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ORIGINAL ARTICLE

Iron kinetics following treatment with sucroferric oxyhydroxide or ferric citrate in healthy rats and models of anaemia, iron overload or inflammation

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ABSTRACT

Background. The iron-based phosphate binders, sucroferric oxyhydroxide (SFOH) and ferric citrate (FC), effectively lower serum phosphorus in clinical studies, but gastrointestinal iron absorption from these agents appears to differ. We compared iron uptake and tissue accumulation during treatment with SFOH or FC using experimental rat models.

Methods. Iron uptake was evaluated during an 8-h period following oral administration of SFOH, FC, ferrous sulphate (oral iron supplement) or control (methylcellulose vehicle) in rat models of anaemia, iron overload and inflammation. A 13-week study evaluated the effects of SFOH and FC on iron accumulation in different organs.

Results. In the pharmacokinetic experiments, there was a minimal increase in serum iron with SFOH versus control during the 8-h post-treatment period in the iron overload and inflammation rat models, whereas a moderate increase was observed in the anaemia model. Significantly greater increases ($P < 0.05$) in serum iron were observed with FC versus SFOH in the rat models of anaemia and inflammation. In the 13-week iron accumulation study, total liver iron content was significantly higher in rats receiving FC versus SFOH ($P < 0.01$), whereas liver iron content did not differ between rats in the SFOH and control groups.

Conclusions. Iron uptake was higher from FC versus SFOH following a single dose in anaemia, iron overload and inflammation rat models and 13 weeks of treatment in normal rats. These observations likely relate to different physicochemical properties of SFOH and FC and suggest distinct mechanisms of iron absorption from these two phosphate binders.

Keywords: ferric citrate, iron uptake, phosphate binder, sucroferric oxyhydroxide

INTRODUCTION

Hyperphosphataemia is an almost inevitable occurrence in end-stage chronic kidney disease (CKD) [1, 2] and dialysis patients require treatment with oral phosphate binders, in addition to dialysis and dietary phosphate restrictions, to achieve control of their serum phosphorus levels [1].

Two recently approved iron-based phosphate binders, sucroferric oxyhydroxide (SFOH) and ferric citrate (FC), have both demonstrated a good efficacy and safety profile in clinical trials [3–9]. However, concerns have been raised regarding the potential for long-term iron accumulation with these agents.

Data from clinical studies indicate differences in the level of iron absorption from SFOH and FC. A Phase 1 study of SFOH demonstrated low iron uptake among dialysis and non-dialysis patients with CKD administered a radiolabelled form of the drug [10]. A Phase 2 study, in which intravenous iron use was not permitted, found no significant changes in iron parameters among haemodialysis patients treated with SFOH [11]. A 24-week Phase 3 study found initial increases in some iron-related parameters with SFOH, including ferritin and transferrin saturation (TSAT) [4]; these increases occurred early and plateaued with continued treatment [3, 4]. A *post hoc* analysis of the Phase 3 study and its 28-week extension study indicated

that the observed increases in serum ferritin with SFOH were primarily driven by concomitant intravenous iron use [12].

In contrast to SFOH, iron absorption from FC can lead to significant elevations in iron parameters. TSAT and ferritin are utilized in clinical practice to determine iron status in patients with CKD, but no upper limits for these parameters are specified by the Kidney Disease: Improving Global Outcomes clinical practice guidelines [13]. Increases in serum ferritin and TSAT were reported in both short-term (8 and 12 weeks) [8, 9] and long-term (52 weeks) clinical studies of FC [5, 6]. As a result, monitoring of serum ferritin and TSAT levels is required prior to and during FC treatment due to the potential risk of iron overload [14]. Despite this risk, there is also evidence that increases in iron stores associated with FC therapy may be beneficial for dialysis patients (in whom iron deficiency anaemia is common), in terms of reducing the need for intravenous iron or erythropoiesis-stimulating agent therapy [15]. FC is currently approved in the USA for iron replacement therapy in the treatment of iron deficiency anaemia in adult patients with CKD not on dialysis [16].

No studies have directly compared iron uptake between SFOH and FC. We conducted *in vivo* studies in rats to evaluate differences in iron absorption resulting from a single dose and 13 weeks of treatment with these two phosphate binders. Because iron absorption from the gastrointestinal tract is regulated by iron stores and inflammatory status [17] (chronic inflammation is common in dialysis patients [18]), pharmacokinetics of iron uptake following a single dose of FC and SFOH were evaluated in anaemic, iron-loaded and inflammatory rat models. A 13-week study also evaluated the effects of daily treatment with these two phosphate binders on iron uptake and accumulation in normal rats. Based on evidence from previous studies, the hypothesis of our study was that SFOH would be associated with lower levels of iron uptake compared with FC.

