PNAS COMMENTARY

Another tool in the toolkit to manage iron overload

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Iron is difficult. It is an essential nutrient for almost every organism; yet, in our oxygen-rich atmosphere, it largely exists in the ferric, Fe^{+3} state, which is practically insoluble in the aqueous milieu that supports life. Most biological systems utilize the ferrous, Fe^{+2} form, which is readily soluble but also highly chemically reactive. Although this chemical reactivity is very useful when present in the form of an iron cofactor in an enzyme or oxygen-carrying molecule, unchaperoned iron can also be highly toxic to cells because it catalyzes the formation of reactive oxygen species that can damage lipids, proteins, and nucleic acids (1). For many organisms, low iron bioavailability limits growth. Humans have long struggled with dietary iron insufficiency because the plant-based diets that sustain most of the peoples of the world tend to be low in iron (2). Thus, it is unsurprising that humans have evolved to be very efficient in their utilization of dietary and bodily reservoirs of iron. We are so efficient in iron reutilization that humans express no effective means of ridding the body of excess iron. Without a means to excrete iron, our systems of iron uptake must be precisely regulated to meet changing metabolic needs and avoid iron overload. Although a healthy human can live for 100 y without developing iron deficiency or iron overload, many disease states are associated with disruption of this balance. Disorders associated with iron overload are both inherited and acquired and caused by intrinsically dysregulated iron trafficking or by iatrogenic iron loading in the form of red blood cell transfusions (3). Excess iron typically accumulates in cells of the reticuloendothelial system, but, in severe iron overload, parenchymal cells, especially of the liver, heart, and kidney, can be affected. These iron stores can be removed by simple interventions, such as phlebotomy, but pharmacologic means are necessary where phlebotomy is not tolerated due to anemia. There are three drugs currently approved for use as chelators to treat iron overload: deferoxamine, deferiprone, and deferasirox (4). Each has its advantages and limitations. Ekaputri et al. (5) discuss, in PNAS, the biological activity and potential therapeutic use of hinokitiol, a small, plant-derived molecule used in traditional Asian medicine that also has the potential to mobilize iron in the setting of iron overload.

In mammals, body iron balance is controlled by the activity of the sole cellular iron efflux pump, ferroportin (6). Uptake of dietary iron in the intestinal epithelium occurs through the iron importer divalent metal transporter 1 (DMT1) (7), and uptake of circulating transferrin-bound iron in the blood occurs through the combined activities of the transferrin receptors (Tfr1 and Tfr2), endosomal iron reductases (Steap3), and importers (DMT1 and Zip14) (8). Although cellular iron uptake is regulated by cell-autonomous systems (Hif-2 in the gut and Irp1 and Irp2 in other cells) (9, 10), body and tissue iron balance is controlled through the activities of ferroportin and the major regulatory hormone, hepcidin (6). Hepcidin is a small peptide synthesized and

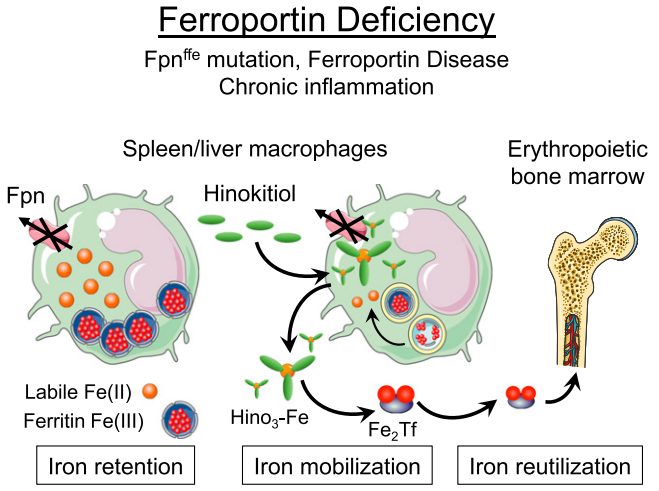

Fig. 1. Hinokitiol mobilizes intracellular iron for reuse. Iron-scavenging macrophages of the liver and spleen accumulate iron in ferritin and the labile iron pool when ferroportin activity is impaired, for example, in mice carrying the flatiron mutation (Fpn^{ffe}), in patients with ferroportin mutations, or in patients with chronic inflammation and excess hepcidin production. Membrane-permeable hinokitiol can bind macrophage labile iron, triggering ferritin degradation in the lysosome. Hino–Fe complexes diffuse out of the cell, and the iron is captured by circulating transferrin. Diferric transferrin can be taken up by erythropoietic tissues and used for hemoglobinization of new red blood cells.

secreted primarily by hepatocytes. Circulating hepcidin binds directly to ferroportin localized on the surface of iron-exporting cells. Hepcidin binding inactivates ferroportin by physically occluding the iron export channel and by triggering internalization and degradation of the exporter. The levels of circulating hepcidin are controlled through the sensing of body iron requirements and the synthesis and release of signaling molecules from the kidney, bone marrow, immune cells, and liver sinusoidal endothelium. These systems largely impact signaling through the bone morphogenetic protein receptor and the IL-6 receptor on hepatocytes, which activate the transcription of hepcidin (11). Any impairment in these sensing or signaling systems can result in inappropriately low levels of hepcidin and inappropriately high levels of iron efflux from the gut or from cellular stores (12). Dietary iron that is taken up by the intestinal epithelial cell may be stored in cellular ferritin

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and not exported through ferroportin; this iron is lost when the epithelial cell is sloughed into the lumen of the gut. Thus, iron efflux from the intestinal epithelium controls the amount of dietary iron that is absorbed in the body. Similarly, iron that is captured from senescent red blood cells by macrophages of the spleen or liver is stored in ferritin or released through ferroportin for reuse in the making of new red blood cells in the bone marrow.

