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Review Article

Hepcidin is a Potential Regulator of Iron Status in Chronic Kidney Disease

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Abstract: Hepcidin is a small defensin-like peptide produced primarily by hepatocytes, but also by other cells, including macrophages. In addition to hepcidin's antimicrobial properties, it is the main regulator of iron metabolism and controls both the amount of dietary iron absorbed in the duodenum and the iron release by reticuloendothelial cells. Hepcidin expression is upregulated by a variety of stimuli, including inflammation and iron overload, and downregulated by anemia, hypoxia, and iron deficiency. Chronic kidney disease (CKD) is associated with increased serum hepcidin levels, and the increased levels may contribute to the development and severity of anemia and to resistance to erythropoiesis-stimulating agents (ESAs). Elevated serum hepcidin levels contribute to the dysregulation of iron homeostasis in CKD patients. Although parenteral iron supplementation can bypass some of the iron-blocking effects of hepcidin in CKD patients with anemia, and free iron and iron stores increase as a result, the anemia is only partially corrected, and the ESA dose requirements remain significantly higher than needed for physiological replacement. Treatment with agents that lower serum hepcidin levels or inhibit its actions may be an effective strategy for restoring normal iron homeostasis and improving anemia in CKD patients. The aim of this article was to review the regulation of hepcidin levels and the role of hepcidin in CKD-related anemia, and to discuss hepcidin's potential as a clinical biomarker and several investigational treatments designed to lower serum hepcidin levels. **Key Words:** Anemia, Chronic kidney disease, Erythropoiesis-stimulating agent, Hepcidin, Iron metabolism.

Hepcidin is primarily produced and secreted by hepatocytes and has emerged as the key regulator of iron homeostasis (1,2). It is encoded as an 84-amino-acid prepropeptide that is processed into the 60-amino acid prohepcidin, which undergoes unregulated proteolytic cleavage to form the 25-amino-acid bioactive hepcidin (3). Circulating hepcidin was recently found to be bound to α2-macroglobulin with relatively high affinity. Based on theoretical calculations, 11% of hepcidin has been estimated to be freely circulating (4). Hepcidin clearance is assumed to occur via cellular codegradation with ferroportin at its sites of action, and via excretion by the kidneys. Because of its low molecular weight and the small radius of the molecule, unbound hepcidin is likely to pass freely into the glomerular filtrate. In human studies, the fractional excretion of hepcidin has been calculated to be as low as 0–5% (5,6).

Hepcidin, prohepcidin, and hepcidin metabolites in the blood increase in chronic kidney disease (CKD) patients and are very high in dialysis patients. Blood prohepcidin levels have been found to be elevated in CKD patients and to be inversely correlated with their glomerular filtration rate (GFR) (7). However, minimal interactions between prohepcidin and iron or inflammatory parameters have been reported in CKD patients (8,9). These findings may be explained by prohepcidin being an intermediate metabolite and devoid of physiologic activity. Since elevated blood hepcidin levels appear to have a major role in the development and severity of anemia in CKD patients (10), there is great interest in hepcidin assays as a diagnostic tool, and in hepcidin as a target for the

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treatment of anemia in CKD patients. The aim of this article was to review the regulation of hepcidin levels and the role of hepcidin in CKD-related anemia, and to discuss hepcidin's potential as a clinical biomarker and several investigational treatments designed to lower blood hepcidin levels.

HEPCIDIN FUNCTION

Hepcidin-25 is thought to be the major regulator of dietary iron absorption and cellular iron release, and it exerts its regulatory function by counteracting the function of ferroportin, the major cellular iron exporter in the hepatocyte cell membrane. Hepcidin-25 induces internalization and degradation of ferroportin (11), which results in increased intracellular iron stores, decreased dietary iron absorption, and decreased circulating iron levels. Hepcidin produced by various cell types may have local effects in different tissues. Hepcidin may protect nearby cells from iron deficiency by interacting in an autocrine manner with ferroportin (12).