MATERIALS AND METHODS

Pharmacokinetic studies

Animal care in pharmacokinetics studies. Studies were performed in a laboratory reviewed and approved by the Veterinary Office of the Canton of St. Gallen, Switzerland, according to the Swiss animal protection regulations (TSchV, SR 455.1) [19] and authorized by the Veterinary Office of the Canton of St. Gallen (SG 12/04 and SG/12/15).

Anaemia model. Healthy male Wistar rats (Janvier Labs, Saint Berthevin, France) ages 3–4 weeks were fed *ad libitum* on a standard diet during the acclimatization period (article 3433, Provimi Kliba, Kaiseraugst, Switzerland) followed by a low-iron diet for 10 days (article 2039, Provimi Kliba). Animals were divided into four groups: three active treatment groups ($n = 5$ per group) and one control group ($n = 2$).

In two treatment groups, animals were treated with FC hydrate (Riona, Torii Pharmaceutical, Tokyo, Japan) or SFOH (Velphoro, Vifor Pharma, Glattbrugg, Switzerland) at 2.5 mg Fe/kg body weight (BW) [0.25 mg Fe/mL in vehicle (1.5% methylcellulose; catalogue #274429, Sigma-Aldrich, St. Louis, MO, USA)], respectively. In the third treatment group, animals received ferrous sulphate heptahydrate in vehicle at 2.5 mg Fe/kg BW. The control group received vehicle alone prepared with deionized water.

Haemoglobin was measured 1 h before treatment in a HemoCue 201 DM analyser (HemoCue, Brea, CA, USA) to verify that animals were anaemic.

Iron-loaded model. Healthy male Wistar rats (Janvier Labs) ages 6–7 weeks, fed *ad libitum* with a standard diet, were divided into four groups ($n = 5$ per group). Twenty-four hours before the experiment, each rat was administered intravenous ferric carboxymaltose (15 mg/kg; Ferinject, Vifor Pharma). In two treatment groups, animals were treated with FC hydrate (Auryxia, Keryx Biopharmaceuticals, Boston, MA, USA) or SFOH (Velphoro, Vifor Pharma) at 40 mg Fe/kg BW [4 mg Fe/mL in vehicle (1.5% methylcellulose)], respectively. The third treatment group received ferrous sulphate heptahydrate in vehicle at 40 mg Fe/kg BW and the control group was treated with vehicle alone.

Inflammation model. Healthy female Lewis rats (Janvier Labs) ages 8–9 weeks and fed *ad libitum* with a standard diet were divided into four groups ($n = 5$ per group). Seventeen hours prior to the experiment, each rat was administered a 0.1 mg/kg dose of lipopolysaccharide (L2630, Sigma-Aldrich) solution in a 0.9% saline vehicle. In two treatment groups, rats were treated with FC hydrate (Auryxia, Keryx Biopharmaceuticals) or SFOH (Velphoro, Vifor Pharma) at 40 mg Fe/kg BW [4 mg Fe/mL in vehicle (1.5% methylcellulose)]. In the third and fourth (control) groups, animals received ferrous sulphate heptahydrate in vehicle at 40 mg Fe/kg BW or vehicle alone, respectively.

Serum iron analyses. Blood was sampled 1 h before treatment administration and at 1, 2, 4 and 8 h post-administration. Serum iron concentrations were measured using the Architect Iron Assay (Abbott, Abbott Park, IL, USA).

Liver iron measurements. At 8 h post-administration, all rats in each study were euthanized. Liver samples were obtained, digested and analysed for iron content by inductively coupled plasma optical emission spectrometry (ICP-OES).

Iron accumulation study

This study was performed using 80 (40 male and 40 female) healthy Sprague Dawley rats (Charles River, Sulzfeld, Germany) ages 7–9 weeks. The animals were derived from a controlled full-barrier maintained breeding system and bred for experimental purposes according to the German Animal Welfare Act [20]. Experiments were performed in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited laboratory, which was reviewed and approved by local authorities. The study underwent ethical review and received approval from the Bavarian animal welfare administration.

Animals were divided into four groups (10 males and 10 females per group). In two groups, the rats were treated with FC hydrate (Riona, Torii Pharmaceutical) or SFOH (Velphoro, Vifor Pharma) at 50 mg Fe/kg BW [10 mg Fe/mL in vehicle (1.5% methylcellulose; catalogue #M0262, Sigma-Aldrich)]. In the third and fourth (control) groups, the rats were treated with ferrous sulphate heptahydrate in vehicle at 50 mg Fe/kg BW and with 1.5% methylcellulose vehicle, respectively.

All animals were treated with a single dose by oral gavage with the test formulation or vehicle daily for 90 days. On Day 91, all surviving animals were euthanized and perfused. Liver and spleen samples were obtained for iron quantification and liver, spleen, kidney, heart, brain and sternum (with bone marrow) samples were obtained for histopathological investigations. These samples were digested and analysed for iron by ICP-OES. At necropsy, blood was collected in serum separator tubes and serum iron was measured using an AU480 Chemistry Analyzer (Beckman Coulter, Brea, CA, USA).

