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Hepcidin agonists as therapeutic tools

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Hepcidin agonists are a new class of compounds that regulate blood iron levels, limit iron absorption, and could improve the treatment of hemochromatosis, β-thalassemia, polycythemia vera, and other disorders in which disrupted iron homeostasis causes or contributes to disease. Hepcidin agonists also have the potential to prevent severe complications of siderophilic infections in patients with iron overload or chronic liver disease. This review highlights the preclinical studies that support the development of hepcidin agonists for the treatment of these disorders. (*Blood*. 2018;131(16):1790-1794)

Iron metabolism and the importance of its regulation

Iron is essential for the development and growth of nearly all living organisms, from bacteria to humans.[1] It plays a role in many vital cellular and organismal functions, from cell division to oxygen transport.[2] As iron is relatively scarce in forms that can be used for biological activities, it is not surprising that organisms developed sophisticated mechanisms for iron acquisition, recycling, and efficient tissue distribution. Although essential, iron in excess can promote the formation of highly toxic reactive oxygen species (ROS), which can damage DNA, protein, and lipid membrane, leading to organ dysfunction.[1-3] Therefore, humans and other vertebrates have evolved regulatory systems to optimize the absorption and organ distribution of iron. These regulatory mechanisms are also used to limit iron availability to invading microbes during infection, as microbes compete with the host for this essential nutrient. However, several genetic or acquired disorders of iron homeostasis dysregulate iron absorption or distribution, causing organ damage and creating conditions for overwhelming infections with associated morbidity and mortality.

Hepcidin: the key factor in iron metabolism

The master regulator of iron metabolism is the 25-aa peptide hormone hepcidin (hepcidin antimicrobial peptide [HAMP]), mainly produced by the liver in proportion to plasma iron concentration and iron stores.[4-7] Hepcidin inhibits the activity of the only known cellular iron exporter, ferroportin-1 (FPN-1), which is expressed on the surfaces of cells that are involved in iron absorption, recycling, and storage.[8,9] The feedback circuitry between hepcidin and iron levels in the body ensures systemic iron homeostasis. Hepcidin is also increased during infection and inflammation, causing reduced intestinal iron absorption and increased iron retention in macrophages.[10-12] This is essential to protect the organism from infection with siderophilic and potentially other gram-negative bacteria, which can grow rapidly in the presence of excess iron.[12-14] In contrast, hepcidin is suppressed during increased erythropoiesis, when more iron is needed to support increased red blood cell (RBC) production.[15-18]

When hepcidin production is chronically decreased, iron absorption and release from body stores are excessive and tissue iron overload occurs.[6,18] Hepcidin deficiency is a common feature of hereditary hemochromatosis (HH) and anemias with ineffective erythropoiesis, including thalassemias and low-risk myelodysplastic disorders.[19-22] In HH, mutations in genes such as *HFE*, *TFR2*, *HJV*, or *HAMP*, which encode proteins in iron-regulatory pathways, lead to reduced hepcidin synthesis and to a phenotype characterized by iron overload from increased intestinal iron absorption and excessive release of iron from macrophages.[22] In β-thalassemia, hepcidin deficiency results from chronically elevated production of 1 or more erythroid suppressors of hepcidin.[23-28] Mutations in the β-globin gene or its regulatory elements cause reduced or absent β-globin synthesis, resulting in the formation of globin tetramers with an excess of α-chains that precipitate in erythroid progenitors causing their premature death.[29,30] This, together with the shorter lifespan of RBCs, results in profound anemia that leads to increased erythropoietin production and consequent expansion of the number of erythroblasts, not only in the bone marrow but also at extramedullary sites, causing hepatosplenomegaly.[19,31] Large numbers of erythroblasts are stimulated by high erythropoietin levels to produce excessive amounts of the erythroid factor erythroferrone, which downregulates hepcidin, leading to increased intestinal iron absorption.[27]

Utilizing hepcidin pathway to treat iron overload disorders

Initially, studies in mouse models of HH (*Hfe*$^{-/-}$) or β-thalassemia intermedia (*Hbb*$^{th1/th1}$ and *Hbb*$^{th3/+}$) demonstrated that transgenic overexpression of hepcidin could prevent iron overload and improve erythropoiesis.[32,33] Similarly, genetic disruption of the hepcidin regulator *Tmprss6* in a β-thalassemia mouse model enhanced signaling by the bone morphogenetic protein (BMP)/small mothers against decapentaplegic (SMAD) pathway and

