Tracking of Intra-Coronary Delivered Mesenchymal Stem Cells Using MRI in a Porcine Model of Myocardial Infarction

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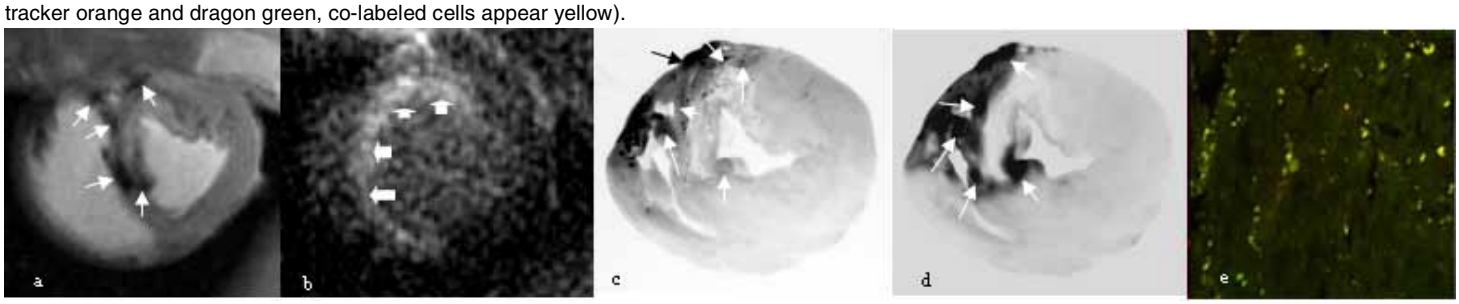
Introduction: The use of precursor stem cells has been shown to regenerate damaged heart muscle in animals.[1] There has been debate over the optimum route of introduction of these stem cells – intra-coronary, systemic intravenous or direct intramyocardial injection. Recent studies have confirmed clinical benefit from intra-coronary injections of progenitor cells derived from both bone marrow and peripheral blood.[2] Local delivery is attractive because of the high concentrations that may be necessary for biological effect while avoiding potential toxicities associated with comparable systemic levels. In vivo tracking of these cells is difficult although initial engraftment rates with PET-imaging have been shown to be very low (1.3 – 2.6%)[3] with the majority of cells engrafting in lung, liver and spleen. The aim of this study was to evaluate MRI as a method of tracking magnetically labeled mesenchymal stem cells (MSCs) in a porcine model of acute myocardial infarction (AMI) following intracoronary delivery.

Materials and Methods: All experiments had the approval of our institution's Animal Care Committee. Reperfused AMI was induced in three Yorkshire pigs (22-28 kg) via a 90-minute percutaneous balloon occlusion of the left anterior descending artery (LAD) distal to the second diagonal branch under an x-ray C-arm system (OEC 9800, GE Healthcare, Milwaukee). Following infarction, the animals were recovered and observed for seven days. Canine MSCs were obtained from bone marrow and expanded in culture. Seven days after AMI creation, the MSCs were co-labeled with Cell Tracker Orange fluorescent dye (Molecular Probes), and superparamagnetic fluorescent microspheres (Bangs Laboratories, Fisher, IN) by means of endocytosis. Following this incubation, the cells were trypsinized, washed twice in phosphate buffered saline (PBS) and resuspended in PBS such that final volume was 4-6 mls. Final cellular yield was $2 – 5 \times 10^6$ and viability was confirmed by trypan blue exclusion. The animal was reinstrumented and an end-hole angioplasty balloon (Opensail, Guidant) was placed at the site of previous balloon inflation. The balloon was inflated to low pressure (2-4 atmospheres) and the cells were infused by hand injection over two 2-minute balloon inflations with an intervening 2-minute period of balloon deflation to minimize ischemia. Following cell delivery the animal was moved to the MRI suite for scanning. MRI techniques: All MRI studies were performed on a GE 1.5T Signa CV/i system (GE Healthcare). Two 5mm short-axis slices, within 25mm of the apex, were selected for monitoring. Bulk motion suppression was achieved using signal averaging and peripheral cardiac gating from the pig's tail. A 5-inch surface coil on the animal's chest provided signal reception. MR signal fluctuations within a stack of 2D fast spoiled gradient recall sequences (FSPGR) were monitored prior to and following cell delivery (TR 7.3ms, TE 3.4ms, FOV 23x23cms, flip angle 90º, matrix 256x256, 4 NEX). 0.2mmol/kg gadolinium DTPA (Magnevist, Schering) was given intravenously to assess for delayed hyperenhancement (DHE) and thus extent of myocardial infarction. Finally, following sacrifice, the heart was excised, sliced into 10mm short axis slices and preserved in 10% formalin. The spatial distribution of MR signal fluctuations was compared with the distributions of cell tracker orange and Bangs fluorescences, both at a tissue level (Kodak Multi-model Imager) and at cellular level using confocal microscopy.

Results: FSPGR imaging immediately following cell delivery revealed signal hypointensity, characteristic of the cells' magnetic label, predominantly in the infarct border rather than within the infarct itself (figure a – arrows). Delayed hyperenhancement confirmed size and location of the infarct (fig b – thick arrows). Post-mortem fluorescent imaging confirmed concordance of fluorescence and MRI hypointensity (fig c – cell tracker orange, fig d – dragon green). Confocal microscopy of tissue from the infarct border reveals concordance of both fluorescent labels (fig e – merged image of both cell tracker orange and dragon green, co-labeled cells appear yellow).



Discussion and Conclusion: Tracking of intracoronary injected, magnetically labeled, stem cells using MRI is possible. Using FSPGR, the cells' magnetic label appears as a hypointensity (negative contrast). Initial results suggest that these cells migrate to the border region between infarcted and viable tissue. MRI has previously been used to guide the injection[4] and subsequent tracking[5] of magnetically labeled cells via the intramyocardial route. Work by Pearlman et al[6] has shown that it is possible to track cells injected via the coronary artery but our work using a FSPGR sequence is the first to show the peri-infarct distribution of the injected cells. Pearlman's model involved a shorter balloon inflation (60 minutes) and larger quantity of cells (20×10^6) which may explain the homogeneous distribution of injected cells they observed. Our model of longer balloon inflation may cause more capillary damage which may limit the injected cells' ability to penetrate the infarcted region and thus explains the peri-infarct distribution seen in our study. Cell engraftment rates are related to subsequent functional improvement. Cunningham[7], using an off-resonance sequence which selectively images the dipolar fields surrounding magnetically labeled cells as a positive contrast, showed positive correlation between signal intensity and cell number. Currently this technique does not have enough sensitivity to quantify cell engraftment within the heart. Further work is ongoing to assess cell engraftment using this off-resonance technique and to assess for long-term functional improvement following administration of autologous endothelial progenitor cells.

References:

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