Iron imports.

IV. Hepcidin and regulation of body iron metabolism

Tomas Ganz and Elizabeta Nemeth

Departments of Medicine and Pathology, David Geffen School of Medicine, University of California, Los Angeles, California
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Ganz, Tomas, and Elizabeta Nemeth. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol 290: G199–G203, 2006; doi:10.1152/ajpgi.00412.2005.—Hepcidin, a small peptide synthesized in the liver, controls extracellular iron by regulating its intestinal absorption, placental transport, recycling by macrophages, and release from stores. Hepcidin inhibits the cellular efflux of iron by binding to and inducing the degradation of ferroportin, the sole iron exporter in iron-transporting cells. In turn, hepcidin synthesis is increased by iron loading and decreased by anemia and hypoxia. Hepcidin is markedly induced during inflammation, trapping iron in macrophages, decreasing plasma iron concentrations, and contributing to the anemia of inflammation. Hepcidin deficiency due to the dysregulation of its synthesis causes most known forms of hemochromatosis.

Iron is an essential element for microbes, plants, and higher animals. It is a component of heme and iron-sulfur centers in many key redox enzymes and is an essential component of oxygen storage and transporting proteins such as hemoglobin and myoglobin. In humans, iron is strictly conserved, in large part, by recycling the iron (about 20 mg/day) from hemoglobin of senescent red blood cells to provide iron for new red blood cells. Smaller amounts of iron from myoglobin and various redox enzymes are also recycled. In most circumstances, human diets contain more iron than is necessary to replace the small daily losses (1–2 mg/day). Dietary iron is absorbed predominantly in the duodenum. Humans and other mammals lack mechanisms to excrete excess iron, and, therefore, intestinal iron absorption must be regulated by a feedback mechanism. Hereditary hemochromatoses are a group of genetic disorders in which dietary iron absorption is dysregulated, resulting in excessive dietary iron uptake and iron deposition in vital organs, including the liver, endocrine glands, heart, and skin. Eventually, the iron-overloaded organs are damaged, presumably through the iron-catalyzed generation of reactive oxygen species. At the opposite end of the spectrum of iron disorders are conditions in which iron absorption, recycling, and distribution from iron stores are not sufficient to meet the needs of hemoglobin synthesis in the bone marrow, despite adequate dietary iron. These “iron-refractory” anemias include most prominently the anemia of inflammation (AI; also called anemia of chronic disease). The discovery of hepcidin, its interaction with the iron exporter ferroportin, and its role in the regulation of iron transport has provided a molecular explanation of the homeostatic regulation of iron absorption and distribution and of its malfunction in hemochromatosis and AI.

Hepcidin and its activities

Forms and distribution. Human hepcidin (Fig. 1) is a 25-amino acid (aa) peptide first identified in human urine (20) and plasma (9). The liver is the main organ in which hepcidin mRNA is expressed (9, 20, 22) and is the main site of hepcidin synthesis. In addition to the 25-aa form, urine also contains 20- and 22-aa forms truncated at the NH2 terminus (20). Closely related hepcidin genes have also been found in the rat and several species of fish (20). The mouse genome contains two hepcidin genes, but only hepcidin 1 appears to have a role in iron metabolism (10).

Structure. Mass spectrometric studies of the 25-aa hepcidin peptide have shown that it contains four disulfide bonds (20), allowing for many potential conformations. By circular dichroism spectrometry, human urinary hepcidin was shown to be rich in β-sheets, and the subsequent NMR structure of hepcidin (Fig. 2) confirmed that hepcidin forms a simple hairpin stabilized by three disulfide bonds and a surprising vicinal disulfide bond in the turn (8). The functional role of this unusual feature remains to be determined. The human hepcidin gene contains three exons that encode a 72-aa preprohepcidin with a characteristic furin cleavage site immediately NH2 terminal to the 25-aa major hepcidin species found in plasma and urine (9, 20, 22).

