MR tracking of magnetofected neural stem cells in the shiverer dysmyelinated mouse brain

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Introduction

Dysmyelination, as caused by defective genes encoding for myelin components, is a rare disorder in humans (Pelizaeus-Merzbacher disease) and can also be found in a variety of small and large [1] animal models. These dysmyelinated animal models have served as a framework to study the myelinating and potentially therapeutic properties of transplanted glial cells and neural stem cells, and may possibly have a wider application in the evaluation of cell therapy for multiple sclerosis (MS). In vivo magnetic resonance (MR) cell tracking of magnetically labeled (magnetofected) cells is a novel research tool, which has been successfully applied to non-invasively visualize transplanted cells. Earlier studies have shown that the migration of magnetically labeled stem and progenitor cells can be accurately determined by both in vivo and ex vivo imaging in two rat models of dysmyelination, the myelin-deficient rat [2] and the shaker rat [3]. In this study, we report on the applicability of MR tracking of magnetofected neural stem cells following transplantation in the dysmyelinated shiverer mouse brain.

Materials and Methods

The *LacZ*-transfected neural stem cell line C17.2 (derived from neonatal mouse cerebellum, courtesy of Dr Evan Snyder) was cultured in standard culture medium. 24 hrs prior to transplantation, cells were magnetofected with dextran-coated Feridex and poly-L-lysine [4], To this end, the growth medium was mixed with 25µg Fe/ml Feridex and 375ng/ml PLL for 60 min, and cells were then incubated in this composition. Neonatal (P1-P3) shiverer mice (C3FeSWVMbp^{shi}/J, Jackson, Bar Harbor, ME) were cryo-anesthetized and placed in a stereotaxic device (Stoelting, Wood Dale, Il). Magnetofected C17.2 cells (80,000 in 2 µl) (n=8 mice) or an equal volume of saline (n=2 mice) were injected into the right ventricle using a micro-injection needle (WPI, Sarasota, FL). In vivo MR imaging was performed at 1, 3, and 5 weeks after cell transplantation using a Bruker 11.7T Avance spectrometer. Mice were anesthetized by isoflurane inhalation (1-2%), and immobilized in a vertical custom-made probe, equipped with a transmitter-receiver coil. The body temperature was maintained at 36°C. In vivo images were obtained using a spin echo sequence (TR=2000 or 3000ms, TE=15ms, thickness=0.8mm, in-plane resolution 117µm, 4 averages). At 30-60 days post-transplantation, mice were transcardially perfused with PBS followed by 4% paraformaldehyde fixation. Brains were then removed and immersed in Fomblin. Ex vivo MR experiments were performed on the same spectrometer using a 10 or 20 mm diameter transmitter/receiver coil. 3D gradient echo images were acquired with TR=100ms; TE=6.2, 11.6, 16.9, 22.3, 10 averages and a resolution of 65x65x65 µm. For (immuno)histochemical analysis, brains were cryopreserved in 20% sucrose, frozen and cryosectioned at 30µm. Three different methods were used to confirm the distribution of transplanted cells: b-galactosidase expression using X-gal histochemistry, immunofluorescent staining for the dextran component of Feridex, and Prussian blue staining for iron.

Results and Discussion

In vivo MR imaging at the different time points revealed that cells appeared to remain within the ventricles for prolonged times; no migration into the brain parenchyma could be observed. In contrast, the ex vivo imaging showed migration of hypointense, labeled cells into the brain parenchyma in close proximity to the ventricles. This mismatch can be explained by the higher sensitivity/detection threshold of the ex vivo MR imaging (both in terms of resolution and signal averaging). The hypointense signal patterns observed on the ex vivo MRI (Fig. 1A and 1D) closely matched the distribution of grafted cells as detected by conventional histological methods: X-gal histochemisty (Fig. 1B) or anti dextran immunohistochemistry (Fig. 1C). The latter staining indicated that, even at 60 days post transplant, the Feridex remained within endosomes as evidenced py the punctate intracellular staining pattern. A Z-projection of 6 merged slices with a total thickness of ~400µm is shown in Fig 1D. The shaking phenotype of the transplanted shiverer mice, apparent at P12, was not altered throughout the course of 60 days as compared to non-transplanted controls. Both the lack of extensive cell migration (i.e., widespread, "global" distribution) and the lack of behavioral improvement is in sharp contrast with a previous report [5]. The reason for this discrepancy is, at present, not known. In order to rule out a possible detrimental effect of PLL-Feridex-labeling, comparison studies with unlabeled C17.2 cells are in progress.

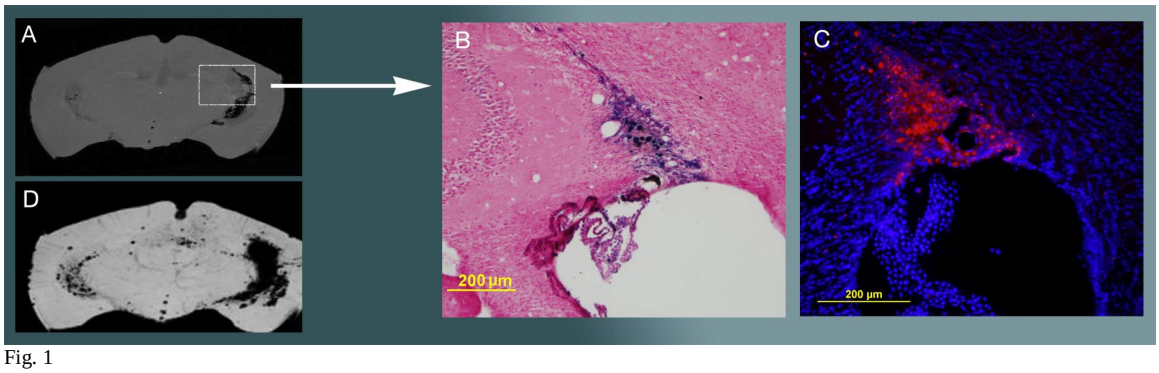

Fig. 1

References

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