Hepcidin

The Key Regulator of Iron in the Blood

St. Dominic Middle School SMART Team

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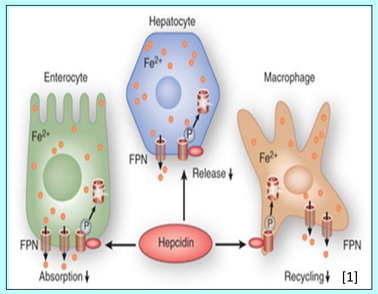
Abstract

Hepcidin, a peptide hormone, is the key regulator of plasma iron levels in humans, and is known to play an important role in various human diseases, such as hemochromatosis. Hepcidin inhibits the entry of iron into circulation by binding to ferroportin, a trans-membrane iron export channel found primarily on enterocytes, hepatocytes and macrophages where iron is sequestered. When hepcidin binds to ferroportin, both are drawn into the cell by endocytosis and degraded in a lysosome. When hepcidin levels increase, ferroportin levels on cells decrease and iron cannot be released from cells into the blood. Hepcidin production by the liver is affected by erythropoiesis in bone marrow, blood oxygenation, certain inflammatory cytokines, intracellular iron storage, and plasma transferrin. The St. Dominic SMART Team (Students Modeling A Research Topic) has modeled hepcidin using 3D printing technology. Hepcidin is a 25 amino acid, β hairpin containing one beta sheet, and four disulfide bonds (Cys1-Cys8, Cys3-Cys6, Cys2-Cys4, and Cys5-Cys7). Removal of the first five amino acids of hepcidin strongly decreases its ability to bind ferroportin and trigger endocytosis. Tests are currently commercially available for measuring both urine and plasma hepcidin concentrations, and research into their clinical applications is underway. Hepcidin is not currently being used to treat iron disorders, but hepcidin agonists and antagonists are being developed and investigated for possible future therapeutic use.

I. Introduction to Hepcidin and Iron Homeostasis

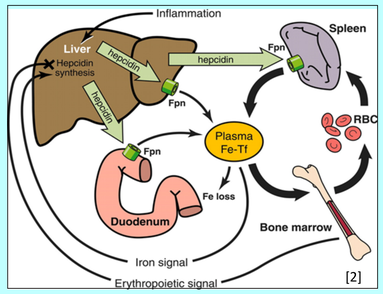
Iron is found everywhere on Earth and is essential to life. The human body contains 3-4 grams of iron and over fifty percent is found in red blood cells (RBCs). Without iron, the oxygen carrying protein hemoglobin that fills RBCs cannot be made and the bone marrow cannot carry out erythropoiesis (RBC production). All iron in the body is absorbed from the diet in the duodenum.

The peptide hormone, hepcidin, controls the release of the dietary iron from duodenal enterocytes into the blood; it is the key regulator of plasma iron levels. When hepcidin levels increase, iron levels decrease and when hepcidin levels decrease, plasma iron increases. Hepcidin controls plasma iron by binding to the iron export channel ferroportin, which is found primarily on enterocytes, hepatocytes, and spleen macrophages. Binding causes both to be drawn into the cell by endocytosis where they are degraded. Hepcidin synthesis in the liver is affected by erythropoiesis, blood oxygenation, certain inflammatory cytokines, intracellular iron storage, and plasma iron (Figure 2). The human body has evolved a very complex system to conserve and recycle iron. However, the body does not regulate iron excretion, which in some circumstances, such as hereditary hemochromatosis, can result in iron overload, a condition in which excess iron is deposited in cells causing severe organ damage.



[1]

Figure 1: Hepcidin binding to ferroportin prevents the release of iron into blood plasma.
Hepcidin is binding to the iron export channel ferroportin (FPN) on the enterocyte, hepatocyte and macrophage. Binding causes both to be pulled into the cells by endocytosis where they will be degraded in a lysosome. Iron atoms (orange spheres) are passing out of the cell via the ferroportins not bound to hepcidin. High hepcidin plasma levels decrease the release of iron from cells by decreasing the number of ferroportins in the membrane. Low hepcidin levels result in more ferroportins in the membrane, which allows iron to enter the blood. High iron plasma levels signal the liver to increase the synthesis of hepcidin.

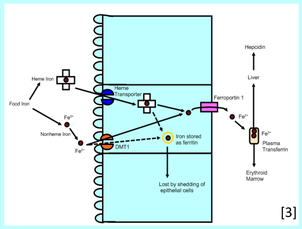


[2]

Figure 2: Hepcidin and Iron Homeostasis

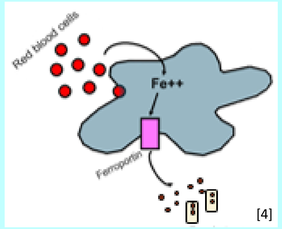
- Hepcidin synthesis in the liver is affected primarily by the iron needs of erythropoiesis, plasma transferrin (Fe-Tf) saturation, and certain inflammatory cytokines.
- Iron enters the blood plasma via ferroportin (Fpn) found on duodenal enterocytes, hepatocytes and spleen macrophages, which are the major sites of iron storage.
- Transferrin, a plasma iron carrier protein, picks up iron as it is entering the blood plasma through ferroportin. High transferrin saturation with iron signals the liver to make hepcidin.
- Spleen macrophages recycle aging red blood cells and the iron from their hemoglobin is released through ferroportin and picked up by transferrin. Transferrin is the only source of iron for erythropoiesis in the bone marrow.

