Emerging Role Of Hepcidin In Pediatric Heart Failure

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Article History: Received 10th June, Accepted 5th July, published online 10th July 2023

Abstract

Background: Hepcidin (hepatic bactericidal protein) was initially identified as a urinary antimicrobial peptide rich in cysteine. Further studies showed that hepcidin is overexpressed with iron overload and that it plays a significant role in iron homeostasis in knockout animals with iron storage disease. The complete abrogation of hepcidin entails excessive intestinal absorption of iron and increased iron release by macrophages, a condition that leads to iron overload. Hepcidin performs its different functions via a single biochemical mechanism: hepcidin-ferroportin interaction. Intestinal epithelial cells and reticuloendothelial macrophages use the same transporter, ferroportin, to transport iron in the plasma. Moreover, macrophages and enterocytes exhibit strong upregulated ferroportin expression in the erythropoietic response in an iron-restricted state. Specific methods were recently developed to measure hepcidin and evaluate its diagnostic potential. The competitive ELISA test uses biotinylated or radioiodinated hepcidin as a tracer. However, mass spectrometry is used for the hepcidin assay; this involves standards labeled with hepcidin isotopically or standards that bind the hepcidin-truncated molecule. Understanding the physiological processes of hepcidin has made it possible to redefine the pathogenetic mechanisms of anemia. There are several physiopathological mechanisms potentially involved in the genesis of anemia associated with heart failure. These include hemodilution, anemia of chronic disease, decreased renal blood flow and iron deficiency. It has also been recognized that iron metabolism may be governed by mechanisms beyond absolute iron store quantities. Hepcidin production can be induced by inflammation, which explains the reduced availability of iron in anemia of chronic disease, whereas anemia and hypoxia have been shown to increase iron absorption and mobilization by decreasing hepcidin production.

Keywords: Hepcidin, iron deficiency anemia, pediatric heart failure

Introduction

Hepcidin is the central regulator of systemic iron homeostasis. Dysregulation of hepcidin production results in a variety of iron disorders. Hepcidin deficiency is the cause of iron overload in hereditary hemochromatosis, iron-loading anemias, and hepatitis C. Hepcidin excess is associated with anemia of inflammation, chronic kidney disease and iron-refractory iron deficiency anemia. Diagnostic and therapeutic applications of this new knowledge are beginning to emerge ***(1).***

Hepcidin synthesis and catabolism:

The hormone hepcidin, a 25-amino-acid (aa) peptide, is the principal regulator of iron absorption and its distribution to tissues. Hepcidin is synthesized predominantly in hepatocytes, but its low levels of expression in other cells and tissues, including macrophages, adipocytes and brain, may also be important for the autocrine and paracrine control of iron fluxes at the local level ***(1).***

Hepcidin is encoded as an 84-aa prepropeptide, containing an N-terminal 24-aa endoplasmic reticulum-targeting signal sequence. The 60-aa prohormone contains a consensus furin cleavage motif, and several proprotein convertases were reported to process hepcidin in vitro including furin, PACE4, PC5/6 and PC7/LPC. The processing step occurs in the Golgi apparatus, does not appear to be regulated, and only the mature peptide, but not the prohepcidin, was shown to be secreted from cells ***(2).***

The mature hormone circulates in plasma and its binding to α2-macroglobulin has been reported. While this interaction was shown to promote hepcidin activity in vitro, the effect on hepcidin clearance is still unknown. A major route of hepcidin clearance is renal excretion. When kidney function is normal, urinary hepcidin concentrations correlate well with circulating hepcidin levels, with no apparent regulation of the excretion process ***(2).***

