A Model of Lysosomal Metabolism of Dextran Coated Superparamagnetic Iron Oxide (SPIO) Nanoparticles: Implications for Cellular Magnetic Resonance Imaging

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We created an in-vitro mechanism for the metabolism of the iron oxide nanoparticles comprising Feridex, an FDA-approved contrast agent for use in magnetic resonance imaging of the liver. We found that the iron oxide nanoparticles were digested into free iron in a sodium citrate buffer solution at pH 4.5, which mimics the environment of the endosomes/lysosomes in mammalian cells. Using ultraviolet (UV) spectroscopy, relaxometry and magnetic resonance imaging analyses, we determined that solubilization of the nanoparticles into free iron occurs between the 6 hour and 96 hour time points. We therefore suggest that a similar metabolic mechanism occurs to break down Feridex in cells.

INTRODUCTION: Superparamagnetic iron oxide (SPIO) Feridex® is an FDA-approved contrast agent for use in magnetic resonance (MR) imaging of the liver. Most of the administered SPIO ends up in the reticuloendothelial system via endocytosis and the iron core released from the SPIO is utilized in normal iron metabolism pathways. Recently, a method has been developed to label different types of mammalian cells with Feridex® complexed with poly-l-lysine (PLL). Preliminary data showed that the iron particles resided in lysosomes and there was rapid clearance of iron particles out of cells by 5-8 cell divisions. However, the physical state of the iron particles during residence in the lysosomes and/or elimination from the cell is unknown. The purpose of this study was to model the lysosomal environment within cells using different buffer systems and pH levels to determine the rate of the physical degradation of the iron particles in relation under varying physiological conditions.

METHODS: Seven incubation mixtures were prepared: purified, deionized water at pH 7, pH 5.5, and pH 4.5, sodium citrate buffer at pH 4.5 and 5.5, and sodium acetate buffer at pH 4.5 and 5.5. A solution of Feridex®, and poly-L-lysine (PLL), a polyamine transfection agent, was prepared on Day 0. A volume of Feridex® was used to achieve a final concentration of 25 µg Fe/ml, and a stock solution of PLL (1.5 mg/ml) was added to achieve a final concentration of 1:2000 (v/v). Equal volumes of Feridex®-PLL were added to each of the seven solutions in individual flasks and incubated at 37° C for up to 3 weeks in 95% air/5% CO_2. The incubation mixtures were analyzed for ionic iron at 0, 6, 24, 48, and 96 hr, and 7, 14, and 21 days. A solution of 4.95 mM bathophenanthroline disulfonic acid (BPS, 4,7-diphenyl-1,10-phenanthroline disulfonic acid) was used in UV spectroscopic measurements. Before analysis at each time point, a fresh 100 mM ascorbate solution was prepared and kept on ice, and the incubation mixtures were diluted 9:1 by combining 100 µl of each of the seven Feridex® solutions and 900 µl of the corresponding buffer/water solution. All diluted samples were measured at each time point at a wavelength of 378 nm to see the spectra of iron complexes. Then, to each cuvette containing the 1 ml of diluted sample, 40 µl of BPS were added. After 90 seconds, the absorbance of each sample was read at 535 nm. 20 µl of ascorbate solution were then added to each cuvette, and after 8 minutes the absorbance was read again at 535 nm. The positive difference of the post-BPS reading and the post-BPS-ascorbate reading was used as the final absorbance value of each sample at each time point and corresponds to the concentration of free iron in the solution. For each of the seven incubation mixtures, 0.5 ml of the mixture was mixed with 0.5 ml 16% gelatin, vortexed, and rapid-frozen on ice to set the gelatin. The solid samples were stored at 4° C. Relaxometry analysis was performed at 23° C on a custom built NMR relaxometer. Longitudinal relaxation time (1/T2) was measured for each sample at 4.2 MHz and 42 MHz, with tau values of 1, 3, and 5. Magnetic resonance imaging of the gelatin-fixed samples was performed on a 1.5 Tesla MR unit.

RESULTS: Spectroscopic data was analyzed using Statview™, and results indicate that the solution of Feridex® and sodium citrate at pH 4.5 resulted in the greatest increase in free Fe^{2+} in solution, compared to relatively no increase in free iron in the other pH/buffer systems (Figure 1). Solubilization (conversion from particle to free ions) occurred when the Feridex solution was added to the sodium citrate pH 4.5 incubation mixture between 6 hours and 96 hours, and the level of free iron released in solution remained steady after 96 hours. Relaxometery (1/T2) and MRI performed on samples of the Feridex®/buffer solutions at each time point indicated that the mixture of the Feridex® solution and the sodium citrate buffer at pH 4.5 contained the greatest amount of free iron between the 6 hour and 96 hour time points.

CONCLUSION: Our results indicate that at the low-pH citrate-based environment model is similar to the in vivo environment of the endosomes/lysosomes. The possible contribution of endogenous iron-chelating agents such as citrate, may contribute to the increased rate of solubilization of the ferromagnetic particles by the cell and may have important implication in the interpretation of numbers of SPIO labeled cells used for cellular MRI.

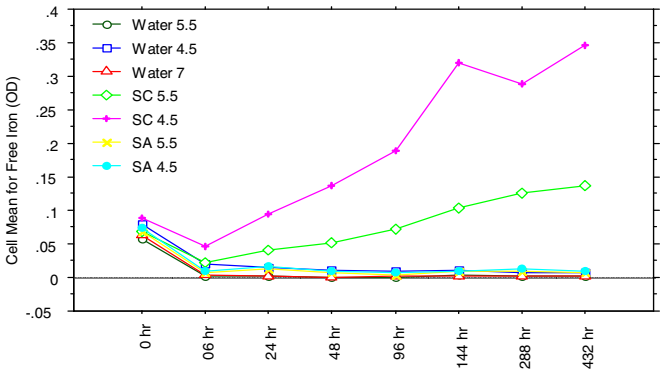

Figure 1: Difference in absorbance values versus time for Feridex® combined with each of the seven incubation solutions. SC= sodium citrate, SA= sodium acetate