Positive Contrast MRI Demonstrates Retention of Mesenchymal Stem Cell Therapy Administered Intramuscularly in Ischemic Skeletal Muscle

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Introduction: Cellular therapies offer a potential source of cells or factors for regenerative therapy. In addition to assessing the outcome of these therapies, it is important to properly deliver and track them over time. Magnetic labeling with superparamagnetic iron oxide (SPIO) nanoparticles has been used to non-invasively monitor stem cell delivery and persistence using MRI [1]. Conventional MR imaging of magnetically-labeled cells is based on T2*-weighted imaging such that the cells appear as signal voids in the images. A limitation of this approach is that not all signal voids in the image are due to the magnetically-labeled cells. Therefore, positive contrast MRI methods, like Inversion Recovery ON-resonance (IRON) water suppression [2], have been proposed for enhanced visualization of magnetically-labeled cells and have shown a good correlation between the volume of enhancement and cellular concentration [3]. A significant limitation of both T2*-weighted and positive contrast imaging is that they cannot discriminate between magnetically-labeled cells and free iron. The purpose of this study was to investigate the difference in signal retention associated with injections of magnetically-labeled cells and the magnetic label alone using positive contrast IRON MRI.

Methods: Intramuscular injections of SPIO-labeled mesenchymal stem cells (MSC) were compared to injections of SPIO alone in an ischemic skeletal muscle bed.

Cell preparation. MSCs were isolated from the bone marrow of male New Zealand White rabbits and cultured-expanded for 3 passages. Magnetic labeling of the cells was performed by electroporating the MSCs with ferumoxides (Feridex, Berlex, Inc), as previously described [4].

Animal Model. Hindlimb ischemia was induced in 13 female New Zealand White rabbits by endovascular placement of 5-6 thrombogenic platinum coils (Vortex, Target-Boston Scientific) in the left superficial femoral artery under X-ray fluoroscopy [5]. At 24h post-coiling, the rabbits were randomized into a treatment group (n=7) which received allogeneic SPIO-labeled MSCs ($12\text{-}13\text{x}10^6$ cells) and control group (n=6) which received only the SPIO (45μL ferumoxides) diluted in phosphate buffer solution (1.5mL). For each animal, six injections of approximately 0.25 mL of either labeled cells or SPIOs were injected into the ischemic adductor compartment of the left hindlimb.

MRI. Prior to MRI, the animals were anesthetized and intubated. Throughout the imaging session, the ECG was monitored, and animals were allowed to breathe freely. All experiments were performed on a 3T Philips Achieva scanner using a 6-element cardiac phased-array receiver coil. 2D and 3D IRON MRI were performed immediately after injections, and 1 and 2 weeks post-injection. IRON imaging was performed using a fast spin echo acquisition with fat saturation and localized shimming. 2D Imaging parameters were as follows: 2000 ms TR, 11.62 ms TE, $0.39\text{x}0.39\text{x}3$ mm^3 spatial resolution, 24 echo train length (ETL), 2 signal averages (NSA), 105° IRON flip angle, and 170 Hz IRON bandwidth. For 3D IRON imaging, imaging parameters were: 1300 ms TR, 12 ms TE, $0.26\text{x}0.26\text{x}1.5$ mm^3, 24 ETL, 2 NSA, 105° IRON flip angle, and 170 Hz IRON bandwidth. Labeled cell distribution and persistence in positive contrast images were evaluated using full-width, half-maximum criteria to determine the total volume of signal enhancement.

Results: Labeled cell viability was 85±9% prior to injection. SPIO-labeled cells and SPIO-only injections appeared as six hyperintense regions in positive contrast MR images. Based on previous ferrozin-based calometric assay studies, intracellular iron in MSCs was estimated at 10 pg/cell. Therefore, the maximum iron content per injection in treated animals was estimated at 0.02 mg. In contrast, injections of SPIO-only contained approximately 0.084 mg of iron. The nearly 4-5 fold greater total administered iron was not directly reflected in the volume of enhancement measurements by IRON MRI analysis. The total volume of enhancement immediately following injections was greater in rabbits receiving SPIO only (Figure 1), but only by 30%. The enhancement volume decreased rapidly and to a greater extent in control animals compared to MSC-treated animals which stabilized from one to two weeks (Figure 1). Furthermore, complete loss of signal enhancement was observed in several control rabbits as early as 1 week post-injection (Figure 2).

Discussion and Conclusions: Free iron oxide nanoparticles were rapidly cleared from the ischemic tissue, while enhancement from labeled MSC injections initially decreased rapidly but stabilized after one week. These findings suggest initial rapid removal of SPIOs when administered alone or when released from MSCs that die in the first days following delivery. However, the stabilization of the signal in MSC-treated animals suggests that a large portion of positive enhancement is due to intact viable MSCs. Immunohistochemistry has confirmed these findings. Lastly, positive contrast MRI proved effective for monitoring the delivery and persistence of MSCs and may be useful in future studies involving cellular therapies.

References: [1] Bulte and Kraitchman. *Curr Pharm Biotechnol*, 2004; 5(5): 567-84. [2] Stuber et al. *J Cardiovasc Magn Reson*, 2006; 8(1): 13-4. [3] Gilson et al. *J Cardiovasc Magn Reson*, 2006; 8(1): 72-3. [4] Walczak et al. *Magn Reson Med*, 2005; 54(4):769-74. [5] Liddell et al. *J Vasc Interv Radiol*, 2005; 16: 991-8.

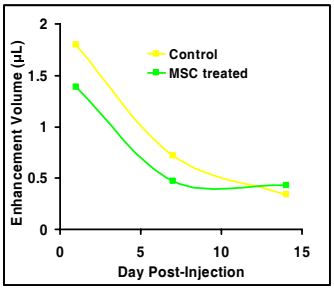

Figure 1. The enhancement volume from 2D IRON images was initially greater in control rabbits than MSC-treated rabbits but continued to decline over the 2 week time course, whereas the enhancement volume stabilized after 1 week in MSC-treated animals.

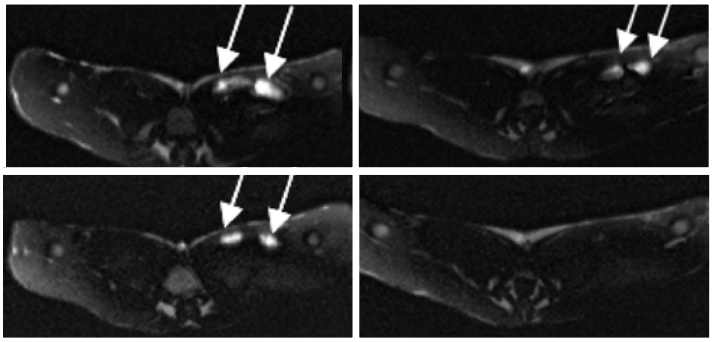

Figure 2. Representative 2D IRON images show that signal enhancement (arrows) was observed in both MSC-treated (top) and SPIO-only (bottom) animals immediately following injections (left). However, signal enhancement rapidly disappeared in several SPIO-only animals as early as 1 week post-injection (bottom right).