Clinica Chimica Acta 412 (2011) 823–830

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Hepcidin: A novel peptide hormone regulating iron metabolism

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ARTICLE INFO

Article history:
Received 29 December 2010
Received in revised form 6 February 2011
Accepted 8 February 2011
Available online 17 February 2011

Keywords:
Hepcidin
Iron metabolism
Hereditary hemochromatosis
Anemia of chronic disease
Anemia of cancer
Rheumatoid arthritis

ABSTRACT

Background: Hepcidin is a low-molecular weight hepatic peptide regulating iron homeostasis. Hepcidin inhibits the cellular efflux of iron by binding to, and inducing the internalization and degradation of, ferroportin, the exclusive iron exporter in iron-transporting cells. It has been recently recognized as a main hormone behind anemia of chronic disease.

Method: A comprehensive literature search was conducted from the websites of Pubmed Central, the US National Library of Medicine's digital archive of life sciences literature (http://www.pubmedcentral.nih.gov/) and the National Library of Medicine (http://www.ncbl.nlm.nih.gov). The data was also assessed from journals and books that published relevant articles in this field.

Result: Hepcidin regulates iron uptake constantly on a daily basis, to maintain sufficient iron stores for erythropoiesis. Hepcidin, by its iron regulatory action on iron metabolism may be expected to have an important role in immune regulation, inflammatory diseases and malignancies. Hepcidin is the underlying cause of anemia in these clinical settings.

Conclusion: Hepcidin analysis may prove to be a novel tool for differential diagnosis and monitoring of disorders of iron metabolism, and establishment of therapeutic measures in various disease conditions like hereditary hemochromatosis, anemia associated with chronic kidney disease, rheumatoid arthritis and cancers.

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doi:10.1016/j.cca.2011.02.014

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1. Introduction

Hepcidin is a recently discovered low-molecular-weight hepatic peptide that plays an important role in iron metabolism. Iron is an essential element and its correct balance is necessary for good health and normal cellular functioning [1]. In recent times hepcidin has been recognized as the main hormone behind the pathogenesis of anemia of chronic disease.

2. Hepcidin: A peptide hormone

Hepcidin is an antimicrobial peptide hormone produced by the liver in response to inflammatory stimuli and iron overload. Hepcidin was first discovered in human urine and serum and later the studies done on the mice models led to most of the information on its structure, function expression and regulation. Originally hepcidin was isolated from plasma ultrafiltrate [2] and was called liver-expressed antimicrobial peptide (LEAP-1). Later it was isolated from human urine and was named hepatic antimicrobial peptide (HAMP). Now it is known as 'hepcidin' because of its hepatic origin and bactericidal effect *in vitro* [3]. This newly discovered peptide has been found to be regulated by inflammation, iron stores, [4] hypoxia and anemia [5].

Hepcidin was discovered inadvertently when hepcidin gene was knocked out in a group of mice and they were noticed to develop iron overload. In contrast when hepcidin was over expressed, mouse fetuses died *in utero* because they developed severe hypoferremia thus it indicates that hepcidin may be involved in maternal–fetal iron transport across the placenta [6,7]. Hepcidin is thought to be the primary regulator of iron homeostasis whose production is mainly controlled by the erythropoietic activity of the bone-marrow, the body iron stores, and presence of inflammation in the body. It is also proved to be a type II acute phase protein [8].

3. Hepcidin: Structure

Hepcidin exists as precursor protein, a full-length preprohepcidin which comprises 84 amino acids (aa). Subsequent to the enzymatic cleavage at the C-terminus, 64 aa long pro-hepcidin peptide is exported from cytoplasm into the lumen of endoplasmic reticulum followed by removal of a 39 aa pro-region peptide by a furin-like proprotein convertase [2,3]. The 25 amino acid form is the mature bioactive hepcidin.

