Hepcidin for Clinicians

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Despite the use of erythropoiesis-stimulating agents (ESAs), the anemia of chronic kidney disease (CKD) can be resistant to therapy. Both absolute and functional iron deficiency along with inflammation can contribute to ESA resistance and can be difficult to identify with current-day markers of iron storage. Hepcidin, a small peptide produced by the liver, is a recently discovered key regulator of iron homeostasis. Via regulation of ferroportin, hepcidin inhibits intestinal iron absorption and iron release from macrophages and hepatocytes. Because of its renal elimination and regulation by inflammation, it is possible that progressive renal insufficiency leads to altered hepcidin metabolism, subsequently affecting enteric absorption of iron and the availability of iron stores. Thus, hepcidin likely plays a major role in the anemia of CKD as well as ESA resistance. This article discusses the biologic actions and regulation of hepcidin along with reviewing studies of hepcidin in CKD.


Erythropoiesis-stimulating agents (ESAs) have revolutionized the treatment of anemia in chronic kidney disease (CKD); however, optimal anemia therapy in CKD must include an analysis of available iron stores and consideration of iron supplementation to ensure that this critical component for hemoglobin synthesis is available. Unfortunately, the two most commonly used markers of iron status, transferrin saturation (TSAT) and ferritin, often lack the needed specificity and sensitivity to predict the response to iron therapy in the CKD population. A common example of this problem is whether an anemic patient with CKD and with a TSAT <20% and a ferritin >500 ng/ml needs iron supplementation. These patients are frequently assumed to have a functional iron deficiency whereby the supraphysiologic rate of red blood cell production driven by ESA therapy has outstripped the ability of transferrin to deliver sufficient iron for hemoglobin synthesis. In this case, iron supplementation may be beneficial; however, an extreme case of functional iron deficiency can occur when increased inflammation results in reticuloendothelial blockade, a state in which iron release from stores is inhibited. Under such circumstances, iron supplementation would be ineffective and could lead to iron overload.

Often the treatment of functional iron deficiency is empiric iron supplementation, because current iron parameters are unable to distinguish reticuloendothelial blockade from functional iron deficiency. In the Dialysis Patients’ Response to IV Iron with Elevated Ferritin (DRIVE) Study, hemodialysis patients with a TSAT <20% and a ferritin >500 ng/ml were randomly assigned to either parenteral iron therapy or no iron therapy (1). Those who received iron therapy had a 25% reduction in their ESA requirement, although a wide variability of patient response was noted. In a follow-up analysis, neither TSAT nor ferritin levels were predictors of a response to parenteral iron (2).

To address the limitations in serum ferritin and TSAT and better target iron therapy, it is crucial to understand the molecular mechanisms behind iron balance, inflammation, and erythropoiesis in CKD. The small peptide hepcidin, primarily produced and secreted by hepatocytes, has emerged as a key regulator of iron homeostasis (3–5). It is encoded as an 84–amino acid (aa) prepropeptide that is then processed into a 60-aa prohormone, prohepcidin. This intermediate then undergoes unregulated proteolytic cleavage to form the 25-aa bioactive hepcidin (5).

Biologic Actions of Hepcidin

Several key animal and human studies have revealed the central role that hepcidin plays in iron metabolism. Knockouts of hepcidin in mice produce a model of hereditary hemochromatosis with severe multiorgan iron overload (6). Conversely, transgenic mice that overexpress hepcidin die at birth as a result of a severe iron deficiency anemia (7). As hepcidin regulation in humans is better understood, it is now apparent that most forms of hereditary hemochromatosis result from a deficiency of hepcidin, either through mutation of the hepcidin gene or through mutations of genes that are suspected to regulate hepcidin expression (8).

Hepcidin’s biologic actions are mediated by its binding to ferroportin, the principal cellular iron efflux channel. Once hepcidin is bound, it causes the rapid internalization and degradation of ferroportin (9). In the case of duodenal enterocytes, high hepcidin prevents the movement of dietary iron through ferroportin into the circulation (Figure 1). In macrophages and hepatocytes, high hepcidin levels similarly prevent the movement of stored iron into the circulation. The rapid sequestration of iron in macrophages and the long-term decrease of enteral iron absorption eventually lead to anemia by decreasing iron...
availability for erythropoiesis. Conversely, the absence of hepcidin leads to unregulated duodenal iron absorption and subsequent iron overload.