For the histopathological examination, paraffin sections from organ samples were stained with Prussian blue for haemosiderin. Iron storage was evaluated with respect to incidence and severity. In the liver, both hepatocytes and Kupffer cells were identified and assessed using a semi-quantitative scoring system of severity as shown in Supplementary data, Table S1. The sum score of iron for each treatment was calculated by multiplying each sample with positive staining by the staining grade. Histological processing and histopathological evaluation were performed on the basis of the Swiss ordinance relating to Good Laboratory Practice adopted on 18 May 2005 [21].

Serum levels of interleukin-6 (IL-6), hepcidin and fibroblast growth factor-23 (FGF23) were also quantified at Week 7 and at the end of the study (Week 13). Serum IL-6 was measured using the V-PLEX Plus Rat IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Meso Scale Diagnostics, Rockville, MD, USA), hepcidin was measured using the ELISA Kit for Hepcidin (Cloud-Clone, Katy, Tx, USA) and FGF23 was measured using the Rat Fibroblast Growth Factor 23 ELISA Kit (Abbexa, Cambridge, UK).

Statistical analysis

In the pharmacokinetic studies, Wilcoxon–Mann–Whitney (exact) tests analysed differences in serum iron (change from baseline at each time point) and liver iron (at 8 h) between treatment groups. In the anaemia model, no statistical comparisons were performed between treatment groups and the control group, because the latter only contained two animals. In the tissue accumulation study, Wilcoxon–Mann–Whitney (exact) tests analysed differences in liver, spleen and serum iron concentrations at 13 weeks between treatment groups. Analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA; P-values <0.05 were considered statistically significant). No adjustment for multiple comparisons was performed. All P-values were considered descriptively considering the exploratory nature of the experiments.

RESULTS

Pharmacokinetics of acute iron absorption

Anaemia model. The animals in all groups were anaemic, with mean haemoglobin values of 62–74 g/L (normal haemoglobin range in age-matched rats: 130–140 g/L). Mean serum iron concentrations increased rapidly from 50 µg/dL to 738 µg/dL and 759 µg/dL by 1 h post-treatment in rats treated with FC and ferrous sulphate, respectively (Figure 1A). In contrast, a smaller increase in serum iron was observed in rats treated with SFOH, peaking at 391 µg/dL at 4 h. Differences in the changes from baseline in serum iron concentrations were statistically significant for the SFOH group versus the FC and ferrous sulphate groups at 1, 2, 4 and 8 h ($P < 0.05$). The mean liver iron concentration at 8 h post-administration was slightly higher for rats treated with ferrous sulphate [36.5 (SD 5.3) µg/g], FC [33.5 (SD 1.4) µg/g] or SFOH [33.8 (SD 7.3) µg/g] compared with the control group [25.8 (SD 0.4) µg/g].

Iron-loaded model. In iron-loaded rats, mean serum iron concentrations increased to the greatest extent in the ferrous sulphate group, peaking at 439 µg/dL after 1 h (Figure 1B). Serum iron also increased in the FC group, peaking at 355 µg/dL at 2 h. In contrast, there was only a minor difference in serum iron in the SFOH group versus the control group during the study, with peak levels of 182 µg/dL versus 166 µg/dL after 4 h. Statistically significant differences between the treatment groups ($P < 0.05$) for changes from baseline in serum iron concentrations were observed for ferrous sulphate versus both the control and SFOH groups at 1, 2 and 4 h versus the FC group at 1 and 4 h, for the FC group versus control at 2 h and for the SFOH group versus control at 1 and 2 h post-administration. In this iron-loaded model, there was no significant difference in liver iron concentrations between any of the treatment groups (data not shown).

Acute inflammation model. Animals in the ferrous sulphate group had the greatest increases in serum iron, peaking at 417 µg/dL after 2 h, followed by the FC group, in which serum iron peaked at 247 µg/dL at 1 h post-administration. The serum iron increase was lower in the SFOH group, rising steadily from 82 µg/dL at baseline to 164 µg/dL after 8 h (Figure 1C). There were statistically significant differences for the changes in serum iron from baseline for the ferrous sulphate group versus the control, SFOH and FC groups (Figure 1C; $P < 0.05$). A significant difference was observed between the FC and the SFOH and control groups post-administration ($P < 0.05$).

The mean liver iron concentration after 8 h was higher in the ferrous sulphate [216 (SD 31.4) µg/g] and FC [204 (SD 32.9) µg/g] groups than in the SFOH [177 (SD 7.4) µg/g] and control [177 (SD 22.8) µg/g] groups. The only statistically significant difference was between the ferrous sulphate and SFOH groups ($P < 0.05$).

