J Biochem Tech (2020) 11(2): 128-134
ISSN: 0974-2328

A saga of Hepcidin anti-microbial Effectiveness as Iron Acquisitor and Anemia Initiator in *Mycobacterium Tuberculosis* Infection

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Received: 03 March 2020 / Received in revised form: 05 June 2020, Accepted: 10 June 2020, Published online: 28 June 2020

Abstract

Hepcidin controls the systemic level of iron as well as the transfer of tissue iron by binding ferroportin to the iron exporter, which allows it to be depleted. Iron is an important element needed by all organisms because of its role in a range of critical cellular biochemical pathways such as DNA production and respiration. Iron is available to both host and pathogen during *Mycobacterium tuberculosis* (TB) infection. Tuberculosis may have an influence on the contagious result. Both the host and *M. tuberculosis* require iron, TB should be capable of trapping iron from the host, and the host must modify its iron spread in consequence to infection. This reallocation could act as a protection mechanism against some infectious agents. Even so, hepcidin exquisites the iron intracellular macrophage and the reticuloendothelial system as a protection mechanism for TB infection. Anemia of chronic disease (ACD) is anemia that occurs in acute or chronic immune disorders such as TB and is the second most common in iron deficiency anemia. Several research has reported the prevalence of anemia in patients with TB, and there is some indication that anemia in the diagnosis of TB is correlated with a higher risk of death, and it is also necessary to identify the factors that lead to TB-related anemia.

Keywords: Hepcidin, anti-microbial, iron metabolism, anemia, *Mycobacterium tuberculosis*

Introduction

Hepcidin is a liver-producing antimicrobial peptide with a wide variety of antibacterial properties. Hepcidin also functions as an iron regulatory hormone by hindering duodenum absorption of iron and macrophage recycling of aged RBCs (Waggiallah, 2020). Hepcidin controls iron excretion by binding ferroportin 1 to cell membranes, leading to internalization and destruction of ferroportin 1. Hepcidin is triggered in response to iron overload and inflammatory stimuli, but is diminished in anemia or hypoxia. While hepcidin is primarily produced in the liver, it has also been found in other organs, including the lungs, the pancreas, and the heart (Sow et al., 2011) (Figure 1).

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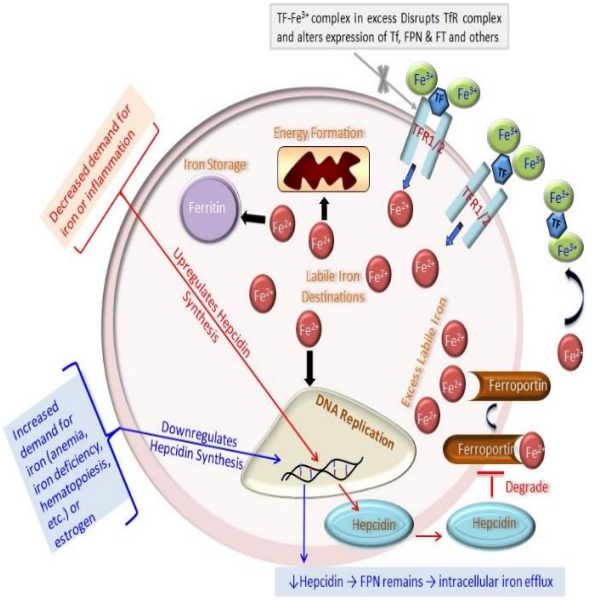

Figure 1. Iron metabolism regulation by hepcidin

Iron is one of the most common elements on earth, but in the physiological pH oxidizing environment of the planet, iron occurs mainly as insoluble ferric salts, such as iron oxide, iron hydroxide, and iron phosphate, which cannot be integrated by bacteria. Free iron ions are rare, therefore. Acquiring iron is even more complicated for pathogenic bacteria since iron ions are engaged to host iron-binding proteins, such as transferrin and lactoferrin, which act as host iron transporters, ferritin-containing iron-containing protein, and hemoprotein-containing iron-protoporphyrins. Throughout infection, the host reduces the quantity of circulated transferrin-bound iron in the body and increases the uptake of dietary iron using iron-deficiency as the body antimicrobial protective mechanism (Fang et al., 2015; Sangkhae and Nemeth, 2017; Silva et al., 2018).

