Review

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Hepcidin: Homeostasis and Diseases Related to Iron Metabolism

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Abstract
Iron is an essential metal for cell survival that is regulated by the peptide hormone hepcidin. However, its influence on certain diseases is directly related to iron metabolism or secondary to underlying diseases. Genetic alterations influence the serum hepcidin concentration, which can lead to an iron overload in tissues, as observed in haemochromatosis, in which serum hepcidin or defective hepcidin synthesis is observed. Another genetic imbalance of iron is iron-refractory anaemia, in which serum concentrations of hepcidin are increased, precluding the flow and efflux of extra- and intracellular iron. During the pathogenesis of certain diseases, the resulting oxidative stress, as well as the increase in inflammatory cytokines, influences the transcription of the HAMP gene to generate a secondary anaemia due to the increase in the serum concentration of hepcidin. To date, there is no available drug to inhibit or enhance hepcidin transcription, mostly due to the cytotoxicity described in the in vitro models. The proposed therapeutic targets are still in the early stages of clinical trials. Some candidates are promising, such as heparin derivatives and minihepcidins. This review describes the main pathways of systemic and genetic regulation of hepcidin, as well as its influence on the disorders related to iron metabolism.

Introduction

Iron is a metal that is required to maintain innumerable functions in the body such as the synthesis and repair of DNA, transport of oxygen, and mitochondrial energetic metabolism, and it is a cofactor for several enzymes [1]. The control and molecular regulation of iron is performed by the hepcidin peptide through the modulation of ferroportin [2, 3]. In addition, disorders related to iron metabolism involve changes between hepcidin and ferroportin, causing damage to the body because these disorders may be related to iron overload or deficiency [4].

Regarding intracellular excess iron, hyperferraemia contributes to the formation of reactive oxygen species (ROS), damaging cell membranes and tissues, especially cardiac, endocrine, and hepatic tissues [5]. However, iron deficiency (hypoferraemia) decreases haemoglobin synthesis, limiting the formation of erythroid precursors,

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with the consequent development of anaemia [6]. Regarding the mechanisms by which hepcidin controls iron homeostasis, it has been demonstrated that it occurs via 3 main routes: inhibition of food absorption of iron in the duodenum, blockage of iron release recycled by macrophages, and control of the movement of iron stocks contained in hepatocytes [7].

Thus, systemic iron stores, elevated concentrations of plasma transferrin, erythropoietic activity and host defences modulate the synthesis of hepcidin [8]. Therefore, when the plasma iron concentration increases, hepcidin mRNA transcription increases in hepatic tissue to decrease iron uptake in duodenal enterocytes and the mobilization of iron stores. When plasma iron decreases, there is also a decrease in hepcidin transcription in the liver [9, 10].

Hepcidin is encoded by the HAMP gene [3]. Stimuli such as hypoxia, erythropoiesis, iron levels, and inflammation control the expression of this gene. During anaemia related to chronic disease, gene expression is increased [11]. Several studies have demonstrated the influence of the serum hepcidin concentration on the pathogenesis of diseases such as juvenile hereditary haemochromatosis, in which a polymorphism present in the HAMP gene promotes hepcidin synthesis deficiency with iron accumulation in the tissues [12–14].

Another important factor that alters iron concentrations is viral infection, such as in cases of HIV-1 infection and hepatitis C virus (HCV), in which iron overload is associated with a poor prognosis and may be, in part, caused by the viruses themselves or by a deregulation of iron homeostasis [15]. Thus, the present review addresses the mechanisms responsible for iron metabolism and the implications of hepcidin for the regulation and development of diseases related to systemic iron homeostasis.

Hepcidin

The hepcidin molecule ("hep" hepatic origin, "cidin" antimicrobial activity) was described in 2000 as a new antimicrobial peptide that functions, in part, in innate immunity. Initially called LEAP-1 (liver-expressed antimicrobial peptide), it was isolated from human blood ultrafiltrates and characterized by mass spectrometry as a cysteine-rich peptide synthesized in the liver. Subsequently, it was purified and isolated from urine in its active form [2, 3].

Early reports of the relationship between hepcidin and iron homeostasis were demonstrated in knockout mice for the gene encoding hepcidin, the HAMP gene, and these animals exhibited iron overload in contrast to transgenic animals for hepcidin, in which the iron concentration resulted from the consequent development of severe microcytic and hypochromic anaemia [16, 17]

Hepcidin: Structure

Hepcidin is encoded by the HAMP gene, mostly by hepatocytes; however, studies have shown that hepcidin can also be produced by adipocytes, macrophages, lymphocytes, neutrophils, pancreatic β-cells, and renal cells, but these findings remain unclear [18–20].

Physiologically active hepcidin originates from a prehepcidin containing 84 amino acids, which after proteolytic cleavage gives rise to prohepcidin, composed of 64 amino acids. Prohepcidin is biologically inactive and is cleaved further by the enzyme furin in a specific NH 2 region to generate biologically active hepcidin composed of 8 cysteine residues bound by 4 bisulphide bridges containing 25 amino acids [2, 3, 16].

At present, 3 other isoforms of hepcidin have been described in the literature, containing 20, 22, and 24 amino acids. Under physiological conditions, these isoforms are found at low concentrations in urine and plasma, which increase during pathological processes such as sepsis. The isoform composed of 20 amino acids is related to the elevated levels of plasma creatinine, but the underlying mechanism has not been elucidated [21, 22].

The isoform composed of 24 amino acids has been described in invertebrate animals, activating several immunological pathways as the main defence mechanism. In humans, this isoform is involved in iron metabolism, similar to an active molecule containing 25 amino acids [23, 24].

Iron Homeostasis: Balance and Renewal

Through our diet, we obtain the iron needed for physiological functions. Enterocytes present in the duodenum and the proximal portion of the jejunum absorb approximately 1–2 mg of iron from our diet, with one man consuming approximately 5 mg iron/day, a growing child approximately 15 mg, and one pregnant woman requiring 5–10 times more iron due to the maternal and foetal requirements [25].

