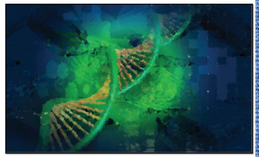
Comparative *In Vitro* Cellular Uptake Study on Reference and Generic Sodium Ferric Gluconate in Mononuclear Phagocyte Systems



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INTRODUCTION

After parenteral administration, the iron nanoparticles of colloidal iron products used in iron replacement therapy are phagocytized by cells of the reticuloendothelial system and become part of the intracellular iron pool. Depending on the body's demand, iron is stored as ferritin/hemosiderin or transported bound to transferrin to areas of need. The reference drug, Ferrlecit®, was approved by FDA in 1999. In 2011, the only generic sodium ferric gluconate complex in sucrose injection formulation was approved.

In 2013, both FDA and EMA have provided industry with guidance on the pathway to generic approval for this product. In contrast with the draft guidance published by FDA, EMA requires biodistribution study in support of generic drug evaluation of intravenous iron-based nano-colloidal drug products. The present collaborative work between the OGD/ORS (Office of Generic Drugs/Office of Research and Standards) and OTS/OCP (Office of Translational Sciences/Office of Clinical Pharmacology) uses the reference and generic pair mentioned above to compare cellular uptake of Ferrlecit® and generic sodium ferric gluconate.

METHODS

Three macrophage derived cell lines (U937, HL-60, THP-1) were selected to conduct the cellular uptake research. Cells were seeded in 24-well plates and treated with two lots of Ferrlecit® (Lot #D2C283A and Lot #D2C593A) and one lot of the generic drug (Lot #132296.1) at the concentrations of 10, 20 and 40 µg/ml for 30, 60, 120 and 240 min. For each treatment, three independent experiments with four replicates were performed. The cell pellets and supernatant were both collected for assay. Either the cell lysate or the supernatant was incubated with the iron-releasing reagent at 60°C for 2 h. After the mixture cooled to room temperature, iron-detection reagent was added and incubated for 30 min. The final solution was transferred into 96-well plate and the absorbance was measured at 550 nm on a plate reader. The iron concentration was calculated according to the iron standard.

The protein concentration was measured for each sample using Bio-Rad protein assay kit II, following the manufacturer's protocol. The final cellular uptake iron was normalized to the protein concentration.

RESULT

Figure 1. Comparison of iron uptake in THP-1 cells. THP-1 cells were incubated with 10, 20 and 40 µg/ml sodium ferric gluconate complex at 37°C for 30, 60, 120 and 240 min. After incubation, cells were harvested in regular Eppendorf tubes or through centrifuge filter tubes. The amount of iron within the cells and retained in supernatant was determined using the ferrizone assay. The measured iron content in each sample was normalized to the amount of protein of the same sample. Data are presented as means±SD (n=3, 3 independent experiments with 4 replicates for each experimental condition). *, p <0.05 between Ferrlecit® and the generic drug product at the given time point and concentration.

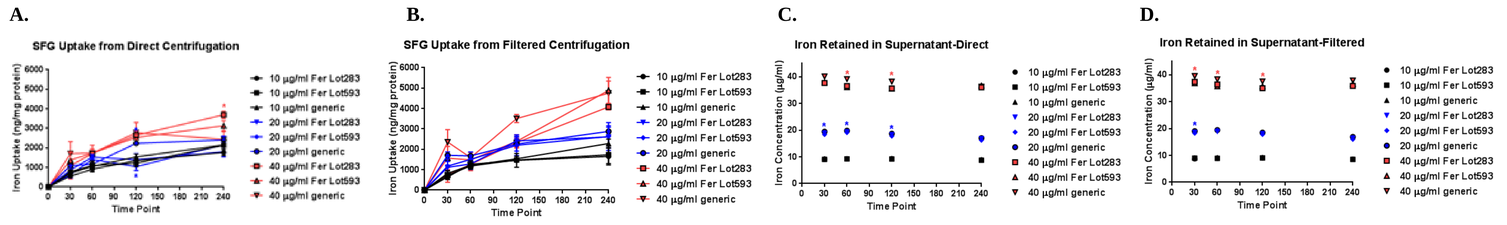


Figure 2. Comparison of iron uptake in HL-60 cells. HL-60 cells were incubated with 10, 20 and 40 µg/ml sodium ferric gluconate complex at 37°C for 30, 60, 120 and 240 min. After incubation, cells were harvested in regular Eppendorf tubes or through centrifuge filter tubes. The amount of iron within the cells and retained in supernatant was determined using the ferrizone assay. The measured iron content in each sample was normalized to the amount of protein of the same sample. Data are presented as means±SD (n=3, 3 independent experiments with 4 replicates for each experimental condition). *, p <0.05 between Ferrlecit® and the generic drug product at the given time point and concentration.

