem Cells in a Canine Model of Reperfused Myocardial Infarction

D. L. Kraitchman[1], I. Izbudak[1], P. Karmarkar[1], L. Tai[2], D. Fritzges[1], R. C. Boston[3], L. Kostura[4], M. Marcelino[4], R. G. Young[4], M. F. Pittenger[4], J. W. Bulte[1,5]

[1]Radiology, Johns Hopkins University, School of Medicine, Baltimore, MD, United States, [2]Bioengineering, Johns Hopkins University, School of Medicine, Baltimore, MD, United States, [3]University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, United States, [4]Osiris Therapeutics, Inc., Baltimore, MD, United States, [5]Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, United States

Introduction: Because of the limited regenerative capacity of the heart, there is enormous interest in using stem cell transplantation techniques to limit infarct size and prevent remodeling after myocardial infarction (MI). While MRI has been used to non-invasively track cardiac function after stem cell therapies, the success of such therapies will require methods to determine stem cell engraftment without post-mortem histology. The recent ability to label mesenchymal stem cells (MSCs) [1] with MR-visible contrast provides an obvious non-invasive pathway for future clinical trials to determine the fate and consequences of MSC therapy after myocardial infarction [2,3].

Methods: Experimental Protocol: Canine MSCs, isolated from bone marrow were magnetically labeled by incubation with ferumoxides injectable solution (Feridex, Berlex Laboratories) and poly-L-lysine (PLL) in the culture medium for 24 hours.

To create an acute MI, mongrel dogs (20-25 kgs, n=9) were subjected to a 90-minute closed-chest left anterior descending coronary artery balloon occlusion using cardiac catheterization techniques, followed by reperfusion. Autologous Feridex-PLL-labeled MSCs (MR-MSCs) were injected intramyocardially (30-150x10^6 MSCs) at 72 hours post-MI under MR guided delivery in 6 animals. MR fluoroscopy was performed using a non-ECG-gated, real-time, steady state free precession (SSFP sequence: 3.4 ms TR; 1.2 ms TE; 45º FA; 128x128 image matrix;0.5 NEX, 10 mm slice thickness; 125 kHz BW; and 30 cm FOV) with interactive scan plan acquisition (i-drive) on a 1.5T MR Scanner (General Electric) using a custom, MR-compatible, active loopless antenna, needle-tipped catheter. MR-MSCs were detected using an ECG-gated, breath-hold, fast gradient echo (FGRE) pulse sequence (6.0 ms TR; 1.6 ms TE; 20º FA; 512x512 image matrix; 5 mm slice thickness; 32 kHz BW; 28 cm FOV; and 4 NSA). MI size and location were determined using a delayed contrast-enhanced MRI (CE-MRI, inversion recovery FGRE, 0.2 mmol/kg Gd-DTPA imaged at 15 minutes post-injection, 7.8 ms TR; 3.4 ms TE; 25º FA; 256x192 image matrix; 5 mm slice thickness; 32 kHz BW; 28 cm FOV; 2 slice averages NSA; and 250 ms TI). Global function measurements (left ventricular ejection fraction [LVEF]; LV stroke volume [LVSV]; end-diastolic volume [EDV]; and end-systolic volume [ESV]) were determined from an ECG-gated SSFP short-axis contiguous stack of images (5.2 TR; 1.8 TE; 45º FA; 256x160 image matrix; 8 mm slice thickness; 125 kHz BW; and 28x21 cm FOV) covering the left ventricle.

Follow-up MRI (ECG-gated SSFP, high resolution FGRE, and CE) was performed at 1, 2, 4, and 8 weeks after the 3-day post-MI scan to determine global function measurements, the presence of MR-MSCs, and infarct size, respectively.

The heart was excised at 8 weeks and sectioned for histological staining to detect MR-MSCs.

Data Analysis: Images were analyzed using custom software to determine global function parameters and infarct size. Endocardial and epicardial borders were manually traced using cinetool (GE) on ECG-gated SSFP images for global function measurements. Hyperenhancing tissue was determined on CE-MRI based on a full-width, half-maximum criteria in cinetool.