A regular diet provides approximately 10–15 mg iron/day from red meat, which contains the haem iron in myoglobin and haemoglobin, and approximately 20–40% of the haem is available for absorption. Non-haem iron is present in cereals, and approximately 10–20% is absorbed. The haem group is a porphyrin ring containing 4

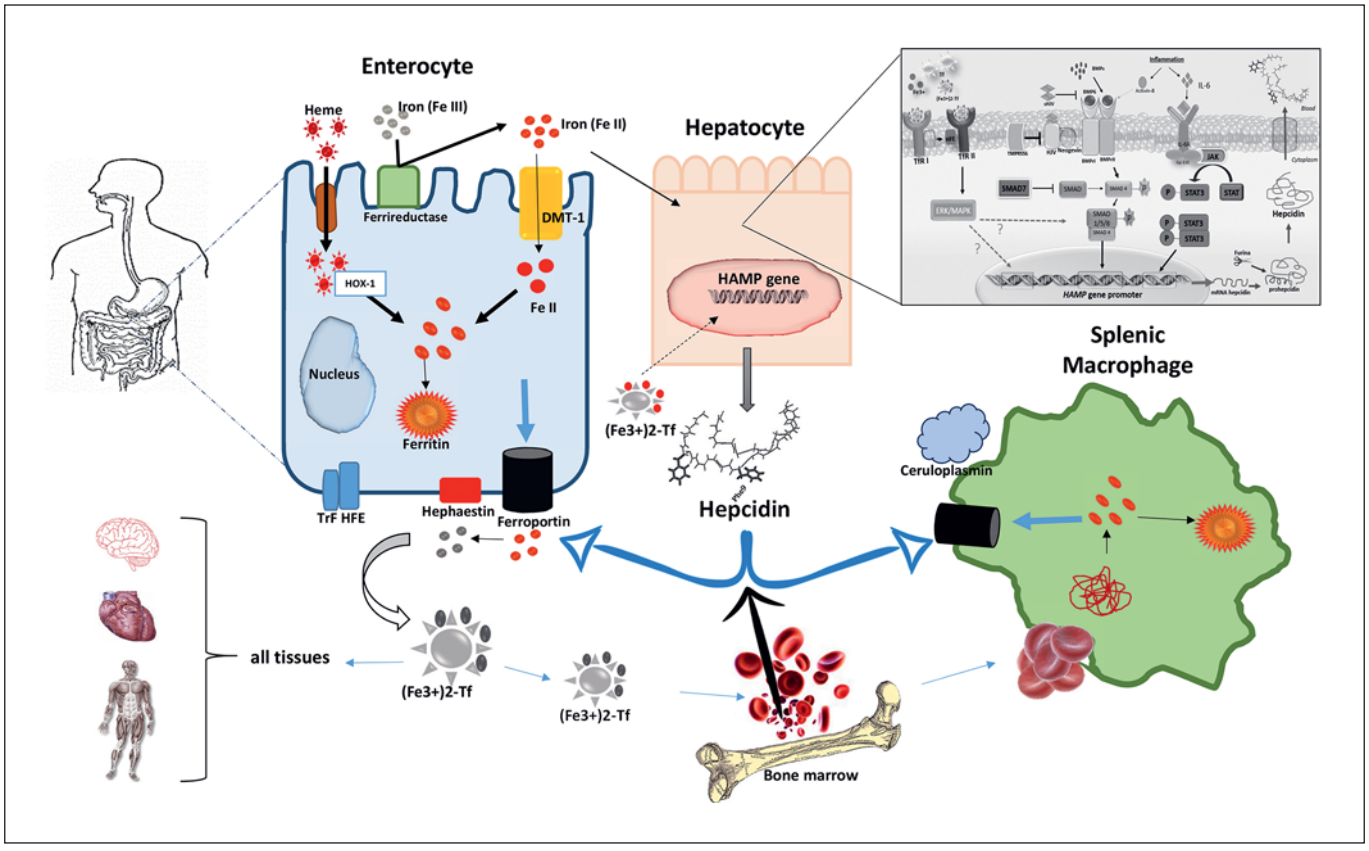

Fig. 1. Iron homeostasis.

pyrrole groups of iron in its ferrous state (Fe^{2+}), with each haem group carrying an O_2 molecule bound to Fe^{2+} and a histidine residue of the globin molecule [26–28].

To obtain more efficient absorption of the iron in the duodenum, it must pass from its ferric state (Fe^{3+}) to the ferrous state [29]. Several factors influence this iron absorption in the duodenum, such as a diet rich in polyphenols and phytic acid, vitamin C deficiency, gastritis caused by *Helicobacter pylori*, and bariatric surgery, among other factors that decrease iron absorption [1].

The organism does not present a mechanism for the elimination of excess iron, and the greatest efflux of iron comes from the recycling of senescent erythrocytes by splenic macrophages, which provide approximately 90–95% of the iron needed for physiological functions and erythropoiesis. Additionally, maintenance of iron stores occurs through the diet [30, 31].

In the cytoplasmic membrane of enterocytes, apical ferric reductase enzymes are present, such as the cytochrome b duodenal enzyme, which promotes the reduction of iron from the ferric state to its ferrous state and consequent mobilization of ferrous iron through the divalent metal type transporter-1. Haem iron from the diet is internalized by the heme-1 carrier protein into the cells where it is stored as ferritin if the plasma iron concentration is elevated; otherwise it is released into the extracellular medium [32].

When the serum iron concentration is low, iron stores are mobilized to the extracellular medium via ferroportin. The released iron in its ferrous state binds to serum transferrin, and it must be oxidized to its ferric state because only Fe^{3+} binds to transferrin. This oxidation reaction occurs through the action of oxidase enzymes: hephaestin present in enterocytes, ceruloplasmin present in hepatocytes and plasma, and zyklopen in the placenta. The iron is then released in the tissues. In the present study, the effect of iron transferrin on iron transferrin and iron transferrin was not significantly different from that of iron [33–35].

Iron recycling by macrophages occurs through the phagocytosis of senescent erythrocytes and of haemoglobin and the haem group from intravascular haemolysis.

Once internalized in the macrophage, the haem group releases ferrous iron via the activity of the enzyme haem oxygenase, which can be exported to the extracellular medium by ferroportin or stored as ferritin [36] (Fig. 1).

