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A review on the role of Hepicidin hormone in beta thalassemia major disease

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Abstract

Thalassemia is a haemoglobin disorder that is inherited. One or more globin chains of haemoglobin tetramers are produced less frequently or not at all, which causes uncontrollably high RBC destruction and severe anemia. An imbalance in globin chain synthesis causes damage to red blood cells in thalassemia. Hepcidin is severely reduced in thalassemia, and any increase in hepcidin function benefits erythropoiesis and iron metabolism. Clinical trials have been conducted on synthetic hepcidin and hepcidin mimetics. Nevertheless, their efficacy/safety profile has not been satisfactory. When taking hepcidin directly as a medication, it appears to be challenging to prevent iron over-restricted erythropoiesis. Indirect methods are numerous and still evolving, each with pros and cons. Targeting erythroferrone, the primary inhibitor of hepcidin expression, is the best strategy because iron-loading anaemias significantly raise its plasma concentrations. The development of pharmacological agonists and antagonists as well as hepcidin diagnostics should enhance the management of iron diseases.

Keyword: Beta- Thalassemia; Hepicidin; Iron; VIT-2763.

1. Introduction

Thalassaemia is a hereditary haemoglobin disease. One or more globin chains of haemoglobin tetramers are produced less frequently or not at all, which causes uncontrollably high RBC destruction and severe anemia [1]. In thalassemia, an imbalance in globin chain synthesis leads to red cell damage. This in turn leads to the destruction of those cells in the bone marrow (i.e., inactive erythrop oiesis) and the peripheral circulation (i.e., hemolysis) [2,3].

Thalassemia are a diverse cluster of genetic diseases. The two most common forms of thalassemia are α and β. The globin chain's involvement is the basis for this [1]. The α-chain is encoded by the Hb α gene (HBA1 and HBA2), which is located on chromosome 16, whereas the β chain is encoded by the Hb β gene, which is found on chromosome 11. The development of two to four distinct globin sequences is either non-functioning or malfunctioning in another complex form of thalassemia [4,5]. In clinical settings, β-thalassemia homozygotes frequently manifest as thalassemia major or intermedia [6].

2. Etiology of beta-thalassemia

Beta-polypeptide chain synthesis is reduced in beta thalassemia. Mild to severe microcytic anemia (thalassemia minor) is asymptomatic in heterozygotes, who are carriers. Homozygotes, also known as Cooley's anemia or beta-thalassemia major, experience severe anemia and hyperactivity of the bone marrow [7].. Patients with thalassemia major type experience severe anemia, hypertrophic swelling, and bone marrow, and they require frequent blood transfusions to maintain a normal life. These symptoms do not manifest at birth, but rather within the first two years of the child's life [8].

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2.1. Pathophysiology of beta-thalassemia