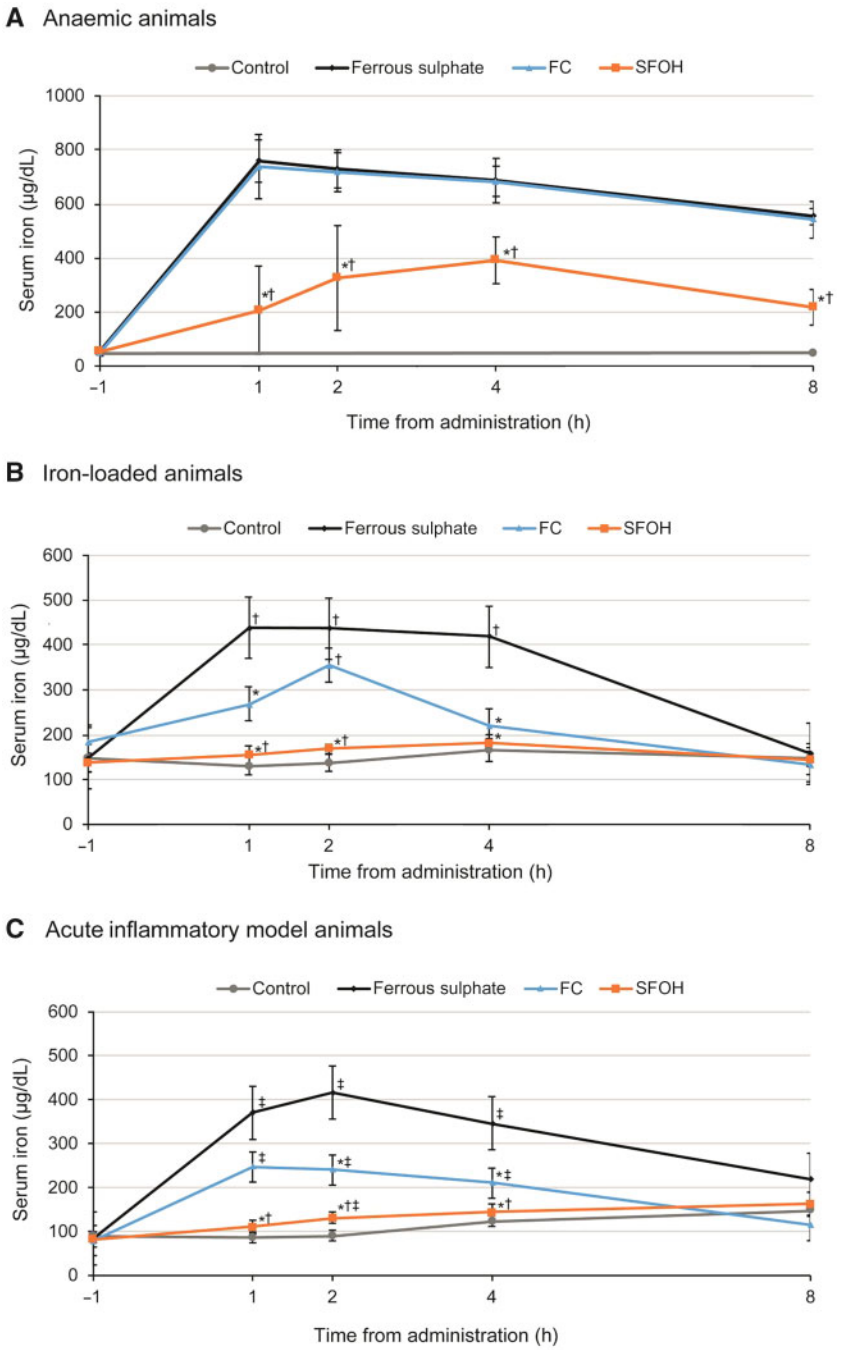

FIGURE 1: Mean (SD) serum iron levels in (**A**) anaemic, (**B**) iron-loaded and (**C**) inflammatory model rats. (A) Comparison of change from baseline: *$P < 0.05$ versus ferrous sulphate and †$P < 0.05$ versus FC. (B) Comparison of change from baseline: *$P < 0.05$ versus ferrous sulphate and †$P < 0.05$ versus control. (C) Comparison of change from baseline: *$P < 0.05$ versus ferrous sulphate; †$P = 0.05$ versus FC and ‡$P < 0.05$ versus control. Error bars represent SDs. BL, baseline.

Organ distribution following 13 weeks of iron administration

Organ iron content. After 13 weeks of treatment, the total liver iron content was similar between the SFOH and control groups (Figure 2). In contrast, there was a statistically significant increase of about 2-fold in liver iron among animals treated with FC and ferrous sulphate versus the control and SFOH groups (all $P < 0.01$). Liver iron content was consistently higher in female compared with male rats (Figure 2).