The highest concentration of iron in the body is stored as ferritin or haemosiderin, in the liver, spleen, duodenum, bone marrow, and other organs. The ferritin molecule is composed of 24 subunits with a spherical "shell" shape, which accommodates about 4,000 iron atoms. Mammals have 3 genes that encode ferritin. The cytosolic heavy chain of the molecule has 183 amino acids and is encoded by the FTH gene, present on chromosome 11. The cytosolic light chain consists of 175 amino acids and is encoded by the FTL gene on chromosome 19, and mitochondrial ferritin is encoded on chromosome 5 in the FTMT intronic gene, showing 242 amino acid residues [37].

Ferroportin mediates the iron efflux of macrophages, enterocytes and hepatocytes into the plasma, maintaining systemic iron homeostasis. This process is negatively regulated by hepcidin, which promotes the endocytosis of ferroportin and then proteolysis in the lysosomes by induced ubiquitination, making it impossible to move ferritin to the extracellular environment [34, 38, 39].

Systemic Regulation of Hepcidin

The regulation of hepcidin synthesis occurs through several mechanisms such as hypoxia, inflammation and endoplasmic reticulum stress, the concentration of serum iron, and increased erythropoiesis [40, 41].

Balance between Hepcidin and Erythropoiesis

Erythropoiesis is the process by which erythrocyte formation occurs. For this to occur, iron is required for erythroblasts and the production of erythropoietin by renal tissue. The erythroid precursors BFU-E (burst-forming unit-erythroid) and CFU-E (colony-forming unit-erythroid) induce the expression of erythropoietin receptors as well as transferrin receptors. Erythropoietin acts on erythroid progenitors to induce differentiation and erythroid maturation pathways such as the STAT5, phosphoinositol-3-kinase, MAP kinase, and protein kinase C pathways, which remain active throughout the process of maturation and differentiation [42–44].

In situations in which erythropoiesis increases, such as haemolytic anaemia and tissue hypoxia, there is an increase in the transcription of erythropoietin to ensure that new cells are viable. However, increased hepcidin synthesis decreases the iron stock that must be available to the erythroblasts for erythropoiesis to occur. Iron is transported in the circulation bound to transferrin and released to erythroblasts by the interaction between holo-Tf and its TFR1 and TFR2 receptors present in erythroid precursors [45].

Another factor that influences the balance between hepcidin and erythropoietin is hypoxia. During the process of hypoxic stress, formation of the HIF complex (HIF-2α, HNF-4, and ARNT or HIF-β), which activates the transcription of the erythropoietin gene, is highly sensitive to oxygen saturation. Hepcidin mRNA levels decrease and iron stores in macrophages and hepatocytes are available for erythropoiesis [46, 47]. The increased iron demand in response to the erythropoietin stimulus and erythropoiesis response may lead to transient anaemia, decreasing the serum concentration of hepcidin [10, 48].

In a study conducted in healthy volunteers to determine the influence of oxygen on the serum concentration of hepcidin, blood samples were collected from volunteers at sea level and then at high altitudes under hypoxic conditions to evaluate serum levels of hepcidin at sites above sea level [8]. The balance between the serum hepcidin concentration and erythropoietin gene transcription has become an alternative for detection of the use (abuse) of recombinant human erythropoietin among athletes in doping tests [49, 50]. The major erythropoietin deficiency occurs in chronic renal disease, with elevated serum levels of hepcidin [51]. Currently, subcutaneous administration of recombinant human erythropoietin as well as erythropoiesis stimulants are the main alternatives for the treatment of anaemia due to a deficiency in the production of erythrocytes [52, 53].

The erythroferrone hormone (ERFE) was described by Kautz et al. [54]. It is a suppressor of hepcidin during erythropoiesis and is produced by erythroblasts in response to erythropoietin. This phenomenon was demonstrated in rats deficient in $ERFE^{-/-}$, in which even the administration of erythropoietin failed to improve anaemia due to haemorrhage. ERFE decreases hepcidin levels in inflammatory anaemia and improves anaemia in haemodialysis patients [55, 56]. This deficiency causes anaemia due to increased hepcidin, and, ERFE deficiency improves the clinical picture in β-thalassemia with increased hepcidin [57, 58].

Regulation of Hepcidin by Inflammation and Oxidative Stress

Inflammation affects iron homeostasis due to the production of interleukin-1 (IL-1), IL-6 and IL-22, acting on the HAMP gene through the SMAD/STAT3 signalling pathway, and increasing hepcidin transcription [59, 12].

Infusion of IL-6 in human volunteers increased hepcidin levels in urine and decreased the serum iron concentration, resulting in a decrease of approximately 30% in transferrin saturation [60].

Endoplasmic reticulum stress induces the UPR (unfolded protein response) pathway, which is responsible for maintaining cell homeostasis after signs of stress [61]. In the model proposed by Oliveira et al. [62] using HepG2 cells, hepcidin mRNA levels are significantly influenced by the UPR pathway, and the C/EBPα transcription factor is the main modulator of this pathway, acting on the HAMP gene with a subsequent increase in hepcidin mRNA levels.

Regulation of Hepcidin by Iron Concentrations and Anaemia

The levels of hepcidin mRNA are controlled by anaemia and serum iron concentration [17]. BMP6 protein and transferrin 1 and 2 receptors (TfR1; TFR2) in response to iron uptake induce intracellular signalling of the BMP pathway, interacting with the haemojuvelin protein (HJV) and the haemochromatosis protein (HFE) to potentiate the transcription of hepcidin [63, 64]. A decrease in serum iron or an anaemic state induces the transcription of the transmembrane serine protease transmembrane matriptase-2 (TMPRSS6) and the multifunctional transmembrane receptor neogenin, which cleaves haemojuvelin to deactivate the BMP pathway [65–68].

Regulation of Hepcidin by Vitamins

Many studies have demonstrated the influence of vitamins on the serum expression of hepcidin, among which antioxidant vitamins are prominent. Vitamin D has a binding site in the promoter region of the *HAMP* gene, and its deficiency is associated with the development of anaemia in elderly people and in haemodialysis patients [69, 70].