In addition to its role in regulating iron metabolism, hepcidin may contribute to host defense. Although in vitro studies have suggested a bactericidal effect of hepcidin, this effect would require a higher concentration than the concentrations measured in the circulation. However, higher concentrations may be achieved locally in the phagosomes of infected macrophages (13). Hepcidin may contribute indirectly to host defense by reducing iron concentrations. Since iron is required for microbial growth, low iron levels are thought to be bacteriostatic. Hepcidin has also been found to modulate lipopolysaccharide-induced transcription in cultured macrophages and in vivo mouse models (14), suggesting that hepcidin plays a role in modulating acute inflammatory responses to bacterial infections.

HEPCIDIN AND IRON ABSORPTION

Most iron absorption occurs across polarized intestinal epithelial cells, or enterocytes, in the duodenum and proximal jejunum. Differentiated duodenal enterocytes express high levels of proteins that are involved in absorption of iron consumed in the diet. The major iron import protein on the brush border is the ferrous iron transporter divalent metal transporter 1 (15). Iron absorption by enterocytes is systemically controlled by hepcidin (16). Iron absorption by enterocytes is also regulated at the local level by their intracellular iron concentration. One major player in this regulation is the iron responsive element/iron regulatory protein system, which operates by affecting the post-transcriptional regulation of proteins involved in iron metabolism (17). Enterocyte ferritin, which stores most of the iron in enterocytes that is not in the labile iron pool, may also play an important role in regulating iron uptake, because intestine-specific knockout of ferritin H protein in mice leads to inappropriately increased iron absorption in view of the iron status in anemia (18). Kuragano et al. reported that serum ferritin levels of 40–130 ng/mL, at which intestinal absorption of non-heme iron is less than 2–5% and does not increase body ion stores, may correspond to hepcidin-25 levels of 10–25 ng/mL in HD patients (19).

REGULATION OF HEPCIDIN LEVELS

Several physiologic and pathologic processes regulate hepcidin synthesis. Hepcidin levels are regulated by several independent mechanisms, as previously reviewed (20) (Fig. 1). Conditions in which demand for circulating iron is increased induce a decrease in hepatocellular hepcidin synthesis, and such conditions include iron deficiency, hypoxia, and conditions in which erythopoietic activity is increased. A decrease in the blood hepcidin level results in the release of stored iron and an increase in dietary iron absorption. Infection and inflammation, on the other hand, cause an increase in hepcidin synthesis that leads to a deficiency of iron available for eryhtropoiesis, and is considered to be the mechanism underlying the reticuloendothelial iron sequestration, impaired intestinal iron absorption, and low serum iron levels that are characteristic of the anemia of chronic disease (21).

Regulation by iron status

Iron stores and circulating transferrin-bound iron (Tf-Fe) provide distinct signals that affect hepcidin expression in hepatocytes (22–24). The response to circulating transferrin appears to be mediated by a hepatocellular complex, which includes transferrin receptor-1 (TfR1), TfR2, and hemochromatosis iron protein (HFE). Defects in TfR2 and HFE lead to decreased hepcidin concentrations via the extracellular signal-regulated kinase pathways: the mitogen-activated protein kinases (ERK/MAPK) and/or the bone morphogenetic protein (BMP)/mothers against decapentaplegic (MAD)-related protein (SMAD). Intracellular iron stores interact with hepcidin via BMPs, particularly BMP-6, in a paracrine or autocrine fashion. These extracellular signaling molecules act on hepatocyte BMP receptors that activate the intracellular SMAD signaling pathway and increase

