Assessing the effects of a contrast agent on the ability of neural stem cell grafts to recover behavioural impairments in a rat model of stroke: A 1 year serial MRI study.

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Transplantation of neural stem cells in ischemia-damaged brains is an attractive novel therapeutic strategy. Still, at present it is unclear how these cells promote behavioral recovery. Cells migrate widely, integrate into host circuits, and differentiate into site-appropriate phenotypes. Nonetheless, from histological studies it is difficult to discern if the migratory pattern or the presence of cells in a particular region impact on behavioral improvements. The dynamic nature of the processes involved in lesion evolution and graft-induced recovery necessitate a repeated assessment of an individual subject over time. The use of contrast agent-loaded cells followed serially over time with MRI provides the opportunity to assess the relevance of the site of these cells to behavior. We report here the use of serial MRI over 1 year with concomitant behavior to evaluate for how long contrast agent-loaded cells were visible on MRI scans and if the presence of the bimodal contrast agent GRID inside transplanted cells affected their ability to promote behavioral improvements after a stroke.

Methods: Sprague-Dawley rats underwent 60 minutes of right intraluminal thread middle cerebral artery occlusion 2 weeks prior to transplantation of 100 000 neural stem cells from the MHP36 stem cell line into the unaffected contralateral hemisphere. MHP36 cells were either labeled with the bimodal contrast agent GRID for 16 h by incubation (Modo et al., 2002, Neuroimage, 17, 803-811) or with the non-MRI enhancing fluorescent dye PKH26 for 4 minutes prior to grafting. Animals were assessed on the bilateral asymmetry test concomitant to MRI examinations 1 week prior to transplantation and 1, 4, 12, 26, 39, and 52 weeks post-implantation. MRI examinations were conducted on a 4.7T Varian system with a spin echo multi echo sequence (TR=4000ms, TE_1=22ms, TE_2=44ms, TE_3=66) affording a spatial resolution of 128 μm in plane with a thickness of 600 μm. To provide an indication as to the volumetric changes transplantation promoted in these animals, lesion volumes were calculated for all time points.

Results: GRID-labeled cells were clearly visible on the MRI scans and by 1 week already appeared to have crossed the corpus callosum into the damaged hemisphere. At 4 weeks after transplantion, the GRID-labeled transplants indicate that MHP36 delineate the area around the lesion and potentially participate in processes of recovery (Fig. 1). No behavioral recovery however was observed at this time point, but previous studies (Modo et al., 2002, Stroke, 33, 2270-2278) suggest that between 4 and 6 weeks a definite improvement in behavior can be observed in these animals. Behavioural recovery therefore appears to lag behind the arrival of transplanted cells in the peri-infarct area. By 3 months following implantation, only cells in the injection tract were still visible on MRI scans suggesting that either the label inside the cells was degraded or that cells have died. Although no behavioral improvement in the removal of 'sticky tape' from the animals' forepaw was observed in animals with GRID-labeled transplants, a definite improvement of animals with PKH26-labeled transplants was observed (Fig. 2A). Nonetheless, GRID-labeled transplants showed some benefit of the transplants on the total time to complete the bilateral asymmetry test. It was only PKH26 labeled transplants that promote a reduction in lesion volumes compared to stroke-only animals, whereas GRID-labeled animals showed no reduction in lesion volumes (Fig. 2B).

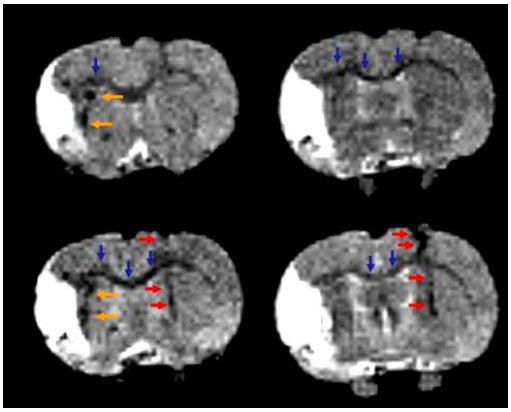

Figure 1. shows that GRID-labeled neural stem cells can still be detected by MRI 4 weeks after transplantation. Some cells still line the injection tract (red arrows), but others appear to migrate or reside in the corpus calousm (blue arrows). A large number of cells appear to reside in the peri-infarct area, but do not appear to form the border of the infarct area at this time point (orange arrows).

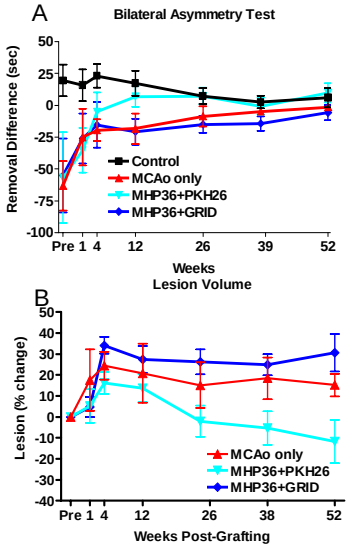

Figure 2. A. Difference in removal of sticky tape on the Bilateral Asymmetry Test. B. % change in lesion volume.

Conclusion: The current study demonstrates the potential of MRI to assess neural transplantation in stroke-damaged brain over extended time periods. We also established that contrast agent-loaded cells were reliably visible for at least up to 4 weeks following transplantation. Some measures suggest that contrast agent-loaded cells promoted improvements in behavior, whereas other measures indicate that these cells did not significantly affect behavior. Additionally, there was no reduction in lesion size in animals with GRID-labeled transplants, whereas an almost 30% reduction of lesion volume was observed in rats with PKH26-labeled cells. These results suggest that loading of cells with contrast agents to assess their in vivo survival provides invaluable information about their migration and location, but will need further investigations to allow contrast-agent labeled cells to fulfill their potential for brain repair.