In an in vitro study, Bacchetta et al. [71] demonstrated a decrease in *HAMP* gene transcription mediated by the vitamin D concentration in HepG2 cells and monocytes. This mechanism occurs through the binding of vitamin D in the promoter region, promoting suppression of the *HAMP* gene. Ferroportin mRNA levels were elevated, and ferritin levels decreased. Zughaier et al. [72] demonstrated that the administration of 1,25 dihydroxy vitamin D_3 promotes a decrease in hepcidin, IL-1B, and IL-6 mRNA, improving anaemia resulting from chronic kidney disease.

In a double-blind pilot study by Smith et al. [73], 28 healthy patients were followed for a period of 1 week. The patients were divided into 2 groups, in which a single oral dose of 250,000 IU of vitamin D_3 or placebo was administered. After 1 week, plasma determination of proinflammatory hepcidin, ferritin, and cytokines was performed. A 73% decrease in the concentration of hepcidin was observed in patients treated with vitamin D_3 compared with the control group. Based on these results, vitamin D analogues are being studied as possible suppressors of the HAMP gene in diseases associated with high levels of hepcidin.

Studies using vitamin C have demonstrated an effect on the synthesis of hepcidin. In HepG2 cell cultures, vitamin C decreased hepcidin mRNA levels and increased the activity of erythropoietin and erythropoietin receptors [74]. Vitamin C improves hepatic injury caused by alcohol abuse by modulating hepatic synthesis [75]. In a study conducted by Díaz et al. [76] with athletes supplemented with vitamin C (500 mg/day) and vitamin E (400 IU/day) over a 28-day period, serum hepcidin and iron concentrations remained the same. The authors attributed this result to the inflammatory processes and the increase in IL-6 in athletes due to the high levels of physical activity.

In other studies investigating vitamin A and its relationship with iron metabolism in *Mus musculus* rats treated with retinol palmitate (4,000 IU/kg) for 22 days, the treatment increased the mRNA levels of hepcidin and ferritin, favouring the accumulation of iron in the liver, as well as increased oxidative and inflammatory stress [77–79].

Gene Regulation of Hepcidin

The HAMP gene located on chromosome 19q13 transcribes hepcidin mRNA through 2 signalling pathways. The first signalling pathway is related to bone morphogenetic proteins (BMPs), and the second JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signalling pathway is related to inflammation [3].

BMPs are a group of cytokines that includes transforming growth factor β, and activation of the BMP pathway requires interactions with its cell surface coreceptor haemojuvelin [80]. This interaction induces the phosphorylation of the activated BMP receptor, promoting an intracellular signalling cascade by binding to a threonine/serine kinase type I and type II receptor complex [81]. The activated type II receptor activates the type I receptor, which then transmits the signal to the SMAD regulatory receiver, (R-SMAD), phosphorylating SMAD-1, SMAD-5, and SMAD-8. In this way, the formation of a transcription complex involving the SMAD-4 factor occurs. The activated complex moves to the nucleus to regulate gene

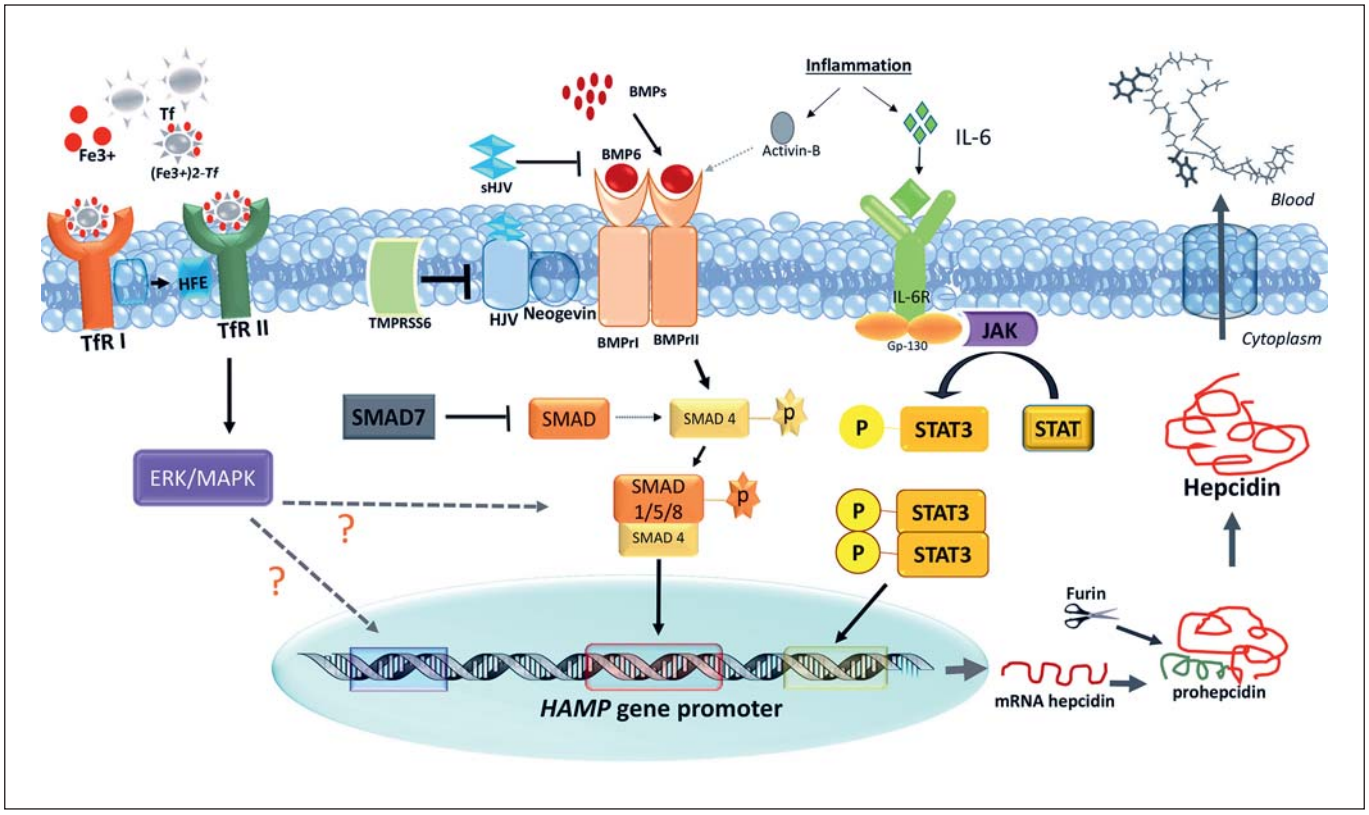

Fig. 2. Regulation of the HAMP gene.

transcription [82]. SMAD-7 acts as a suppressor of hepcidin mRNA levels in the liver [83].

