P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2021; 9(1): 2469-2474

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Received: 01-10-2020

Accepted: 04-11-2020

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International Journal of Chemical Studies

Hepcidin: An emerging hormone in iron homeostasis: A review

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DOI: <https://doi.org/10.22271/chemi.2021.v9.i1ai.11600>

Abstract

Hepcidin, is a peptide hormone, which is a key regulator of systemic iron homeostasis and its unbalanced production contributes to iron disorders is derived from liver. Hepcidin was discovered by Krause and coworkers in the year 2000. The name 'Hepcidin' was given, from the place of synthesis in liver hepatocytes (hep-) and its antimicrobial activity (-cidin). The gene encoding hepcidin is expressed in various organs like liver, heart, lungs, brain, spinal cord, intestine, stomach, pancreas, adipocytes, skeletal muscles, testis and macrophages.

Hepcidin is a 25 amino acid peptide hormone which inhibits entry of iron into the plasma compartment from the three main sources of iron: dietary absorption in the duodenum, the release of recycled iron from macrophages and the release of stored iron from hepatocytes. This blocking of iron flow is achieved by Hepcidin functions by causing degradation of iron receptor, via an iron transporter ferroportin. Hepcidin production is tightly regulated by (1) increased plasma and liver iron as a feedback mechanism to maintain stable body iron levels, (2) decreased by erythroid activity to ensure iron supply for erythropoiesis and (3) increased by inflammation as a host defense mechanism to limit extracellular iron availability to microbes. Hepcidin levels reflect the integration of signals involved in iron regulation and it directly controls iron absorption and bioavailability in circulation. Its measurement is a useful clinical tool for the management of iron disorders in the body. Recent evidence shows that the deficiency of hepcidin may cause iron overloading even in the much milder common form of hemochromatosis, from mutations in the *HFE* gene. The discovery of hepcidin and its role in iron metabolism could lead to new therapies for hemochromatosis and anemia of inflammation.

Keywords: Hepcidin, role, iron metabolism, anemia

Introduction

Hepcidin was first discovered in human blood ultrafiltrate and urine samples as a small bactericidal peptide (defensin and cathelicidin) and named liver-expressed antimicrobial peptide (LEAP-1). The name 'hepcidin' originates from the place of synthesis in hepatocytes (hep-) and its antimicrobial activity (-cidin). The gene encoding hepcidin (*HAMP*, 19q13) is expressed in the liver, heart, lungs, brain, spinal cord, intestine, stomach, pancreas, adipocytes, skeletal muscles, testis and macrophages. The hepcidin genes have been also found in mice, pigs, birds and fish. The antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp. group B) and antifungal activity (*Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*) is one of the important property of Hepcidin. This protein is a key regulator of iron level; it decreases the iron absorption from the duodenal enterocytes, iron release from macrophages and its transport across the placenta (Bansal *et al.*, 2009) [4]. Hepcidin production is regulated by (1) Increased plasma and liver iron as a feedback mechanism to maintain stable body iron levels, (2) Decreased by erythroid activity by ensuring iron supply for erythropoiesis (3) Increased by inflammation as a host defense mechanism to limit extracellular iron availability to microbes (Girelli *et al.*, 2016) [11]. The main role of hepcidin in iron metabolism was confirmed on animal models and *in vitro* studies. The synthesis of hepcidin in hepatocytes can be regulated by iron overload, inflammatory signals, increased erythropoiesis, hypoxia and anemia (Ruivard *et al.*, 2009) [22]. Human hepcidin, a 25-amino acid peptide made by hepatocytes, may be a new mediator of innate immunity and the long-sought iron-regulatory hormone.