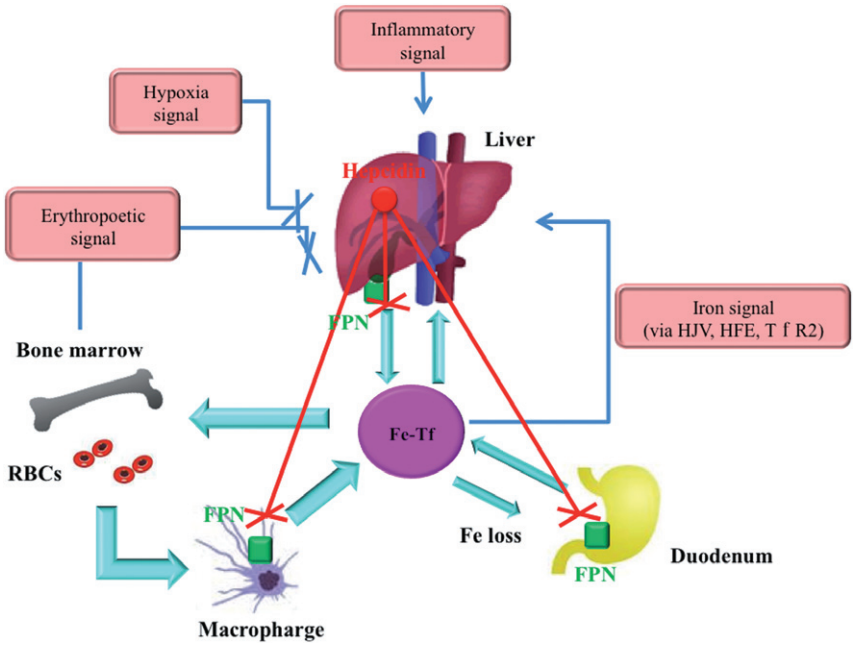

FIG. 1. Hepcidin is a central regulator of systematic iron homeostasis. Hepcidin levels are regulated by at least four independent mechanisms. Both inflammation and iron loading induce hepcidin production, whereas hypoxia and erythropoietic activity suppress its production. Hepcidin production is stimulated by iron through hemojuvelin (HJV), hemochromatosis iron protein (HFE), and transferrin receptor 2 (TFR2). Hepcidin controls iron release into the plasma by downregulating the expression of ferroportin (FPN) on macrophages, hepatocytes, and enterocytes. (Modified from (10)). RBCs, red blood cells.

hepcidin transcription. The BMP receptor, hemojuvelin (HJV), is crucial for hepcidin expression, because various hepcidin regulatory pathways converge at this membrane-bound protein (25). Under low iron conditions, membrane-bound HJV is cleaved by a transmembrane protease (26). The signaling pathways for hepcidin transcription have been reviewed by Coyne (27) (Fig. 2).

Regulation by hypoxia

Decreased hepcidin expression has been reported in response to hypoxia in vivo (28,29), and the decrease in expression may be attributable to the effect of hypoxia on erythropoietin (EPO) expression and erythropoietic activity via a direct interaction with hepatocyte EPO receptors (30). The lower hepcidin concentrations observed in response to hypoxia may also be associated with liver-specific stabilization of hypoxia-inducible factor (HIF)-1 (31) and downstream effects on the BMP/SMAD signaling pathway (32,33). Whether HIFs directly bind to the hepcidin promoter is a matter of controversy, but there are indirect mechanisms by which HIFs may regulate hepcidin expression. Increased HIF activity

