The ins and outs of ferric citrate

Volker H. Haase[1,2,3,4]

Ferric citrate is used clinically for the treatment of hyperphosphatemia in patients with chronic kidney disease and is approved as an oral iron replacement product for patients with iron-deficiency anemia. In this issue of *Kidney International*, Hanudel and colleagues take advantage of genetic models with and without chronic kidney injury to demonstrate that the enteric absorption of iron delivered by ferric citrate is dependent on ferroportin expression and does not involve paracellular iron transport.

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In addition to erythropoietin (EPO) deficiency, absolute iron deficiency and functional iron deficiency have been identified as key contributors to anemia associated with chronic kidney disease (CKD).[1] Ferric citrate, an iron-containing phosphate binder, is effective in treating hyperphosphatemia and has been approved in the US for the treatment of iron-deficiency anemia (IDA) in patients with CKD not on dialysis, and more recently in Japan for patients with IDA. In randomized clinical trials, ferric citrate raised transferrin saturation, serum ferritin, and hemoglobin (Hb) levels, compared with placebo in non-dialysis-dependent CKD patients,[2] and decreased i.v. iron and erythropoiesis-stimulating agent use in patients with end-stage kidney disease.[3]

A major clinical challenge in treating CKD patients with anemia is the presence of functional iron deficiency—that is, the inability to absorb and mobilize sufficient amounts of iron for erythropoiesis.[1] A key mediator of this condition is hepcidin, a small peptide produced by the liver that promotes the degradation of ferroportin, the only known transmembrane protein that transports ferrous iron from the inside to the outside of a cell. Serum hepcidin levels are elevated in patients with CKD, due to inflammation and reduced clearance, resulting in diminished intestinal iron uptake and release from iron-storing cells, such as macrophages and hepatocytes.[4]

Recent studies in animals showed that more iron was taken up in ferric citrate–treated rats under conditions of IDA, iron overload, and inflammation, compared with those treated with sucroferric oxyhydroxide, another iron-based phosphate binder.[5] These findings raised the possibility that the presence of citrate in the intestinal lumen may have enhanced iron uptake through paracellular absorption, as citrate can disrupt tight junction integrity between enterocytes, via calcium chelation, and thus promote, for example, the uptake of aluminum in patients with CKD.[6]

In this issue of *Kidney International*, Hanudel and colleagues (2022) investigate the mechanism by which iron, administered as ferric citrate (Auryxia, Akebia Therapeutics), is absorbed under conditions of elevated serum hepcidin and chronic kidney injury.[7] Specifically, Hanudel and colleagues set out to test whether citrate promotes paracellular iron absorption, using a genetic approach.

Hanudel and colleagues first investigated iron parameters in mice globally deficient for transmembrane serine protease 6 (TMPRSS6), also known as matriptase-2, a type II transmembrane protease that negatively regulates the production of hepcidin in the liver. In humans, inactivating mutations in TMPRSS6 result in iron-refractory IDA, which is usually unresponsive to oral iron therapy and only partially responsive to i.v. iron administration.[8] IDA is also observed in *Tmprss6* knockout mice, which are characterized by elevated hepcidin levels, low duodenal ferroportin expression, decreased liver and serum iron levels, and microcytic anemia. Treatment of *Tmprss6* knockout mice with 0.1% ferric citrate for 3 weeks (mixed with rodent chow) improved Hb and iron parameters.[7] Ferric citrate administration also improved Hb levels in *Tmprss6* knockout mice with adenine-induced nephropathy, which were characterized by more pronounced increases in serum hepcidin levels.[7]

Hanudel and colleagues then investigated the effects of ferric citrate on erythropoietic and iron parameters in mice with enterocyte-specific ablation of ferroportin, employing a conditional gene-targeting approach based on tamoxifen-inducible *villin*-regulated Cre recombinase. Not surprisingly, loss of enteric ferroportin resulted in IDA, which was associated with a severe reduction in serum iron levels. In contrast to *Tmprss6* knockout mice, mice that lacked enteric ferroportin did not respond to ferric citrate administration with an increase in Hb or serum iron levels (1% of ferric citrate for 3 weeks).[7] This lack of response to ferric citrate was observed in ferroportin-deficient mice with and without chronic kidney insufficiency. The

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authors concluded from their studies that ferroportin was necessary for the absorption of iron delivered by ferric citrate, and they further suggested that paracellular citrate-induced iron transport did not play a role in this model.

