Monitoring Stem Cell Migration in the Normal Mouse Brain by MRI

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Introduction: Delivery of therapeutic molecules by stem cells opens a possible alternative mechanism for the treatment of neuronal diseases. Cell tracking has been performed in the rat brain using a variety of custom made MRI contrast agents [1-3]. Recently, clinically approved super paramagnetic iron oxide (SPIO) particles have been used to label stem cells *in vitro* [4]. *In vivo* MRI detection of "homing in" of stem cells has been reported for stroke [5] and photo-dynamically induced lesions [6] in the rat brain. We implanted neural stem cells (NSC, C17.2) labeled with clinically approved SPIO particles to examine the extent of stem cell migration in the normal mouse brain. Serial MR imaging of the brain was performed after implantation in the adult and neonatal mouse. *Ex vivo* MR images were correlated with Prussian blue staining for iron detection.

Methods: *Labeling of cells with iron oxide particles:* C17.2 cells were labeled with Feridex (25µg Fe/ml) as reported earlier [4]. *Intra-cranial implantation:* Neonatal C3H/SCID mice (n=10) were cryo-anaesthetized and injected on the day of birth. Two µl of the labeled cell suspension ($5x10^4$ cells/µL) was injected into each lateral ventricle. Adult female C3H mice (n=5) were anaesthetized and labeled NSC's ($4x10^4$ cells/µl) were implanted in the hippocampus region. *In vivo imaging:* 2D gradient-echo images were acquired on a 4.7T magnet using a 3 cm birdcage coil. Adult mice were imaged at five time points after cell implantation (1, 7, 16, 21 and 32 days) and neo-natally injected animals were imaged after 6-7 weeks of implantation. Imaging parameters: TR/TE=330/4 ms, thk=0.2 mm, matrix=128x128, FOV=2.0 cm, nt=16, total acquisition time ~25 min, spatial resolution -156µm. At the end of *in vivo* experiments, mice were sacrificed and perfused with 4% paraformaldehyde. Brains were removed and 3D gradient-echo imaging was performed on a 9.4T vertical bore magnet using a 20 mm Helmholtz NMR probe. Imaging parameters: TR/TE=100/10 ms, matrix=256x128x128, slab thk~20 mm, FOV=2x1x1 cm, nt=28, total acquisition time ~13 h, spatial resolution -78 µm. *Histology:* 20 µm-thick sections of the brain were stained with Prussian blue for detection of iron particles.

Results: As shown in Figure 1, SPIO labeled C17.2 cells were detected near the injection site of the adult brain up to 32 days after injection with a gradual decrease of the hypo-intensity from the labeled cells. About two weeks after implantation, there seemed to be moderate degree of relocation of stem cells along the white matter tracts as shown in Figure 2. The relocation/migration of these cells was confirmed by *ex vivo* images (2B) and Prussian blue staining for iron (2C). In contrast, when SPIO labeled C17.2 cells were injected neo-natally, a widespread migration of cells was observed after 7 weeks of implantation (Figure 3). Small clusters of these cells were distributed widely throughout the brain and appeared as hypo-intense regions on *ex vivo* MRI images (3B). A good correlation was observed between Prussian blue staining and MRI in these animals (3A, C).

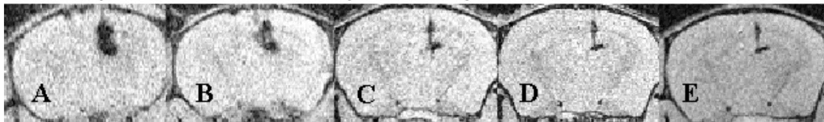

Fig.1 Transverse sections 2D multi-slice gradient echo images of an adult mouse brain after implantation of SPIO labeled C17.2 cells. (A) 1 day, (B) 7 days, (C) 16 days, (D) 21 days, (E) 32 days. Imaging parameters: TR/TE = 330/4 ms, thk = 0.2 mm, matrix = 128x128, FOV = 2.0 cm, nt = 16, acquisition time ~ 25 min.

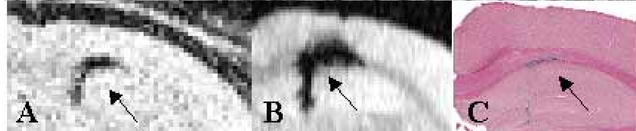

Fig.2 *In vivo* (A), *ex vivo* (B) and corresponding Prussian blue (C) image of adult mouse after 16 days of NSC injection. Imaging parameters: *In vivo*: same as Fig.1, *Ex vivo*: TR/TE = 330/10 ms, thk = 0.2 mm, matrix = 256x256, FOV = 1.5 cm, nt=64. Arrow depicts SPIO labeled cells (A, B) and iron staining (C)

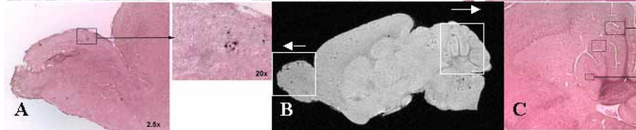

Fig.3 (B) *Ex vivo* MR image of a mouse brain after neonatal implantation of C17.2 stem cells labeled with SPIO particles and corresponding Prussian blue staining (A, C). MR images were acquired after 7 weeks of NSC implantation. MRI parameters TR/TE = 100/10 ms, matrix = 128x128x128, slab thk = 20 mm, FOV = 2x1x1, nt = 28 acquisition time ~ 13 h.

Discussion and Conclusion: Longitudinal screening of SPIO labeled stem cells by *in vivo* MRI opens the possibility to detect the migration ability of these cells non-invasively. Our studies demonstrate the ability of MRI in detecting engrafted SPIO labeled C17.2 stem cells up to 32 days after injection in the adult brain, or 7 weeks in the neonatal brain. The gradual decrease of hypo-intensity from the injection site may be due to cell migration, dilution of iron by cell division or loss of iron-oxide particles by cell clearance pathways. Widespread migration and differentiation of these cells was observed when the cells were injected neo-natally. To minimize the possible toxicity of labeling agent, we used a very low concentration of clinically approved iron oxide particles (25 µg/ml), as compared to previous studies (140 - 2800 µg Fe/ml, [5,6]). MRI studies of cell tracking have shown the migration of stem cells to the site of the lesion [5,6]. We performed our experiments in normal mouse brain where the implanted cells did not have any preferences to migrate. This approach may assist in a treatment of global brain disorders, such as, mucopolysaccharidosis (MPS VII) [7].

References:

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