Among the male rats, total iron spleen content was significantly higher in those treated with ferrous sulphate, FC and SFOH compared with the control, but there was no significant difference between any of the treatment groups among the female rats (data not shown).

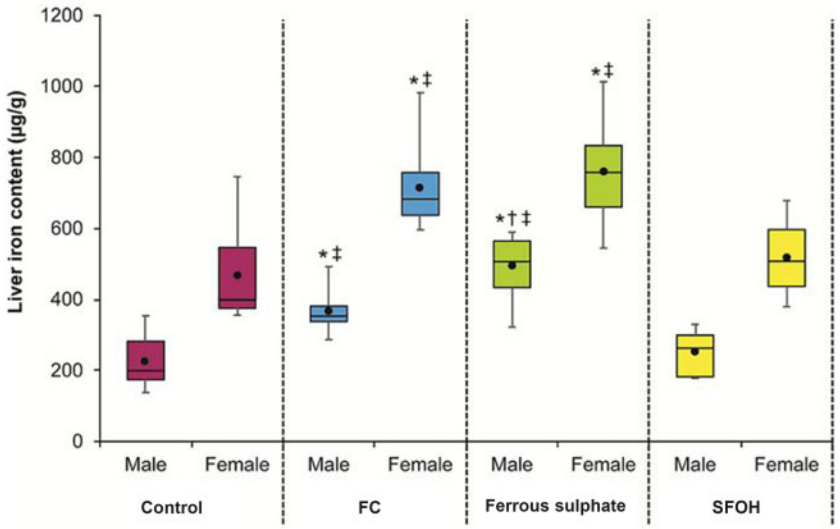

FIGURE 2: Total liver iron content in rats treated with FC, ferrous sulphate, SFOH or control for 13 weeks. *$P < 0.05$ versus control; $^{\dagger}P < 0.05$ versus FC and $^{\ddagger}P < 0.05$ versus SFOH. Bars represent Q1 and Q3; error bars represent minimum and maximum values; black dots represent the mean and horizontal lines represent the median.

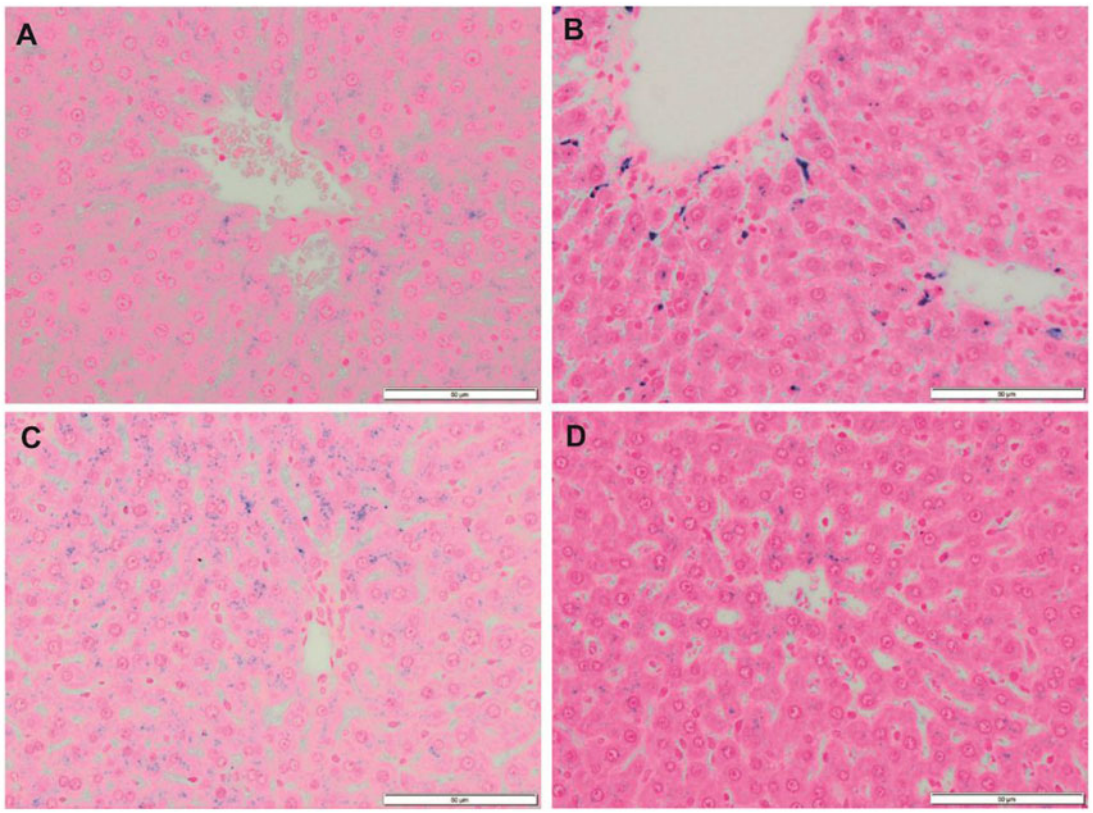

FIGURE 3: Representative images of Prussian blue staining in the liver from male rats treated with (**A**) saline (Grade 1), (**B**) FC (Grade 2), (**C**) ferrous sulphate (Grade 2) and (**D**) SFOH (Grade 1).