Table 1. Classification of hepcidin agonists

Hepcidin agonists	Company	Drug	Target	Clinical trials	Reference
Class 1: hepcidin mimetics	University of California, Los Angeles	MHs (PR65, PR73, M009, M012)	Ferroportin	Validated in preclinical studies	See articles cited in the review
	La Jolla Pharmaceutical Company	LJPC-401 (hepcidin formulation)	Ferroportin	Phase 1: no toxicity reported; expected hypoferremia observed	45
	Protagonist Therapeutics	PTG-300	Ferroportin	Phase 1: no serious adverse events reported; expected hypoferremia observed	58
Class 2: stimulators of hepcidin production	Ionis Pharmaceuticals	Tmprss6-ASO	Tmprss6	Phase 1 ongoing	59
	Alnylam Pharmaceuticals	Tmprss6-siRNA	Tmprss6	Validated in preclinical studies	See articles cited in the review
Class 3: ferroportin inhibitors	Vifor Pharma	VIT-2763	Ferroportin	Phase 1 planned in 2018	60

ASO, antisense oligonucleotide; siRNA, small-interfering RNA.

increased hepcidin levels with consequent prevention of iron overload and improved erythropoiesis.[34,35] These genetic studies prompted the exploration of other approaches to increasing circulating hepcidin in iron-overloaded patients with hepcidin deficiency. Synthesis of full-length hepcidin is relatively inefficient, and the half-life of hepcidin in circulation is short because of rapid renal clearance.[36] To overcome these limitations, several molecules have been designed to mimic hepcidin activity or stimulate endogenous hepcidin production.[2,37] We developed minihepcidins (MHs), short peptides based on the 7-9 N-terminal amino acid segment of hepcidin. We first showed that this N-terminal segment of hepcidin is sufficient to induce FPN-1 internalization and degradation in vitro.[38] We then engineered these peptides to increase their half-life and potency, and demonstrated that administration of MHs to mice mimics the iron-restrictive effect of endogenous hepcidin.

Studies in a severe mouse model of HH (*Hamp*$^{-/-}$) showed that the use of MHs could prevent or limit liver iron accumulation and reduce iron level in the heart, while increasing iron sequestration in splenic macrophages.[39] Subsequent studies in a mouse model of β-thalassemia intermedia showed that the iron-restrictive effect of MHs improved anemia, iron overload, ineffective erythropoiesis, and splenomegaly.[40] Treated animals also showed significant reduction in hemichrome formation and ROS as well as improved lifespan of circulating RBCs.[40] However, this study also demonstrated that high doses of MHs could cause anemia from excessive iron restriction, highlighting the need to titrate hepcidin agonists to the desired effect.[40]

Utilizing hepcidin agonists to treat siderophilic infections

MHs were also highly effective in preventing mortality from infections with siderophilic pathogens in mouse models. Patients with iron-overload disorders are known to be susceptible to severe and lethal infections with siderophilic bacteria such as gram-negative *Vibrio vulnificus* and *Yersinia enterocolitica*.[41,42] Mouse models of hepcidin deficiency (*Hamp*$^{-/-}$) reproduced the susceptibility to lethal infection not only with *V vulnificus* and *Y enterocolitica*, but also with another gram-negative pathogen, *Klebsiella pneumoniae*.[12-14,43] Even wild-type mice after administration of parenteral iron showed greater infection burden with *Y enterocolitica* and *K pneumoniae*.[12,14] In mouse models, the pathogenicity of these gram-negative bacteria was dependent on extracellular iron concentrations, specifically on the availability of nontransferrin-bound iron (NTBI). NTBI appears in circulation when transferrin saturation exceeds ~70%, and stimulates extremely rapid proliferation of these bacteria.[14] Administration of MHs to mice rapidly lowered the concentration of extracellular iron, and prevented formation of NTBI. As a result, MHs treatment prevented or reduced mortality from *V vulnificus*, *Y enterocolitica*, and *K pneumoniae* in *Hamp*$^{-/-}$ mice, and decreased abscess formation in iron-loaded wild-type mice infected with *Y enterocolitica*.[12-14] Based on these studies in mouse models, patients with siderophilic infections in the setting of iron-overload disorders and chronic liver disease associated with high transferrin saturation and the presence of NTBI could benefit from treatment with hepcidin agonists.[44]