II. Iron Absorption and Recycling



[3]

Figure 3: Duodenal enterocytes
Enterocytes that line the duodenum absorb iron from the diet. Iron enters enterocytes through membrane transporters. Heme iron enters through the heme transporter (blue) Nonheme iron enters using the Divalent Metal Transporter. Iron can be stored in the spherical protein ferritin (yellow) for later use. Iron moves out of the cell into the blood through iron export channel ferroportin. The plasma iron transport protein, transferrin, picks iron from ferroportin. Hepcidin controls iron entering the blood through ferroportin.



[4]

Figure 4: Spleen Macrophage
Macrophages in the spleen recycle old red blood cells after their life span of about 120 days. The red blood cells are engulfed by macrophages and degraded in lysosomes. The iron is recycled and leaves the cell through ferroportin. Transferrin (yellow rectangle) transports the recycled iron in the plasma. Transferrin is the only source of the iron needed for the production of red blood cells. High iron levels in the plasma stimulate hepcidin production in the liver. Hepcidin binds ferroportin and slows the release of iron from macrophages.

III. Hepcidin Structure & Function

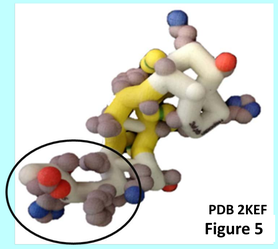


Figure 5

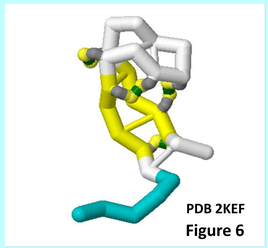


Figure 6

Hepcidin is a 25 amino acid peptide that regulates plasma iron levels by binding to its receptor, ferroportin (an iron export channel). Hepcidin has a ϑ-hairpin structure, which is stabilized by four disulfide bonds. The first five amino acids are the most important for binding to ferroportin. In the E. Nemeth et. al. experiments [5], removing N-terminal amino acids progressively, one by one, decreased hepcidin's ability to trigger ferroportin's endocytosis both *in vitro* and *in vivo*. All bioactivity was lost when the first five amino acids (Asp1, Thr2, His3, Phe4, and Pro5) were removed.

Figure 5: The St. Dominic SMART Team designed the physical model of hepcidin shown here using 3-D printing technology. The model displays all 25 amino acid sidechains. Sidechains are colored CPK (chemist's preferred colors) in which oxygens are red, carbon are gray, nitrogen are blue, and sulfur atoms are yellow. The alpha carbon backbone is white except for the ϑ sheet, which is yellow. The four disulfide bonds are green and the hydrogen bonds in the sheet are white. [6]

Figure 6: The shape of hepcidin is shown in alpha carbon backbone in this Jmol image to highlight its underlying structure. The five N-terminus amino acids are colored cyan.

IV. Iron Overload and Hereditary Hemochromatosis

Hereditary Hemochromatosis is a group of genetic diseases characterized by too much iron absorption and storage. Over time, iron overload develops as iron accumulates in major organs, like the liver, heart, pancreas and spleen, disrupting their normal function. The liver biopsy in Figure 7 shows severe iron overload. The iron deposits in the hepatocytes are stained blue.

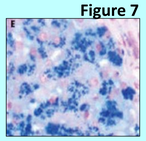


Figure 7

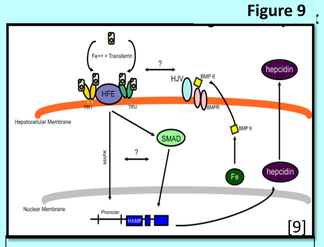
Figure 8

Disease	Disease Onset	Hepcidin Level/ Phenotype	Mutated Gene/Protein
Hereditary Hemochromatosis (HH)			
Type 1	**adult**	**Low hepcidin/Iron overload**	**HFE (Human Hemochromatosis)**
Type 2a	**juvenile**	**Low hepcidin/Iron overload**	**HJV (Hemojuvelin gene)**
Type 2b	**juvenile**	**Low hepcidin/Iron overload**	**HAMP (Hepcidin gene)**
Type 3	**intermediate**	**Low hepcidin/Iron overload**	**TfR2 (Transferrin Receptor 2)**
Type 4a Ferroportin Disease	**adult**	**Low hepcidin/Iron overload**	**SLC40A1 (Ferroportin gene)**
Type 4b 'Ferroportin Disease	**juvenile**	**High hepcidin/Iron overload**	**SLC40A1 (C326S mutation in Ferroportin gene)** [8]