Although the genetic studies by Hanudel and colleagues established that transcellular ferroportin-mediated iron transport is the predominant mechanism for the enteric absorption of iron administered as ferric citrate, the studies are indirect. They did not include cell-based assays that take advantage of transwell or microfluidic bilayer systems to investigate paracellular iron transport directly. Paracellular transport assays with intestinal cells isolated from healthy and CKD mice are needed to validate the *in vivo* findings and would also be useful in characterizing any effects on epithelial barrier integrity and permeability resulting from ferroportin gene inactivation. Furthermore, given the relatively high data variability, an increase in the number of experimental animals might reveal statistically significant differences between ferric citrate–treated versus non-treated knockout animals. Therefore, whether and to what degree paracellular iron transport plays a role in iron uptake following ferric citrate administration remain open questions that warrant further investigation.

In their studies, Hanudel and colleagues reported that duodenal ferroportin protein expression was increased in ferric citrate–treated *Tmprss6* knockout mice with and without chronic kidney injury, compared with respective controls not receiving ferric citrate. This finding is important and provides additional insights into ferric citrate's mode of action; it warrants further mechanistic examination.

The regulation of enteric iron uptake is complex and involves the integration of multiple systemic and cellular iron- and oxygen-dependent signals, which can have both synergistic and antagonistic effects. For example, under conditions of increased systemic iron, ferroportin cell surface expression is decreased due to elevated serum hepcidin, which promotes ferroportin internalization and degradation, whereas ferroportin translation is increased when cells are iron-replete (Figure 1). Analogous to *ferritin* mRNA, the 5' untranslated region of *ferroportin* mRNA contains an iron-response element, which binds iron regulatory proteins when cellular iron levels are low, repressing ferroportin translation. When cells are iron-replete, iron regulatory proteins dissociate from the iron-response element, resulting in derepression of *ferroportin* mRNA translation, which in turn leads to increased ferroportin expression. Therefore, a plausible possibility is that a rise in intracellular iron concentrations was responsible for the increase in duodenal ferroportin expression observed in ferric citrate–treated *Tmprss6* knockout mice. To what degree ferric citrate administration modulates the expression of divalent metal transporter 1 (DMT1), which is oxygen- and iron-regulated and imports ferrous iron into cells,[1] is unclear and requires further investigation.

To add complexity to ferroportin regulation, ferroportin transcription is oxygen-sensitive and increases with hypoxia-inducible factor 2 (HIF2) activation, which itself is iron-regulated.[1] The complexity and context dependence of enteric ferroportin regulation is illustrated under conditions of iron deficiency. In IDA, HIF2 increases duodenal *ferroportin* transcription, whereas *ferroportin* mRNA translation is repressed by iron regulatory proteins. On the other hand, duodenal ferroportin internalization and degradation is reduced due to hepcidin downregulation in the liver, the net result being a physiologic increase in ferroportin surface expression that promotes