Histopathology. Prussian blue staining of the liver showed iron loading in all rats treated with FC or ferrous sulphate compared with 7/10 male and 8/10 female rats treated with SFOH and 2/10 and 9/10 in the control group, respectively (Supplementary data, Figure S1). The sum iron scores for the liver were higher for FC and ferrous sulphate than for SFOH or the control group. This was the case for both hepatocytes and Kupffer cells within the liver (Figures 3 and 4). In all locations and groups, female rats tended to exhibit similar or higher scores than male rats (Figure 4). A greater intensity of Prussian blue staining was recorded in the spleen sections from rats treated with FC and ferrous sulphate compared with those treated with SFOH (data not shown). There were no clear differences for haemosiderin deposition in the other organs assessed (kidney, heart, brain and sternum).

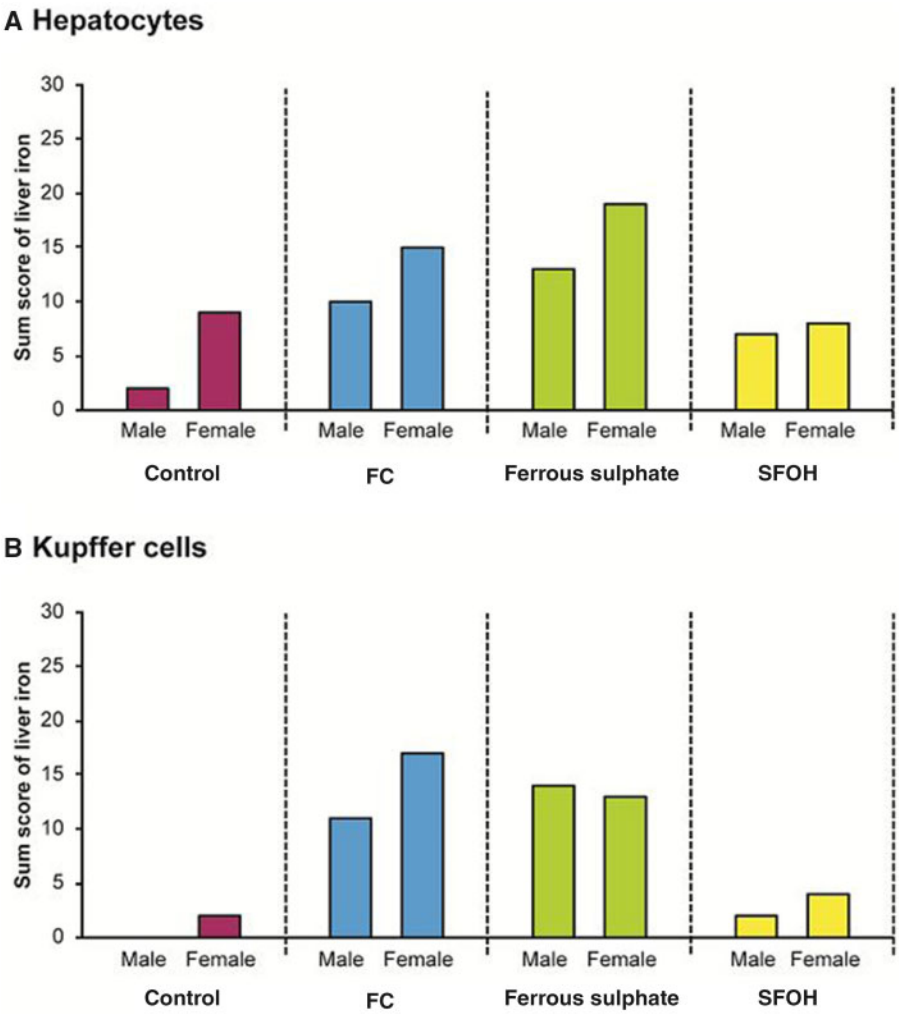

FIGURE 4: Sum score of iron in (**A**) hepatocytes and (**B**) Kupffer cells based on the incidence and severity of Prussian blue staining in samples from 10 male and 10 female rats treated with FC, ferrous sulphate, SFOH or control for 13 weeks.

Serum iron, TSAT and reticulocyte count. On Day 91, serum iron concentrations were not significantly different between the ferrous sulphate or SFOH group versus the control group. There were small, statistically significant ($P < 0.05$) increases in mean serum iron in the FC group [239 (SD 111) µg/dL in males and 426 (SD 49) µg/dL in females] compared with the levels in the control group [169 (SD 30) µg/dL in males, 329 (SD 50) µg/dL in females].