Antimicrobial activity. In vitro, human hepcidin exerts antibacterial and antifungal activities (9, 20) at 10–30 μM concentrations. As is the case with many other cationic peptides, antimicrobial activity is favored by low-ionic-strength media. Urinary hepcidin concentrations are typically in the 3–30 nM range (10–100 ng/ml) and can be at least 10-fold higher during infections (13). It is thus unlikely that hepcidin can exert antimicrobial activity in urine. The concentrations of hepcidin in its other potential sites of activity, the liver and plasma, are not yet known with certainty. It is not clear whether the antimicrobial activity of hepcidin is an important biological function, a residuum of its evolutionary origin, or a consequence of the structural constraints dictated by its activity as an iron-regulatory hormone.

Hormonal activity. The involvement of hepcidin in iron metabolism was suggested by the observation that hepcidin synthesis is induced by dietary iron (22). The specific role of hepcidin was then examined by assessing the effects of its deficiency or excess in transgenic mouse models. Fortuitously, a mouse lacking hepcidin already existed (14) as a by-product of targeting a nearby gene, upstream stimulatory factor 2 (USF2), and was found to have hemochromatosis with iron deposition in the liver and pancreas and sparing of the macrophage-rich spleen. This phenotype indicated that hepcidin...
controlled intestinal iron uptake and the retention of iron in macrophages. The phenotype was not due to USF2 disruption because an independent USF2 knockout line expressed normal amounts of hepcidin mRNA and had normal iron metabolism (15). In contrast to hepcidin-deficient mice, mice that overexpressed hepcidin 1 under the control of a liver-specific promoter were born with severe iron deficiency, suggesting that hepcidin inhibited placental transport of iron (15). The mice died of iron deficiency unless supplemented with parenteral iron, indicating that hepcidin also blocked intestinal iron uptake. These observations suggested that hepcidin was a negative regulator of iron transport in the small intestine and placenta and that it induced iron retention in (mainly splenic) macrophages engaged in the recycling of iron from senescent erythrocytes. Our own experiments in mice (23) suggested that the export of iron from hepatocytes is also negatively regulated by hepcidin. The bioactive form of hepcidin is the 25-aa form, as evidenced by the ability of an injected synthetic 25-aa hepcidin (but not its 20-aa NH2-terminally truncated variant) to induce within 1 h profound and prolonged hypoferremia in mice (24). The regulatory effects of hepcidin are summarized in Fig. 3. Mice have a second hepcidin gene that encodes a peptide less similar to human hepcidin. Overexpression of hepcidin 2 in mice had no effect on iron metabolism, suggesting that it may have another function (10).

REGULATION OF HEPCIDIN SYNTHESIS

Regulation of hepcidin synthesis by iron and oxygen. Most of the iron absorbed from the diet or recycled from hemoglobin is destined for developing erythrocytes. The production of erythrocytes is physiologically increased in response to blood loss or hypoxia. It is therefore not surprising that hepcidin production is also homeostatically regulated by anemia and hypoxemia (16). When oxygen delivery is inadequate, the homeostatic response is to produce more erythrocytes. Thus hepcidin levels decrease, its inhibitory effects diminish, and more iron is made available from the diet and from the storage pool in macrophages and hepatocytes. Although the human hepcidin promoter contains several consensus binding sites for hypoxia-inducible factor, these are not typical and not conserved in other mammals, and their role if any has not yet been experimentally tested. Neither is it known how hepcidin is regulated by iron. The hepcidin gene and mRNA lack any canonical binding sites for iron-regulatory proteins, and a study of patients with hemochromatosis whose hepcidin regulation is defective (discussed in more detail later) suggest the involvement of a previously uncharacterized pathway.