TB is a life-threatening infectious disease (Rahmanian et al., 2018; Qattan and Khattab, 2020; Qanbarnezhad et al., 2018), and is associated with a wide range of clinical conditions (Jannah et al., 2019). It has been reported that one-third of the world's inhabitants are diagnosed with TB, a tuberculosis leading cause that continues to kill more than 1.7 million people per year (Qin et al., 2015). *Mycobacteria*, like many other living organisms, need iron to

perform many necessary functions. Iron is the critical catalytic core of the binding site of different enzymes, which facilitates enzymatic reactions (Lorent et al., 2014). The capacity of iron to acquire and give an electron that oscillates between ferrous (Fe2) and ferric (Fe3) oxidative conditions allows the enzyme to catalyze redox reactions (Fatima et al., 2014). Of this reason, iron is commonly associated with cytochromes essential of oxidative phosphorylation and energy generation. To resolve this, iron-deprivation mycobacterial pathogens have formed iron pathways that are more effective than those of their vertebrate hosts. (Minchella et al., 2014).

In this review, we want to enumerate the role of hepcidin as a powerful immune substance that plays a key role in iron metabolism and as well as has an active role in anemia associated with TB infection.

Iron hemostasis in TB

Iron metabolism is related to the development of TB in infectious cases. TB improvement at any point was correlated with decreased basal concentrations of transferrin, and early TB-progressors had higher basal levels of ferritin and hepcidin relative to delayed TB-progressors (Minchella et al., 2015). Heme has been used as an alternate source of iron to save their *M. Tuberculosis* mutants in which the biosynthetic pathway of mycobactin has been impaired in low iron states. Heme is an iron-containing prosthetic group present in many hemoproteins, like hemoglobin that is plentiful in RBCs. Two-thirds of the iron in vertebrates is integrated into the heme. Heme is thus a source of iron for several pathogenic bacteria, and its heme storage mechanisms have been well described (Sutherland et al., 2011). *M. Tuberculosis* may encounter heme or heme-containing protein, both intracellular and extracellular, during infection. RBCs are destroyed in macrophage phagosomes resulting in heme release (Ganz, 2012; Soe-Lin et al., 2009) and *M. tuberculosis* is exposed that occurs in the phagosome directly in the heme. Extracellular M. tuberculosis may have access to hemoglobin in the bloodstream where hemolysis formed by *M. tuberculosis* promotes the freeing of hemoglobin from RBCs (Rahman et al., 2010). A variety of studies have shown the potential of *M. tuberculosis* to use heme as an iron supply in low iron culture media (Jones and Niederweis, 2011; Tullius et al., 2011).

Within *M. tuberculosis*, heme may either be destroyed to produce iron or can be used to synthesize heme-containing proteins that have yet to be identified. Heme destruction is catalyzed by heme oxygenase (HO) in a well-defined mechanism in which heme is degraded into biliverdin, generating carbon monoxide (CO) and iron ions. The equivalent of HO in *M. tuberculosis*, MhuD (Mycobacterial heme utilization Degrader, Rv3592) does not share a sequence homology with HO proposing a unique catalytic process, even so, deteriorates heme to mycobilin without releasing CO. As CO causes *M. tuberculosis* to reach the inactive state, this specific heme destruction process does not interact with the CO sensing of the bacteria to the host environment (Nambu et al., 2013).

Because MhuD may bind to two heme molecules in its inactive form, it can act as a supplementary heme storage molecule (Chim et al., 2013).

Even though *M. tuberculosis* prefers heme as an iron supply, it produces a heme biosynthetic enzyme, ferrochelatase, encoded with hemZ (Rv1485). This protein includes a cluster of [2Fee2S] and catalyzes the last stage of heme biosynthesis in which ferrous iron is incorporated into protoporthyrin IX to shape a protoheme (Parish et al., 2005). This gene is important in vitro, which means that heme is not only utilized as an iron source by *M. tuberculosis*, but it is also an important cofactor for other metabolic enzymes. For instance, catalase-peroxidase (KatG) and DosS/DosT redox sensing two constituent system proteins contain heme at their binding sites (Boradia et al., 2014).

Iron acquisition pathway

M. tuberculosis occurs within the macrophage of the phagosome and inhibits phagosome-lysosome fusion through an unknown mechanism. The *M. tuberculosis*-containing phagosome has early endosomal properties, integrates with early endosomes, and does not acidify below pH 6.3–6.5. *M. tuberculosis* recognizes the phagosome as a low-iron environment: thus the necessity of iron acquisition strategies in the face of host iron retention processes (Boelaert et al., 2007).

A primary route used by *mycobacteria* to battle for scarcely available iron is the use of high-affinity iron chelators, siderophores, mainly produced during iron deprivation (Jones et al., 2014). There are three types of siderophores, mycobactin, carboxymycobactin, and exochelin, where mycobactin and carboxymycobactin share a central structure that is different from exochelin. Mycobactin is a cell envelope-associated and promotes the transfer of iron via the cell envelope to the cytoplasm, while carboxymycobactin and exochelin are secreted by iron chelators which obtain iron in the extracellular environment and transfer iron to the bacterial cytoplasm (Jones et al., 2014).

