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Hepcidin in β-thalassemia

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Abstract

Iron overload is the principal cause of morbidity and mortality in β-thalassemia with or without transfusion dependence. Iron homeostasis is regulated by the hepatic peptide hormone hepcidin. Hepcidin controls dietary iron absorption, plasma iron concentrations, and tissue iron distribution. Hepcidin deficiency is the main or contributing factor of iron overload in iron-loading anemias such as β-thalassemia. Hepcidin deficiency results from a strong suppressive effect of the high erythropoietic activity on hepcidin expression. Although in thalassemia major patients iron absorption contributes less to the total iron load than transfusions, in non-transfused thalassemia, low hepcidin and the consequent hyperabsorption of dietary iron is the major cause of systemic iron overload. Hepcidin diagnostics and future therapeutic agonists may help in management of patients with β-thalassemia.

Keywords

hepcidin; β-thalassemia; iron overload

Hepcidin regulates iron homeostasis

Hepcidin is a small peptide hormone secreted by hepatocytes, circulating in blood plasma and excreted in urine1. Hepcidin regulates iron concentration in the plasma and the distribution of iron among different tissues. Dysregulation of hepcidin production underlies many iron disorders. Chronic excess of hepcidin causes iron-restricted anemia2 whereas hepcidin deficiency results in iron overload with iron deposition in the liver and other parenchyma3.

Hepcidin acts by regulating the cellular concentration of its receptor, ferroportin. Ferroportin is the sole known cellular iron exporter4 and it exports iron into plasma from duodenal enterocytes which absorb dietary iron, macrophages of the spleen and liver which recycle iron from old erythrocytes, and hepatocytes which release stored iron according to body needs5–7. Hepcidin binding to ferroportin triggers internalization and degradation of the receptor8. Removal of ferroportin from the membrane stops cellular iron export leading to decreased supply of iron into plasma. Conversely, when hepcidin concentration is low, ferroportin remains on the cell surface, leading to increased iron absorption and export from macrophages. If uncontrolled, increased iron absorption eventually causes iron overload.

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Conflicts of Interest: Dr. Elizabeta Nemeth is a co-founder and the Chief Scientific Officer of Intrinsic LifeSciences, LLC, a biotech company developing iron-related diagnostics.

Regulation of hepcidin

Hepcidin is homeostatically regulated by iron and erythropoietic activity, but similar pathways are involved in hepcidin dysregulation in thalassemia.

Increased plasma and stored iron stimulates hepcidin production, which in turn blocks dietary iron absorption and further iron loading. Conversely, hepcidin is suppressed in iron deficiency9, allowing increased absorption of dietary iron and replenishment of iron stores. Hepcidin appears to be regulated by both plasma iron-transferrin and intracellular iron stores, and these signals likely utilize the bone morphogenetic protein (BMP) pathway to alter hepcidin expression. Although several BMPs can increase hepcidin production in vitro and in vivo10, BMP6 has recently emerged as the principal endogenous BMP that regulates hepcidin. BMP6 knockout mice develop severe iron overload but no other significant abnormalities11،12. BMP signaling to hepcidin is modulated by hemojuvelin (HJV), a co-receptor of the BMP pathway13. HJV mutations in humans or mice result in severe iron overload similar to that caused by hepcidin mutations14. It is possible that another HJV-interacting protein, neogenin, participates in hepcidin regulation by iron15 because neogenin-deficient mice also displayed low hepcidin expression and developed liver iron overload16.

Hepcidin regulation by extracellular iron is thought to depend on sensing of iron-transferrin concentrations by transferrin receptor 2 (TfR2) and HFE. Mutations of TfR2 and HFE lead to hepcidin deficiency and the adult form of hereditary hemochromatosis17،18. Increase in iron-transferrin concentration appears to promote HFE/TfR2 interaction19،20 and likely potentiate BMP pathway signaling. The mechanism by which intracellular iron regulates hepcidin expression is less understood. HFE and TfR2 do not appear to be required for hepcidin regulation by iron stores, as mice and humans with HFE and TfR2 mutations are still capable of decreasing hepcidin levels after iron depletion18. Expression of BMP6 mRNA was recently shown to increase with iron loading in mice, raising the possibility that BMP6 is a signal reflecting iron stores21.