Haemoglobin is composed of four identical protomers, two to two. Each protomer is made up of heme, which contains an iron atom, and globin, which is an alpha or beta chain of globular glycoproteins [9]. Haemoglobin synthesis is regulated by two multigene clusters: on chromosome 16, which consists of three homologous genes (the zeta (δ) gene, alpha 1 (HBA1), and alpha 2 genes (HBA2), and on chromosome 11, which has five functional genes (ε (HBE), Gγ (HBG2), Aγ (HBG1), δ (HBD), and β (HBB)). These genes are located along the chromosome in the following order of development: foetal (α2γ2), adult (HbA, α2β2 and HbA2, α2δ2), and embryonic (Hb Gower-1 (δ2ε2), Hb Gower-2 (α2ε2), and Hb Portland (δ2β2) [10]. Upstream of the entire β globin complex is the locus control region (LCR), which consists of five DNase hypersensitive (HS) sites (referred to as HS 1–5). The LCR is crucial for gene expression because it maintains an open chromatin state and acts as a strong promoter of globin gene transcription. Without it, beta globin gene expression is relatively low. Four of the sites (HS 1) are erythroid-specific and include bin sequences for erythroid-restricted transcription factors (GATA-1 and NF-E2), despite HS5 being widely dispersed [11]. A balanced generation of non-α globin chains that guarantees a reciprocal pairing into the typical tetramers characterizes the healthy state. Due to inadequate beta globin chain formation caused by mutations in the beta-globin gene, which accumulate globin chains and induce anemia as well as an increase in HbF and HbA2, this equilibrium is broken in β-thalassemia. The human β-like globin gene is expressed during the embryonic stage and is replaced by a γ-to-β-globin switch after birth, according to the developmental patterns of this gene. The expression of the adult δ-globin gene is low. Five DNase hypersensitive (HS) sites (designated HS 5) make up the locus control region (LCR), a pentameric complex that facilitates prolonged interactions between the LCR and globin complex. The LCR is essential for the low-level expression of the β globin gene because it maintains an open chromatin state and acts as a powerful promoter of globin gene transcription. The physiological condition is defined by a balanced production of the α and the that guarantees a pairing into the usual tetramers. Limited transcription E2 is one of the four unique sites (HS 1–4). The poor generation of β-mutations in thalassemia disrupts this equilibrium, resulting in a buildup of α that causes anemia and an increase in HbF and HbA2 due to fewer beta chains for HbA synthesis [9]. More than 200 β-thalassemia mutations that affected any of the phases—transcription, RNA processing, and translation of β-globin mRNA—have been found on the beta gene cluster. Frame shifts are caused by simple nucleotide substitutions, oligonucleotide insertions, or deletions in the great majority of mutations. β-thalassemia is seldom caused by extensive gene deletions [12]. Based on the degree of β-globin chain output decrease, the mutations are divided into three groups: β0, β+, and β+.+ thalassemia alleles. β0 (severe) or beta thalassemia major mutations completely stop the formation of β-globin, while β+ A less severe reduction in β-globin is caused by mutations. beta thalassemia. A group of mutations referred to as β++ has very little effect on β production [13]. The globin gene locus comprises five β-like globin genes, which are related to globin genes and their patterns of expression during development. During the embryonic stage, ε-globin is expressed, and during fetal life, γ-globin takes its place. The globin gene is poorly expressed when the globin transition takes place and β-globin is mostly expressed in adults. Long-term interactions between the LCR and γ- and β-globin promoters in fetal and adult erythroblasts are mediated by a pentameric complex. Simple nucleotide substitutions, oligonucleotide deletions, or insertions that result in frame shifts account for the vast majority of mutations. Thalassemia are rarely caused by gross genes. The three types of mutations are β0, β+, and β+.+ thalassemia alleles based on the level of globin chain production. Mutations that result in a milder reduction in βthalassemia are referred to as beta thalassemia major or β0-thalassemia (severe). A collection of β+The β-globin chain is only slightly affected by +-thalassaemia. In β-thalassemia major, excess unpaired and insoluble α globin chain precipitate at the membrane of red cell precursors, and free iron promotes the creation of reactive oxygen species that induce oxidative cell damage and early cell death by apoptosis. this occurs within the erythropoietic tissue, leading to hemolysis of mature red blood cells and inefficient erythropoiesis (IE) [14].

2.2. Clinical signs and symptoms of beta-thalassemia

One of the most frequent side effects of thalassemia is iron overload, which is caused by frequent blood transfusions and can harm the liver, heart, and endocrine system [15]. The liver is where iron mostly builds up, followed by the heart and endocrine organs. Iron excess can cause growth hormone insufficiency, hypoparathyroidism, hypogonadotropic hypogonadism, hypothyroidism, and diabetes mellitus [16]. Additionally, iron overload in the heart can be lethal and result in arrhythmias and cardiac failure. To avoid these problems, chelation therapy is required, and iron excess should be routinely checked for sufficient chelation [17].. Thalassemia patients are more susceptible to infections, which can be detrimental to their organs.This illness affects how the body develops. Patients with thalassemia may then experience it. The skull bone is visible in the majority of cases. Skeletal anomalies are also caused by the thickening of the skull and face bones [18]. Numerous bacterial, viral, and infectious factors can induce spleen enlargement, which also happens accidentally as a result of blood flow issues and liver failures. The spleen will be compressed when the liver gets inflamed. One condition that causes the spleen to expand is thalassemia. Anemia symptoms include, but are not limited to, shortness of breath, cold hands and feet, pale skin, irritability, dark urine, and fatigue [19].