The regulation of the HAMP gene through the JAK/STAT pathway begins when specific ligands act on the JAKs, resulting in a multimerization of their subunits. Erythropoietin and growth hormone associate with the receptor to form a homodimer, whereas inflammatory cytokines and interferons form a heterodimer [84].

IL-6 binds to its receptor, which is formed by 2 subunits: an α-subunit (IL-6-R) and a β-subunit (gp130). When IL-6 binds to IL-6-R, gp130 dimerization occurs, which recruits cytoplasmic JAK to phosphorylate the gp130 protein. After phosphorylation, STAT proteins (STAT1 and STAT3) bind to gp130 and autophosphorylate. Subsequently, they migrate to the nucleus, bind to specific transcription sites of the gene, and promote increased transcription of hepcidin mRNA in hepatocytes [85, 86] (Fig. 2).

Ferroportin: Hepcidin Receptor

Ferroportin is a transmembrane protein that is present in hepatocytes, enterocytes, macrophages, spleen, and bone marrow, which regulates the amount of iron present in the extra- and intracellular medium. Increased transcription of the *HAMP* gene increases the serum levels of hepcidin, which binds to ferroportin in the extracellular medium through its N-terminal portion, promoting its phosphorylation, internalization, and ubiquitination in lysosomal endosomes [4, 87].

De Domenico et al. [207] suggest that the internalization of ferroportin occurs after the binding of JAK2 to tyrosine 302 and 303 residues present in the intracellular monomers of ferroportin. However, Ross et al. [88] showed that this phenomenon does not occur via the JAK2/STAT3 pathway, but is due to a mutation in 2 ferroportin lysine residues, which may be targets of ubiquitination.

Primary Hepcidin-Related Disorders

Primary hepcidin-related disorders refer to changes or genetic mutations in coding genes responsible for iron homeostasis. Tomas Ganz [12] classified disorders related to serum hepcidin concentrations as primary and secondary. Primary changes involve mechanisms

involved in the synthesis and control of hepcidin production, in which an increased serum concentration or total inhibition and/or deficient production of hepcidin may occur.

Decreased Serum Hepcidin

Hepcidin deficiency leads to inadequate absorption and an accumulation of iron in the body. Mutations present in the HFE genes of HJV, TFR2, and SLC40A1 and in the HAMP gene are the causes of hereditary haemochromatosis formation [89–93].

Among the forms of haemochromatosis, juvenile hereditary haemochromatosis predominates, which is associated with the deletion of a guanine at position 93 of the HAMP gene (93delG) and the C → T substitution at position 166 in exon 3 of the cDNA. This substitution encodes a prohepcidin of 179 amino acid residues with mismatched cysteine bridges by altering the prohormone convertase-binding site, yielding a truncated prohepcidin that lacks the complete peptide sequence. This truncation prevents the binding of hepcidin to ferroportin, with a consequent decrease in serum hepcidin [94].

Hattori et al. [95] discovered a mutation in exon 3 of the gene p.R75X in a Japanese patient with juvenile haemochromatosis and without circulating hepcidin, as a consequence of erroneous formation of the prohepcidin molecule. Studies have shown that after liver transplantation in patients with haemochromatosis, the serum concentration of hepcidin returns to its physiological state, improving the clinical picture of the disease [96].

Descriptions of the mutant variants of FPN, Y64N, N144D, and C326Y are associated with the resistance of hepcidin binding to FPN [97]. Mutations present in the C326S residue of the ferroportin SCL40A1 gene produce a severe form of haemochromatosis with excessive iron deposition in early-age tissues due to the loss of hepcidin binding to ferroportin; the thiol form of the C326 residue is essential for the interaction between the 2 molecules and subsequent ubiquitination of ferroportin [98, 99].

Studies of mice homozygous for C326S revealed iron overload and death between 7 and 14 months of age due to oxidative damage caused by iron, weight loss, and pancreatic insufficiency [100]. Thus, iron accumulation occurs mainly in macrophages, liver, spleen, and heart. The N144D and Q248H variants of ferroportin are referred to as "partially resistant to hepcidin," with iron accumulation in tissues, but presenting different characteristics of hereditary haemochromatosis [101, 102].

In a study by Praschberger et al. [103], the D181V and A69T variants were described to functionally characterize the resistance of ferroportin to hepcidin binding. The D181V variant is associated with poor iron exportation, and the A69T variant is less sensitive to low hepcidin concentrations, attenuating the symptoms of ferroportin disease, with elevated iron concentrations in the spleen and liver. In the same study, the authors suggest that the binding of hepcidin to ferroportin occurs during the internalization of iron in the ferrous state.

Excess Hepcidin

Iron-refractory iron deficiency anaemia (IRIDA) is an autosomal recessive disease characterized by microcytic and hypochromic congenital anaemia, low transferrin saturation, low iron concentrations, elevated serum ferritin, and excess hepcidin due to mutations present in the TMPRSS6 gene [104].

The TMPRSS6 gene is highly polymorphic with 17 variants, among which the described polymorphisms are K225E, K253E, G228D, R446W, V736A, and V795I. Of these, polymorphism rs855791, with a substitution of the amino acid alanine for a valine at position 736 in the serine protease domain of matriptase-2 (p.Ala736Val), is the most frequent, being responsible for the effects observed in IRIDA [105, 110]. Mice with the Tmprss6$^{-/-}$ phenotype present severe alopecia and severe iron deficiency anaemia, with elevated serum hepcidin levels and decreased expression of ferroportin [105].

The T287N variant contributes to microcytic anaemia and increases in hepcidin levels due to the inactivation of haemojuvelin cleavage, preventing the binding of haemojuvelin and hepcidin. Thus, it activates the BMP pathway by increasing hepcidin transcription in the HAMP gene [106–111].