Changes in global function parameters and infarct size were determined by fitting a general cross-sectional time-series linear model using feasible generalized least squares (Stata Corporation, College Station, TX). A P value <0.05 was considered statistically significant.

Results: All hypointense lesions or susceptibility artifacts from MR-MSCs that were present at 72 hrs post-infarction were still visible at 8 weeks. However, the degree of expansion of the artifacts was variable among different animals.

MI size, as a percentage of left ventricular (LV) mass, decreased significantly over the 8 week period in both MSC-treated (MSC) and control dogs (Fig 1). This pattern of MI size reduction over 8 weeks was similar among all animals. Concurrently, LV mass was maintained up to 8 weeks in MSC whereas controls showed a decline in LV Mass at 2-8 weeks post-MI (Fig 2). The pattern of the change in LVSV over time was significantly different between MSC and controls groups. MSC animals showed an improvement from baseline LVSV up to 4 weeks whereas controls demonstrated a decline in LVSV up to 4 weeks (Fig 3). However, LVSV was similar between groups at 8 weeks post-MI. A similar pattern was observed with LVEF such that was LVEF improved significantly in MSC vs. controls up to 4 weeks, but was not different between groups at 8 weeks post-MI.

*Fig 1: MI size in untreated control animals (left) and MSC-treated (MSCs) animals (right) decreased significantly over 8 weeks post-MI relative to baseline (*P<0.04). The decrease in MI size was similar between MSCs and controls. (P=NS).*

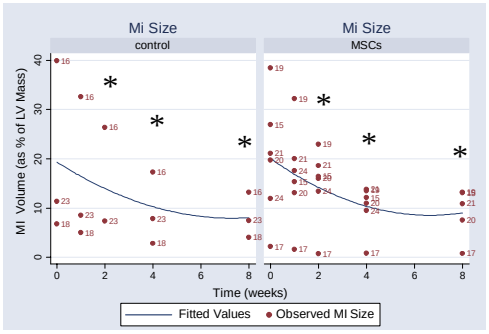
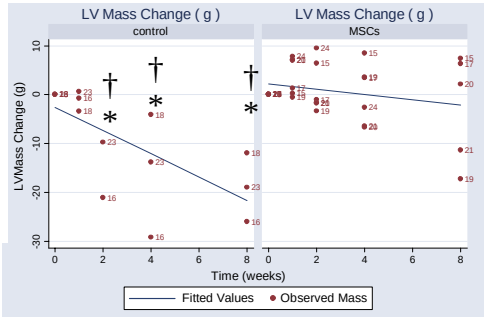


*Fig 2: Change in LV mass in untreated control animals (left) decreased significantly over 8 weeks post-MI relative to baseline (†P<0.001) and was decreased relative to MSC treated dogs (right, *P<0.002 vs. same week post-MI)*



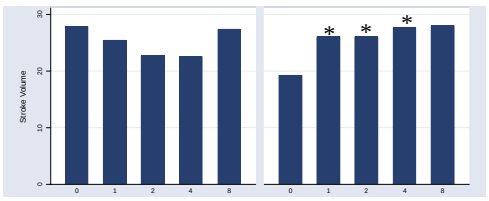


*Fig 3: LV stroke volume (SV) demonstrated improvement in MSC-treated dogs up to 4 weeks relative to untreated controls (*P<0.04 vs. LVSV same week in control dogs).*

Discussion:

MR-MSCs offer a method to target and confirm the delivery of stem cell therapies and track their migration and engraftment up to 8 weeks post-MI. MI size declined in both treated and untreated dogs, however myocardial mass was preserved in MSC-treated animals. This preservation of myocardial mass was related to functional improvements in LVSV and LVEF up to 4 weeks post-MI. Improvements beyond 4 weeks, if they exist, may become apparent with a higher statistical power (e.g., more animals) or more sophisticated regional functional analysis techniques.

References:

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[3] Garot et al. J Amer Coll Cardiol. 2003 **41**(10):1841-6.