Author's response to reviews

Title: Determination of Iron Sucrose (Venofer) or Iron Dextran (DexFerrum) removal by hemodialysis: An In-Vitro Study

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Thank you for your comments regarding our manuscript entitled "Determination of iron sucrose (Venofer) or iron dextran (DexFerrum) removal by hemodialysis: An in-vitro study".

Below please find our replies to each raised concern. We trust that each concern is adequately addressed and look forward to hearing back from you in due course.

Sincerely,

Harold J. Manley, Pharm.D., BCPS

Major:
1) The most important point to be clarified relates to the analysis of mass balance of iron during the in-vitro test (Results, pages 11 and 12; Discussion, page 14). For example, the authors observed loss of iron from the SBS without any appearance of iron in the dialysate. More interestingly, the authors also observed a gain in iron in the SBS in other tests. Could it be that the gain of iron measured as an increase in iron concentration in the SBS was due to hemoconcentration caused by uncontrolled ultrafiltration from the SBS? And could it be that the loss of iron in the other experiment was due to uncontrolled "backfiltration" and volume gain in the SBS thus diluting the iron concentration? It is very probable that these paradoxical findings have their origin in volume changes in the SBS. Even with a volumetrically controlled machine such as the 2008H the dialysate flow into and out of the dialyzer is subject to some "balance chamber error". Typically, this error is in the range of 0.3 parts per thousand, thus 4 h of dialysate flow at 800 mL/min will lead to a typical volume shift into (or out of) the SBS of approximately 60 mL, i.e. 1% of the SBS in this study. In the in-vitro system (with untypical line pressures) the error could be larger. The question thus arises, did the authors measure the balancing error or correct for unwanted volume changes in the SBS system?

As eluded to, hemoconcentration of the SBS did occur and accounted for the measured gain of iron in the SBS. However, this was accounted for in the analysis of the raw data. The data that we present in the results and tables are appropriately adjusted for any hemoconcentration of the SBS that occurred. Regarding "back filtration", we did not account for the balance chamber error. As pointed out though, this would account for approximately 60 ml or only 1% of the SBS. Therefore the effects would be minimal on our calculations.

2) Introduction, page 4: Sucrose is a disaccharide so it is not really clear where the molecular weight of 34000 to 60000 comes from in Venofer? Furthermore, sucrose is be easily dialyzed. Please, clarify.

The molecular weight of iron sucrose ranges 34000 to 60000 Daltons as during the manufacturing
process, the number of sucrose molecules bound to iron varies. It is not a simple, standard ratio.

3) Are the solutions of iron stable in normal saline and in dialysate or do they form the typical Fe(OH)₃ gels?

Both iron dextran and iron sucrose are stable in and are recommended for dilution by normal saline. (DexFerrum and Venofer package inserts) We do not know if iron dextran or iron sucrose is stable in hemodialysis dialysate. However, iron dextran is stable in peritoneal dialysate (Manley HJ, Grabe DW, Norcross M, Bailie GR. Stability of iron dextran (DexFerrum) in peritoneal dialysis bags. Perit Dial Int 1998; 18 (5): 538-540.) of which constituents are similar to that appears in hemodialysis dialysate solution. Nonetheless, our intent was to determine whether any iron was detected in the dialysate compartment. As such we measured the presence of iron by atomic absorption spectrophotometry which will detect any iron regardless if it was bound to dextran or sucrose. Given that iron was found in non-clinically significant amount in the dialysate compartment, knowledge of whether the iron was bound (i.e., stable) to sucrose or dextran is moot.

4) Materials & Methods, page 5: Which base was used in the dialysate bath, acetate or bicarbonate? Would the different solubility of iron-acetate or iron-carbonate play a role in the result of the experiment?

At both centers, Albany, NY and Kansas City, MO, bicarbonate dialysate bath was utilized. We do not think that use of a bicarbonate bath will affect the results. Again, we measured the presence of iron by atomic absorption spectrophotometry which will detect any iron regardless if it was bound to carbonate. Given that iron was found in non-clinically significant amount in the dialysate compartment, knowledge of whether the iron was bound (i.e., stable) to carbonate is moot.

5) Page 19, Table 1: It is not clear which experiments were done twice and which were re-run. Please, clarify (in the Table and in the Materials & Methods section) whether the re-run was also done in duplicate or whether the re-run was part of the duplicate measurements.

The re-run samples were also done in duplicate.

Minor:
6) Abstract, page 2, line 5 from bottom: change "effect" to "affect".

Changed as suggested.

7) Materials & Methods page 6, last paragraph: use uniform abbreviations and change "cc" to "ml".

Changed as suggested.

8) Page 9, line 5 from top: change "1% x 100" to "1% / 100"

Changed as suggested.