The synthesis of hepcidin is greatly stimulated by inflammation or by iron overload. Evidence from transgenic mouse models indicates that hepcidin is the predominant negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages. The key role of hepcidin is confirmed by the presence of nonsense mutations in the hepcidin gene, homozygous in the affected members, in 2 families with severe juvenile hemochromatosis. The discovery of hepcidin and its role in iron metabolism could lead to new therapies for hemochromatosis and anemia of inflammation. (Ganz and Nemeth, 2012) ^[7]. Iron is an essential trace metal involved in oxygen transport, cellular metabolism, DNA synthesis, innate immunity, growth, and development. The ability of iron to cycle between 2 stable oxidation states, ferrous iron [iron (II) or Fe^{2+}] and ferric iron [iron (III) or Fe^{3+}], equips iron to participate in a wide array of biochemical processes. The delivery of iron to every cell in the body depends on circulating iron, bound to the plasma protein transferrin. Therefore, the rate of iron entry into the circulation is proportional to the amount of ferroportin on iron-exporting cells. The key iron-regulatory hormone, hepcidin (HAMP or HEPC), controls systemic iron homeostasis through its ability to regulate ferroportin, its cognate receptor (Sangkhae, and Nemeth, 2017) ^[23], negatively.

Aim

The aim of this review is to refresh the knowledge regarding new hormone Hepcidin for the regulation and maintenance of iron in Physiological system.

Hepcidin

The Hepcidin was discovered by Krause and coworkers in the year 2000. Hepcidin, a small peptide secreted mainly by the liver, plays a central role in iron status regulation. The experiments on Hepcidin seemed very promising and gave new life to understanding iron metabolism (Jordan *et al.*, 2009) ^[15].

Hepcidin is a 25 amino acid peptide hormone that inhibits iron entry into the plasma compartment from the three main sources of iron: dietary absorption in the duodenum, the release of recycled iron from macrophages and the release of stored iron from hepatocytes. Multiple signals reflecting systemic iron stores and concentrations, erythropoietic activity and host defense converge to regulate Hepcidin production and thereby affect iron homeostasis. Hepatocytes have evolved as the predominant producers of the iron-regulatory hormone Hepcidin, perhaps because of their location astride the portal venous system that delivers iron absorbed in the intestine, because of their involvement in iron storage, or because of their proximity to kupffer cells that sense pathogens and recycle erythrocytes. The production of Hepcidin is regulated by iron, so that more Hepcidin is produced by hepatocytes when iron is abundant, limiting further iron absorption and release from stores. When iron is deficient, hepatocytes produce less or no Hepcidin, allowing more iron to enter plasma. Both diferric plasma transferrin and stored iron in hepatocytes can stimulate hepcidin synthesis, by distinct mechanisms (Ramos *et al.*, 2011) ^[20].

In addition to iron, Hepcidin is homeostatically regulated by the erythropoietic requirement for iron. During active erythropoiesis the production of Hepcidin is suppressed,

making more iron available for hemoglobin synthesis. Apart from hepatocytes which are the main source of circulating Hepcidin, other cell types such as macrophages and adipocytes express Hepcidin mRNA, but at a much lower level.

The human Hepcidin gene (HAMP) is located on chromosome 19q13.1. It is 2637 base pairs long and composed of three exons and two introns. It contains binding sites for such regulatory factors as HNF3 β , C/EBP β and NF- κ B. HAMP gene expression was detected mainly in the liver, but also in heart, brain, lung, prostate gland, tonsils, salivary gland and trachea. HAMP encodes a precursor of Hepcidin – preprohepcidin, which is 84 amino acids protein comprised 24 aa leader peptide at the N- terminal, a 35 aa proregion, and the C- terminal 20 or 25 aa mature peptide. Preprohepcidin is cleaved to 60 aa prohepcidin which is further amino- terminally processed and gives rise to Hepcidin. There are three forms of Hepcidin with 25 aa, 22 aa and 20 aa peptide. All three forms are detectable in urine, but only hepcidin- 25 and hepcidin- 20 are present in human serum (Kemna *et al.*, 2008) ^[17].

The structure of hepcidin- 25, which is a major form of Hepcidin, contains eight cysteine residues connected by disulfide bonds. Analysis of Hepcidin structure by NMR spectroscopy showed that this peptide forms a simple hairpin stabilized by four disulfide bonds between the two anti- parallel strands. Unusual vicinal disulfide bridge found at the turn of the hairpin probably plays significant functional role.