However, based on the comparison between serum and urinary concentrations, it appears that only 5% of hepcidin from plasma filtered in the kidneys ends up intact in the urine, suggesting that hepcidin may not be freely filtered in the glomerulus and/or that filtered hepcidin is reabsorbed and degraded in proximal tubules similarly to other small peptide hormones. Hepcidin may also be cleared by receptor-mediated endocytosis in tissues expressing its receptor ferroportin, as indicated by the accumulation of radiolabeled hepcidin in ferroportin-rich tissues and the degradation of the endocytosed ferroportin-hepcidin complex in cultured cells. How much hepcidin catabolism occurs by renal clearance or by degradation in target tissues remains to be determined ***(3).***

Hepcidin Structure:

Structurally, the hepcidin peptide resembles a bent hairpin held together by four disulfide bonds. The disulfide connectivity was recently revised. NMR spectroscopy, partial reductive alkylation and Fourier transform mass spectroscopy were used to resolve ambiguities arising from the proximity of the four disulfides. The new model indicates that two bonds stabilize the antiparallel β-sheet, and two tether the bent conformation of the peptide ***(4).***

Recent data indicate that hepcidin binding to its receptor requires the involvement of one of the disulfide bonds. However, considering that removal of individual bonds does not dramatically decrease hepcidin activity in vitro, multiple disulfide bonds must be capable of forming a contact with ferroportin ***(5).***

Apart from the disulfide bonds, structure-function studies also revealed that the N-terminus of hepcidin is important for its iron-regulatory activity. The N-terminally truncated human 20-aa peptide was inactive both in vitro and in vivo indicating that this region may also contain contact residues for hepcidin interaction with its receptor ***(1).***

The amphipathic structure of hepcidin and its extensive disulfide bonding are common characteristics of antimicrobial and antifungal peptides. However, hepcidin has only displayed modest antimicrobial properties in vitro at very high concentrations (10–30 μM), and the significance of its antimicrobial properties in vivo remains to be determined ***(6).***

Patients with hereditary hemochromatosis, a disease generally resulting from relative hepcidin deficiency, are reported to develop infections caused by unusual microorganisms (Vibrio, Yersinia and Listeria), but this susceptibility could be related to the bacteria benefitting from increased iron levels rather than from the loss of any direct antibacterial effect of hepcidin ***(7).***

Mechanism of Hepcidin action:

Hepcidin acts by modulating cellular iron export through ferroportin to plasma and extracellular fluid. Ferroportin is both the hepcidin receptor and the only known cellular iron exporter. Ferroportin is expressed on cells that act as professional iron handlers in the body: duodenal enterocytes absorbing dietary iron, macrophages in liver and spleen recycling old erythrocytes, hepatocytes storing iron and placental trophoblasts transferring iron to the fetus during pregnancy ***(8).***

Ferroportin is also expressed in erythroid precursor cells, and it has been proposed that its presence enhances the sensitivity of precursors to systemic iron levels and helps determine their commitment to expansion and differentiation. The complete loss of ferroportin expression was shown to be embryonic lethal due to the inability of embryonic trophoblasts to transfer iron from the mother to the embryo ***(2).***

Posttranslational control of ferroportin levels by its ligand hepcidin is the major mode of ferroportin regulation. The binding of hepcidin to ferroportin triggers the internalization and degradation of the receptor-ligand complex. The binding likely involves disulfide exchange between one of disulfide bonds of hepcidin and the exofacial ferroportin thiol residue Cys326 ***(4).***

Hepcidin (hepatic bactericidal protein) was initially identified as a urinary antimicrobial peptide rich in cysteine. Further studies showed that hepcidin is overexpressed with iron overload and that it plays a significant role in iron homeostasis in knockout animals with iron storage disease. The complete abrogation of hepcidin entails excessive intestinal absorption of iron and increased iron release by macrophages, a condition that leads to iron overload ***(7).***

Hepcidin performs its different functions via a single biochemical mechanism: hepcidin-ferroportin interaction. Intestinal epithelial cells and reticuloendothelial macrophages use the same transporter, ferroportin, to transport iron in the plasma. Moreover, macrophages and enterocytes exhibit strong upregulated ferroportin expression in the erythropoietic response in an iron-restricted state ***(9).***

