Direct Incubation of PBMCs with Ferumoxides Labels Monocytes and B cells

J. S. Pratt[1], A. Moore[1], and A. G. Sorensen[1]

[1]Radiology, Massachusetts General Hospital, Charlestown, MA, United States

Introduction

Non-invasive radiological methods for monitoring cell transplants might augment the development of effective therapies for many diseases by allowing detailed evaluation of transplant location, survival, and rejection. Some advantages of MRI include high spatial resolution, unlimited depth penetration, and stability of contrast agents. Superparamagnetic iron oxide nanoparticles (SPIOs) are commonly used in the laboratory and in preclinical studies to label cells for MR detection (1, 2). SPIO-loaded cells produce signal voids on T2 /T2* weighted MR images and are detectable after in vivo transplantation. In the majority of studies, cell labeling with SPIOs had no significant effects on cell survival, growth, differentiation, or in vivo migration. If feasible, translation of MRI-based cell monitoring to the clinic may be simplified by a method involving direct incubation of therapeutic cells with SPIOs. PBMCs are used clinically for CML treatment, are of interest in a number of illnesses, and could be used to investigate a wide range of immunologic questions in humans. Ferumoxides (Feridex/Endorem) is a commercially available preparation of SPIO approved as an MRI contrast agent for intravenous use. The purpose of our study was to determine the feasibility of labeling PBMCs by direct incubation with ferumoxides, and to evaluate the types of leukocytes labeled by this method for the eventual development of MRI-based clinical monitoring of PBMCs in patients.

Methods

PBMC labeling: PBMCs were isolated from IRB approved buffy coats by centrifugation over Ficoll-Paque Plus using the manufacturers protocol. Cell viability was evaluated with trypan blue staining. PBMCs were incubated at 10^6 -10^7 cells/ml of media with 400 μg/ml for 2 hours in tissue culture conditions (37°C, 5% CO2, humidified chamber). Immediately after incubation with ferumoxides, cells were diluted 10x with RPMI 1640/2% serum and washed by centrifugation thrice at 4°C. Cells were resuspended in D-PBS(-Ca^{2+}-Mg^{2+})/10% CPDA/1% Human AB serum for FACS or magnetic separation. Cells were fixed with buffered 2% PFA for light microscopy, iron assay, and MRI.

Iron content determination: Labeled PBMC iron content was determined using the Total Iron Reagent Kit (Pointe Scientific, Canton, MI). Known quantities of cells or ferumoxides standards were incubated in 6 N HCl at 70°C for 90 minutes. The reaction consisted of 10 μl aliquots of cell lysate or iron standard combined with 100 μl buffer, 10μl of 6 N NaOH and 2 μl iron color reagent incubated at 37°C 30 minutes, and absorbance was read at 560 nm. The frequency of cell labeling was estimated by Prussian Blue (PB) staining of PBMCs. Intracellular localization of ferumoxides was determined by transmission electron microscopy conducted by a core facility.

MRI: Images were acquired on 4.7 Tesla and 9.4 Tesla horizontal bore scanners from Bruker BioSciences, Billerica, MA. Cell phantoms comprised of different combinations of ferumoxides-incubated and non-incubated PBMCs were formed by centrifugation of cells in 2% agarose molds. T2 values were calculated from multi-echo spin echo images using Paravision software.

Cell Sorting: Magnetic sorting was done by applying 10^7 cells/500 μl labeled PBMCs to pre-washed LS columns on a MidiMACs magnet. The columns were washed thrice. The differences between magnet bound and non-bound cells were determined by for flow cytometry, hemacytometer counting, PB staining and iron assay. Fluorescence cell sorting of specific cell populations from labeled PBMCs was conducted using a FACSAria. Cells were stained with either anti-CD3 FITC and anti-CD4 PE, or anti-CD19 FITC, or anti-CD56 PE. Iron content of collected cells was determined by iron assay and PB staining.

Specific cell isolation and labeling: For monocytes, PBMCs were incubated in vented flasks for 2 hours in media, and attached cells were recovered. B cells were isolated from PBMCs using the B cell Isolation Kit II (Miltenyi Biotech, Auburn, CA) according to the manufacturer's protocol. B cells or monocytes were labeled ± 400 μg/ml ferumoxides, 10^6 cells/ml for 2 hours in tissue culture conditions. After 3 washes, cells were processed for PB staining and MRI phantoms.

