MR Microscopy of Magnetically Labeled Neurosphere Transplants in the Lewis EAE Rat.

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Abstract

(Stem) cell transplantation is being explored as a new paradigm for the treatment of demyelinating diseases. We have transplanted magnetically labeled neural precursor cell spheres into the ventricles of rats with acute experimental allergic encephalomyelitis (EAE). Migration patterns of cells into the brain could be easily visualized using high resolution MR imaging, suggesting that this technique can be used to guide the development of succesful transplantation protocols.

Introduction

Multiple sclerosis (MS) is a multifocal, immune mediated chronic disease. in which demyelination persists as an important pathological component. Recent research has suggested that it is possible to promote (re)myelination in animal models of abnormal myelination or demyelination, either by endogenous oligodendrocytes or exogenous myelinating cells (1,2). For the latter repair mechanism, the immediate dispersion, migratory capacity, and long-term survival of transplanted cells are critical factors in determining whether there will be a beneficial clinical effect.

Using MR tracking of magnetically labeled oligodendrocyte progenitors (3) and neural stem cell-derived oligospheres (4) as a new technology, we have recently shown that it is possible to monitor the fate of transplanted cells non-invasively. We present here our initial experience with magnetically labeled neural precursor cell spheres following intraventricular transplantation in rats with acute experimental autoimmune encephalomyelitis (EAE), a commonly used animal model for MS.

Materials and Methods

Neural precursor cell spheres were prepared from newborn rat striatal stem cells (5), and labeled for 24-48 h at 25 μg Fe/ml with either OX-26-MION-46L (3) or MD-100 (4) as the magnetic probe. Cells were co-labeled with Hoechst and BrdU in order to co-evaluate cell migration by conventional histologic techniques. Approximately 1.0-1.5 x 10^4 neurospheres (corresponding to 5 x 10^5 cells) in 20 μl medium were injected into the lateral ventricle of EAE Lewis rats (n=8), at the peak of their disease, as well as into normal, non-diseased animals (n=9). Control transplantations of magnetically labeled dead cells (3 cycles of freeze/thawing) were included (n=6). The animals were perfused at 1 week following transplantation, and brains were removed and further fixated in 4% paraformaldehyde. Following embedding in Fomblin®, 3D multi-gradient echo MR images were obtained at 104 μm resolution using a 4.7T Bruker NMR spectrometer and a 25 mm DOTY Litz coil. The scan parameters were: FOV=30x20x20 mm; matrix=288x192x192; NEX=12; TR=100 msec; TE=6 msec; n echoes=6, flip angle=15°.

Results and Discussion

The neural cell spheres were efficiently labeled with either magnetic probe. Fig. 1 shows cells grown for 5 days following labeling with MD-100; a normal proliferation and differentiation pattern was seen. Following transplantation into EAE rats, magnetically labeled cells were found to migrate out of the ventricle into the following white matter structures: corpus callosum, internal capsule, cerebral peduncles, fimbria, medial forebrain bundle and optic tracts (Fig. 2). The MR images showed dissemination of the graft throughout the entire ventricular system, including 3rd and 4th ventricles and cisterns. Thus, one single injection of cells is able to spread throughout the entire neuroaxis close to the primary sites involved in demyelination (i.e peri-ventricular white matter and brain stem). The hippocampus, striatum, cerebral cortex and other gray matter structures did not show the presence of any labeled cells. MD-100 labeled cells appeared to induce somewhat better contrast as compared to OX-26-MION-46L labeled cells. For the naïve (non-diseased) animals, the labeled cells appeared to stay mainly within the ventricles and no widespread migration into white matter structures was seen. The latter pattern was also observed for the dead labeled control experiments. We conclude that MR imaging appears wel suited to evaluate cell transplantation protocols.

References

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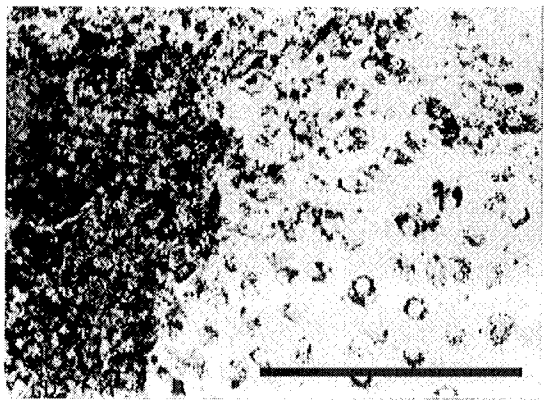

Fig. 1 DAB-enhanced Prussian blue stain of MD-100 labeled neural precursor cell spheres. Note the migration of cells away from the sphere. Bar represents 500 μm.

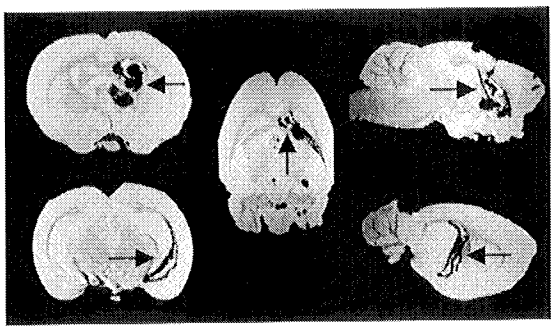

Fig. 2: MR microscopic images of EAE rat brain 7 days following transplantation of MD-100 labeled neural spheres. Arrows indicate parenchymal integration of labeled cells around the ventricles.