Introduction

Demyelination is frequently encountered in various neurological diseases and is often considered a major cause of the associated morbidity and death. It has recently been demonstrated (1) that allografted oligodendrocytes and their precursor cells can (re)myelinate axons, and this has raised hope for possible therapeutic intervention. The survival, acute dispersion and migratory pattern of these transplanted cells is of crucial importance for the extent and limit of remyelination, and, at present, cannot be determined until invasive, irreversible procedures are carried out. We hypothesized that tagging oligodendrocyte progenitors with a magnetic label might allow MR tracking of their cellular translocation and induced remyelination following neurotransplantation.

We have explored an endogenous iron transport system for efficient delivery of MION-46L into cultured oligodendrocyte progenitors. The method uses a monoclonal antibody (mab) directed against the transferrin receptor (Tfr) that induces internalization of the Tfr-mab complex. The Tfr is a ubiquitous, high density receptor that is recycled back to the cell membrane within minutes following endocytosis. This rapid recycling enables multiple delivery of magnetic nanoparticles through use of a single receptor. A similar mab-Tfr delivery system has previously been used (2) to shuttle drugs across an intact blood-brain barrier. We report here on our initial experience with magnetically labeled oligodendrocyte progenitors, which were transplanted in the spinal cord of neonatal myelin-deficient (md) and normal rats.

Materials and Methods

Using the peroxidate-oxidation and borohydride reduction method, MION-46-L was covalently linked to the mouse anti-rat Tfr mab OX-26 (3). The oligodendrocyte precursor cell line CG-4 (4) was cultured for 48 h in the presence of MION-46L-OX-26 (1, 3, 6, 12, 25, and 50 μg Fe/ml medium) or unconjugated MION-46L (50 and 500 μg Fe/ml medium). Following proper washing steps, cells were examined with immunohistochemistry, Prussian Blue staining, electron microscopy, and variable-field T1 and T2 relaxometry. In addition, cells were recultured for another 5 days following magnetic labeling.

Approx. 5 x 10^4 magnetically labeled CG-4 cells in 1.5 μl medium were grafted into the T13/L1 region of the spinal cord of 5- to 7-day-old md rats (n=5) and normal littermates (n=2) as described previously (5). Grafting experiments with unlabeled cells were included as control (n=5). At each time point (14 = 14 (n=8) days following neurotransplantation, paraformaldehyde-perfused spinal cord specimens were placed in 5 mm NMR tubes, filled with a perfluoropolyether (devoid of proton signals). 3D multi gradient echo MR images were obtained on a GE Signa 0.5 T scanner (1024 x 128 x 40, FOV=20 x 5 mm, matrix 384x96 or 256x64; NEX=100; TR=100 ms; TE=2 or 6 msec; n echoes=6, flip angle = 15 deg. From the raw dataset, both amplitude images, quantitative R2* maps, and differential phase maps were created using IDL processing software. For histopathological correlation, deparaffinized 10 μm tissue sections of imaged spinal cord specimens were stained for ferric iron (Prussian Blue stain), myelin (anti-PLP), astrocytes (anti-GFAP), microglia (Isoclin B4), and oligodendrocytes (anti-CNP).

Results and Discussion


![Figure 1](image1)

**Figure 1**

MR microscopy of spinal cord specimens showed extensive migration (up to 10.1 mm) of grafted cells, in particular around the area of the dorsal column. There was no difference in migration and parenchymal integration of transplanted cells between md rats and rats with normal myelination. The average migration distance was 6.4 ± 2.9 and 6.8 ± 2.9 mm for 10 and 14 days post neurotransplantation, respectively. Figure 2 shows the three planes of view for an md spinal cord specimen 10 days following grafting. In the control graft images, no contrast was observed except a fine hairline representing the 30 μm needletrack of the microspipette. (Immu)nocytochemistry of the specimens revealed a close association of Prussian Blue positive cells and newly formed myelin sheaths, that did not overlap with GFAP and isoclin B4, and that corresponded to the MR images.

![Figure 2](image2)

**Figure 2**

The above results suggest that MR tracking of neurotransplanted cells is feasible, and may be useful to accurately determine the achieved extent of remyelination.

References