Iron-regulatory activity of Hepcidin:

Hepcidin is the main regulator of plasma iron concentrations. Injection of hepcidin resulted in a dramatic drop in serum iron within just 1 h. Even though hepcidin is rapidly cleared from the plasma, the effect of a single dose was apparent for up to 72 h, likely because of the time required to resynthesize sufficient amounts of the hepcidin receptor, ferroportin ***(1).***

Chronic overexpression of hepcidin causes iron-restricted anemia, typically manifested as microcytic, hypochromic anemia. Conversely, hepcidin deficiency results in iron overload with iron deposition in the liver and other parenchyma, and sparing of the macrophage-rich spleen. Complete absence of hepcidin in humans causes juvenile hemochromatosis, the most severe form of hereditary hemochromatosis ***(8).***

The phenotypes of hepcidin excess and deficiency indicate that hepcidin inhibits intestinal iron uptake and the release of iron from macrophages recycling old red blood cells. When hepcidin was overexpressed during embryonic development, fetuses developed severe iron deficiency anemia and most died at birth indicating that hepcidin also inhibited the placental transport of iron ***(2).***

Hepcidin derived from extrahepatic sources may also exert control over local iron fluxes within tissues in which hepcidin is produced. For example, the central nervous system is separated from the plasma by the blood-brain barrier, and circulating hepcidin may not be transported across this barrier. However, brain tissue itself was reported to express hepcidin, allowing the possibility of iron regulation independent of the systemic control ***(9).***

Hepcidin measurement and related problems:

Specific methods were recently developed to measure hepcidin and evaluate its diagnostic potential. The competitive ELISA test uses biotinylated or radioiodinated hepcidin as a tracer. However, mass spectrometry is used for the hepcidin assay; this involves standards labeled with hepcidin isotopically or standards that bind the hepcidin-truncated molecule ***(3).***

The use of different methods for the determination of hepcidin levels mainly depends on the employment of different hepcidin standards as well as the varying abilities of different methods to detect the hepcidin-20 and hepcidin-22 isoforms in addition to the hepcidin-25 bioactive isoform ***(10).***

Despite the varying abilities of the methods mentioned above, there is no difference between the samples and analytical methods used. This indicates that all methods are appropriate for detecting hepcidin isoforms in samples ***(4).***

Many studies concluded that the differences in the levels of hepcidin isoforms between methods could be due to the use of different calibrators with assigned levels obtained by different techniques, hepcidin aggregation of either the standard solution or sample, binding of hepcidin to α2-macroglobulin or albumin, or existence of 3 hepcidin isoforms (hepcidin-25, hepcidin-22, and hepcidin-20) ***(11).***

Since approximately 90% of circulating hepcidin is linked to α2-macroglobulin, it is important to consider whether it is necessary to measure total or unbound hepcidin and which current valuation methods can be used. The difference between immunochemical (IC) methods and mass spectrometry is another

important issue. IC methods cannot selectively distinguish hepcidin-22 and hepcidin-25 from hepcidin-20 ***(1).***

However, the reasons for including hepcidin-20 and hepcidin-22 in the total hepcidin level for determining different iron-related diseases remain unclear. The coefficient of variation among samples is lower for the plasma hepcidin assay than that for urinary ***(6).***

This suggests that the difference between measured levels in the urine and plasma is not due to the method used, but due to biological mechanisms such as excretion. Hepcidin levels measured using different methods vary considerably, but the analytical variance is low and similar for other methods in general ***(9).***

In order to harmonize the various methods used to measure hepcidin, it is recommended to introduce internal standards for all basic mass spectrometry methods used for clinical trials, reach assigned consensus levels, adjust the levels of calibrators used in each procedure, produce a calibrator that mimics a patient's serum, periodically analyze shared samples and/or interchangeable calibrators, and have a value assigned for quality control ***(7).***