Multiple other strategies have been developed to mimic hepcidin activity or increase production of endogenous hepcidin. These include synthetic full-length human hepcidin,[45] another peptide-based hepcidin mimetic,[46] small molecule FPN-1 inhibitors,[47] or pharmacologic inhibition of *Tmprss6* by in vivo targeting and degradation of *Tmprss6* messenger RNA,[48,49] all displaying similar beneficial effects in preclinical models of hepcidin deficiency. *Tmprss6* antisense oligonucleotide or small-interfering RNA approaches were used in mouse models of β-thalassemia intermedia and hereditary hemochromatosis (*Hfe*$^{-/-}$).[48,49] Successful silencing of *Tmprss6* led to increased hepcidin and reduced serum and liver iron concentration in both mouse models. In particular, in β-thalassemia models, increased hepcidin concentration levels were associated with improved anemia, ineffective erythropoiesis (with improved RBC maturation), and reduced splenomegaly. Treated mice also showed reduction in hemichrome formation, apoptosis, and ROS as well as improved RBC life span.[48,49] Additional studies using MH or *Tmprss6* inhibitors in combination with the iron chelator deferiprone demonstrated a

more powerful effect of the combined therapy on iron overload and ineffective erythropoiesis than the single agents alone.[40,50,51]

Hepcidin agonists could also be beneficial for the management of β-thalassemia major (TM). In patients affected by TM, the major cause of iron overload is the iron burden of repeated blood transfusions.[19,52] Although transfusion therapy transiently increases hepcidin levels, hepcidin decreases toward the end of the transfusion cycle when hemoglobin levels decrease.[53] Therefore, increased intestinal iron absorption may also contribute a small but potentially important component of iron overload. In addition, low or relatively low levels of hepcidin in this condition could lead to the generation of NTBI and labile plasma iron, which trigger oxidative stress and tissue injury in many organs.[44] Therefore, in these disorders, the use of hepcidin agonists could help limit or prevent intestinal iron intake when the synthesis of endogenous hepcidin is not sufficient, and prevent formation of labile plasma iron by sequestering iron in macrophages. Furthermore, the restrictive effect of iron deficiency on ineffective erythropoiesis could also decrease the number of erythroid progenitors and splenomegaly. A smaller spleen should, in turn, reduce the rate of destruction of transfused RBC and reduce the transfusion frequency. To test these hypotheses, we are now conducting a series of studies in a new mouse model of TM.

Another disease in which iron restriction might be beneficial is polycythemia vera (PV).[40] Most of the clinical characteristics of PV are triggered by the excessive number of erythrocytes, leading to an increased risk of pulmonary hypertension and thrombosis.[54] The principal therapy for PV is phlebotomy to reduce the hemoglobin concentration and reduce the risk of thrombosis.[54] Because phlebotomy does not suppress the production of RBCs in the bone marrow, the effect of each phlebotomy is transient, until patients become iron deficient. As an alternative, systemic iron restriction induced by administration of MHs in a mouse model of PV limited iron supply to erythropoiesis and thereby reduced hemoglobin concentrations and decreased splenomegaly.[40] All of these studies also indicate that overadministration of hepcidin agonists can cause suppression of intestinal iron uptake and macrophage iron recycling, with potential exacerbation of anemia, as in β-thalassemia, or suboptimal production of RBCs, as in PV. These observations stress the notion that careful titration of these compounds is needed to fully benefit from their therapeutic properties, while avoiding undesirable side effects.

Nevertheless, based on the positive results from preclinical studies, several hepcidin agonists are being tested in clinical trials (Table 1).

Conclusions and future directions

Hepcidin agonists have been developed to control iron absorption and ameliorate iron overload in multiple iron disorders, but their therapeutic efficacy alone or in combination with existing therapies remains to be tested in clinical trials.

In patients with hereditary hemochromatosis, hepcidin agonists may become useful in conjunction with phlebotomy during the treatment phase, or as a stand-alone treatment during the maintenance phase. Furthermore, hepcidin agonists may effectively treat severe siderophilic infections affecting patients with iron overload or chronic liver disease.

Based on preclinical studies, hepcidin agonists could improve not only iron overload but also the anemia in patients with β-thalassemia intermedia. Future studies will explore whether hepcidin agonists could also ameliorate splenomegaly and decrease the need for blood transfusion in individuals affected by β-thalassemia major. Because of their distinctive effect on iron metabolism, hepcidin agonists may also complement other novel treatments that increase RBC production, such as agents that target ligands of the transforming growth factor-β family.[55-57]

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