**Regulation and Metabolism of Hepcidin**

Hepcidin levels are regulated by at least three independent mechanisms (Figure 1). Whereas both inflammation and iron loading induce hepcidin production, erythropoietic activity suppresses its production. In the case of inflammation, the primary mediator seems to be increased IL-6 levels, which in turn cause the binding of Signal Transducer and Activator of Transcription 3 to the hepcidin promoter, increasing its activity (8,10). Studies of humans with chronic infections and severe inflammatory disease have shown markedly increased levels of hepcidin, strongly suggesting that elevated hepcidin levels play a key role in the anemia of inflammation and reticuloendothelial blockade (10).

The regulation of hepcidin via iron loading seems to be mediated by the bone morphogenetic protein receptor complex at the surface of hepatocytes (11–13). This complex includes two proteins that are known to be mutated in various forms of hereditary hemochromatosis: HFE and hemojuvelin. Although the exact molecular mechanism is not yet completely understood, this bone morphogenetic protein receptor complex interacts with transferrin receptors 1 and 2, likely linking the sensing of serum iron with hepcidin production (14,15).

Finally, the regulation of hepcidin production by erythropoiesis remains poorly understood. One or more unidentified bone marrow–derived signals generated during increased erythropoiesis cause decreased hepcidin production (16,17). Thus, the increased demand for iron incorporation into hemoglobin is met by increased enteral iron absorption and release of stored iron from the reticuloendothelial system. This signal transduction mechanism seems very robust and is able to keep hepcidin levels low even in the face of systemic iron overload as is seen in the iron-loading thalassemias (8,18). In this particular subset of patients, growth differentiation factor 15 has been suggested as the bone marrow signal causing hepcidin suppression (19).

The exact metabolism of hepcidin remains to be determined. Two N-terminally truncated forms of hepcidin (hepcidin-20 and hepcidin-22) have been identified and are likely degradation products that are biologically inactive (3). Hepcidin-25 seems to be filtered and reabsorbed in the proximal tubule in a manner similar to β2-microglobulin (20). As indicated by the presence of measurable amounts of hepcidin-25 in urine, renal excretion may play a major role in its eventual clearance.

**Hepcidin Levels in CKD**

The initial measurements of bioactive hepcidin in patients without CKD examined spot urine hepcidin normalized to urine creatinine as a suitable serum assay was not available (21). Because of concerns that a urine assay would not be accurate in CKD, the first published studies in CKD used serum prohepcidin as a surrogate for bioactive hepcidin (22–24). Prohepcidin was found to be elevated in patients with CKD and inversely correlated with GFR (23–25); however, prohepcidin had minimal interaction with iron or inflammatory parameters in CKD (23,24,26,27) and patients without CKD (28,29). This may be explained by prohepcidin being an intermediate metabolite without physiologic activity. Moreover, it now seems that prohepcidin levels do not mirror bioactive hepcidin production (30).

Fortunately, two major types of bioactive serum hepcidin assays have recently become available. In the first type, mass spectrometry (MS) is used to estimate levels of hepcidin peptides, typically after adjustment with internal standards to improve accuracy. Increased bioactive serum hepcidin levels in CKD were originally demonstrated in hemodialysis patients using MS (31). Unlike with assays of prohepcidin, an expected correlation of hepcidin with ferritin was documented in hemodialysis patients with this MS technique (27,31). An MS study of predialysis patients with CKD and with a GFR <30 ml/min also showed elevated hepcidin levels (32); however, because quantitative MS-based assays require an internal isotopic hepcidin standard that is not yet readily available and costly equipment that is not generally accessible, immunochemical methods have been developed in parallel. In this second type of hepcidin assay, an antihepcidin antibody is used in a competitive binding assay between a labeled hepcidin and the serum sample. Our group used a competitive ELISA to demonstrate that serum hepcidin is elevated in pediatric and adult patients with stages 2 through 4 CKD and pediatric peritoneal dialysis patients compared with respective control subjects (33). Among all groups, peritoneal dialysis patients had the highest hepcidin level. Multivariate analysis showed that hepcidin strongly correlated with ferritin in each study group and inversely correlated with GFR in adults with stages 2 through 4 CKD. Comparable findings were reported by Ashby et al. (34) with a different serum hepcidin radioimmunoassay. Ashby et al. showed adult CKD and hemodialysis groups had increased hepcidin concentrations, with the hemodialysis group having the highest level. Similarly, hepcidin inversely correlated with
GFR and directly correlated with ferritin in stages 2 through 4 CKD, although the association with ferritin was lost in the hemodialysis group. Target-driven therapy with supplemental intravenous iron to predefined goal ferritin levels may have confounded the relationship in this hemodialysis group.