In addition to measuring hepcidin, it is possible to measure prohepcidin. However, the measurement of the latter does not appear to be biologically relevant with respect to hepcidin ***(5).***

Another problem is the fluctuation of diurnal hepcidin values. Hepcidin levels are lower in the morning and increase in the afternoon. In addition, assay sensitivity is related to the amount of iron introduced by the diet. Various types of anemia exhibit different iron-related parameters including hepcidin, transferrin saturation, ferritin, and soluble transferrin receptor ***(10).***

Hepcidin and anemia:

Understanding the physiological processes of hepcidin has made it possible to redefine the pathogenetic mechanisms of anemia ***(7).***

Iron deficiency anemia:

In pure iron deficiency anemia (IDA), serum and urinary hepcidin concentrations are significantly decreased and are even undetectable by some methods currently in use. Even in the absence of anemia, hepcidin appears to be a sensitive indicator of iron deficiency ***(7).***

Moreover, compared to hematocrit or hemoglobin, a decrease in hepcidin is an early marker of iron deficiency together with transferrin saturation and decreased ferritin. Since hepcidin in the urine may also be measured, samples can be collected easily from babies and children ***(4).***

Iron-refractory iron deficiency anemia:

Iron-refractory iron deficiency anemia (IRIDA) is a genetically transmitted hypochromic microcytic anemia. It is characterized by increased hepcidin production due to a gene mutation in the suppressor matriptase-2 (TMPRSS6). Extracellular BMP2, BMP4, and BMP6 bind to the co-membrane receptor m-HJV as well as BMP receptor (BMPR) ***(8).***

This condition triggers the phosphorylation of SMAD1, SMAD5, and SMAD8 as well as the formation of heteromeric complexes with SMAD4 as the common mediator. After nuclear translocation, heteromeric SMAD complexes stimulate the transcription of the Hamp gene, which is responsible for hepcidin production ***(2).***

Hepcidin transcription is negatively regulated by soluble HJV (s-HJV), which acts as an antagonist of the BMP pathway, competing with m-HJV for BMP ligands. When matriptase-2 is mutated, hepcidin increases, resulting in the chronic inhibition of iron absorption and consequent anemia ***(9).***

Anemia with iron overload:

In β-thalassemia and congenital dyserythropoietic anemia, anemia is characterized by iron overload. Patients who do not receive transfusions have greatly reduced serum and urinary hepcidin levels. Increased erythropoietic activity and the lack of hepcidin adjustment due to the iron overload suppress the signal for the production of hepcidin itself ***(3).***

In ineffective erythropoiesis syndromes, the suppression of hepcidin production is regulated by GDF15 and TWSG I. Hepcidin levels are much higher in chronically transfused patients than that in non-transfused patients due to iron overload and ineffective erythropoiesis ***(1).***

In non-transfused thalassemic patients, iron is stored in hepatocytes rather than macrophages, similar to that in transfused thalassemic patients. The consequence of this different iron cellular distribution is that serum ferritin is much lower in non-transfused patients and does not adequately reflect liver iron storage ***(8).***