Fig 1: Structure of Hepcidin (Kemna *et al.*, 2008) ^[17]

Mechanisms of Hepcidin action and regulation

Hepcidin is a well known iron- regulatory hormone. Generally, it causes a decrease in serum iron concentration by regulating its absorption. The mechanism of Hepcidin activity depends on its interactions with Ferroportin. A mammalian cellular iron exporter, which is expressed on the surface of reticulo- endothelial macrophages, hepatocytes, duodenal enterocytes and placenta cells. Hepcidin regulates post-translational Ferroportin expression. Hepcidin binds to Ferroportin and causes its internalization and degradation in Endolysosomes, what in turn blocks the iron transport via Ferroportin. When iron stores are adequate or high, increased Hepcidin expression inhibits intestinal iron absorption, release of recycled iron from macrophages and its transport across the placenta. On the other hand, when iron stores are low, Hepcidin production is suppressed. By modulating Hepcidin expression, organism can control plasma iron level and maintain iron metabolism homeostasis (Atanasiu *et al.*, 2007, Camaschella, 2013) ^[3, 5].

Fig 2: Regulation of systemic iron homeostasis by Hepcidin (Sangkhae, and Nemeth, 2017) ^[23]

Positive and negative regulators of hepcidin

Hepcidin is important iron regulatory hormone, which is responsible for the regulation of systemic iron homeostasis. The regulation of this master iron regulator is complex, with a number of positive and negative regulators. This is probably

because of its wide-ranging roles in modulating iron availability for various biological processes and in different disease states. Some of the main regulatory pathways involving iron, inflammation, erythropoiesis, hypoxia, growth factors and hormones have been studied and the mechanisms of regulation deciphered. However, there are likely to be many more additional regulatory factors and pathways that will be uncovered in the future and cement the place of this small molecule as a major player in many homeostatic processes (Gautam *et al.*, 2015). Disturbance in the regulation of hepcidin production disturbed the iron homeostasis may leads to iron disorders like infection and inflammation, hypoxia and erythropoiesis.

Hepcidin deficiency causes iron overload in certain diseases like hemochromatosis, non-transfused β -thalassemia, whereas overproduction of hepcidin is associated with iron-restricted anemias seen in patients with chronic inflammatory diseases and inherited iron-refractory iron-deficiency anemia (Roth *et al.*, 2019) ^[21]. Type I hemochromatosis, the most common form of hereditary hemochromatosis, is caused by loss-of-function mutations in the Hfe gene. HFE protein regulates hepcidin expression in response to increased extracellular iron. Loss of Hfe decreases hepcidin expression, increases iron absorption and extracellular iron concentrations, and releases iron from macrophages (Michels *et al.*, 2015) ^[19].

concentrations increase hepcidin transcription, as in inflammation, whereas increased erythropoietic activity suppresses hepcidin production. In turn, hepcidin regulates plasma iron concentrations by controlling ferroportin concentrations on iron-exporting cells including duodenal enterocytes, recycling macrophages of the spleen and liver, and hepatocytes (Sangkhae, and Nemeth, 2017) [23].

Iron storage

In response to the poor bio availability of iron in many environments, humans and animals have evolved highly efficient mechanisms for iron conservation. The daily losses of iron, 1–2 mg in adults, represent less than 0.1% of the 3–4 g of total iron in the human body, and must be replaced from dietary sources to maintain iron balance. Daily dietary iron requirements are about 8 mg for adult men and 18 mg for adult women with menstrual iron losses (Trumbo *et al.*, 2001) [24]. Non-menstrual iron losses occur predominantly through desquamation of epithelial cells in the intestine and the skin, and through minor bleeding. Importantly, the losses of iron cannot substantially increase through physiologic mechanisms hemoglobin of red cells which contain about 1 mg of iron per ml of erythrocytes, or about 2–3 g of iron total. In contrast, blood plasma contains only 2–3 mg of iron, bound to transferrin, the plasma iron carrier that is the exclusive source of iron for erythropoiesis.

The life span of human erythrocytes is about 120 days, so every day the oldest 1/120 of erythrocytes is degraded by macrophages and their iron content is returned to plasma transferring (Ganz and Nemeth, 2012) [7]. The recycling of erythrocytes generates a stream of 20–25 mg of iron a day, causing plasma iron to turn over every two hours or so.

Fig 4: Hepcidin has a central role in maintenance of iron homeostasis (Ganz, 2003) [8].

