Comparison of Magnetic Labeling Methods of Neural Cells for MR Cell Tracking

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PURPOSE:

Tracking migration of progenitor cells within the living brain is essential to the understanding of certain neurological pathology and treatment developments. We investigated a method to label neural cells for *in vivo* MR imaging. LipofectAMINE 2000 is a liposomal reagent for excellent cellular transfection and labeling, but it has been associated with an increase in reactive oxygen intermediates[1]. To avoid toxicity, we used paramyxovirus envelopes of the hemagglutinating virus of Japan (HVJ-Es) to label rat pheochromocytoma (PC12) cells[2] with Feridex I.V.[3], a superparamagnetic iron oxide (SPIO) solution. This technique was compared to magnetic labeling by LipofectAMINE 2000.

MATERIALS AND METHODS:

In this study we examined cell labeling by 1) transfecting cells with SPIO particles using HVJ-Es and LipofectAMINE 2000; 2) iron staining and microscopic observations; 3) differentiations of cells; 4) quantifying cell iron content; and 5) using MR imaging. HVJ-Es were incubated with Feridex I.V. at concentrations of 1, 3.2 and 10 µL for five minutes to encapsulate the SPIO particles. LipofectAMINE 2000 was incubated with Feridex I.V. at concentrations of 3.2, 10 and 32 µL for ten minutes. PC12 cells were incubated with either the SPIO-HVJ-Es or the SPIO-LipofectAMINE mixture for six hours and then washed. Prussian blue staining was used with light microscopy to evaluate cell labeling and transfection efficiency. SPIO-labeled PC12 cells were differentiated using 100 ng/mL nerve growth factor (NGF) for six days. The iron content of labeled cells was determined by atomic absorption spectrophotometry. A 1.5T MR scanner and a custom birdcage volume coil were used to image labeled cells embedded in 4% gelatin using a 3D steady-state free precession (SSFP) pulse sequence, and signal intensity was calculated.

RESULTS:

Microscopic examination with Prussian blue staining showed SPIO localization within the cell. For both methods of transfection, the amount of superparamagnetic agent within the cell, as well as the number of cells labeled, increases with the concentration of Feridex I.V. (Figure 1). A labeling efficiency of >90% was observed for SPIO-HVJ-E transfected cells with 10 µL of Feridex I.V. and for SPIO-LipofectAMINE transfected cells with 32 µL of Feridex I.V. Transfection was not observed upon incubation with only Feridex I.V. The iron content of SPIO-HVJ-E transfected cells with 10 µL of Feridex I.V. and of SPIO-LipofectAMINE transfected cells with 32 µL of Feridex I.V. was 3.87±0.51 pg/cell and 5.54±0.44 pg/cell, respectively (Figure 2). Labeled and native PC12 cells had identical morphological features and dendritic growth after induced differentiation into sympathetic nerves by NGF (Figure 3). The MR signal intensity of SPIO-HVJ-E transfected cells with 10 µL of Feridex I.V. and of SPIO-LipofectAMINE transfected cells with 32 µL of Feridex I.V. (one million cells/mL) was 73.7% and 64.2%, respectively, less than that of unlabeled control cells.

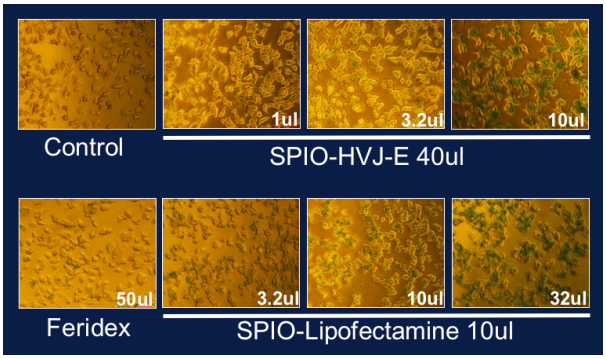

Figure 1. Photomicrographs of cells containing Prussian blue staining of control cells, SPIO-HVJ-E transfected cells, cells incubated with Feridex I.V. only and SPIO-LipofectAMINE transfected cells. Volume of Feridex I.V. used is shown in the lower right corner.

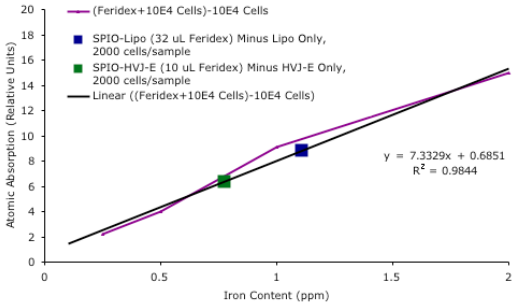

Figure 2. Linear relationship between iron content at atomic absorption values. Iron content of labeled cell counts can be extrapolated along this calibration curve.

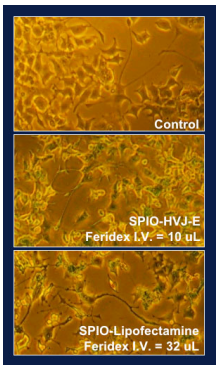

Figure 3. Photomicrographs of Prussian blue staining of control and transfected cells after NGF-induced differentiation.

SUMMARY AND CONCLUSIONS:

Cell transfection with SPIO-HVJ-Es represents a more efficient and equally effective technique of magnetically labeleling neural cells as compared to LipofectAMINE 2000. This method can be applied to non-toxic, *in vivo* cell tracking in the brain.

REFERENCES: 1. Dokka S et al. Pharm Res. 2000; 2. Hawrylak N et al, Exp Neurol. 1993; 3. Berlex Laboratories, Inc., Wayne, New Jersey, USA.