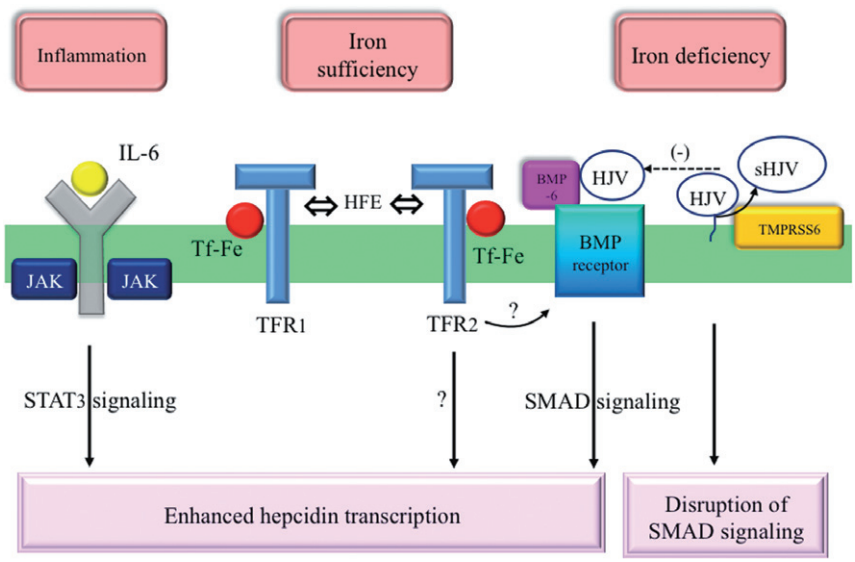

FIG. 2. Signaling pathways for hepatic hepcidin transcription. In inflammatory conditions, interleukin (IL)-6 binds to its receptor and then activates STAT3 signaling via Janus kinase (JAK). In iron-sufficient conditions, transferrin-bound iron binds to the transferrin receptor 1 (TFR1) and displaces hemochromatosis iron protein (HFE). Then, HFE and transferrin-bound iron bind to the transferrin receptor 2 (TFR2) and promote hepcidin transcription with or without interaction with bone morphogenetic protein (BMP)-6 and hemojuvelin (HJV). Iron deficiency impairs hepcidin transcription via the liver transmembrane serine protease (TMPRSS6) cleaving HJV, which produces soluble HJV (sHJV), which in turn impairs BMP receptor complex signaling. (Modified from (27)).

is associated with protease-mediated cleavage of HJV and a subsequent decrease in hepcidin expression (33). Mice lacking intestinal HIF2α express lower amounts of ferroportin and metal transporter 1, and thus they fail to induce iron absorption even when hepcidin expression is reduced (34). These findings suggest that expression of HIF2α promotes iron absorption by the intestine via increased activity of metal transporter 1 and ferroportin under hypoxic and/or iron-deficient conditions.

Regulation by inflammation

Interleukin-6 is the major inflammatory cytokine that mediates the increase in blood hepcidin levels under inflammatory conditions (35). Previous studies have shown very high blood hepcidin levels in humans with a chronic infection or a severe inflammatory disease, suggesting that elevated hepcidin levels play a key role in the anemia of inflammation and reticuloendothelial blockade (29). IL-6 causes signal transducer and activator of transcription (STAT)-3 to bind to the hepcidin promoter, thereby increasing hepcidin's activity (36). The heptocellular interaction between IL-6 and its receptor activates the Janus kinase (JAK)/STAT-3 signaling pathway (37,38). Administration of lipopolysaccharide to healthy volunteers has been shown to induce increased blood IL-6 levels, and the increase is followed by increased blood hepcidin levels, and then hypoferremia (39).

Regulation by erythropoietic signals

Administration of erythropoiesis-stimulating agents (ESAs) has been found to decrease hepcidin production by hepatocytes (30,40,41). Erythropoiesis requires considerable amounts of iron, and suppression of hepatic hepcidin synthesis by erythropoietic signals is of great physiological importance. However, how erythropoiesis regulates hepcidin has not been clearly determined. The hypothesis that erythropoietin (EPO) acts directly on hepatocyte EPO receptors in vitro (30) has not been supported by studies in animal models of anemia, which showed that decreased hepcidin expression depends on erythropoiesis and that erythropoiesis is not directly mediated by EPO (41,42).

SERUM HEPCIDIN LEVELS IN CKD PATIENTS

No standard assay for serum hepcidin levels has ever been established, and some of the problems with comparing assay methods have been reviewed in previous articles (43,44). The most informative assays determine hepcidin-25 and can be used to measure hepcidin in blood samples. Two major types of bioactive serum hepcidin assays are currently available. In the first type, mass spectrometry is used to estimate the hepcidin level, typically after adjustment with internal standards to improve accuracy (45). Unlike prohepcidin, the expected correlation between serum hepcidin levels and ferritin levels was documented in HD patients (45). In the second type of hepcidin assay, an antihepcidin antibody is used to perform a competitive binding assay between labeled hepcidin and the serum sample (46).

The kidney is the major route of hepcidin clearance. Recent studies have demonstrated higher serum hepcidin concentrations in CKD patients than in healthy controls (5,45,47,48). We measured serum hepcidin-25 levels in healthy volunteers and CKD patients using mass spectrometry by a method reported previously (45). Serum hepcidin levels increased significantly as the CKD stages progressed (Fig. 3). Previous studies on hepcidin levels revealed a strong positive correlation between serum hepcidin and ferritin concentrations in CKD patients. The serum hepcidin levels in CKD patients have also been shown to be associated with iron-restricted erythropoiesis, as reflected by the relation of high serum hepcidin levels and low hemoglobin concentrations and/or reticulocyte counts (47,48). The serum hepcidin levels have been found not to predict HD patients on ESA therapy whose hemoglobin concentration increased after iron loading (49). Several investigators have reported a decrease in serum hepcidin concentration after EPO administration to CKD patients (47,48,50). These findings suggest that

