Ultrastructural Characteristics of Feridex Endocytosis by Human Peripheral Mononuclear Cells and Mesenchymal Stem Cells

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Introduction:

Tracking of peripheral blood mononuclear cells (PBMCs) would be an invaluable asset for monitoring the safety and efficacy of donor infusion therapies in cancer treatment paradigms. For clinical applications, minimal cell manipulation is desirable. Feridex (Endorem), an MRI T2 contrast agent approved for intravenous use, is taken up by a variety of cells via endocytosis (1-4). In humans, parenteral Feridex (Endorem) is cleared from the blood stream primarily through endocytosis by liver resident macrophages (Kupffer cells). The intracellular localization of Feridex in Kupffer cells provides a darker T2 contrast in healthy livers compared to tumor bearing livers with altered cellular makeup. Feridex has also proven effective in multiple animal models for labeling and tracking many types of transplanted cells (1). The superparamagnetic properties of Feridex result from its small size, 60 nm on average (3). The dextran coating of Feridex provides a neutral hydrophilic interphase, allowing the large, charged nanoparticles of iron oxide to remain separated and suspended in solution (3). During the internalization process, Feridex particles form larger agglomerates, and thus have altered physical properties (4). This study aimed to use transmission electron microscopy to compare the agglomeration state of internalized Feridex in human PBMCs, with comparison to human mesenchymal stem cells (HMSCs).

Methods:

PBMCs were isolated from "Buffy Coat" obtained from the MGH blood bank. Buffy Coat was diluted 4 fold in RPMI 1640 (BioWhittaker). Cells were centrifuged over Histopaque®-1077 (Sigma) at 400 x g for 30 minutes. The PBMC interlayer was removed, diluted, and washed by three additional dilution and centrifugation steps. PBMCs at a concentration of 5 million cells/ml were labeled ± 400 μg/ml Feridex in RPMI media containing 10% human AB serum, 1% pen/strep at 37°C, 5% CO_2 for 2 hours. PBMCs were then iced 1 minute, and washed 3 times with Ca^{2+}/Mg^{2+} free HBSS.

HMSCs, obtained from Cambrex, were grown according to the supplier's protocol. HMSCs were grown at 5000 cells/cm^2 in MSC Basal Medium supplemented with Growth Supplement, L-glutamine, and 1% pen/strep. On the 6th passage, attached cells were incubated in growth media ± 200 μg/ml Feridex at 37°C, 5% CO_2 for 18 hours. Cells were washed once with Ca^{2+}/Mg^{2+} free HBSS, trypsinized 30 seconds, and diluted in growth media. Centrifugation and washing steps were repeated 4 additional times to ensure complete removal of unbound Feridex.

PBMCs and HMSCs were immediately fixed with 2% gluteraldehyde, 3% paraformaldehyde in 0.1M cacodylate and 5% sucrose pH 7.4 for 2 hours at room temperature. For HMSCs, transmission electron microscopy (TEM) was performed by a core facility. For PBMCs, TEM was performed by a separate unaffiliated core facility.

Iron content of Feridex labeled and control cells was determined with the Total Iron Reagent Set (Pointe Scientific). Cells were dissolved in 6N HCl at 70°C for 1-3 hours. A standard curve consisted of various concentrations of $FeCl_2$ dissolved in 6 N HCl.

PBMCs were analyzed by FACS using mouse anti-human CD14-FITC, and CD3-FITC with CD4-PE or CD8-PE (BD Pharmingen) by incubation of 1x10^6 cells with 10 ml antibody in 50 ml buffer (HBSS, 1% Human Albumin, 0.1% Azide) for 20 minutes at 6°C. Cells were washed, stained 10 minutes with 20 ml 7-AAD and fixed in 1% fresh paraformaldehyde in PBS. A minimum of 10,000 events were recorded per sample on a BD FACSCalibur.

Results and Discussion:

The iron content of PBMCs was 0.8 pg/cell (±0.2 S.D.) after 2 hours incubation with 400 μg/ml Feridex as determined by the iron assay. MRI T2 weighted measurements of PBMCs suspended in agar demonstrated a reliable detection limit of 500 cells/μl that corresponded to 1 cell/voxel (2 nl). HMSCs had an iron content of 64.4 pg/cell (± 5.5 S.E.) after 18 hours incubation in 200 μg/ml Feridex. No iron was detectable in PBMCs or HMSCs that were not incubated with Feridex.

Monocytes and neutrophils are considered phagocytes because of their high rate and capacity for nonspecific endocytosis of micro- and nano-sized particles in comparison to lymphocytes and other cells. Monocytes may have a higher contribution to the iron content of a Feridex labeled PBMC preparation. FACS analysis of PBMCs demonstrated that for living cells 21-26% were $CD14^+$ monocytes, 5-8% were polymorphonuclear cells, and 25% were $CD3^+$, consisting of 70% $CD4^+$ and 23% $CD8^+$.

Transmission electron microscopy revealed dense labeling of lysosomes in both PBMCs (Fig.2) and HMSCs (Fig.1). The primary cell type labeled by Feridex in PBMCs was monocytes. In both monocytes and HMSCs, linear bands of agglomeration were present. These bands represent the early stages of Feridex accumulation at the cell surface, and entry into early lysosomal compartments. The banding patterns were observed less frequently in HMSCs than in PBMC monocytes. HMSCs had more loosely associated agglomerates present within lysosomal vacuoles in comparison to PBMC monocytes. Regions were observed in HMSCs where disrupted linear bands of Feridex were closely associated with the edges of lysosomal vacuoles (Fig.1). Of note, in both PBMC and HMSC images, agglomeration bands were visible at the cell surface (Fig.2). These banding patterns were seen in two different cell types handled by two different microscopy facilities, and therefore, are not likely to be experimental artifacts. Taken together, these observations suggest that the linear bands of Feridex agglomerates form at the cell surface in sizes as high as 2 microns, are endocytosed as a unit, and become dissociated in later stage lysosomal compartments. This pattern of endocytosis was not observed in other cell types present in PBMC preparations.

Conclusions:

In this study, unique findings regarding the agglomeration state of internalized Feridex were observed in both PBMCs and HMSCs using TEM. The observation of high concentrations of internalized bands of Feridex ranging from 0.5 - 2 microns suggest that the magnetic properties of Feridex in cells are vastly different from Feridex in solution. Finally, the broad distribution of concentration of Feridex label per cell is an imporant consideration for interpretation of cell tracking studies.

References:

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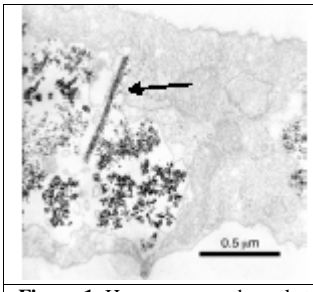

Figure 1. Human mesenchymal stem cells labeled by direct incubation with Feridex 200 μg/ml for 18 hours at 37°C. Feridex was evident in agglomerate bands ranging from 0.5-1 μm in length (arrow).

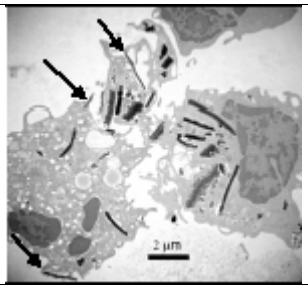

Figure 2. Human peripheral blood monocytes labeled by incubation with Feridex 400 μg/ml for 2 hours at 37°C. Numerous bands of Feridex agglomerates are evident with some forming on the cell surface (arrows).