Finberg et al. [104] described SNP-like polymorphisms in 5 families and in 2 sporadic cases in the protease region of TMPRSS6, in which the development of IRIDA occurred with a predominance of a small cell, low corpuscular volume, low haemoglobin, and low levels of serum iron and transferrin saturation. No improvement after oral or parenteral iron treatment was observed, or after the administration of recombinant erythropoietin in patients with IRIDA [112, 113].

However, a genome-wide study conducted by Galesloot et al. [114] showed no direct link between HFE and TMPRSS SNPs, based on serum hepcidin levels and plasma iron parameters, suggesting that other variables that influence the transcription of the HAMP gene.

The balance between hepcidin and TMPRSS6 influences certain diseases related to iron homeostasis, such as haemochromatosis, with iron accumulation and hepci-

din deficiency, and anaemia related to chronic disease, with a decrease in serum iron and an increase in plasma hepcidin. Thus, the modulation between both can impact therapeutic targets, either as a TMPRSS6 receptor agonist or as an antagonist.

Hepcidin-Related Secondary Disorders

Secondary disorders related to hepcidin refer to primary pathogens and consequently lead to disturbances in serum hepcidin concentrations and changes in iron metabolism.

Chronic Liver Disease

Hepatocytes produce the major portion of serum hepcidin. Dysregulation of hepcidin expression during chronic liver disease is an aggravating factor in hepatic iron overload, which may cause insulin resistance, fibrogenesis, and increased susceptibility to hepatocellular carcinoma development [115].

Decreased serum hepcidin levels directly reflect the function of hepatic markers, contributing to increased fibrosis, as well as cytotoxic free iron, generating reactive species that damage the phospholipids present in the cell membrane [116]. The accumulation of iron in hepatocytes prevents the binding of transcription factors to DNA, decreasing the hepatic synthesis of hepcidin [117].

In an animal model with non-alcoholic fatty liver disease suggested by Ye et al. [118], serum hepcidin levels decreased with hepatic injury, increased iron and free cholesterol, and haemosiderin deposition in hepatocytes, with no change in transferrin receptors. Decreased hepcidin synthesis leads to impairment of the pathogenesis of alcoholic cirrhosis and autoimmune hepatitis, with increased production of hydrogen peroxide in which alcohol functions as negative regulator of hepcidin [119–123].

Chronic Kidney Disease

The kidney is the major clearance organ of hepcidin. In renal disease, there is a decrease in the glomerular filtration of hepcidin and consequent anaemia [124]. Recent studies have shown that elevated concentrations of hepcidin in the serum of patients with chronic kidney disease contribute to increased serum ferritin, decreased iron availability for erythropoiesis, and low haemoglobin levels [125]. Because renal disease is an inflammatory process, the production of IL-6 and IL-1β maintains an activated JAK/STAT pathway with elevated hepcidin transcription of the HAMP gene, contributing to anaemia via 2 mechanisms: iron restriction and increased inflammation [126–128].

Cancer and Hepcidin

Excess iron in the free ferrous state contributes to cancer mutagenesis, given the formation of reactive oxygen species through the Fenton reaction; these reactive oxygen species interact with the DNA double helix and modify nitrogenous bases [129, 130]. The lack of iron during the development of cancer causes anaemia due to overactivity of the JAK/STAT pathway via IL-6 in Hodgkin lymphoma and activation of the BMP pathway in prostate cancer [131, 132].

Some authors suggest that the increase in hepcidin during cancer is a defence mechanism for iron restriction during the disease and that p53 protein positively regulates hepcidin, binding in the promoter region of the HAMP gene [133]. However, other studies have shown that hepcidin/ferroportin regulation may be an alternative for breast cancer because the reduction of hepcidin and the increase in ferroportin decreases the metastasis of tumour tissue and is an alternative for cancer treatment [134–136].

Anaemia of Inflammation

Anaemia of inflammation (AI) is a moderate to severe anaemia, with haemoglobin levels of approximately 8 g/dL, which develops due to infections, chronic inflammation, and malignant disease. The condition is characterized by low iron concentrations in serum and high levels of ferritin [137]. Increased production of IL-6 and BMP-2 in these diseases stimulates hepcidin synthesis [138]. In addition, the proliferation of erythrocytes is reduced by the direct action of inflammatory cytokines in the progenitors of red blood cells, which activate macrophages to promote erythrophagocytosis, and by the production of erythropoietin [139, 140].

In addition, the elevated production of IL-6 in Crohn disease increases serum levels of hepcidin and decreases haemoglobin, aggravating coronary diseases and favouring the development of atherosclerosis [141, 142]. Anti-IL-6 antibodies are an alternative in inflammatory diseases to decrease the hepcidin production by inhibiting the STAT pathway [143].

Neurodegenerative Diseases and Hepcidin

Iron is a necessary cofactor for metabolic processes of the central nervous system, including oxidative phosphorylation, myelin synthesis, and the production of neurotransmitters, and it contributes to the metabolism of nitric oxide and the transport of oxygen. With aging, iron accumulation occurs in the motor cortex, the prefrontal cortex, sensory cortex, and the thalamus [144]. The iron deposited in these regions contributes to the lipid peroxidation of neural cells

and to the activation of the immune response in the nervous system as one of the aggravating factors of neurodegenerative diseases induced by oxidative stress [145, 146].

In Alzheimer disease, iron is deposited in cortical plaques and in the neurofibrillary tangles. Histochemical analysis of the brain of patients with Alzheimer disease has shown that the concentration of hepcidin and ferroportin is decreased in damaged neurons, with haem group deposition in regions with blood vessel lesions [147]. In rats with iron overload in the central nervous system, treatment with hepcidin showed decreased concentrations of proteins involved in iron metabolism in the brain (TfR1, DMT1, and Fpn1). This is a possible pharmacological alternative for diseases with iron overload in the brain [148].

Diabetes Mellitus Type 2 and Obesity

Diabetes mellitus and obesity have become chronic diseases associated with insulin resistance and mild chronic inflammation. Adipose tissue secretes chemokines, cytokines, and growth factors [149]. Studies have demonstrated a relationship between increased levels of iron stores and diabetes mellitus [150, 151]. The main relationship is activation of the STAT pathway and elevated HAMP gene transcription [152].