3. Hepcidin

Hepatocytes secrete the 25-amino-acid peptide hormone known as hepcidin [20]. The release of stord iron from hepatocytes, the absorption of dietary iron in the duodenum, and the release of recycled iron from macrophages are the three primary iron fluxes that enter the plasma compartment and are all negatively regulated by hepicdin [21]. Iron and hepcidin regulate each other via a conventional endocrine feedback loop. When iron is abundant, hepatocytes produce more hepcidin, which limits further iron absorption and iron release from storage. When iron levels are low, hepatocytes make less hepcidin, allowing more iron to enter plasma. The specific forms of iron that increase the formation of hepcidin are stored iron in hepatocytes and diferric plasma transferrin. In addition to hepatic and extracellular iron, hepcidin is homeostatically controlled by the erythropoietic need for iron as well as the elevated iron requirements of the mother and fetus during pregnancy. Vigorous erythropoiesis increases the amount of iron available for haemoglobin synthesis by inhibiting the generation of hepicidin [22]. Erythroferrone, a hormone released by erythropoietin-stimulated erythroblasts that works on the liver to reduce hepcidin production, is the main erythropoietic suppressor of hepcidin (erythroid factor). Hepcidin is markedly reduced in the second and third trimesters of pregnancy, which raises the amount of maternal erythrocytes and boosts iron mobilisation for fetal growth [23].Although the inhibitory signal has not yet been molecularly identified, it most likely starts in the placenta. In keeping with its probable evolutionary origin as an antibacterial peptide, hepcidin also serves as a mediator of innate immunity. Interleukin (IL)-6 and other cytokines enhance hepcidin synthesis during infections and other inflammatory processes [24]. Increased hepicidin levels prevent iron from being recycled from damaged tissues during infection by causing macrophages to retain iron. Transferrin may absorb any Non-transferrin bound iron (NTBI), an iron species that is extremely accessible to gram-negative bacteria and other microorganisms and promotes their fast growth, by slowing the entrance of iron into plasma and extracellular fluid [25].

3.1. Role of hepicidin in the iron metabolism

Hepicidin is a protein that is synthesized by the liver, and it is responsible for the regulation of iron metabolism [26]. by directly binding to ferroportin, an iron export transmembrane protein present in red blood cells, macrophages, enterocytes, and hepatocytes, hepcidin facilitates its cellular lysosomal breakdown [27]. Iron mobilisation in macrophages, hepatocytes, and/or bone marrow, as well as iron absorption from enterocytes, were thus impaired when ferroportin was degraded [26]. The amount of iron in the body, including the liver and plasma, is intimately related to hepicidin homeostasis in healthy individuals. Iron excess starts a feedback mechanism to control iron levels by increasing hepcidin concentration, whereas an increase in erythroid activity can significantly reduce hepicidin concentration to maintain a sufficient iron supply for erythropoiesis [27].Additionally, studies have shown that hepcidin levels rise dramatically during either acute or chronic inflammation, which may be the reason for anemia in long-term conditions [27]. Patients with β-thalassemia may have higher erythropoietic activity because of their chronic anemia. Therefore, poor erythropoiesis contributes to a drop in hepcidin concentration in order to maintain plasma iron levels high enough for the production of erythrocytes [28]. Iron overload is more likely as a result of increased intestinal absorption and higher release of iron from intracellular reserves [29]. The hormone erythroferrone functions as an erythroid modulator of hepcidin [30]. The bone marrow produces erythroferrone to suppress hepcidin gene expression during increasing erythropoietic demand or hypoxic conditions, hence promoting greater iron availability [27]. thus, erythroferrone expression is significantly stimulated by thalassemia-related inefficient erythropoiesis [31]. Numerous investigations have shown that people or animal models with β-thalassemia have extremely high amounts of erythroferrone, which suppresses hepcidin and causes iron overload [32]. In patients with β-thalassemia major, elevated iron loading is the primary cause of morbidity and mortality [33], but its harmful effect in transfusion-independent β-thalassemia intermedia should therefore not be overlooked [34]. Therefore, in the current care of β-thalassemia, regulating both erythroferrone and hepcidin is essential [32].