Hereditary hemochromatosis refers to a genetically diverse group of clinical disorders in which iron overload occurs through mutations in the genes that impact the levels of circulating hepcidin (3, 6). These mutations include loss of function mutations in *HFE*, the major hemochromatosis gene and the most common cause of hereditary iron overload, as well as rare mutations in other genes involved in iron sensing and signaling through the bone morphogenetic receptor. Each of these mutations results in inappropriately low levels of hepcidin, which results in high levels of ferroportin activity and systemic iron overload. The most serious disorders of iron overload, however, are associated with dyserythropoietic or transfusion-dependent anemias, especially thalassemia major. These disorders require regular transfusions of red blood cells, and patients rapidly develop life-threatening iron overload. Iron overload occurs both through the infusion of red cells at 250 mg of iron per unit of packed cells, which is more than 10 times the daily dietary requirement for iron, and through the down-regulation of hepcidin through the homeostatic responses to dyserythropoietic anemia. The excess iron exceeds the normal capacity for iron storage in macrophages and accumulates in multiple tissues, including cardiomyocytes where it is exceptionally toxic. Iron is stored in ferritin as Fe^{+3}, which is relatively unreactive. The remaining intracellular iron is largely the highly reactive Fe^{+2}, which reacts with endogenous hydrogen peroxide and lipid peroxides to form highly damaging oxygen and lipid radicals. Uncontrolled lipid peroxidation can lead to mitochondrial dysfunction (13) and cell death (14). Cardiomyopathy is the most serious form of iron toxicity seen in patients with thalassemia major and the most common cause of death if iron levels are not controlled (15).

In addition to the systemic iron overload associated with hemochromatosis and iron-loading anemias, chronic inflammatory diseases can be associated with iron mislocalization and an associated anemia referred to as the anemia of inflammation (16). In this setting, inflammatory stimuli activate hepcidin expression and trigger the sequestration of iron within the reticuloendothelial system. Here the problem is the mislocalization of iron rather than overload, as the iron sequestration leads to anemia due to iron-limited erythropoiesis. Chronic inflammatory diseases of the liver, such as chronic hepatitis C and nonalcoholic steatohepatitis, are associated with liver iron loading that contributes to more severe disease and poorer outcomes (17). Thus, therapeutic strategies aimed at relieving iron overload or correcting iron mislocalization are needed in multiple clinical settings. The iron chelators in current clinical use work by binding intracellular or extracellular iron with relatively high affinity so that the low molecular weight iron chelate can be filtered and excreted by the kidney (4, 18). The studies of hinokitiol described by Ekaputri et al. (5) indicate this small molecule works in a fundamentally different way.

In 2017, Grillo et al. (19) demonstrated that a small molecule with known metal-binding activity could correct growth defects in mutant yeast lacking the endogenous high-affinity iron uptake system. This molecule, hinokitiol, was also shown to improve the dietary iron absorption defects of rodents carrying mutations in DMT1 or ferroportin and to improve hemoglobin formation in fish with impaired mitochondrial iron uptake. Mechanistically, hinokitiol functions by binding iron and mediating the diffusion of the iron–hinokitiol (iron–hino) complex down a concentration gradient. Because it is highly lipid soluble, it readily crosses cellular membranes. As an iron–hino complex, it can mobilize iron across a cellular membrane that lacks efficient endogenous iron transport. As an apo molecule, hinokitiol can access intracellular iron pools and mediate iron efflux. In the studies by Ekaputri et al. (5), the authors use a mouse model of ferroportin deficiency in which the animals exhibit macrophage iron overload, low levels of iron in serum, and mild anemia (5). Treatment of these animals with hinokitiol results in the mobilization of iron from the liver and spleen with concomitant increases in serum iron and improvements in anemia (Fig. 1). Ex vivo studies on liver macrophages from these animals confirm that hinokitiol promoted iron release from intracellular stores. In the blood, iron bound to hinokitiol is rapidly transferred to serum transferrin. This prevents the renal excretion of iron complexes and facilitates the delivery of iron to erythropoietic tissues. Finally, these authors use monocyte-derived macrophages from patients that carry mutations in ferroportin. They demonstrate that hinokitiol could deplete intracellular labile iron pools and trigger ferritin degradation in the hemoglobin-loaded macrophages from both healthy donors and patients.

Because the hinokitiol iron mobilized from ferroportin-deficient cells was rapidly exchanged and converted to transferrin iron in the blood, hinokitiol did not facilitate iron excretion from the ferroportin-deficient mice (5). It did, however, facilitate the redistribution of iron from intracellular stores to the blood where the iron could then be presented to the erythron. This type of redistribution could, for example, alleviate the iron-limited erythropoiesis that results in anemia of inflammation (16). Current iron chelation protocols for patients with iron-loading anemias have focused on the capacity to improve iron mobilization and excretion using a combination of iron chelators (4, 18). The combination of an orally active, cell-permeable, iron-binding molecule, such as hinokitiol, with a higher-affinity extracellular chelator could prove to enhance both iron mobilization and excretion. Because of the clinical necessity of developing effective iron chelation protocols that minimize the toxicities of current regimens, another arrow in the quiver of iron chelation therapy would be welcome, indeed.

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