Results

The optimal concentration of ferumoxides was determined to be 400 μg/ml for 10^6 PBMCs/ml; this gave an average incorporation of 0.435 ± 0.025 pg iron/cell. No difference was observed in the iron content per cell from 15 min to 2 hours for 2 separate donors. The frequency of PB staining in labeled PBMCs was 18.3% (n=1000). TEM images showed ferumoxides primarily compartmentalizated in late endosomes/phagosomes.

T2 measurements of various mixtures of labeled and non-labeled PBMCs were calculated to investigate the dynamic range of measurements at 4.7T and 9.4T. The lowest tested dilution, a 1% labeled/non-labeled cell mixture (29 pg iron/voxel), had a significant difference in T2 value compared to non-labeled cells ($p<0.001$). Saturation of T2 measurements occurred for concentrations of 20% labeled/non-labeled cells (>588 pg iron/voxel at 4.7T).

Sorting of ferumoxides labeled PBMCs on magnetic columns enabled identification of the types of cells that engulfed the SPIOs. PB microscopy showed visible iron deposits in 71.0% of ferumoxides-incubated cells that bound to magnetic columns. Cells that washed through magnetic columns negligible PB deposits, and had undetectable levels of iron in the iron assay. The total iron content of ferumoxides-incubated PBMCs was significantly enriched from 0.195 pg iron/cell before column separation to 0.385 pg iron/cell after binding to the column. Flow cytometry was conducted after ferumoxides labeling and magnetic separation to analyze the differences between cell types in the magnet bound and wash fractions. For labeled PBMCs, 94.0% of monocytes, 63.5% of polymorphonuclear cells (PMNs), and 25.5% of lymphocytes were retained on magnetic columns. Measurements of lymphocyte subsets showed 79.7% of $CD19^+$ B cells were retained on the magnetic column, but 93.7% $CD3^+$ T cells and 87.7% of NK cells washed through the column.

FACS sorting was used to quantify the degree of labeling of individual leukocyte subtypes in a single donor. PB staining of FACS sorted fractions showed 66.7% of $CD19^+$ B cells contained small clusters of iron staining, and 70.3% of monocytes contained dense ferumoxides deposits. However, 97.6% of $CD3^+$ T cells, and 98.8% of $CD56^+$ cells were negative for PB staining. Only 6% of PMNs were labeled but those labeled had a high density of PB deposits. Iron assay confirmed that monocytes contained the highest average level of iron per cell (0.414 pg iron/cell ± 0.06 s.e.m.). $CD19^+$ B cells, PMNs had moderate iron levels (0.191 ± 0.006, and 0.166 ± 0.01 pg iron/cell, respectively). $CD3^+$ T cells and $CD56^+$ NK cells had nearly undetectable iron content.

Purified B cells labeled with ferumoxides were approximately 64% (n=1000) positive for PB, whereas 100% non-labeled purified B cells were negative for PB (n=1000). MRI T2 measurements were decreased in phantoms of purified B cells or monocytes labeled with ferumoxides vs. non-labeled cells.

In summary, magnetic cell sorting and FACS sorting confirmed that monocytes, $CD19^+$ B cells, and PMNs were labeled after ferumoxides incubation, whereas $CD3^+$ T cells and $CD56^+$ NK cells were not labeled.

Discussion

Direct incubation of PBMCs with the clinically approved ferumoxides preparation, Feridex, was sufficient for detection of <100 labeled PBMCs/voxel (29 pg iron/voxel) at 4.7T. With this method, monocytes (94% column bound, 70% PB positive), and B cells (79.7% column bound, 66.7% PB positive) were preferentially labeled as determined by magnetic column separation and FACS sorting. Our study suggests that, in addition to monocytes, direct incubation of PBMCs with ferumoxides can be used to monitor B cells in vivo using MRI. As B cells are not considered to be phagocytic, we suggest receptor mediated endocytosis as the mechanism of ferumoxides internalization.

References

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