3.2. Hepicidin inactivates Ferroportin to Decrease Iron Transfer to Plasma

Hepcidin inhibits cellular iron efflux not only by directly binding to and occluding ferroportin [35] Hepicidin also prevents cellular iron efflux by causing ferroportin to undergo a conformational change that leads to ferroportin ubiquitination [36], endocytosis of both molecules, and subsequent lysosomal destruction [37]. Due to ongoing plasma iron consumption, mostly for the manufacture of haemoglobin by erythrocyte precursors, the loss of iron efflux into plasma from macrophages, hepatocytes, and enterocytes in vivo causes hypoferremia. Recent cryoelectron microscopic investigation of the hepcidin–ferroportin complex and thorough structure–function studies have shed light on the structural foundations of the hepcidin–ferroportin interaction [38]. Minihepcidins and other small drug-like hepicidin agonists have been made using the N-terminal 9-amino-acid portion of hepcidin, which is both necessary and sufficient for the hepcidin–ferroportin interaction [39].

3.3. Regulation of iron absorption by hepicidin

The oxygen- and iron-dependent prolyl hydroxylases that target hypoxia-inducible factors (HIFs) for proteasome degradation are less active and the HIFs are stabilised when enterocyte iron levels are low. Low hepcidin levels cause duodenal enterocytes to have more ferroportin activity, which lowers enterocyte iron levels when there is an iron deficit. HIF2α controls the transcription of iron reductase DCYTB (duodenal cytochrome b), apical iron importer DMT1 (divalent metal transporter 1), and basolateral exporter ferroportin [40].

3.4. Hepcidin function

Hepatocytes secrete the 25-amino acid peptide hormone known as hepcidin [47]. The absorption of dietary iron in the duodenum, the release of recycled iron from macrophages, and the release of stored iron from hepatocytes are the three primary iron fluxes that enter the plasma compartment and are adversely regulated by hepcidin [48]. Through a traditional endocrine feedback loop, hepcidin and iron control one another. Hepatocytes create more hepicidin when iron is plentiful, which restricts additional iron absorption and release from storage. Hepatocytes make less hepcidin when iron levels are low, which permits more iron to enter plasma [49]. Iron stored in hepatocytes and diferric plasma transferrin are the particular types of iron that boost the production of hepcidin. Hepicidin is homeostatically regulated not just by hepatic and extracellular iron but also by the erythropoietic need for iron and during pregnancy to fulfil the fetus's and mother's higher iron requirements. Hepcidin production is inhibited during vigorous erythropoiesis, which increases the availability of iron for haemoglobin formation [50]. The primary \ erythropoietic suppressor of hepicidin (erythroid factor) is erythroferrone, a hormone secreted by erythropoietin-stimulated erythroblasts that acts on the liver to decrease hepcidin production. During the second and third trimesters of pregnancy, hepicidin levels are considerably lower. which increases iron mobilisation for foetal growth and increases the number of maternal erythrocytes [51]. Although the inhibitory single has not yet been molecularly identified, it most likely starts in the placenta. In keeping with its probable evolutionary origin as an antibacterial peptide, hepicidin also serves as a mediator of innate immunity.Interleukin (IL)-6 and other cytokines enhance hepcidin synthesis during infections and other inflammatory processes [52]. Increased hepcidin levels prevent iron from being recycled from damaged tissues during infection by causing macrophages to retain iron. By limiting the entry of iron into plasma and extracellular fluid, transferrin can absorb any NTBI, an iron species that is particularly accessible to gram-negative bacteria and other microbes and encourages their rapid growth [53]. Hepicidin derived from cardiomyocytes appears to preserve the baseline iron homeostasis of the heart [54]. The primary producers of hepcidin, which controls systemic iron homeostasis, are hepatocytes. Hepicidin mRNA, which has both autocrine and paracrine effects on the local iron distribution, is also expressed by cell types such as keratinocytes, dendritic cells, and cardiomyocytes. Hepcidin, which is produced by keratinocytes and dendritic cells, aids the body's reaction to infection and inflammation [55,56].