Regulation of hepcidin synthesis by inflammation. Hepcidin is not only an iron-regulatory hormone but also importantly links iron metabolism to host defense and inflammation. Hepcidin synthesis is markedly induced by infection and inflammation (13, 16, 22). The cytokine IL-6 is the key inducer of hepcidin synthesis during inflammation (12) because 1) IL-6 but not IL-1α or TNF-α induce hepcidin synthesis in human hepatocytes; 2) anti-IL-6 antibodies block the induction of hepcidin mRNA in human primary hepatocytes treated with LPS or peptidoglycan; 3) anti-IL-6 antibodies block the induc-
tion of hepcidin mRNA in human hepatocyte cell lines treated with supernatants of LPS- or peptidoglycan-stimulated macrophages; 4) IL-6 knockout mice (unlike control mice) fail to induce hepcidin in response to turpentine; and 5) in human volunteers, urinary hepcidin excretion is increased an average of 7.5-fold 2 h after IL-6 infusion.

**IL-6, hepcidin, and hypoferremia of inflammation.** During inflammation induced by subcutaneous injections of turpentine, normal mice show a marked decrease in serum iron (hypoferremia) (12, 16). This response is completely ablated in hepcidin-deficient mice and IL-6-deficient mice. In humans, the hepcidin increase elicited by IL-6 infusion is accompanied by a 30% decrease in serum iron and in transferrin saturation (12). It therefore appears that the IL-6-hepcidin axis is critically important for this response and that hepcidin is the main mediator of hypoferremia of inflammation, at least acutely. However, other cytokines may also contribute to this response.

It is worthwhile to consider briefly how and why the hypoferremia develops so rapidly (within hours of the inflammatory stimulus). The plasma transferrin compartment contains about 3 mg of iron and functions as a transit compartment through which flows about 20 mg of iron each day, largely generated by recycling of senescent erythrocytes. This means that plasma iron turns over every 3–4 h. If hepcidin could completely block iron recycling, this would result in a 25% drop in plasma iron in an hour. As to the purpose of hypoferremia, it is hard to attribute to this response any role other than as an acute response directed at eliminating microbial infections.

**MECHANISM OF ACTION OF HEPCIDIN**

*Cellular iron transport.* Depending on the cell type, iron can be taken up by several distinct pathways. Bioavailable iron in the diet is mostly present either in its ferric (Fe$^{3+}$) form or as heme. The uptake of ferric iron is mediated by a combination of a ferric reductase (duodenal cytochrome b), which reduces iron to its ferrous (Fe$^{2+}$) form, and a ferrous iron transporter, divalent metal transporter 1 (DMT1), which moves iron across the cell membrane. The absorption of heme is less completely characterized. Macrophages that recycle iron from senescent erythrocytes first phagocytose erythrocytes, lyse them, and then extract the iron from hemoglobin using heme oxygenase. Other cells import iron using transferrin receptors (TfRs) that capture and endocytose diferric transferrin and then use low vacuolar pH to strip the iron from the transferrin-TfR complex. The transport of iron across vacuolar membranes of macrophages probably involves DMT1. In the cytoplasm, iron is stored bound to ferritin. Ferroportin is the sole known exporter of iron in all of these cell types, and it requires a ferroxidase (hephaestin in enterocytes and ceruloplasmin in macrophages) to deliver ferric iron to transferrin.

**Hepcidin directly regulates the expression of ferroportin on cell membranes.** A recent study (12) has indicated that 1) hepcidin directly binds to ferroportin, 2) the binding of hepcidin causes ferroportin to be internalized and degraded, and 3) the loss of ferroportin from the cell membrane ablates cellular iron export. This mechanism is sufficient to explain the regulation of iron absorption, because absorptive enterocytes only perform their function for 2 days before being shed from the tips of the villi into the intestinal lumen. Therefore, the transport of iron by ferroportin across the basolateral membrane determines whether the iron is delivered to plasma transferrin or removed from the body with shed enterocytes. When iron stores are adequate or high, the liver produces hepcidin, which circulates to the small intestine. There, hepcidin causes ferroportin to be internalized, blocking the sole pathway for the transfer of iron from enterocytes to plasma. When iron stores are low, hepcidin production is suppressed, ferroportin molecules are displayed on basolateral membranes of enterocytes, and there they transport iron from the enterocyte cytoplasm to plasma transferrin (Fig. 4). Similarly, the hepcidin-ferroportin interaction also explains how macrophage recycling of iron is regulated and accounts for the characteristic finding of iron-containing macrophages in inflammatory states characterized by high production of hepcidin. In the presence of hepcidin, ferroportin is internalized, iron export is blocked, and iron is trapped within macrophages.