It is interesting that the absolute values of hepcidin may vary as much as 10-fold depending on the hepcidin assay used (35). Possible reasons for the wide ranges include hepcidin-binding factors in the serum, differing hepcidin standards, cross-reaction with hepcidin metabolites, and hepcidin adsorption by assay surfaces. Efforts are being undertaken to resolve these discrepancies and provide technical standardization of the hepcidin assays. Of utmost importance, however, the two types of assays seem to correlate extremely well despite intersay differences in absolute values; therefore, the associations and conclusions based on these studies are likely to be reproducible and valid.

In this context, it seems clear that serum hepcidin levels increase across the spectrum of CKD. Compared with healthy control subjects, predialysis patients with CKD have an approximately two- to four-fold elevation in serum hepcidin, whereas dialysis patients may have a six- to nine-fold increase. In the majority of CKD studies, hepcidin has correlated very well with ferritin, which is both a measure of iron stores and an acute-phase reactant. In our study, multivariate analysis demonstrated that hepcidin was independently predicted by dialysis status, ferritin, and C-reactive protein, suggesting that inflammation may also regulate hepcidin levels in CKD (33). Correlations of hepcidin with inflammatory markers in CKD have also been found in other studies (31), although not all (27,34). It therefore seems that diminished renal clearance and increased inflammation in the setting of renal failure may result in elevated serum hepcidin in patients with CKD. Future studies to assess the impact of residual renal function and clearance by dialysis on hepcidin levels are needed.

Potential Diagnostic and Therapeutic Uses of Hepcidin

With further validation, hepcidin may become an important biomarker of iron status in CKD, especially because current markers such as TSAT and ferritin appear suboptimal. For example, hepcidin may hypothetically distinguish patients with simple functional iron deficiency from those with reticuloendothelial blockade. In the former, hepcidin would be expected to be low as a result of the decreased availability of iron for erythropoiesis. Conversely, in reticuloendothelial blockade, hepcidin, as the pathogenic mediator of inflammation, would be expected to be high. Furthermore, because hepcidin levels increase with iron loading, monitoring its rise may help to delineate when sufficient iron stores have been achieved and alert physicians to those who are at risk for possible harmful effects of iron overload. Finally, low hepcidin levels may identify patients who are most likely to respond to oral iron. Whether hepcidin measurements can guide iron supplementation requires further study.

Limited data are available on whether hepcidin levels can predict a response to ESA therapy. Using MS, Kato et al. (27) studied 24 hemodialysis patients and compared hepcidin levels in patients who were responsive versus unresponsive to recombinant erythropoietin (rhEPO). rhEPO unresponsiveness was defined to be present in anemic, iron-replete patients when weekly rhEPO requirement was >9000 IU. No difference in hepcidin levels was revealed between the two groups. The interpretation of this result may be limited by assay variability (intra-assay precision 7.5 to 9.5%; interassay precision 25.7 to 27.5%) and small sample size. Ashby et al. (34) also noted a negative correlation of hepcidin and rhEPO dosage, suggesting that rhEPO suppressed hepcidin, a finding supported after observing decreased hepcidin levels after initiation of rhEPO in seven ESA-naive patients. Thus, larger scale studies will be valuable in determining whether hepcidin levels can predict and monitor responsiveness to ESA therapy.

In the future, it is possible that a hepcidin antagonist could be developed as a therapeutic tool in CKD. Specifically, lowering hepcidin levels or antagonizing its actions would reverse the negative effects of inflammation on erythropoiesis by allowing mobilization of stored iron and improved enteral iron absorption. Both effects could reduce or even eliminate the need for expensive and potentially toxic parenteral iron repletion along with decreasing ESA requirements.

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Disclosures

None.

References