Mycobacteria use complex molecular pathways to establish iron homeostasis. Iron acquisition pathways are designed in response to low levels of intracellular iron to obtain iron from the environment. If there is adequate intracellular iron, excess iron is retained to prohibit Fenton from taking part in a reaction that would create dangerous hydroxyl radicals (Pandey and Rodriguez, 2014). *Mycobacteria* have evolved complex gene control processes that react to different concentrations of iron to maintain iron homeostasis. Iron-dependent regulator, Ider, in *M. tuberculosis* was defined based on homology to the Diphtheria Toxin Repressor (DtxR), an iron-dependent gene expression regulator in Corynebacterium diphtheria. Ider is important in the in vitro TB (Gold et al., 2001). The removal of the iron-responsive regulator contributes to the attenuation of *M. tuberculosis* in macrophages in the mouse pattern. Ider attachment sites have been recognized around the *M. tuberculosis* of H37Rv. Expression of 153 *M. tluberculosis* genes is regulated in response to iron level and 44 of these genes are expressed directly by IdeR (Gold et al., 2001).

In addition, there is a different iron-dependent regulator in *M. tuberculosis* called FurA (ferric uptake regulator A, Rv1909c). FurA, whose gene is upstream of katG in the same operon, is capable of negatively regulating the expression of KatG (Chen et al., 2012). The FurA mutant displayed improved tolerance to H_2O_2 and isoniazid vulnerability (INH) due to the upregulation of KatG (Chen et al., 2012).

Role of hepcidin as an antimicrobial in TB

Hepcidin also can modify immunological reactions. Ferroportin (a target for hpcidin) high expression on macrophages has been shown to dramatically inhibit intracellular *M. tuberculosis* growth throughout the initial phases of in vivo infection (Johnson et al., 2010). Hepcidin inhibits lipopolysaccharide-induced IL-6 and tumor necrosis factor-alpha transcription and development in macrophages (De Domenico et al., 2010). Human hemochromatosis protein, another iron metabolism peptide, also has different immunological tasks (Reuben et al., 2017). Such results pose the issue of whether iron-related factors affect immunological reactions and the clinical course of action in *M. tuberculosis* infection. Hepcidin concentrations have also demonstrated predictive capacity for the possible risk of *M. tuberculosis* infection (Qanbarnezhad et al., 2018) and death with active *M. tuberculosis* infection (Kerkhoff et al., 2016) in patients with HIV infection. In fact, hepcidin concentrations are stable over age in healthy subjects excluding premenopausal women. Diurnal variations in hepcidin rates have been reported (Schaap et al., 2013); nevertheless, the spectrum of fluctuations in this diurnal variability is hard to detect by the enzyme-linked immunosorbent assay (ELISA) (Galesloot et al., 2011).

Some characteristics of peptide tend to be suitable for adaptation in medical practice. Even so, there are few studies on the predictive efficacy of peptide in TB patients without HIV co-infection. In research involving only a tiny percentage of HIV co-infected participants, hepcidin rates of active TB patients at diagnosis were considerably greater than those of both tuberculin skin tests (TSTs)-negative and positive contacts without the active disease (Minchella et al., 2015). The research in Tanzania examined household TB interactions observed that those who acquired active disease had higher rates of hepcidin than those who did not display active disease, even those who did not develop HIV co-infection (Hella et al., 2018).

Hepcidin may have a direct effect on the development of IFN- γ in T cells. IFN- γ is important for the protection of the host against mycobacterial infection, including *M. tuberculosis* infection (Kampmann et al., 2005), as well. In HIV-infected persons, the probability of TB incidence increases with a decreasing CD4+ cell count, a significant source of IFN- γ (Ellis et al., 2017). Decreased IFN- γ production in T cells triggered by hepcidin may affect protection against *M. tuberculosis*, culminating in a long time of culture-negative. This inhibiting effect of hepcidin on the development of IFN-γ in peripheral blood T cells in patients with PTB was detected only during activation with ESAT6 and not with CFP10 (Ellis et al., 2017).

Interaction between immune system and TB relying on iron

Protection toward TB includes the immunization of macrophages and dendritic cells, leading to tumor necrosis factor (TNF) – a development and collaborative association with T cells, mainly CD4+ T lymphocytes and NK cells. The subsequent pro-inflammatory reaction of type Th1 involves interleukin IL-18, IL-12, interferon IFN- γ, and IL-1b development and is responsible for killing or monitoring *M. Tuberculosis.* Impact factors include reactive oxygen and nitrogen intermediates and apoptosis (Rook et al., 2005). Anti-inflammatory cytokines such as IL-4, IL-10, and transforming growth factor–b, the latter 2 of which are generated by regulatory T (T_{reg}) cells, suppress Th1 type reactions, and intervene with effector T cell activation. In *M. tuberculosis*-infected people, 5%–10% develop clinical TB, while the rest acquire latent infections marked by quiescent *M. Tuberculosis* with a powerful Th1 and likely suppressed Th2-cytokine response. Clinical TB is associated with mixed Th1/Th2 response and Th2 dominance, and curative TB treatment typically includes restoring Th1 superiority over Th2 (Rook et al., 2005)

