In vivo MRI long term follow up of the iron labelled hRPE cells implanted in rat brain

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Introduction

Parkinson's Disease (PD) is an incurable, progressive neurodegenerative disease with motor symptoms mostly associated with the progressive loss of nigro-striatal dopamine. The currently available therapies are associated, on a short to long-term range, with numerous side effects. Recently, intra-striatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin micro-carriers have been shown to provide sustained therapeutic benefit in PD patients as well as in rodent and non-human primate models of PD. Long term efficacy studies of this therapy require non invasive monitoring of the implanted cells. Recently, cell labeling methods have been described that allow the in vivo follow up of the transplanted cells using MRI [1]. In this pilot study we examine a possibility of using MRI for a long term follow up of the iron labeled hRPE cells implanted in a rat brain.

Methods

Iron labeling of the hRPE cells was carried out using Ferumoxide-Protamine Sulfate (Fe-Pro) preparation as described before [2]. 18-24 hours post labeling, Fe-Pro labeled cells were attached to gelatin micro-carriers (GM) using previously described technique [3]. For in vitro study five groups of cells (ca. 10^6 cells each) with varying amounts of Fe and Pro were prepared and sandwiched between two layers of 6% gelatin. For in vivo measurements a normal rat was prepared with 2 implants of SPIO-labeled hRPE-GM cells (50µg/ml Fe, 1µg/ml Pro) in the left striatum and 2 implants of unlabeled hRPE-GM cells in the right striatum. Each track received approximately 10,000 cells. The rat was then imaged at 3 and 8 weeks post implant.

MRI experiments were carried out on a 7T animal scanner (Bruker, Germany). Rat brain images were acquired using a quadrature volume coil for excitation and a 4-element phased array coil for signal reception. T_2-weighted images were acquired in coronal and axial orientations with RARE sequence (FOV=4cm, slice thickness=1mm, 256x256 matrix, TR/TE=2335/42ms or 2000/76ms, NA=4, total acquisition time = 8.5 min per image).

Results and Discussion

Gradient echo images acquired from the labelled cells in vitro (Fig. 1) clearly show the dependence of image intensity on the iron content within the cells. Figure 2 shows T_2-weighted coronal cross-sections through a rat brain acquired at 3 and 8 weeks following implantation of the hRPE-GM cells. SPIO-labelled cells can be easily identified as hypo-intense areas in the left striatum surrounded by a characteristic "blooming" artefact (red arrows). Hypo-intense areas in the contra-lateral striatum corresponding to the location of the unlabelled cells result likely from the scar tissue formed following implantation. From the T_2-weighted axial cross-sections (Fig. 3) it is apparent that the majority of the SPIO-labelled cells are located at the bottom of the implantation track in the right striatum both at 3 and 8 weeks post-implantation. Several dark spots along the implantation area and the white matter tracks suggest that some of the iron label has migrated from its original location in the right striatum. hRPE are anchorage dependent cells and undergo apoptosis in the absence of a support matrix (i.e. the gelatine carriers). They have been shown to neither migrate nor replicate. Thus, the movement of iron along the tract is likely due to the phagocytosis of dead RPE cells by macrophages and iron-filled macrophages movement along the implantation area and the white matter tract. We are currently investigating this hypothesis and the actual cellular location of iron particles, post mortem in similarly implanted animals using both immunohistochemistry and electron microscopy techniques.

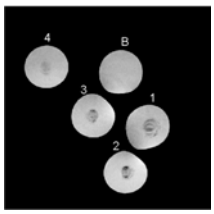

Fig. 1 MRI image of hRPE cells labelled with iron oxide particles acquired with FLASH sequence (TR/TE=500/6ms).
B – unlabelled cells;
1 – 50µg/ml Fe, 1.5µg/ml Pro;
2 – 50µg/ml Fe, 1.0µg/ml Pro;
3 – 50µg/ml Fe, 0.5µg/ml Pro;
4 – 25µg/ml Fe, 1.0µg/ml Pro;

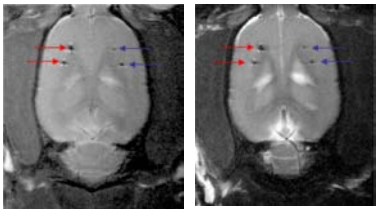

Fig. 2 MRI images acquired from a rat brain 3 weeks (left) and 8 weeks (right) following intra-striatal implantation of hRPE cells. The rat received 2 tracks of SPIO-labeled cells in the left striatum (red arrows) and 2 tracks of unlabeled cells in the right striatum (blue arrows). The number of implanted cells was about 10,000 per track.

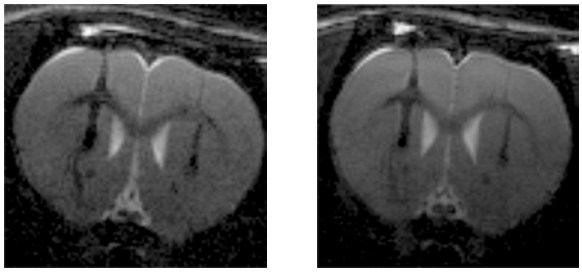

Fig. 3 Axial cross-sections acquired from a rat brain 3 weeks (left) and 8 weeks (right) following intra-striatal implantation of hRPE cells. Lack of signal due to presence of iron suggests that the SPIO-labelled implanted cells are located at the bottom of the track in the right striatum. Dark spots along the implantation area and white matter tracks are likely due to the dead hRPE cells being phagocytized by macrophages and the iron-labelled macrophages migrating with time. The effect seems to be more pronounced at 3 weeks post implantation.

Conclusions

In this pilot study we have shown that the in vivo long term MR imaging of the SPIO-labelled hRPE-GM cells is possible. This technique may prove to be a viable method to follow up the hRPE cells for studying the efficacy of the implantation based treatment of PD.

Acknowledgments

This study has been supported by the Vancouver Coastal Health Research Institute.

References: [1] Frank JA, et al. *Radiology*, 2003: **228**, 480–487; [2] Arbab AS, et al. *Blood* 2004: **104**, 1217-1223; [3] Doudet DJ, et al. *Exp Neurol*, 2004: **189**, 361-368;