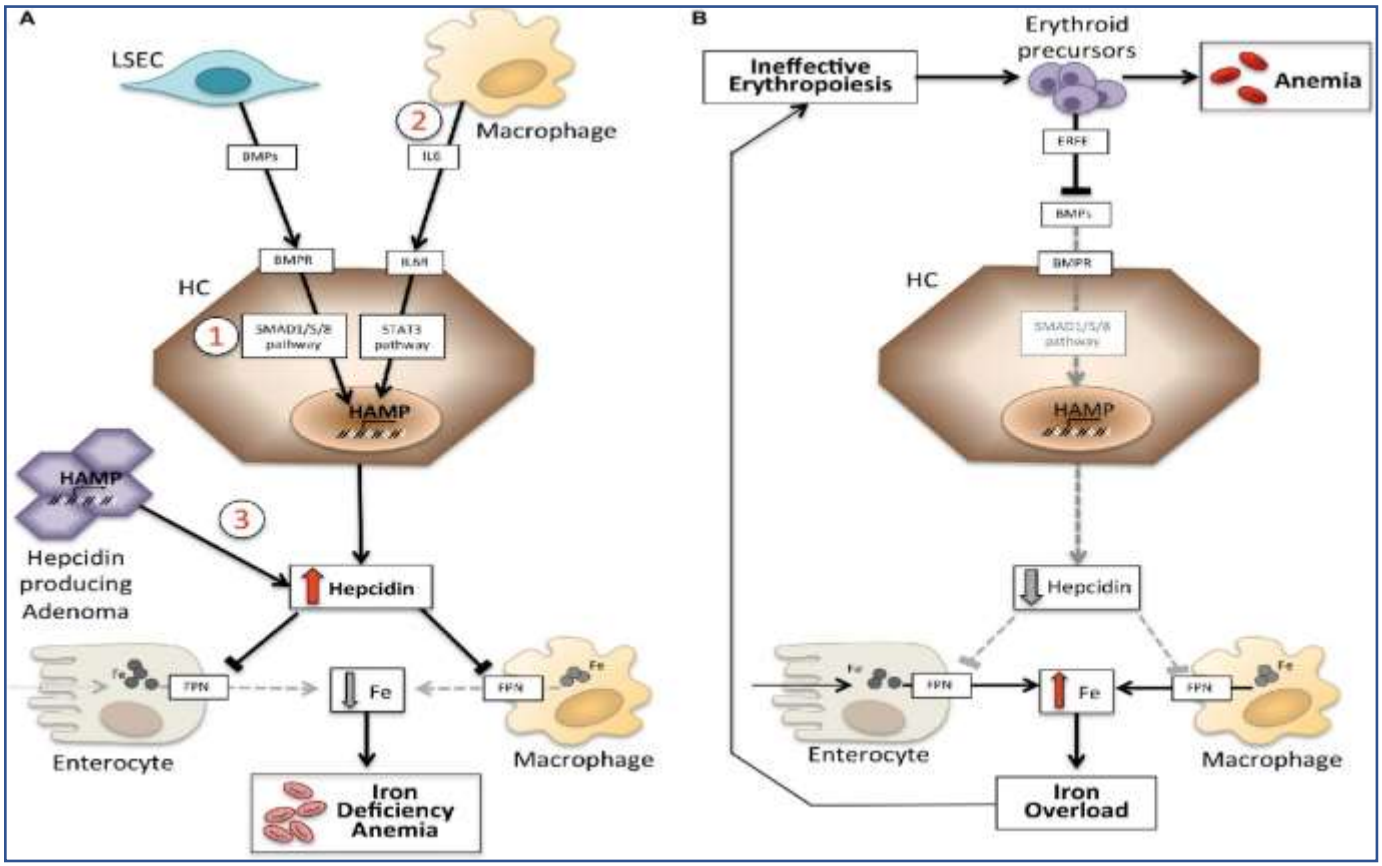

Figure (1): Schematic representation of mechanisms of anemias with high (left panel) and low hepcidin (right panel). Panel (A). Molecular pathogenesis of anemia associated with high hepcidin levels. LSEC, liver sinusoidal endothelial cells producing bone morphogenetic proteins (BMPs); BMPRs, BMP receptors; IL6, interleukin 6; HC, hepatocytes; HAMP, hepcidin gene. Fe, iron; FPN, ferroportin; 1, IRIDA; 2, Anemia of inflammation; 3, hepcidin producing adenoma. Panel (B). Molecular pathogenesis of hepcidin variation in anemias due to ineffective erythropoiesis. ERFE, erythroferrone sequestering BMPs. Other mechanisms inhibiting hepcidin in this type of anemia, as decrease of transferrin saturation and hypoxia, are not shown ***(12).***

Anemia of chronic disease/inflammation:

Patients with infections, chronic inflammatory disorders, and cancers have "anemia of chronic disease/inflammation" (ACD). Hepcidin is elevated in the following inflammatory conditions: rheumatic diseases, inflammatory bowel disease, chronic infections, multiple myeloma, and critical disorders ***(11).***

There is a rare form of iron-refractory hyposideremic anemia generally present in hepatic adenoma. The early removal of the adenoma resolves the hypoferremia and the consequent anemia, probably because the tumor is the cause of autonomous hepcidin production ***(6).***

Obesity can also be considered a chronic inflammatory state that can cause hyposideremia. In both anemia and hyposideremia, the elevated hepcidin level helps differentiate ACD from IDA. A condition of "mixed anemia" can arise in chronic inflammatory diseases involving bleeding and/or malnutrition ***(5).***

Under these conditions, the hyposideremia could counteract the hepcidin increase mediated by inflammation. A true iron deficiency from non-intestinal absorption by hepcidin may develop when inflammation is present for years ***(10).***

- Anemia in chronic kidney disease:

In patients with anemia in chronic kidney disease (CKD), the anemia is mainly due to the lack of EPO. Kidney function (i.e., excretion) plays an important role in hepcidin clearance. Kidney dysfunction results in decreased hepcidin clearance and consequent hepcidin storage with hyposideremic anemia development ***(3).***

As CKD progresses, hepcidin level increases regardless of the inflammatory state. Hepcidin production may be considered to mirror genetic hemochromatosis and chronic diseases. Hemochromatosis is characterized by low hepcidin production, which increases intestinal absorption of iron and iron release by macrophages. This inevitably leads to progressive iron storage in tissues ***(9).***

In chronic diseases, high hepcidin production inhibits iron release from macrophages and intestinal absorption of iron. This consequently induces an anemic condition. The interaction between hepcidin and ferroportin determines the plasma iron transport. Hepcidin concentration is regulated by iron, erythropoietic activity, and inflammation ***(2).***

Hepcidin and pediatric heart failure:

Anemia is frequently observed in patients with heart failure. Its prevalence varies according to cutoff values for its definition and patients' functional status. Indeed, previous studies of children with heart failure report anemia rates from 4 to 23%. In patients hospitalized for decompensated heart failure, anemia rates may reach 70% ***(13).***

Regardless of the child's clinical condition, it has become increasingly recognized that anemia is associated with worse outcomes in heart failure. In addition, it is well known that anemia further deteriorates the already impaired functional capacity of individuals with heart failure ***(14).***

There are several physiopathological mechanisms potentially involved in the genesis of anemia associated with heart failure. These include hemodilution, anemia of chronic disease, decreased renal blood flow and iron deficiency. It has also been recognized that iron metabolism may be governed by mechanisms beyond absolute iron store quantities ***(2).***

Indeed, the availability of iron stores appears to be as important as the quantity of iron. Apparently, the iron availability needed for erythropoiesis is blunted or reduced due to inflammation. It has also been described that, in patients with heart failure, there may be blunted erythropoietin production possibly caused by inflammatory cytokines or associated with chronic kidney disease and erythropoietin resistance in target organs ***(8).***

A low-grade inflammatory status is thought to accompany heart failure across a wide spectrum of disease severity and potentially modulate iron metabolism and anemia. Hepcidin appears to be pivotal in iron homeostasis with regards to inflammatory signals. Hepcidin modulates iron availability by promoting the internalization and degradation of ferroportin, a key iron transporter that is essential for iron absorption in the duodenum and recycling of iron by macrophages ***(1).***

Hepcidin production can be induced by inflammation, which explains the reduced availability of iron in anemia of chronic disease, whereas anemia and hypoxia have been shown to increase iron absorption and mobilization by decreasing hepcidin production ***(13).***

Moreover, hepcidin is a negative regulator of iron absorption and mobilization. Thus, serum hepcidin is expected to be high in iron-overload states and diminished in iron-deficient states ***(9).***

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