The Role Of MRI For The Evaluation Of Spinal Cord Injury And Stem Cell Transplantation In Mice.

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Introduction: Among the treatments being studied for spinal cord injury (SCI) an area of great interest is stem cell therapy. In animal models of SCI stem cells are commonly transplanted directly into the injured cord. The success of this transplantation depends on the expertise of the technician. Whether transplanted stem cells survive in the host tissue and/or home to and engraft in tissues other than the target are important factors in the development of effective stem cell treatment protocols. The effectiveness of stem cell transplantation in preclinical models is usually evaluated by locomotor testing and, in some cases evoked potentials, to assess functional recovery, in addition to histopathological analysis to measure tissue sparing, cell survival, and tissue regeneration. Since conventional histology looks at only a limited amount of tissue, typically at the endpoint of the study, it is not possible to have a complete picture of the initial stem cell distribution or to understand how it changes over time. In this study we use high resolution MRI together with iron nanoparticles to label stem cells for the *in vivo* evaluation of SCI in mice. We show that the noninvasive and 3D nature of MRI is crucial for gaining an appreciation of the location of transplanted stem cells and their fate over time.

Methods: A severe clip compression SCI was induced in 18 female black mice at the level of the 4th thoracic vertebrae. Bone marrow-derived mouse multipotent stromal cells (MSC) were isolated by adherence and labeled with micron-sized iron oxide (MPIO) particles (SiMAG, 0.75-1 μm; *Chemicell*). SCI mice received an intra-spinal MPIO-labeled MSC transplant within a week after the injury using a standard configuration with a Hamilton syringe. Mice were scanned at 3T using a custom built gradient coil insert and a 3D balanced steady state free precession imaging pulse sequence with RF phase cycling (FOV: 4x2.6cm, TR/TE = 3.8/1.8ms, 128 locs/slab, 16 NEX). Acquisition time was 31 min.

Results: Excellent 3D images were obtained with a 200x200x200μm resolution. Successful transplants (Fig. 1) were observed as distinct areas of signal loss within the cord in 14 mice. However, in 4/18 mice no signal loss was observed within the spinal cord. Closer inspection of the 3D image data revealed that signal loss was outside of the intended transplant location, in the surrounding tissues (Fig. 2). In another example, where the transplant was successful, additional regions of signal loss were observed in the hindbrain in close proximity to ventricles. This signal void diminished in size over time but persisted in the same location until the end of the study (Fig. 3). An additional interesting result was the observation of cysts forming rostral to the epicenter of the injury in 2/18 mice. While in one case the cyst was close to the epicenter (Fig. 4) in another it fell outside of the segment of tissue that would be included in traditional histological assessment.

Discussion: We are the first to have used cellular MRI to study stem cell therapy in a mouse model of SCI. MRI is an invaluable tool to learn more about cell therapy and to consider better methods to exclude subjects and avoid bias before comparing treatments. MRI allows us to verify precise delivery of the cells to the intended target *in vivo*, which is crucial when comparing delivery routes. Including unsuccessful transplants during analysis can produce erroneous or biased results. The ability to observe the overall distribution at different time points beyond the tissue or organ of interest also provides important information regarding cell trafficking within the body. Longitudinal studies supplies additional important information such as the development of cysts, which are not usually present in mice with SCI. Cellular MRI allows us to observe cell delivery, distribution and changes in the tissue over time providing a better understanding of models of disease and improve how we approach and compare potential treatments.

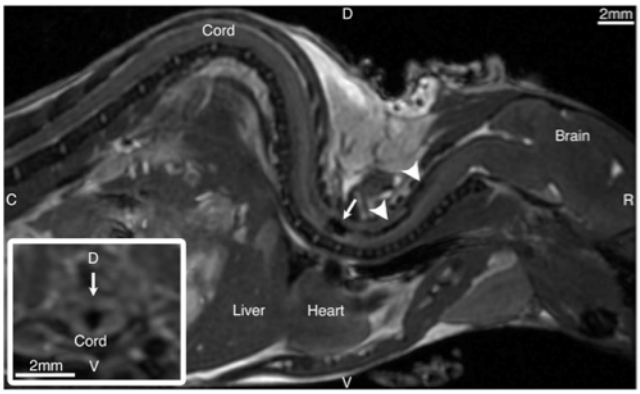

Figure 1. Sagittal view of the mouse cord. Areas of signal loss are seen where MPIO-labeled MSC were transplanted (arrow) and rostrally (arrowheads). Insert shows oblique axial view of the transplant site (arrow sagittal view).

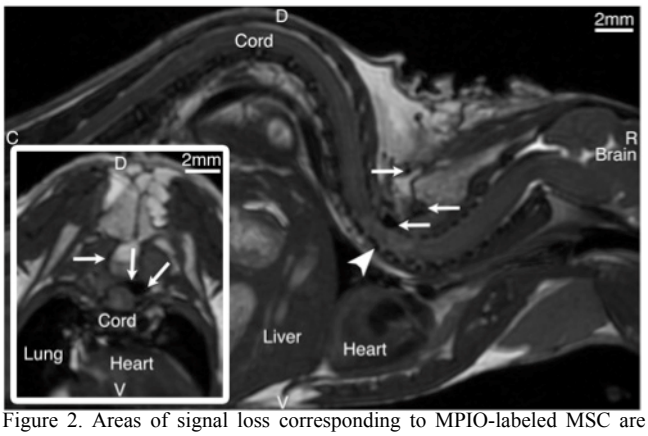

Figure 2. Areas of signal loss corresponding to MPIO-labeled MSC are observed outside of the cord in the surrounding tissue (arrows) but not within the cord or at the epicenter (arrowhead).

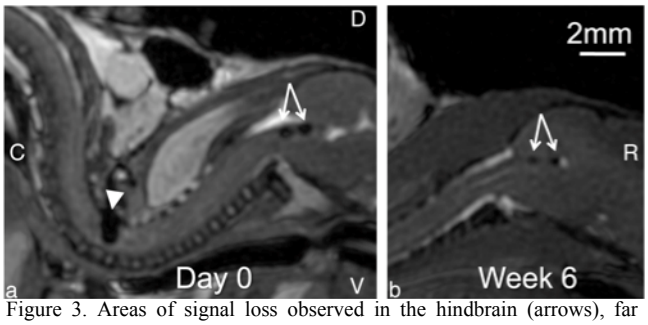

Figure 3. Areas of signal loss observed in the hindbrain (arrows), far rostrally from the epicenter (arrowhead), as early as the day of the transplant (a) that, while decreasing in size, persisted throughout the study (b).

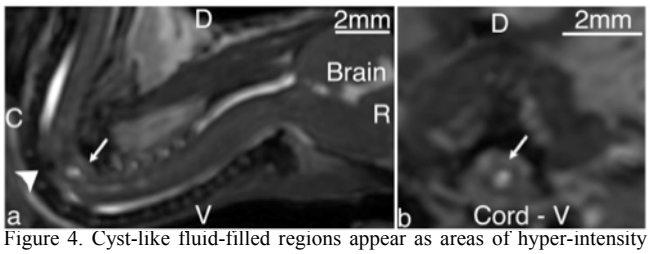

Figure 4. Cyst-like fluid-filled regions appear as areas of hyper-intensity (arrows) in a sagital view (a) and in an axial view (b) rostral from the epicenter (arrowhead).