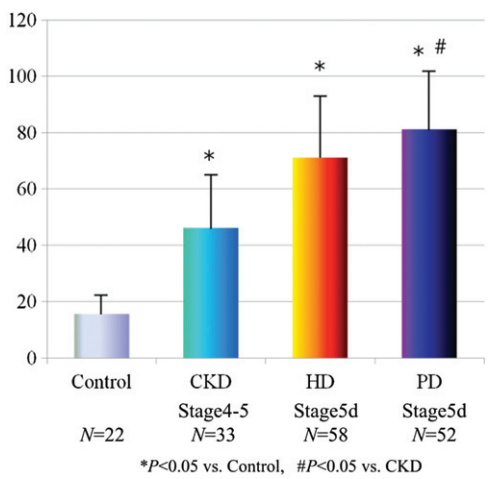

FIG. 3. Serum hepcidin-25 levels in healthy controls and chronic kidney disease (CKD) patients. The serum hepcidin-25 levels were quantified by mass spectrometry. Serum hepcidin-25 levels increased significantly as the CKD stages progressed.

the erythroid series demand for iron might be a more powerful regulator of hepcidin expression than iron-induced hepcidin formation.

HEPCIDIN AS A DIAGNOSTIC TOOL IN CKD PATIENTS

A major diagnostic problem in managing CKD-related anemia is determining whether or not patients require iron supplementation. Studies of serum ferritin levels and transferrin saturation have failed to find a reliable predictor of the iron response in dialysis patients (51,52) or non-dialysis CKD patients (53). Because hepcidin is the main regulator of iron stores, the serum hepcidin level has been speculated to be the best predictor of iron-restricted erythropoiesis.

Several studies have shown a relationship between the serum ferritin and hepcidin levels of dialysis patients. Ashby et al. reported an inverse correlation between serum hepcidin levels and epoetin doses and a decline in the hepcidin level after the start of epoetin therapy (47), and Weiss et al. obtained similar findings (48). The above findings are consistent with hepcidin being a biomarker of iron status and iron demand in dialysis patients, and they suggest that serum hepcidin levels may have predictive value in individual CKD patients.

Although serum hepcidin levels are correlated with iron status, they have a high short-term intra-patient coefficient of variation and are influenced by inflammation (54). Ferritin has been shown to be an inadequate predictor of response to iron (55). Moreover, Kato et al. found no difference between the hepcidin levels of epoetin-responsive and epoetin-resistant dialysis patients (56), and Ford et al. found no relationship between blood hepcidin levels and epoetin doses (54). When CKD patients have an inflammatory condition, it is unclear whether the timing of iron administration is adequate or not. Also, the intra-patient coefficient of variation is even higher for hepcidin than for ferritin, undermining the value of a single hepcidin determination as an indicator of iron status or iron requirement (49).

When an ELISA method was used to measure the serum hepcidin levels, greater intra-patient variability was observed in their hepcidin levels than ferritin levels of HD patients, thereby indicating that hepcidin assays are unlikely to be useful diagnostic tools for anemia in CKD patients. Consistent with this impression, a recent study showed that serum hepcidin levels measured by a mass spectrometry method were not predictive of an erythropoietic response by HD patients to intravenous iron administration (49). However, further investigation of the relationship between hepcidin assay methods and the predictive value of the measurements is needed. Measurements of the serum hepcidin levels of stage 2–4 CKD patients may be of greater diagnostic value, because their values are lower and may be more stable.