The expression of genes involved in the immune response, TNF-α, IL-6, NF-kB, and TLR-2/4, in patients with diabetes and obesity alters the metabolism of glucose and fatty acids, increasing oxidative stress and levels of micronutrients such as iron [153]. Thus, elevated serum concentrations of ferritin and hepcidin lead to insulin resistance in type 2 diabetes mellitus due to the sensitization of peripheral glucose receptors. Current pharmacotherapy with metformin has not demonstrated improvement in hepcidin levels, but hypocaloric diets facilitate the reduction of hepcidin in serum [154, 155].

Hepcidin in Infections

Iron is essential for the development of certain pathogens during infection [156]. Hepcidin is a β-defensin of the immune system that possesses antimicrobial activities [157]. Microorganisms have developed several mechanisms to acquire intra- and extracellular iron; however, as a host response, the organism has mechanisms for iron retention, such as increased iron binding protein production; reduction of dietary iron absorption; increased production of haptoglobin, haemopexin, and haemoglobin; and the release of apolactoferrin from neutrophils to sequester free iron and reduce microbial proliferation [158, 159].

Increased transcription of hepcidin is responsible for the hypoferraemia present in acute or chronic infections. Iron sequestration by macrophages and iron deposition in hepatocytes aggravates viral, bacterial, parasitic, and fungal infections [160–162]. In addition to the development of anaemia associated with inflammation, the pathogens use intracellular iron to replicate, reducing the availability of iron for erythropoiesis [15].

Research conducted with patients with HIV-1, HBV, and/or HCV during the acute phase of the disease has shown increased levels of plasma iron during HIV-1 infection and of serum hepcidin. Hepcidin levels remained elevated in patients in the chronic phase of the disease without the use of HAART, increasing the viral load. This effect was not found in patients with HBV or HCV, indicating that hypoferraemia is pathogen-specific and that the HIV-1 virus utilizes intracellular iron for viral replication [163–166].

Cunha et al. [167] have demonstrated that reduced levels of hepcidin, iron, and ferritin are associated with a reduced number of CD4+ T lymphocytes in HIV-1-infected individuals not using antiretroviral therapy, whereas patients using antiretroviral therapy had a restored immune system and recovered CD4+ T cells with an undetectable viral load. Thus, the HIV-1 virus interferes with serum hepcidin levels, the major regulator of iron metabolism. Experiments in vitro and in mice have demonstrated a role for hepcidin as part of the innate immune system during Gram-positive and Gram-negative bacterial infections [168].

Chen et al. [169] infected hepcidin-deficient rats with *Vibrio vulnificus* bacteria and observed an increase in bacteraemia with decreased animal survival. However, when administering hepcidin agonists in rats, hypoferraemia and bacteraemia control was observed. Thus, based on the contradictory results, the role of hepcidin as an integral part of innate immunity is not yet well understood [170].

Hepcidin: Therapeutic Targets

Modulation of the hepcidin/ferroportin axis is one of the main therapeutic targets for the treatment of diseases related to iron overload or deficiency. In pathologies associated with iron overload, haemochromatosis is prominent. Currently, the treatment is based on iron chelators and bleeding of the patients, but these strategies are not effective. Thus, a stimulus to increase the production of hepcidin or a hepcidin analogue may aid in the treatment of hereditary haemochromatosis. However, IRIDA and iron deficiency anaemia stand out as the main iron deficiency diseases. In this sense, the use of hepcidin inhibitors or TMPRSS6 analogues are effective choices for the treatment of these pathologies.

Hepcidin Agonists

Ramos et al. [171], optimized a prototype of hepcidin containing the amino acid residues DTHFPICIF in its N terminal portion. This amino acid sequence is required to bind to ferroportin and promote its ubiquitination, but it did not present in vivo activity. After molecular improvement, PR65, a minihepcidin, was synthesized. PR65 was administered twice weekly, subcutaneously, in knockout mice (*HAMP*$^{-/-}$), which presented an iron overload and haemochromatosis phenotype. Iron deficiency was observed in cardiac and hepatic tissue, intestinal adsorption and iron retention in the spleen and duodenum. When high doses of PR65 were administered to rats, anaemia was established.

Thus, minihepcidins can be used as an adjunct to diseases with iron overload and ineffective erythropoiesis, such as β-thalassemia and polycythemia vera [172]. Other experiments are being conducted with the aim of increasing the utility of the minihepcidin molecule by improving the ease of its synthesis, reducing costs, and improving its bioavailability [173, 174].

Other alternatives for the treatment of iron overload would be the application of an inhibitor of TMPRSS6, which would increase serum levels of hepcidin, reducing iron overload [175, 176]. The use of siRNA to suppress TMPRSS6 along with chelation therapy is promising, as experimental data have shown that hepcidin levels in β-thalassemia rats increase after applying siRNA [177]. Therefore, modulation of the BMP/SMAD pathway by exogenous BMP6 treatment stimulates transcription of the *HAMP* gene [178].

Hepcidin Antagonists

In inflammatory diseases with high levels of cytokine production, anti-IL-6 antibodies are targeted so the JAK/STAT pathway will not be activated, decreasing serum levels of hepcidin. In a model of collagen-induced arthritis in monkeys, Hashizume et al. [179] demonstrated that a 1-week treatment with an anti-IL-6 receptor antibody, tocilizumab, decreased C-reactive protein (CRP) levels and improved iron deficiency anaemia induced by IL-6 [180, 181].

In a randomized phase II study by Casper et al. [182] including 79 patients with Castleman multicentre disease using siltuximab, haemoglobin levels rose to 15 g/dL after 1 week of treatment. The researchers attributed this increase to the dilution of hepcidin synthesis by blocking the JAK/STAT3 pathway.

The antibody infliximab (anti-TNF-α) has also been shown to be efficient for the treatment of inflammatory anaemia in cancer patients [181]. Inhibition of the STAT3 pathway by AG490 (Calbiochem), by the peptide PpYLKTK, and by curcumin, was observed in rat hepatocyte cell culture, even when stimulated with IL-6, as well as in vivo in rats [183, 184].