3.5. The Impact of Hepcidin gene Polymorphisms on the Development of Beta thalassemia

Many studies have connected varying degrees of iron overload in beta thalassemia to distinct single nucleotide polymorphisms in the promoter of the hepcidin-encoding HAMP gene [41–44]. In research conducted by Zarghamian et al. [42] and Andreani et al. [41]. Increased iron loading was linked to the c.-582 A>G HAMP-P variation; iron loading was determined by measuring blood ferritin, liver iron concentration, and cardiac iron deposition [41, 42]. Higher iron loading activity appears to have been caused by low hepcidin levels, as the c.-582 A>G HAMP-P variation is known to downregulate HAMP gene transcription [41,43]. It's interesting to note that the impact of the polymorphism varies depending on the chelating therapy regimen used. The c.-582 A>G HAMP-P variation was found to be clinically significant in patients receiving regular chelating therapy, according to one research group [42], whereas another group only showed an improvement in iron loading when chelation therapy was not given frequently [41]. In order to further understand these linkages, future studies on a wider population should be carried out. The subset of beta-thalassemia patients with the c.-582 A>G HAMP-P variation may benefit more from the development of related treatments in the future if growing data confirms these first findings. Furthermore, in a patient with beta-thalassemia major, a -72 C>T mutation in the HAMP promoter region was reported to exacerbate iron overload [37]. Defective hepcidin molecule production resulted in significantly high blood ferritin (4323 ng/mL), liver iron concentration (5230 _g/g liver dw), nontransferrin-bound iron (3.66 _M), and transferrin saturation of 110% [44]. Notably, although it is outside the purview of this research, polymorphisms of additional genes involved in maintaining iron homeostasis may have an impact on the state of beta thalasemia patients. Among these, GDF15, along with HFE1 and HFE2, which are commonly associated with hereditary hemochromatosis, have been shown to increase iron overload in individuals with beta thalassemia [45,46].

4. Hepicidin treatment beta thalassemia

4.1. Hepcidin Agonists

In hepcidin deficiency disorder, such as the majority of β-thalassemia, treatment with hepcidin agonists should avoid or reduce iron excess. Hepcidin can theoretically be increased by giving exogenous hepcidin or hepcidin agonists, or by increasing endogenous hepcidin production (for example, by deactivating TMPRSS6). Any of these techniques should be used for reverse the iron's hyperabsorption and maldistribution. Indeed, it has been shown that a number of hepcidin mimetics can prevent iron overload in mouse models of hemochromatosis, manage polycythaemia in a mouse model of polycythaemia vera, and reduce anaemia in a mouse model of thalassaemia intermedia [57–59]. Rusfertide, a hepcidin agonist peptide, appears to effectively substitute phlebotomy in human clinical trials for the treatment of polycythaemia vera without worsening iron deficiency symptoms. [60].

4.2. Hepcidin Antagonists

In addition to potentially releasing iron from storage and enhancing the effects of erythropoiesis-stimulating medicines even when hepcidin concentrations are within the normal range, antagonists should be helpful in treating iron-restrictive anaemias with elevated circulation hepcidin concentrations. These agents are envisioned in four classes [61,62]. Hepcidin synthesis is inhibited by class I agents. They might, for instance, target the stimulatory cytokines BMP6 or IL-6 as well as related pathways. Anti-IL-6 antibodies and anti-IL-6 receptor antibodies are promising strategies that have already been licensed for the treatment of Castleman's disease and rheumatoid arthritis [63]. anti-IL-6 shown exceptional efficacy in treating CKD anaemia in a clinical investigation [64]. It remains to be seen if the toxicity of their immunosuppressive effects would prohibit more widespread usage in contexts other than those that have already been licensed for the treatment of anemia. Additionally, Monoclonal antibodies against hemojuvelin, soluble hemojuvelin, which functions as an antagonist of BMP signalling, and recombinant erythroferrone—all of which have been demonstrated to reduce hepcidin levels and are now undergoing preclinical development—may target the BMP pathway [65–67]. Although they would need to be quite selective for the hepcidin-regulatory system, small-molecule inhibitors of the kinase activity of BMP type I receptors might be helpful therapeutically [68]. Class II hepcidin antagonists either inactivate or sequester the hepcidin molecule. Research teams at Lilly and Amgen have reported hepcidin-neutralizing monoclonal antibodies [69,70]. In a mouse model of inflammatory anaemia, the latter antibody boosted haemoglobin either alone at higher doses or in combination with inadequate levels of erythropoiesis-stimulating drugs. Monoclonal antibodies that block ferroportin's hepcidin binding site are class III medicines that have demonstrated some efficacy in treating CKD anemia [71].