On Day 91, there was no significant difference in mean TSAT levels or reticulocyte count between the animals treated with SFOH, FC or ferrous sulphate and the control group (data not shown).

Inflammatory markers and FGF23. Serum IL-6 concentrations were generally very low and below the detectable range in the majority of animals. At Week 13, there was no significant difference in mean hepcidin or FGF23 levels between the animals treated with SFOH, FC or ferrous sulphate and the control group (data not shown).

DISCUSSION

Overall, our *in vivo* studies demonstrated higher iron uptake with FC than with SFOH following a single dose in anaemic, iron-loaded and inflammation rat models.

In anaemic rats, a dose of 2.5 mg Fe/kg BW was administered, which corresponds to a low dose as is used for the treatment of iron deficiency anaemia in humans. The iron uptake profile was nearly identical for the rats treated with ferrous sulphate and FC, and the peak serum iron concentration in these groups was nearly 2-fold higher than in rats treated with SFOH. Even at this low dose, there was an increase in liver iron detected 8 h after treatment.

In iron-loaded rats, a higher dose (40 mg Fe/kg BW), corresponding to the maximum daily dose of SFOH (3000 mg/day) [22] , was administered. In iron-loaded conditions, increased hepcidin levels would be expected to block further iron uptake from the gastrointestinal tract. This appeared to be the case for SFOH, where iron uptake was low and serum iron levels peaked at 182 µg/dL at 4 h after treatment, comparable with serum iron increases observed in the no-

treatment control group. These findings are consistent with pharmacokinetic studies conducted in healthy iron-replete rats receiving SFOH at a dose of 50 mg Fe/kg BW, in which minimal iron uptake was also observed [23]. Similarly, in a Phase 3 study of SFOH, greater increases in iron-related parameters during SFOH treatment were observed among dialysis patients with lower baseline serum ferritin levels, whereas minimal changes were observed in patients with elevated baseline serum ferritin levels. These findings suggest iron uptake from SFOH is mainly regulated by body iron stores and inflammation [12]. In contrast, increased levels of iron uptake were apparent with ferrous sulphate and FC, despite the high iron load in the model. Liver iron concentrations were not significantly influenced by oral iron, most likely because they were already elevated due to the intravenous iron treatment.

In the acute inflammation model, iron uptake was minimal in rats administered SFOH, with serum iron remaining close to baseline levels. In contrast, serum iron increased >2-fold with FC and 3-fold with ferrous sulphate, in a similar manner to the iron-loaded model. There were apparent differences in iron storage, because significantly more iron was stored in the liver of rats treated with ferrous sulphate compared with those receiving SFOH.

Enteric iron absorption is complex and tightly controlled by the actions of the iron-regulatory hormone hepcidin and iron transport proteins. These include the divalent metal ion transporter 1 (DMT1), which transports ferrous (Fe^{2+}) iron from the lumen into the enterocyte across the apical membrane, and ferroportin, which transports iron from the enterocyte into the plasma across the basolateral membrane [24]. The ferric reductase duodenal cytochrome B (DcytB), which is present on the apical surface of enterocytes, is responsible for the reduction of non-haem ferric iron (Fe^{3+}) into the ferrous (Fe^{2+}) form required for uptake by iron transport proteins and subsequent absorption [25].

Under conditions of iron deficiency, as evaluated in our model of anaemia, stabilization of hypoxia-inducible factor-2 alpha leads to the upregulation of DcytB and DMT1 in the apical enterocyte membrane and increased expression of ferroportin on the basal membrane [26]. This results in increased transfer of dietary iron into the plasma at the expense of iron depletion within the enterocytes. In conditions of iron overload, increases in hepcidin levels control iron efflux from enterocytes post-translationally by inducing endocytosis and the degradation of ferroportin. Under conditions of inflammation, hepcidin expression is increased, resulting in ferroportin degradation as well as additional reduction in ferroportin transcription and a block of iron absorption [27].

Observed differences in iron uptake may be due to different physicochemical properties and mechanisms of absorption between the iron compounds under study. Specifically, higher solubility and the uptake of iron through alternative, non-controlled absorption pathways could potentially explain the increased serum iron levels in animals treated with FC and ferrous sulphate versus SFOH. For ferrous sulphate, active iron uptake via DMT1 in humans, has been calculated as limited to ~1 mg from an ~5 mg dose, and at higher doses, additional uptake by passive diffusion may occur [28]. The FC complex is highly soluble in water [29]. Furthermore, citrate can disrupt the intercellular tight junctions in the small intestine epithelium by chelating calcium ions [30], which could open an additional route of iron absorption, in an analogous manner to that observed with aluminium absorption and aluminium citrate [31]. This may explain why in our experiments marked iron uptake from FC occurred even in the presence of inflammation or in iron-loaded conditions. In contrast, the iron oxyhydroxide moiety of SFOH is practically insoluble, and iron release is minimal under physiological conditions [32].

