

Expression of Transferrin Receptor and Ferritin Following Ferumoxides- Protamine Sulfate Labeling of cells: Implications for Cellular Magnetic Resonance Imaging.

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Background:

Ferumoxides-protamine sulfate (Fe-Pro) complexes are being used for intracellular magnetic labeling of cells to non invasively monitor cell trafficking and tissue distribution by *in vivo* MRI (1). Although no short or long- term toxic effects on cell's viability and biological functions including differentiation capacity of stem cells have been reported (2), the effects of Fe-Pro labeling on a cellular expression of transferrin receptor (TfR-1) and ferritin, (Fer), proteins involved in iron transport and storage, are unknown.

Objective:

To evaluate the effect of using Fe-Pro for magnetic labeling in tumor cells, mesenchymal stem cells (MSCs), and primary macrophages on the gene and protein expression of TfR-1 and Fer.

Methods:

Primary human macrophages, MSCs and adherent human cervical carcinoma (HeLa) cells were labeled with Fe-Pro complexes at the ratio of 100 µg/ml to 6 µg/ml of Pro (1), washed and primary macrophages were cultured for 7 days, while HeLa and MSCs for 28 days. TfR-1 and Fer expression were evaluated at the gene and protein levels in total cell lysates, on days 1,3, 7, 14, 21 and 28, by real-time RT-PCR and Western blotting. Both gene expression levels were normalized to the endogenous control, 18S ribosomal RNA and protein levels to beta-actin. MTT (proliferation assay) and reactive oxygen species (ROS) analysis was also performed.

Results:

Fe-Pro labeling of HeLa cells and MSCs resulted in transient decrease in TfR-1 mRNA and protein levels. TfR-1 mRNA in labeled HeLa cells was decreased on day 1, 3 and 7 ($p < 0.005$), while TfR-1 protein levels were decreased on day 3 and 7 ($p < 0.05$) when compared to unlabeled cells. TfR-1 mRNA in labeled MSC was decreased on day 1, 3, 7 and 14 ($p < 0.005$), while protein levels on day 3 and 7 ($p < 0.05$) when compared to unlabeled MSC. In contrast, Fe-Pro labeling of primary macrophages resulted in an increase in TfR-1 mRNA on day 1, 3 and 7 ($p < 0.005$), but no in TfR-1 protein levels. Fer mRNA and protein levels increased transiently in labeled HeLa cells (mRNA on day 7, $p = 0.002$; and protein levels on days 1, 3, 7, 14, ($p < 0.05$, see Fig.1) and macrophages (mRNA on day 1, 3, and 7 ($p < 0.005$), and Fer protein on day 1 and day 7, ($p < 0.05$) (Fig.1) when compared to unlabeled cells. In contrast, Fe-Pro labeled MSCs showed sustained increased Fer mRNA (day 14 and 28 ($p < 0.05$) and protein levels (day 1, 3, 7, 14, 21 and 28 ($p < 0.005$) (Fig 1). No changes in MTT and ROS analysis were noted for labeled cells compared to controls.

Conclusions:

Labeling of cells with Fe-Pro increased the gene and protein expression of Fer and at the same time decreased the gene and protein expression of TfR-1. These changes however, do not appear to be clinically significant. This study provides further evidence on the lack of toxicity of labeling cells with ferumoxides and that cellular iron metabolic pathways can process the increase in intracellular iron load. The dissolving of ferumoxides in the endosome/ lysosome compartment, as seen in our previous studies (3), should increase ferritin levels within slowly replicating or differentiated cells resulting in a continued shortening of T2 and T2* of the cells in the tissue and a persistent decrease in signal intensity on T2 and T2* weighted images in areas containing labeled cells.

References: (1) Arbab, AS *et al.* Blood 2004; 104: 1217, (2) Arbab, AS *et al.* NMR Biomed, 2005 Oct 17 (on line), (3) Arbab, AS *et al.* NMR Biomed, 2005 Oct 18(6): 383-9.

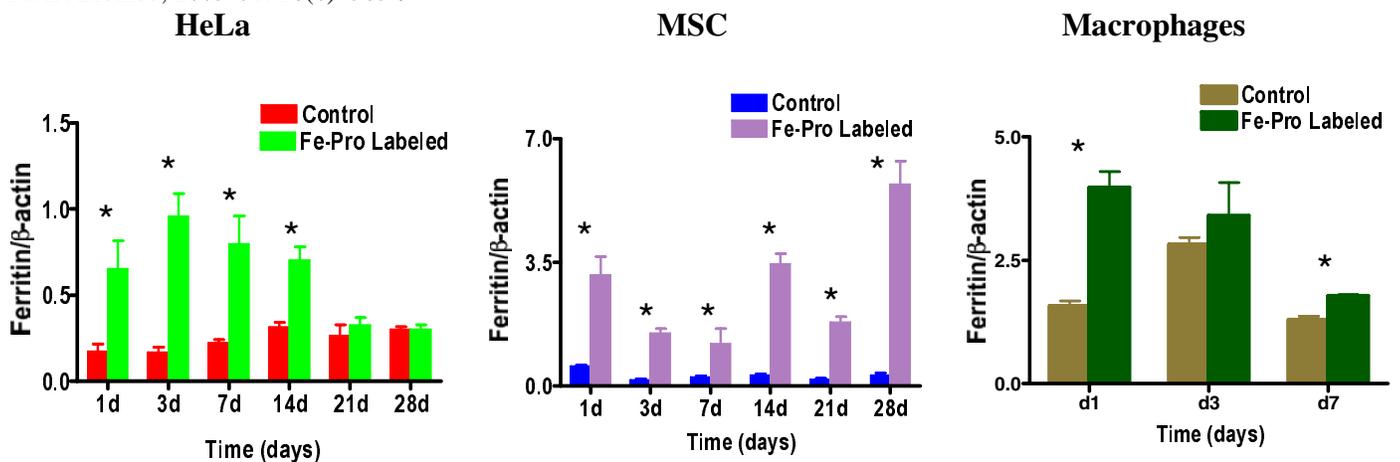


Fig. 1. Ferritin protein expression assessed by Western Blot in Fe-Pro labeled HeLa cells, MSCs 1 to 28 days post-labeling and primary macrophages 1 to 7 days post-labeling.