Delayed T2 changes following cerebral ischaemia and cell transplantation with ferumoxide-labelled neural stem cells

R. Dobson[1,2], P. Ferretti[2], and M. F. Lythgoe[1]

[1]Insitute of Child Health, RCS Unit of Biophysics, University College London, London, United Kingdom, [2]Institute of Child Health, Developmental Biology Unit, University College London, London, United Kingdom

Introduction: Transplantation of neural stem cells following cerebral ischaemia has been shown to improve functional outcome and reduce lesion volume (Kelly et al, 2005). Ferumoxide-based MRI contrast agents have been used to track the migration of transplanted cells from the injection site towards the lesion (Hoehn et al, 2002). The protamine sulphate-ferumoxide complex (FePro), has been used to enhance ferumoxide cell labelling *in vitro*(ref). In this study, the migration of transplanted fetal neural stem cells (NSC), labelled *in vitro* with the FePro complex, was monitored using MRI in a model of cerebral ischaemia, and animals were imaged serially up to four weeks.

Methods: *Middle cerebral artery occlusion (MCAO):* Animals were anaesthetised, and the left common carotid artery exposed. A suture was inserted into the carotid artery and advanced 17 mm into the circle of Willis to occlude the MCA for 30 minutes, followed by reperfusion. *Stem cell culture and labelling:* Cortical and striatal tissue from embryonic day 13.5 mice was cultured in DMEM:F12 with N2 supplement, FGF, EGF and heparin to form neurospheres. Neurospheres were labelled with the FePro complex or Endorem. For FePro labelling, 9 µl Endorem was incubated with 5 µl protamine sulfate per ml medium to form the FePro complex. For Endorem labelling, neurospheres were incubated with 28µl Endorem per ml medium. Neurospheres were incubated with the FePro complex or Endorem for 18 hours, washed and dissociated into a single cell suspension. Iron uptake by cells was quantified using a superquantum interference device (SQUID). *Intracerebral microinjection*: At 48 hours post-ischaemia, animals were anaesthetised and placed on a stereotactic frame. The skull was exposed and a small bore hole drilled. Cell treatment group (n=6), 1.25X10^5 FePro-labelled cells were injected into the ipsilateral corpus callosum (AP+0.08; ML+0.14; DV-2.0). The control group (n=5) received no cell injection. *MRI*: MRI was performed at 72 hr, 7, 14, 21 and 28 days post-ischaemia, using 2D spin and gradient echo sequences on a 2.35T SMIS system. Spin echo: TR 1500 ms, TE 120 ms. Gradient echo: TR 500 ms, TE 30 ms, flip angle 30°. *Histology*: Animals were transcardially perfused fixed with 4% paraformaldehyde after the final imaging timepoint, brains removed and frozen. Coronal 30 µm cryosections were taken. Prussian blue stain for iron with hematoxylin-eosin staining was used to detect FePro. Sections were fluorescence immunostained against doublecortin, nestin, GFAP and OX-42.

Results: FePro-labelled NSC were capable of differentiation into oligodendrocyte, astrocyte and neuronal lineages. Labelled stem cells were capable of proliferation, and retained the FePro label through cell divisions. Iron uptake was increased fourfold in FePro-labelled cells (16 pg/cell) compared to Endorem (4.1 pg/cell), as measured by SQUID. Little migration of NSC was observed outside of the injection site on MRI and histology. In both control and cell treatment groups, hyperintensity was observed in the lesioned area of the brain, consistent with many previous studies. In addition, however, hypointense regions on spin echo images developed within the lesioned striatum, relative to the contralateral striatum in both control and cell treatment groups (Fig 1A; t-test, p=0.001). Prussian blue stain was observed within the lesioned striatum and cortex, for both the cell treatment and control groups (Fig1C,D). Cell treatment may provide a mild protective effect compared to control, reducing lesion volume evolution over time (Fig 1B; t-test, p=0.06).

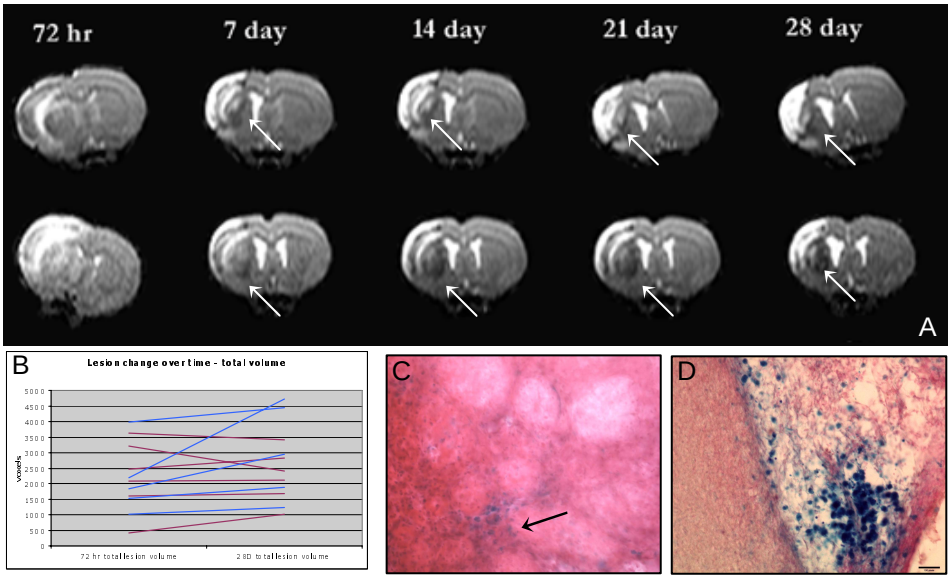

Figure1: A, Lesion evolution timecourse in individual animals, T2-weighted spin echo. Top row: cell treatment. Bottom row: control. Arrows, denote hypointense regions within the lesion. B, Lesion volume change over time in control and cell treatment group. Blue, control. Pink, cell treatment. Mild protective effect of cell treatment (t-test; p=0.06). C,D, Prussian blue stain in control (C) and cell treatment (D) lesioned striatum.

Conclusion and Discussion: FePro labelling reduces the concentrations of ferumoxide required for incubation compared to Endorem alone, while increasing cell labelling efficiency. Neural stem cells labelled with FePro retain their capacity to proliferate and differentiate. Hypointense regions developed within the ischaemic lesion on MRI images, and the presence of iron was identified on histological sections; however no contrast agent was administered to the control group. A possible interpretation of the MRI and histological data is that endogenous iron may accumulate at the lesion site as a result of pathological processes following ischaemia. This may include degradation of blood products at the lesion site in both groups, and macrophage uptake of FePro in the cell treatment group. Other pathological activity that affect T2 may be involved. These results highlight difficulties in the interpretation of data from cell tracking studies using MRI contrast agents in animal models of disease.

References

Kelly S, et al. Proc Natl Acad Sci U S A. 2004 Aug 10;101(32):11839-44.
Hoehn M, et al. Proc Natl Acad Sci U S A. 2002 Dec 10;99(25):16267-72.
Arbab AS, et al. NMR Biomed. 2005 Dec;18(8):553-9.
Wang S, et al. Magn Reson Med. 2006 Sep;56(3):474-80.