Figures 8 - 9: Molecular Basis of Hereditary Hemochromatosis [8][9]

- **Type 1** The HFE gene codes for a membrane protein on hepatocytes, which signals the nucleus to make hepcidin when transferrin binds to transferrin receptors 1 and 2 (TRf1 and TRf2). Mutant HFE is ineffective in signaling the nucleus to express the hepcidin gene.
- **Type 2** The HJV gene codes for a membrane protein that interacts with BMP (Bone Morphogenic Protein) to signal the nucleus to make hepcidin. Mutant HJV results in early onset hemochromatosis.
- **Type 2b** is caused by a rare mutation in the hepcidin gene (HAMP), resulting in a partially functional hepcidin.
- **Type 3** is caused by mutations in TRf2, which helps iron enter hepatocytes. Both TRf1 and TRf2 associate with HFE to signal the nucleus to make hepcidin.
- **Type 4a and 4b** both result from mutations in the SLC40A1 gene, the only gene which codes for ferroportin, a transmembrane iron export channel. In Type 4b, ferroportin is resistant to hepcidin due to a C326S mutation. In Type 4a, ferroportin contains various missense mutations that affect export of iron from spleen macrophages causing iron to accumulate.

Figure 9

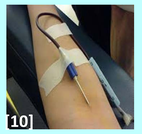


[9]

HFE= Membrane Signaling Protein
HJV= Hemojuvelin (Membrane Signaling Protein)
HAMP= Hepcidin Gene
TfR1= Transferrin Receptor 1
TfR2= Transferrin Receptor 2
BMP6= Cytoplasmic Signaling Molecule
Fe= Iron
Tf-Fe=Transferrin (Blood Plasma Iron Carrying Protein)

V. Current Treatments for Iron Overload

There are no cures for iron overload but there are treatments to prevent and halt damage. Excess iron can be removed by phlebotomy (blood donation) or it can be removed using iron chelation drugs that bind iron so that it can be excreted. Phlebotomy is a preferred treatment because regularly schedules blood donation can keep iron levels normal. Chelation is used when blood donation is not possible. The drug deferoxamine binds iron so that it can be excreted in the urine. However, it must be given as a subcutaneous infusion taking 8-12 hours. A wearable pump is usually worn. A newer drug, deferasirox, can be taken orally.


[10]
Phlebotomy or blood donation can be used to keep iron levels normal.


[11]
Deferoxamine is given as a subcutaneous infusion using a wearable pump.


[12]
Deferoxamine is used to chelate iron. When it is binding iron well, urine takes on an orange color.

VI. Hepcidin Agonists and Antagonists Research

Figure 10

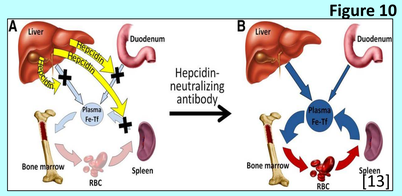


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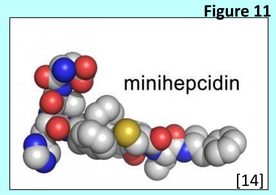
Figure 11



[14]

Figure 10: Hepcidin Antagonists Reduce the Effects of Hepcidin
In inflammation and several other conditions, hepcidin production increases causing iron to be sequestered and the resulting low plasma iron can contribute to the development of anemia because the bone marrow needs a supply of iron to produce red blood cells. New animal research is showing that anti-hepcidin antibodies can increase plasma iron levels by preventing hepcidin from lowering plasma iron. [13]

Figure 11: Hepcidin Agonists Increase the Effects of Hepcidin
Scientists are researching the use of hepcidin agonists, which increase the effects of hepcidin. A minihepcidin (PR65) was developed to treat hepcidin deficient mice. PR65 administration decreased iron loading. PR65 was given to mice with pre-existing iron overload but had a more moderate effect, causing partial redistribution of iron from the liver to the spleen. The study showed that minihepcidins could be beneficial in iron overload disorders either used alone or possibly as used in conjunction with phlebotomy or chelation. [14]

VII. Summary

Hepcidin is the key regulator of plasma iron levels in humans and plays a central role in hereditary hemochromatosis (HH). Since the body does not regulate iron excretion, iron overload can develop over time in HH patients as excess iron accumulates in major organs, such as the liver, heart, pancreas and spleen, disrupting their normal function. Phlebotomy and iron chelating drugs are currently being used to keep plasma iron at normal levels. Research is underway to develop hepcidin agonists and antagonists to treat hereditary hemochromatosis and other iron overload conditions.

VIII. References

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