The direct interaction of hepcidin with ferroportin need not be the only pathway by which ferroportin density on cell membranes is regulated. There is evidence that ferroportin mRNA levels are also regulated by iron (6, 11). In addition to direct effects on ferroportin and iron export, hepcidin would be expected to have secondary effects on cellular iron intake. These indirect effects would be activated by rising intracellular iron concentrations in enterocytes, macrophages, or hepatocytes that would, for example, suppress the synthesis of the iron-regulatory element-containing DMT1 splice variant (5, 6).

**ROLE OF HEPCIDIN IN DISEASE**

*Hereditary hemochromatosis.* Humans and other mammals lack the capacity to excrete excess iron, so iron balance is achieved almost exclusively by regulating iron uptake. Hereditary hemochromatosis (4) is a group of disorders characterized by excessive and dysregulated intestinal absorption from the diet, leading to accumulation of iron and eventually to the formation of iron deposits in the pancreas, liver, heart, and endocrine glands. Iron accumulation is caused by a defect in the synthesis or function of ferroportin or by a defect in the control of iron export. These defects can be due to mutations in the gene encoding ferroportin (4, 14) or to the production of a hepcidin analog that abolishes hepcidin function. Hepcidin is the only known regulator of iron export, and the only known mechanism for the control of iron export is the internalization of ferroportin. Therefore, hepcidin deficiency, whether due to mutations in the hepcidin gene or to hepcidin analog formation, causes iron overload and the characteristic findings of iron deposition in the liver, heart, and endocrine glands.
saturation of transferrin, ferritin, and other iron-binding proteins and the deposition of iron in vital organs. Free iron is toxic, probably due to its ability to catalyze the production of reactive oxygen products. Hemochromatosis may progress to liver failure, cardiomyopathy, destruction of endocrine glands, and damage to joints. The specific genetic defects that cause this group of disorders have been discovered over the last decade, but the understanding of how these defects lead to iron overload has been much more elusive. The most common form of hereditary hemochromatosis in populations of European origin is due to mutations in the hereditary hemochromatosis gene (*HFE*), which result in an autosomal recessive disorder of low penetrance that clinically affects predominantly older men. Mutations in *TfR2* are much rarer but cause a similar phenotype. Autosomal recessive diseases due to mutations in the hepcidin gene (*HAMP;* hepcidin antimicrobial peptide) or the hemoujuelin gene most often cause a much more severe phenotype (“juvenile hemochromatosis”), which affects young men and women equally. The autosomal dominant hemochromatosis due to mutations in the ferroportin gene differs from other hemochromatoses by causing early iron overload in Kupffer cells (liver macrophages) rather than hepatocytes, but more recent evidence suggests that some ferroportin mutations cause the classical pattern of parenchymal iron overload. Several models have been proposed to account for the role of these molecules in normal and aberrant regulation of intestinal iron absorption (21).