Other factors that could explain differences in iron uptake between the study compounds include the functionality of the ferric reductase, DcytB, which catalyses the reduction of ferric (Fe^{3+}) to absorbable ferrous (Fe^{2+}) iron [24]. While the ferrous iron in ferrous sulphate is available for enteral absorption, ferric iron from SFOH and FC requires DcytB-mediated reduction. Under the iron-deficient conditions of the anaemia model, in which DcytB expression is increased, levels of iron uptake from ferrous sulphate and FC were similar, whereas serum iron levels were significantly lower with SFOH. It is possible that some of the observed difference in iron absorption between SFOH and FC was due to the contrasting solubility profiles of these two compounds, resulting in slower DcytB-mediated reduction of SFOH versus FC. In the models of inflammation and iron overload, reduced DcytB activity may also have contributed to the lower levels of iron uptake from FC versus ferrous sulphate.

A 13-week iron-accumulation rat study examined the effect of iron uptake over time in a range of organs. The physiological iron levels are regulated by iron absorption in the small intestine and absorbed iron is, to a large extent, stored in the liver [33, 34]. This organ was therefore of primary interest for the assessment of iron overload. The iron uptake was assessed by measuring total iron content in organ samples and using immunohistochemical staining to detect haemosiderin, an intracellular iron storage complex. In iron-loaded conditions when transferrin iron capacity is saturated, most non-transferrin-bound serum iron is deposited in the liver [35], causing haemosiderin levels to increase [36, 37]. Elevated haemosiderin has been associated with iron-related tissue damage [38], and the liver is the organ most likely to be affected [39]. By the end of our present treatment period, total iron content was significantly higher in the livers of rats treated with ferrous sulphate and FC than in those treated with SFOH. There was no significant difference in total iron content between the SFOH group and controls. This finding is consistent with the results of a study evaluating iron accumulation in healthy rats receiving 40 mg Fe/kg BW as SFOH for 26 weeks or 2 years [23]. These results were also reflected in the histopathology examinations of liver samples, which showed a greater degree of haemosiderin staining with ferrous sulphate and FC compared with the SFOH and control samples. Evaluation of liver enzymes found no major difference in serum levels of serum aspartate aminotransferase or alanine aminotransferase in any of the treatment groups versus controls, indicating that liver function was unaffected by treatment.

The degree of organ iron accumulation observed in this study did not exceed toxic levels in any of the treatment groups. The acute toxic dose of iron reported in infants is 20 mg/kg BW, which leads to local physiological effects, but systemic effects do not generally occur at doses <60 mg/kg BW [40]. The lethal dose of iron reported in children and adults is ~200–300 mg/kg BW and ~1400 mg/kg BW, respectively [40].

The long-term impact of iron accumulation at the level shown in the current studies is unclear. Effects of long-term iron accumulation have been mainly described for genetically determined disturbances of iron metabolism, in particular, hereditary haemochromatosis [31].

In studies of iron supplementation conducted in patient populations at risk of iron deficiency, potential adverse effects other than gastrointestinal side effects have generally not been addressed.

Study limitations included the small number of animals per treatment group and the relatively short (13 weeks) study duration, which may not reflect very long-term iron exposure levels. The rats did not have renal impairment, hence the results may not translate to patients with CKD and further work is needed in renal impairment models. Changes in enteric levels of ferroportin and other iron transport proteins were not measured.

In conclusion, the single-dose and 13-week exposure studies in rats indicate iron uptake from FC is higher than for SFOH. This is likely due to different physicochemical properties of SFOH and FC and suggests different mechanisms of action are involved for iron uptake between these two phosphate binders.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

F.F. designed the study. All authors contributed to data analysis, data interpretation, drafting the manuscript, revising manuscript content and approval of the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

J.F. has received consulting fees or lecture fees from AbbVie, Amgen, Chugai, Fresenius Medical Care, Sanofi, Shire and Vifor Pharma. M.K. has received consulting fees or lecture fees from Vifor Pharma, Fresenius Medical Care, Amgen, AbbVie, Medice, Mitsubishi Pharma, Sanofi and Shire. A.R. has received consultancy fees from Fresenius Medical Care and Vifor Pharma and lecture and consultancy fees from Sanofi, Cytochroma/OPKO Health, Vifor Pharma, Satellite Healthcare and Deltanoid. S.W. and F.F. are employees of Vifor Pharma. A.C.C. has received consultancy fees or lecture fees from Vifor Pharma, Fresenius Medical Care and Amgen. S.M.S. has received consultancy fees from OPKO, Vifor Pharma, Amgen, Fresenius Medical Care, Litholink and NPS Pharma and research funding from Abbott, Amgen and Shire. The results presented in this article have not been published previously in whole or part.

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