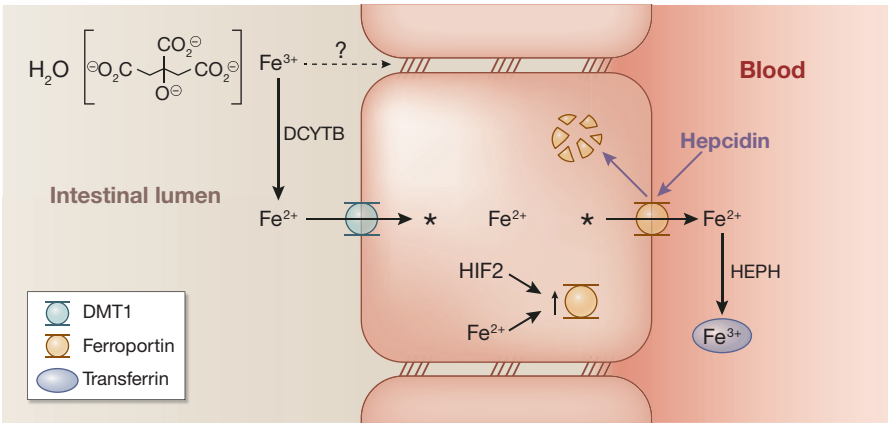

Figure 1 | Simplified overview of enteric iron absorption and ferroportin regulation. Ferric iron administered orally as ferric citrate is converted to ferrous iron by duodenal cytochrome B (DCYTB) and then transported into the cell by divalent metal transporter 1 (DMT1). DMT1 is hypoxia-inducible factor (HIF)–2-regulated and iron-regulated. Ferrous iron is transported out of the cell via ferroportin and then oxidized to its ferric form by the copper-dependent transmembrane ferroxidase hephaestin (HEPH). In the circulatory system, iron is transported in its ferric form by transferrin. HIF2 increases the transcription of ferroportin, and iron regulates ferroportin translation via iron regulatory protein–dependent mechanisms. Hepcidin promotes the internalization and degradation of ferroportin. The study by Hanudel and colleagues[7] suggests that iron, delivered by ferric citrate, is not taken up by a paracellular route; however, a citrate-mediated paracellular contribution cannot be completely excluded (dashed arrow). Asterisks indicate that transcellular transport is the predominant mechanism by which iron is absorbed following ferric citrate administration.

enteric iron uptake when iron deficiency is present. Although ferric citrate administration likely resulted in the derepression of iron regulatory protein–mediated translational inhibition in enterocytes, effects on other signaling pathways cannot be excluded and may have contributed to a shift in the balance between ferroportin synthesis and degradation, toward increased duodenal ferroportin expression. Also unclear is the degree to which other oral iron preparations, such as ferrous sulfate and ferrous gluconate, modulate ferroportin expression, and whether the effects on ferroportin expression are specific to ferric citrate alone. In this regard, additional studies are justified to gain more insights into the molecular mechanisms by which ferric citrate increases duodenal ferroportin expression in the presence of elevated hepcidin levels.

More recently, pharmacologic activation of HIF signaling with oral compounds that inhibit the degradation of the oxygen-regulated HIFα-subunit has been shown to be efficacious in correcting and maintaining Hb and improving iron parameters in patients with CKD anemia.[9] Because HIF regulates the expression of several iron metabolism genes, including *DMT1* and *ferroportin*,[1] a point of clinical interest to investigate is whether the combination of pharmacologic HIF activation and ferric citate administration synergistically increases cellular ferroportin expression and transcellular iron transport under high-hepcidin conditions.

In summary, the study by Hanudel and colleagues establishes that iron, administered as ferric citrate, enters the systemic circulation predominantly via ferroportin-dependent transcellular transport. However, a contribution of intestinal paracellular transport could not be completely excluded. Furthermore, the investigators provide evidence that ferric citrate modulates duodenal ferroportin expression under conditions of elevated serum hepcidin, which is of significant clinical interest. This finding could provide a rationale for clinical studies that investigate ferric-citrate therapy in conjunction with therapies that modulate or target the hepcidin–ferroportin axis.

DISCLOSURE

The author declared no competing interests.

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Semaphorin class 3C, vascular permeability, and the swollen injured kidney

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Class 3 semaphorins (SEMA3) are secreted glycoproteins with established roles in the developing brain, heart, and kidney. In this issue of *Kidney International*, Cai *et al.* show that in acutely injured kidneys, semaphorin isoform SEMA3C is expressed *de novo* in glomeruli and the nephron, secreted into the circulation, and excreted into the urine. Compelling evidence is provided for SEMA3C promoting microvascular permeability, kidney swelling, and acute injury. SEMA3C antagonism may be a treatment option for acute kidney injury.

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Semaphore is the sign bearer, and so the semaphorins were first identified as a class of secreted molecules and membrane proteins, originally found to be axonal growth-cone guidance molecules. They serve as short-range inhibitory molecules and signal through multimeric receptor complexes. Semaphorins not only guide axons in development, but also have major roles in immune function (classes 4, 6, and 7) and the development of bones. Class 3 semaphorins (SEMA3)

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