As would be expected for the iron-regulatory hormone, hepcidin production is also regulated by the process which consumes the most iron, erythropoiesis22. Increased erythropoietic activity suppresses hepcidin production which allows the release of stored iron from macrophages and hepatocytes, and increased absorption of dietary iron, all resulting in greater supply of iron for hemoglobin synthesis. How erythropoiesis affects hepcidin production is not clear. Although injection of erythropoietin in humans and mice decreased hepcidin expression23،24, Epo by itself does not appear to be a direct regulator of hepcidin because pretreatment of mice with carboplatin, a cytotoxic inhibitor of erythropoiesis, completely abrogated the effect of Epo on hepcidin22. Similarly, mouse models of anemias caused by bleeding or hemolysis showed that hepcidin suppression depended on intact erythropoietic activity22،25. Erythropoietin administration in mice was found to reduce Smad signaling24 suggesting that erythroid activity may affect hepcidin expression by modulating the BMP pathway.

The mediators of hepcidin suppression may include the production of soluble factors by the erythroid precursors in the bone marrow, decreased circulating or stored iron, and hypoxia. Two proteins produced by erythroid precursors, growth differentiation factor 15 (GDF15) and twisted gastrulation protein (TWSG1), have been proposed to mediate hepcidin suppression in anemias with ineffective erythropoiesis26،27. GDF15, a member of the TGF-β superfamily, and TWSG1, a BMP-binding protein, are both produced by developing erythroblasts and were shown to suppress hepcidin mRNA in vitro26،27. Very high levels of GDF15 were detected in β-thalassemia and congenital dyserythropoietic anemia type I, and

elevated Twsg1 expression was found in a mouse model of thalassemia. However, whether these factors are the main suppressors of hepcidin in β-thalassemia remains to be determined. Furthermore, there is no evidence that these factors mediate physiologic hepcidin suppression in response to bleeding or to anemias with effective erythropoiesis.

HIF pathway was proposed as another regulator of hepcidin in anemias, and in vivo alterations of this pathway can affect hepcidin expression28. Hypoxia may exert its effect on hepcidin by increasing erythropoietin production in the kidney, and subsequent proliferation of erythroblasts in the bone marrow, but whether hypoxia can suppress hepcidin directly is still unresolved28,29.

Hepcidin production is also pathologically increased in inflammation and infection9,30–33. The resulting hypoferremia may represent a host defense strategy to limit iron availability to microorganisms, but can also lead to iron dysregulation and iron-restricted anemia in inflammatory diseases. Of inflammatory mediators regulating hepcidin, IL-6 was shown to be a prominent inducer in vitro and in vivo, but other cytokines such as IL-1 may also contribute.

The role of hepcidin in β-thalassemia

In iron-loading anemias not treated by frequent transfusions, such as β-thalassemia intermedia and congenital dyserythropoietic anemias, urinary and serum hepcidin are severely decreased34–36. Hepcidin deficiency in turn allows excessive iron absorption and development of systemic iron overload. The liver is most commonly affected by iron overload due to the avid uptake of non-transferrin bound iron by hepatocytes. Iron overload of other organs appears to correlate broadly with the rate of iron absorption. Rapid accumulation of iron, such as seen with severe hepcidin deficiency (juvenile hemochromatosis, thalassemia intermedia) is associated with prominent deposition of iron in the heart and some endocrine organs.

In β-thalassemia major, transfusions rather than dietary iron absorption are the predominant cause of iron overload. In chronically transfused patients, hepcidin concentrations are significantly higher than in nontransfused patients, presumably due to both increased iron load and the alleviation of ineffective erythropoiesis34,36. However, hepcidin concentrations decrease in the intervals between transfusions, as the effect of each transfusion wears off34–36. During those periods, decreased hepcidin and the resulting increase in intestinal iron absorption may be significant contributors to patients' iron load.