The iron-transferrin is mostly destined for erythrocyte production in the bone marrow. Other cells contain and require much less iron, and some are able to utilize non-transferrin bound iron as well. In the average adult male, about 1 g of iron is held in storage mostly in the hepatocytes and macrophages in the liver and red pulp macrophages in the spleen but the amount stored is much lower in most women of reproductive age, in part due to blood losses from menstruation and parturition. Hepatocyte and macrophage iron is stored in cytoplasmic ferritin and is readily mobilized during period of high iron demand.

Species variation in iron metabolism

Other vertebrate species have similar distribution of body iron, but the relative proportion of daily iron absorption,

recycling and losses may differ from those observed in humans. These variations are of particular importance in species used as models for studying iron metabolism. In laboratory mice, for example, dietary iron absorption and losses seem to be proportionally far greater than those in humans. The average life span of mouse erythrocytes is close to 40 days and the adult mouse has about 0.6–1 ml of packed erythrocytes (assuming blood volume of 7% of 20–30 g adult mouse weight and hematocrit of 45–50%) or about 0.6–1 mg of iron in hemoglobin. Each day about 15–25 µg of iron is recycled and used for the production of new RBCs. Regarding the daily losses, when mice are placed on iron-deficient diet (~4 ppm Fe) for 2 weeks, at least 200–250 µg Fe is depleted from their iron stores (Ramos *et al.* 2011) [20], indicating that they normally lose ~15–20 µg Fe per day, i.e. an amount similar to their daily erythropoiesis needs. Thus on an iron-sufficient diet, similarly high amount of iron will be absorbed each day. In contrast, daily iron absorption and losses in humans represent 5–10% of the iron recycling amount. Furthermore, standard mouse chow has high iron content (about 350 ppm or about ten times the daily dietary requirement), and leads to significant iron loading even in healthy mice, potentially confounding studies of iron regulation. Therefore, studies in animal models of iron homeostasis and its disorders need to consider the species differences in iron stores and fluxes, and take into account the disproportionately strong effect of diet on iron homeostasis in mice.

Impaired iron homeostasis

Both iron deficiency and iron excess cause cellular and organ dysfunction. Low plasma iron concentrations (hypoferremia) restrict iron uptake by erythrocyte precursors, limiting hemoglobin synthesis and causing anemia. In nonerythroid cell types, the synthesis of other ferroproteins may be compromised affecting muscle performance and the maintenance of epithelia undergoing rapid turnover. At the other extreme, high plasma iron concentrations that exceed the iron-binding capacity of transferrin generate complexes with other plasma proteins as well as with organic anions such as citrate. The non-transferrin bound iron (NTBI) is avidly taken up by hepatocytes and other parenchymal cells by as yet poorly understood pathways.

In humans, rapid and excessive accumulation of intracellular iron causes cell and tissue damage, presumably by iron-catalyzed generation of reactive oxygen species, with specific tissue toxicities dependent on both the rate and the extent of iron accumulation. Cardiac and endocrine tissue damage is characteristic of rapid iron accumulation while slower iron accumulation predominantly targets hepatocytes. Laboratory rodent models, with the exception of the Mongolian gerbil appear resistant to iron toxicity (Kaiser *et al.*, 2009, Ambachew and Biadgo, 2017) [16, 1].

Iron regulation and host defense

Iron is essential for nearly all microbes, and microbial pathogens utilize multiple and often complex iron uptake mechanisms to obtain it. Disruption of these uptake mechanisms attenuates microbial viability and pathogenicity (Weinberg, 2009, Hernik *et al.*, 2019) [26, 13]. Multicellular hosts limit iron availability to microbes by coupling it to protein carriers (e.g. ferritin, transferrin, lactoferrin, ovotransferrin) or utilizing it in ferroproteins, all forms of iron not readily accessible to most invading microbes. Further targeting microbial vulnerability to iron deprivation, the host

rapidly responds to microbial invasion by decreasing total iron concentration in extracellular fluids, presumably to slow down microbial proliferation. The response appears to be evolutionarily ancient as infection-related mechanisms of iron sequestration have been described not only in humans, mice and other vertebrates but also in invertebrates including echinoderms. As will become clear, the molecular mechanisms of these responses are closely tied to homeostatic iron regulation (Micheles *et al.*, 2015) [19].