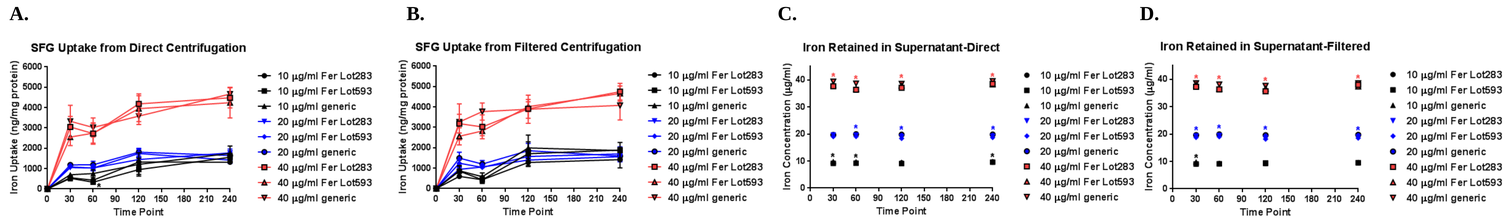
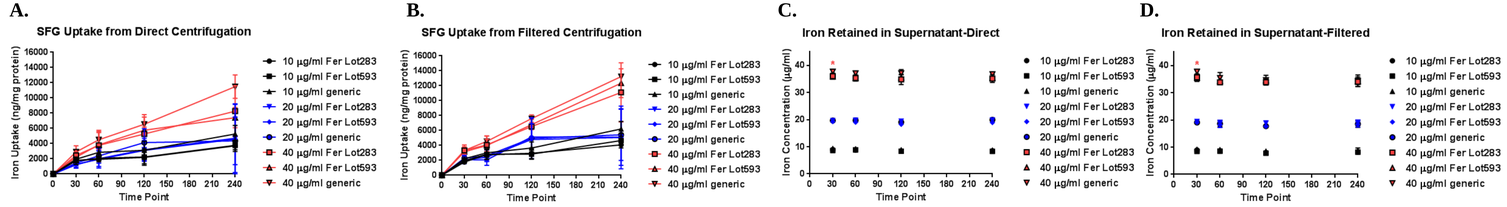


Figure 3. Comparison of iron uptake in U-937 cells. U-937 cells were incubated with 10, 20 and 40 µg/ml sodium ferric gluconate complex at 37°C for 30, 60, 120 and 240 min. After incubation, cells were harvested in regular Eppendorf tubes or through centrifuge filter tubes. The amount of iron within the cells and retained in supernatant was determined using the ferrizone assay. The measured iron content in each sample was normalized to the amount of protein of the same sample. Data are presented as means±SD (n=3, 3 independent experiments with 4 replicates for each experimental condition). *, p <0.05 between Ferrlecit® and the generic drug product at the given time point and concentration.



REFERENCE: EMA, March 2015. Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product (EMA/CHMP/SWP/620008/2012) European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/03/WC500184922.pdf.
FDA, June 2013. Draft guidance on sodium ferric gluconate complex, U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER)
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358142.pdf

DISCUSSION

The NDA holder of Ferrlecit® conducted a post-approval pilot study (PER9801) in adults. In the study, the peak drug levels (Cmax) were significantly affected by both dosage and rate of administration. The Cmax was 20.6 mg/L with 125 mg dose administered undiluted over 7 min infusion. The terminal elimination half-life for the drug bound iron only depended on the dosage. It ranged from 0.85 to 1.45 hours. Using that information, three doses (10, 20 and 40 µg/ml) and four time points (30, 60, 120 and 240 min) were established for the cellular uptake protocol.

The attached cell lines such as RAW264.7 have been widely used in studying nanoparticle cellular uptake. However, the effect of sedimentation can result in values greater than actual uptake. Therefore, suspension cell lines were chosen for this study. The more matured U937 cells exhibited higher iron incorporation than THP-1 and HL-60 as might be expected in a more phagocytic phenotype. The sodium ferric gluconate complexes are stable in water or saline, but they can self-agglomerate or form agglomerates with the serum proteins in cell culture medium. This agglomeration can co-precipitate with cell pellets during the centrifugation. In order to minimize this effect, centrifuge tube filters were applied when collecting cell pellets. Iron uptake values in cell pellets obtained from direct centrifuge and via filter centrifuge tubes were largely consistent.

CONCLUSION

Studies performed in all three macrophage cell lines showed identical results. Iron was predominantly retained in the supernatant. The overall trends of cellular iron uptake are very similar between the generic drug and its reference listed drug (Ferrlecit®) in three human macrophage cell lines. The results support the sufficiency of current FDA guidance to demonstrate the bioequivalence for generic sodium ferric gluconate in sucrose complex. There is no significant difference in cellular iron uptake between Ferrlecit® and the generic copy of sodium ferric gluconate complex products approved using the current FDA's bioequivalence approach.