4.3. Developmental therapy of Hepicidin treatment beta thalassemia

VIT-2763, a new small molecule oral ferroportin inhibitor, is one such treatment. The only known iron transporter in animals, ferroportin is primarily expressed in the duodenum, liver, spleen, and mononuclear phagocyte system, which are organs involved in iron absorption, recycling, and storage. Ferroportin mediates the transfer of iron into the blood [72–75]. VIT-2763 showed good oral absorption in preclinical investigations and prevented cellular iron efflux in vitro with a potency similar to that of hepcidin, the natural ferroportin inhibitor [76]. Additionally, a synthetic human hepcidin called LJPC-401 was evaluated on healthy people to see if it improved the participants' iron profiles [77]. Subjects showed decrease serum iron levels and elevated serum ferritin, suggesting that LJPC-401 may successfully control iron concentration. A hepcidin peptidomimetic (PTG-300) was created and tested in a murine model of beta thalassaemia despite the encouraging phase 1 results on LJPC-401 [77]. Similar to hepcidin, PTG-300 works by inhibiting ferroportin expression on cells that store or recycle iron, which lowers the saturation of serum iron and transferrin [78]. Because they increase the formation of hepcidin, natural substances like icariin and astragalus polysaccharide have been proposed in some earlier studies to treat iron problems [79,80]. This certainly merits more research. Astragalus polysaccharide works by stimulating p38 mitogen-activated protein kinases and increasing interleukin-6, both of which are involved in the activation of the HAMP gene [80].

5. Conclusions

Hepicidin plays a significant role in controlling iron homeostasis. Its concentration decreases during beta thalassaemia as a result of increased but inefficient erythropoiesis. Elevated serum erythroferrone is noted in the interim. Patients suffer from iron excess and its consequences as a result. Therefore, improving high erythroferrone and low hepcidin appears to be essential for creating novel therapeutic approaches. Additionally, regular serum hepcidin and erythroferrone measurements may be used as a diagnostic tool for the early prediction of iron accumulation, while more research is required for a therapeutic application. In addition to luspatercept's encouragement of erythroid maturation,

a number of substances that target hepcidin and erythroferrone have been proposed thus far to combat iron overload in individuals with beta thalassemia. They include a classes of hepcidin-related drugs that directly imitate the effects of natural hepcidin (minihepcidins, LJPC-401, PTG-300, VIT-2763), as well as indirect hepcidin activators that work by inhibiting erythroferrone or TMPRSS6. Furthermore, it has recently been determined that a few naturally occurring bioactive substances, such as icariin and astragalus polysaccharide, stimulate the expression of hepcidin. VIT-2763 is an oral ferroportin inhibitor. In line with the iron-lowering PD impact seen in preclinical models, VIT-2763 rapidly and noticeably decreased circulating iron levels the decreased transferrin saturation. According to the current study's findings, treating NTDT and other conditions involving inadequate erythropoiesis, excessive iron absorption, or excessive erythropoiesis may include reducing iron availability for erythropoiesis by inhibiting ferroportin activity. Future studies should clarify the safety and effectiveness of the aforementioned medications in individuals with beta thalassemia. Concurrently, iron metabolism regulators must to be thoroughly investigated as possible targets of innovative therapeutic approaches. Furthermore, the discovery of polymorphisms affecting iron homeostasis may provide context for developing a customised strategy for beta thalassemia treatment.

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