Different levels of hepcidin in thalassemia intermedia and major result in different cellular distribution of iron. Origa et al36 showed that nontransfused patients have liver iron concentrations similar to those of regularly transfused thalassemia patients. However, in nontransfused thalassemia, iron was deposited in hepatocytes, whereas higher hepcidin levels in transfused patients resulted in macrophage iron loading. As a consequence of this difference in cellular iron distribution, serum ferritin levels were much lower in nontransfused patients, and did not adequately reflect the patients' liver iron load. The specific pattern of cellular iron deposition may be important because different cell types exhibit differential tolerance to iron-mediated toxicity37.

Hepcidin diagnostics and therapeutics in β-thalassemia

Hepcidin measurements have only recently become possible with the development of assays for bioactive mature hepcidin in serum and urine. The methodologies include competitive ELISAs9,38, and mass spectrometry-based assays (MALDI-TOF MS, SELDI, and LC MS/MS)39–41. Determining hepcidin concentrations in patients with iron-loading anemias may

be useful to identify the patients at higher risk of iron toxicity due to severely decreased hepcidin levels. However, the utility of hepcidin for the management of iron overload in β-thalassemia is not known and needs to be evaluated in larger clinical studies.

Continuous or nearly continuous chelation is the major therapeutic modality for β-thalassemia, either with parenteral agents and/or with oral chelators. However, compliance with chelation therapy is often inadequate and iron overload remains a major cause of morbidity and mortality. Hepcidin-targeted therapeutics may help improve treatment of iron overload in β-thalassemia. In thalassemia intermedia, hepcidin therapy would be expected to have beneficial effects by curbing hyperabsorption of dietary iron. It is possible that hepcidin agonists could be useful even in transfused thalassemia patients. Although intestinal iron absorption contributes less to the total iron load in these patients, hepcidin therapy may help in the intervals when endogenous hepcidin falls and intestinal iron uptake increases. Furthermore, considering that high hepcidin shifts iron distribution to macrophages where it is less toxic, pharmacologic doses of hepcidin may postpone the iron deposition and consequent damage in the parenchyma.

The first indication of the beneficial effect of hepcidin in β-thalassemia came from the group of Dr. Stefano Rivella (Weill Medical College of Cornell University, New York). They used the t*h3*/+ mouse model of beta-thalassemia, which exhibits features comparable to those of patients affected by beta-thalassemia intermedia. Th3/+ hematopoietic stem cells were transplanted either into transgenic mice expressing hepcidin or into wild-type mice. After several months, mice with moderate overexpression of hepcidin had lower spleen and liver iron content, decreased inefficient erythropoiesis in the spleen, lower spleen weight and even improvement of hematological parameters (Gardenghi et al, submitted). Thus transgenic hepcidin overexpression in β-thalassemia intermedia mice not only decreased iron overload but also ameliorated ineffective erythropoiesis and anemia.

Although these proof-of-principle studies show promise, no hepcidin therapies are yet available. Hepcidin has several characteristics that limit its potential use as a therapeutic. Because of its length and complex 4-disulfide structure it is very expensive to produce. Its relatively large size and susceptibility to inactivating proteolytic degradation at the N-terminus preclude efficient oral absorption. Rapid clearance of hepcidin through renal excretion may limit its efficacy *in vivo*. We have undertaken structure-function analysis of the hepcidin-ferroportin interaction and based on those findings developed small (7–9 amino acid) peptide agonists that mimic the activity of full-length hepcidin (E. Nemeth, unpublished). In preliminary experiments using wild-type mice, we showed that minihepcidin acted as agonists in vivo. The small size should allow eventual development of orally available agonists.

Future preclinical and clinical studies will be necessary to assess the risks and relative benefits of hepcidin-targeted treatment approaches.

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