**Pathogenesis of hereditary hemochromatoses.** It now appears that most forms of hemochromatosis are due to hepcidin deficiency, and the autosomal dominant form is due to the dysfunction of the main target of hepcidin, the cellular iron exporter ferroportin. In acquired (nongenetic) iron overload due to frequent transfusions, urinary hepcidin excretion and, presumably, hepcidin production is appropriately increased (Ref. 13 and T. Ganz, unpublished observations). This is not the case with the common form of hereditary hemochromatosis, due to mutations in the (Human Leukocyte Antigen)-related gene *HFE*. This form of hemochromatosis affects older adults and has low clinical penetrance. Several studies (1, 7, 13) have indicated that hepcidin is inappropriately low in iron-overloaded patients with this disorder. Moreover, in the mouse model of *HFE* hemochromatosis, correction of the defect is achieved by overexpression of hepcidin 1 (17). This would suggest that the *HFE* gene is required for normal regulation of hepcidin synthesis and that the deleterious effects of *HFE* mutations are caused by hepcidin deficiency. This notion is reinforced by two gene defects that lead to juvenile hemochromatosis, a more severe form of the disease. One set of defects involves the hepcidin gene itself (25), and the other affects a newly discovered gene, hemojuvelin (18). In patients with either of these defects, urinary hepcidin is absent or nearly absent. Hepcidin is also deficient in patients with homozygous *TfR2* mutations but is elevated in some patients with autosomal dominant ferroportin mutations (19). Genetic lesions in ferroportin that decrease its ability to export iron would be expected to cause iron overload of macrophages, predominantly those recycling large amounts of iron, accompanied by mild anemia, whereas mutations that cause hyporesponsiveness of duodenal ferroportin to hepcidin should cause hepatocyte and other parenchymal iron overload. Early studies (2, 3) have confirmed that this is indeed the case.

**Anemia of inflammation.** AI is a common consequence of chronic infections including human immunodeficiency virus, tuberculosis, bacterial endocarditis, and osteomyelitis, but AI can also develop within days during sepsis. AI is also seen in noninfectious generalized inflammatory disorders including rheumatological diseases, inflammatory bowel disease, multiple myeloma, and other malignancies. These anemias are characterized by decreased iron and iron-binding capacity (transferrin), increased ferritin, and the presence of iron in bone marrow macrophages, indicating impaired mobilization of iron from stores. The link among infections, hypoferremia, and AI suggests that hypoferremia and AI are a part of the host defense response to infection. The induction of hepcidin by IL-6 and other inflammatory cytokines and the resulting limitation of iron supply to the bone marrow is a major contributor to the pathogenesis of AI. Because most of the iron in the transferrin compartment is destined for the bone marrow, hypoferremia resulting from excess hepcidin diminishes the amount of iron available for hemoglobin synthesis and erythrocyte production. Indeed, clinical and experimental situations in which hepcidin is overproduced are commonly associated with anemia. In addition to transgenic mice that overproduce hepcidin 1 and suffer from lethal anemia (15), severe anemia is also seen in rare patients with liver tumors that autonomously produce hepcidin (26). Patients with hypoferremia and anemia due to infections or inflammatory disorders have increased urinary hepcidin excretion (13). It is interesting to note that anemia is commonly associated with experimental IL-6 therapy as well as diseases associated with excess IL-6 such as Castleman’s syndrome, multiple myeloma, and juvenile rheumatoid arthritis. On the basis of these observations, we and others have proposed that the pathogenic (as well as host defense) cascade that produces AI leads from IL-6 to hepcidin to hypoferremia and then to AI.

In summary, hepcidin is the long-anticipated hormone responsible for the regulation of iron recycling and iron balance. It may have evolved from an antimicrobial peptide such as those expressed in the fat body (liver) of insects. Like the synthesis of insect antimicrobial peptides, hepcidin synthesis is induced by infection and inflammation. Specifically, IL-6, produced early during host defense, induces hepcidin production, which then inhibits iron recycling by macrophages, leading rapidly to hypoferremia. The contribution of hypoferremia in defense against microbes is not well understood and almost certainly varies depending on the capability of the invading microbial species to obtain iron in infected tissues. Hepcidin excess may be the key pathological feature of AI, and hepcidin deficiency may be responsible for most cases of familial hemochromatosis. The development of pharmacological hepcidin agonists and antagonists should be useful in the treatment of these conditions.

**REFERENCES**