CLINICAL APPLICATION OF HEPCIDIN-MEDIATED IRON BLOCKADE

Elevated serum hepcidin levels mediate iron-restricted erythropoiesis and contribute to inducing anemia in CKD patients. Short-term increases in serum hepcidin levels impair the release of storage iron, and long-term increases in serum hepcidin levels result in iron deficiency. The results of experimental studies have suggested that elevated serum hepcidin levels may also directly contribute to the development of anemia in CKD patients by inhibiting erythroid colony formation when blood erythropoietin concentrations are low, and by impairing red blood cell survival (57). Thus, lowering serum hepcidin levels may improve anemia or reduce the ESA requirement. Parenteral iron administration reverses anemia-induced overexpression of hepcidin, but oral iron supplementation does not (58–61). Studies in pre-dialysis CKD patients and dialysis patients have shown that intravenous iron administration improved anemia in most patients, including in patients with elevated blood hepcidin levels (49) and in patients with markedly elevated blood C-reactive protein levels (52,53).

Although intravenous iron therapy clearly improves hepcidin-mediated iron blockade to some degree, there are several reasons for concern (62). Iron therapy would further increase blood hepcidin levels and thereby increase the subsequent iron blockade. Consistent with continued dysregulation of iron metabolism following intravenous iron administration, a high iron concentration was found in the liver of dialysis patients who received intravenous iron therapy. The long-term effects of intravenous iron administration and increased iron stores have not been rigorously investigated (63). Free blood iron levels increase after intravenous iron administration, and the higher iron concentrations may increase susceptibility to infections and oxidative stress, whereas long-term iron therapy promotes cellular iron storage because of the continuously high hepcidin levels. Consistent with dysregulation of iron metabolism following intravenous iron administration, although the ESA dose requirements fall, the iron doses required are significantly higher than physiological replacement doses.

Since overexpression of hepcidin in animals impairs their response to even supraphysiological doses of ESA (64), high serum hepcidin levels may contribute to the ESA resistance observed in many dialysis patients. Thus, lowering the serum hepcidin level or inhibiting hepcidin signaling would release iron from stores, promote erythropoiesis, and prolong red cell survival, and that might be safer than treatment with high doses of ESAs and repeated parenteral doses of iron. Administration of an anti-hepcidin antibody in combination with ESA therapy has been shown to be effective against inflammation-induced anemia in an animal model expressing human hepcidin (64). Interestingly, the animals did not respond to intravenous iron administration in the absence of the neutralizing antibody, indicating that the effects of the antibody are mediated by other factors besides iron. Directly suppressing hepcidin transcription with small-interfering RNA has been shown to lower hepcidin levels, increase erythropoiesis, and increase serum iron levels in preclinical studies (64).

Hypoxia-inducible factor suppresses hepcidin expression and stimulates endogenous erythropoietin production (31). Early human studies have shown that HIF prolyl hydroxylase inhibitors increase HIF, improve anemia in CKD, increase endogenous erythropoietin production, and lower blood hepcidin levels. However, in some tumor models, stabilization of HIF has been found to increase tumor growth. Clinical trials with this class of agents are in progress. Treatment directed at other targets, such as treatments designed to interrupt the binding of hepcidin to ferroportin, may result in an increase in iron absorption and mobilization (65).

These investigational treatments of anemia in CKD patients are not risk-free. Sequestration of iron during inflammation and infections may provide distinct benefits that may be negated by the neutralizing effects of hepcidin. Since lower serum iron and higher serum hepcidin levels can be antimicrobial, reducing the hepcidin level of dialysis patients might increase their susceptibility to infections. Stabilization of HIF may improve anemia but facilitate tumor growth. Further interventional treatments intended to overcome hepcidin blockade must be independently evaluated for safety.

CONCLUSIONS

Hepcidin is a key regulator of iron balance, and high serum hepcidin levels cause iron blockade and anemia in chronic disease. Chronic kidney disease patients with anemia have been found to have elevated serum hepcidin levels, and the high levels are likely to contribute to anemia in CKD and to ESA hyporesponsiveness. Serum hepcidin measurements in dialysis patients may not be of greater diagnostic value than serum ferritin measurements. Given the limited number and size of studies to date, the unresolved issues surrounding hepcidin assays themselves, and the numerous factors that can modulate hepcidin levels in the CKD/CKD-D population, including iron and ESA administration, body iron burden, inflammation, renal clearance, and dialysis, more studies will be needed to determine whether hepcidin will have diagnostic utility as a measure of iron status, inflammatory status, and/or ESA responsiveness and to investigate the hepcidin lowering agents may have a role in treating anemia in CKD patients.

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