The mature hepcidin molecule exists as a simple hairpin structure with disulfide bridges linking the two arms in a ladder like configuration. Structural analysis of hepcidin by NMR spectroscopy has revealed that there are four disulfide bonds present between the cysteine molecules in the mature hepcidin. The hairpin loop has an indistinct β-sheet structure steadied by four disulfide bonds between the two anti-parallel strands (Fig. 1). An unusual feature is the presence of a cysteine bridge between two adjacent cysteines near the turn of the hairpin that might be acting as a vital domain in the activity of the molecule [9]. Like other antimicrobial peptides, hepcidin displays spatial separation of its positively charged hydrophilic side chains from the hydrophobic ones, a characteristic of peptides that disrupt bacterial membranes.

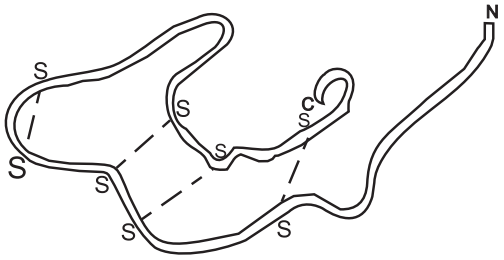

Fig. 1. Schematic diagram of the structure of hepcidin showing 4 disulfide bonds.

Although the predominant form of hepcidin in human urine is the 25 amino acid (aa) long (hepcidin-25), two peptides shorter at the amino terminus have also been found, hepcidin-22 and hepcidin-20 [3]. These isoforms are truncated at the N-terminus of hepcidin-25. Both 25 aa and 20 aa forms are detectable in human serum and urine while the 22 aa isoform has been identified only in the urine [10] suggesting that it may be a urinary degradation product of hepcidin-25 [11]. Recent studies have shown that the iron regulating bioactivity is almost exclusively due to the 25 aa peptide, signifying that the five N-terminal amino acids are essential for this activity [12,13]. The 25 aa form has been proved to have both antibacterial [3] and antifungal activities [2] making hepcidin a member of the family of cysteine rich, cationic, antimicrobial peptides (AMP) which includes the defensins and cathelicidins [14] and is responsible for providing first-line of defense at mucosal barriers [2,3].

4. Hepcidin: Genetics

Human hepcidin is encoded by a 0.4-kilobase (kb) mRNA generated from 3 exons of a 2.5-kb gene on chromosome 19q13.1. In humans, *HAMP* is the gene that encodes for hepcidin. The development of severe iron overload by knocking out the gene in mice suggested that hepcidin is involved in iron metabolism [6]. Animal models of iron overload include mice deficient in Upstream factor 2 (*Usf2*) that do not express the antimicrobial peptide hepcidin [6]. Constitutive overexpression of hepcidin in *HAMP* transgenic mice leads to iron-deficient anemia [15]. These findings indicate a key role for hepcidin in the regulation of iron absorption in mammals and make *HAMP* a functional candidate for association with juvenile hereditary hemochromatosis that is not linked to 1q.

5. Maintenance of normal iron homeostasis in the body

During absorption dietary non heme iron (Fe^{3+}) is reduced to Fe^{2+} by ferric oxidoreductase "duodenal cytochrome B" (DcytB) for transport across apical brush border. Iron is absorbed through the transporter DMT1 (divalent metal transporter 1) also called Nramp2 (natural-resistance-associated macrophage protein 2) [16,17] present in apical intestinal epithelial cells. DMT1 mediates transport of non-transferrin bound iron (NTBI) along with other divalent metals (Zn^{2+} and Mn^{2+}). This is a proton dependant Fe^{2+} import; therefore conversion of Fe^{3+} to Fe^{2+} is necessary. In enterocytes, iron can be stored in ferritin or moves to the basolateral surface of the cell from where it is transported out by the iron exporter ferroportin. It is reoxidized by hephaestin which is a homolog of serum multi-copper oxidase ceruloplasmin that oxidizes Fe^{2+} to Fe^{3+} and facilitates incorporation of iron into transferrin. Iron is then collected by transferrin for distribution to tissues [18]. Ferroportin is a 571 amino acid protein that is present in basolateral membrane of enterocytes and macrophages and is involved in iron-recycling in senescent erythrocytes and reticuloendothelial macrophages. The export is linked to a ferroxidase. Iron in RBCs is phagocytosed by macrophages during