Research using L-oligoribonucleotide antihepcidin has demonstrated good results for the treatment of the disease. Schwoebel et al. [185] described the use of Spiegelmer NOX-H94, an L-oligoribonucleotide that binds to hepcidin, preventing the degradation of ferroportin. In the experiment, monkeys were used in an IL-6-induced inflammation amenity model. These animals were treated with intravenous NOX-H94 and showed significant improvements in anaemia and increased serum iron concentrations.

In a randomized study of healthy volunteers, in which endotoxaemia was induced from *Escherichia coli* lipopolysaccharides after induction of inflammation, volunteers received an intravenous dose of Spiegelmer lexaptepid, and then biochemical parameters were determined, among which the group that received Spiegelmer lexaptepid presented serum iron levels within normal ranges with low plasma concentrations of hepcidin [185]. Spiegelmers are specific, have demonstrated good safety and efficacy results in healthy patients, and are promising targets for the treatment of anaemia [187].

In an in vivo rat study, Mayeur et al. [188] demonstrated that oral administration of a BMP type I receptor inhibitor, LDN-193189, which inhibits BMP/SMAD signalling in hepatic tissue, reduced expression of the *HAMP* gene, improving anaemia in these animals. The administration of LDN193189 facilitated the movement of iron stores in rats with AI and increased haemoglobin levels, favouring erythropoiesis and reticulocytosis [189, 190].

Hepcidin is regulated by ALK2 (activin-like receptor), which is a receptor for BMPs [191]. TP-0184 is an inhibitor of the ALK2 receptor, and in preclinical phase studies, it has been shown to be a negative regulator of hepcidin expression. The monoclonal antibodies ABT-207 and h5F9-AM8, which inhibit haemojuvelin, have been shown to inhibit hepcidin expression in rats [192].

Sclerostin, a domain-containing protein (SOSTDC1), is an antagonist of the BMP pathway and has been shown to negatively regulate hepcidin in prostate cancer cells. SOSTDC1 antagonizes BMP signalling by sequestering BMP-like linkers, similar to sHJV [132, 193]. The soluble portion of HJV was fused to the Fc portion of an IgG, resulting in sHJV.Fc, which binds to BMPr, inhibiting the transcription of the HAMP gene via the BMP6/SMAD pathway [194].

Hepcidin/ferroportin binding has become one of the major therapeutic targets; it has been identified that the thiol form of Cys326 in ferroportin is required for binding to hepcidin. Thus, fursultiamine prevents the interaction between ferroportin-hepcidin by sequestering the Cys326-HS residue and blocking the internalization of hepcidin ferroportin [195]. Another humanized antibody was developed for the same purpose, LY2928057, which is currently in phase I clinical trials of haemodialysis patients [193].

Antihepcidin antibodies have been described and purified by Sasu et al. [196]. Their efficacy was demonstrated in an AI model induced by *Brucella abortus*, in which hepcidin neutralization was observed and anaemia improved. The humanized antibody, 12B9m, has been shown to be a potent hepcidin inhibitor [197, 198].

The first evidence for the ability of heparin to control hepcidin expression was demonstrated by Poli et al. [199] in HepG2 cells and in rats provided with pharmacological doses of heparin. In that study, hepcidin mRNA expression decreased following BMP-6 protein sequestration and subsequent phosphorylation of the SMAD1/5/8 complex, with a reduction of the iron concentration in the spleen and increased serum iron. Based on these results, structural modifications in the heparin molecule were performed to decrease its anticoagulant activity for clinical use to treat disease with elevations in hepcidin levels. In clinical trials, modified heparins, by oxidation/reduction, glycol-split heparins (GS-heparin), or sulphation (SS-heparins), demonstrated little or no toxicity as potent inhibitors of hepcidin in vitro in HepG2 cells and primary hepatocytes, and in vivo in rats, even without presenting anticoagulant activity [200, 201].

Completely humanized LY2787106 is currently in phase I trials for the treatment of cancer-related anaemia. Proteins that block hepcidin were obtained by modifying lipocalins, which are natural proteins that bind cell surface receptors [202]. Anticalin SPR-080, which exhibits subnanomolar affinity for human hepcidin, when administered in monkeys, has been shown to be efficient for mobilizing iron. Studies are currently underway to examine the safety and in vivo tolerability of SPR-080 anticalin for the treatment of AI [203].

Growth differentiation factor 15 has been shown to negatively regulate hepcidin mRNA expression in humans [204]. Fujiwara et al. [205] has described the activity of compound K7174 in HepG2 cells and rats, in which an elevation of growth differentiation factor 15 and reduced expression of hepcidin were observed. The ERFE erythroid hormone has shown suppressive activity towards hepcidin mRNA in a rat model of β-thalassemia, positioning it as a new alternative for the treatment of diseases associated with hepcidin plasma elevations [58].

Interfering microRNAs (siRNAs) negatively regulate gene expression through sequence complementarity and are used to block the synthesis of proteins that cause disease [206]. During the negative regulation of hepcidin synthesis, siHJV, siTRF2, and siHepcidin are key participants. In this way, suppression of the gene occurs, with inhibition of hepcidin synthesis.

Perspectives

The molecules involved in iron metabolism are the main targets for new drugs. However, despite favourable results, the studies are in phase I or II trials, and the number of volunteers for clinical trials has diminished. The precise adverse reactions associated with such drug candidates in a clinical trial with large numbers of individuals are not yet known. Therefore, even if the proposed alternatives are feasible, there is a need for continuous research in the field of iron homeostasis.

The main molecular target of hepcidin in iron metabolism is the internalization of ferroportin. However, the role of hepcidin in innate immunity is known. The modulation of hepcidin synthesis pathways is important as a therapeutic target in the development of agonists and antagonists that specifically interact at their binding sites to minimize adverse effects. Studies and research are needed to clarify such signalling pathways and their respective regulation for the treatment of pathogenetic mechanisms related to iron metabolism.

Conclusion

Since the discovery of hepcidin, its mechanism of action has been elucidated as a hormone that regulates iron metabolism; however, many studies have described its activity in several pathologies. Some cases have presented beneficial results, whereas others have indicated a poor prognosis. Further studies describing the mechanism of action of hepcidin in diseases are needed to develop drugs with specific activity.

Disclosure Statement

The authors state that there are no conflicts of interest in the publication of this article.

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