Regulation of Hepcidin production

Like other hormones, Hepcidin is feedback-regulated by the substance whose concentration it controls, iron. In principle, the feedback requires molecules that function as intracellular or extracellular iron sensors coupled to one or more transduction pathways that regulate Hepcidin synthesis or secretion by hepatocytes. Genetic and biochemical evidence suggests that the two transferring receptors, Tfr1 and Tfr2, together with the membrane protein HFE that interacts with both receptors, may serve the function of Holotransferrin (Diferric transferring) sensors (Gao *et al.*, 2009) [9]. Hepcidin levels reflect the integration of multiple key signals involved in iron regulation and Hepcidin directly controls iron absorption and bioavailability in circulation, its measurement should be a useful clinical tool for the management of iron disorders (Girelli *et al.*, 2016) [11].

HFE is structurally related to MHC class I molecules. Its binding to Tfr1 is competitively inhibited by holotransferrin. With increasing concentrations of holotransferrin, HFE is displaced from the complex with Tfr1, as the binding site of HFE overlaps with that of holotransferrin. Free HFE interacts with Tfr2, which itself is stabilized by holotransferrin binding. The FeTf/HFE/Tfr2 complex then stimulates hepcidin expression.

The Bone Morphogenetic Proteins (BMP) pathway

The BMP pathway with its canonical signaling system utilizing cytoplasmic Smads is the key pathway for the regulation of hepcidin transcription. The BMP pathway regulates many other processes, including embryonic morphogenesis, bone development and remodeling and tissue repair. In the liver, this pathway appears to have been specifically adapted for iron regulation through a combination of factors, including a membrane anchored coreceptor hemojuvelin and, in mice, an iron-specific ligand BMP6 (Meynard *et al.*, 2009) [18].

Hemojuvelin is not required for other, iron-unrelated BMP functions, and the nonredundant function of BMP6 in bone development appears to be minor. In contrast, BMP6 and hemojuvelin are essential for normal iron homeostasis in mice as their loss ablates the hepcidin response to acute iron loading and impairs the response to chronic iron loading (Andriopoulos *et al.*, 2009) [2].

BMPs other than BMP6, including BMP2, 4, 5, 7, 9, are also able to induce hepcidin expression *in vitro*, but their physiological role in iron regulation remains to be determined. Along with stimulation of hepcidin synthesis by holotransferrin, hepatocytes can also increase hepcidin synthesis in response to stored intracellular iron.

Iron-dependent ubiquitin ligases and prolyl hydroxylases are candidates because of their dependence on iron and their association with hypoxia-related regulatory processes. In contrast, iron regulatory proteins IRP1 and IRP2 involved in posttranscriptional regulation of many iron- and erythrocyte-

related proteins do not directly regulate hepcidin (Hentze *et al.*, 2010) [12].

Hepcidin Clearance

It is found in Recent studies that hepcidin may circulates in association with $\alpha 2$ - macroglobulin in blood plasma, but the affinity of hepcidin with binding protein is relatively low (about 200–300 nM) so that a substantial proportion of hepcidin will be unbound at physiologic concentrations. Due to its small size (2.7 kD), hepcidin readily passes through the glomerular membrane but like other small proteins is then taken up and degraded in the proximal tubule. A small fraction of the filtered hepcidin passes intact into urine where it is readily detectable. Chronic kidney diseases impair the clearance of hepcidin leading to its accumulation in pasma where it may contribute to iron sequestration in macrophages and limit the availability of iron for erythropoiesis. This mechanism may contribute significantly to anemia of chronic kidney diseases. Hepcidin is efficiently cleared during hemodialysis suggesting that iron utilization could be improved by more frequent or more effective hemodialysis (Zaritsky *et al.*, 2010) [27].

Regulation of Hepcidin-independent ferroportin

Although hepcidin has only been found in vertebrates (Hilton *et al.*, 2008) [14], ferroportin is present already in simple invertebrates such *C. elegans*, as well as in plants. Invertebrate ferroportins generally lack the extracellular cysteine (C326 in humans) shown to be essential for hepcidin binding, indicating that the two molecules coevolved to interact. Consistent with its earlier evolutionary history, ferroportin expression is also regulated in hepcidin-independent cellautonomous manner by heme and iron (Delaby *et al.*, 2008) [6].

Conclusion

From the above study it is concluded that the Hepcidin is an important hormone for the regulation of the iron metabolism and host defense mechanism.

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