IFN- γ, which is essential to TB defense, also affects the status of cellular iron. Moreover, IFN-γ stimulation of human monocytes decreases the number of TfRs on the cell surface (Boelaert et al., 2007) and the level of macrophage iron acquisition of holotransferrin (Olakanmi et al., 2002), and reduces the accessibility of iron to intracellular microorganisms that use transferrin iron, such as *Legionella pneumophila* and *M. tuberculosis* (Boelaert et al., 2007).

M. tuberculosis appears to be able to reach other iron sources, at least in vitro, despite the IFN- γ -induced restriction in the acquisition of macrophage iron (Olakanmi et al., 2002). Macrophage *M. tuberculosis* infection does not subvert this TfR-induced reduction in TfR production (Olakanmi et al., 2002; Kahnert et al., 2006).

Unlike normal macrophage activation by bacterial products or IFN-γ, alternate macrophage activation by Th2 cytokines has 2 possible consequences for the status of macrophage iron. First, during experimental TB, alternatively enhanced mouse macrophages express the arginase-1 encoding gene (Kahnert et al., 2006). Arginase-1 rivalry with inducible nitric oxide synthase (iNOS) for l-arginine results in reduced NO output and, thus, probably less iron efflux (Mulero et al., 2002) and more iron accessible to TB. The differential expression of arginase-1 versus iNOS is applied to humans is uncertain. Second, additional stimulation of uninfected macrophages leads to higher production of both TfR and CD163, leading to increased TfR-related and CD163-mediated accumulation of iron (Kahnert et al., 2006; Mosser, 2003). Besides, in the comparative gene expression analysis of TB-infected murine macrophages triggered by either IL-4 or IFN- γ, IL-4 resulted in increased TfR of host and TB iron accessibility as shown by reduced mycobactin and improved bacterioferritin synthesis, while IFN- γ was correlated with contrary results (Kahnert et al., 2006). Since these important experimental findings are applicable to human *M. tuberculosis* diseases is speculative. The finding that serum ferritin levels raised in a dose-dependent manner throughout recombinant human IL-10 treatment of patients with Crohn's disease (proven grade 1) (Tilg et al., 2002) is indicative of the fact that immunosuppressive cytokines improve the accessibility of macrophage iron.

In some cases, reticuloendothelial iron storage derives from the gastrointestinal tract, respiratory tract, or hemolysis, and in others, it results from chronic inflammation. The treatment of iron-dextran to rats results in the filling with broncho-alveolar macrophages with iron (Boelaert et al., 2007).

It is, therefore, possible that human loading conditions for iron will boost TB development in alveolar macrophages. Iron storage impacts the immune system in a manner similar to alternate macrophage activation (Boelaert et al., 2007).

Anemia in TB correlated with hepcidin

Hepcidin levels are closely correlated with the mycobacterial burden and spread of tuberculosis. Levels of hepcidin were also strongly correlated with higher anemia incidence in tuberculosis patients. Excessive levels of hepcidin significantly predict poorer short-term survival amongst hospitalized patients. In tuberculosis patients, increased levels of hepcidin were closely correlated with more serious anemia, and the highest concentrations of hepcidin in both in and outpatient patients were in those with acute anemia.

Because hepcidin plays a well-defined, central role in ACD (Weiss and Goodnough, 2005), in which its development is dominantly enhanced by IL-6 in response to infections like tuberculosis (Piperno et al., 2009; Armitage et al., 2011), these findings suggest further evidence indicating that ACD is the dominant process underlying anemia in HIV-associated tuberculosis patients (Wisaksana et al., 2011; Lee et al., 2006). Besides, the finding that tuberculosis therapy with or without ART results in stabilization of hepcidin levels and is correlated with anemia resolution in the most of patients offers further observational evidence of ACD as the predominant cause of anemia in TB patients (Wisaksana et al., 2011; Lee et al., 2006; Kerkhoff et al., 2014).

Iron deficiency (ID) identification in chronic inflammatory diseases like TB is difficult. For instance, utilizing ferritin as a single measurement could underestimate ID because ferritin is adjusted in infections (Thurnham et al., 2010). Helminths diseases, in particular Strongyloides stercoralis, poor dietary intake of iron and consumption of monotonous, cereal-based diets lead to the burden of anemia (Zimmermann et al., 2005), and that may be the explanation for the IDA being detected in the control group. There was no strong correlation between TB disease and IDA, possibly due to strong control of iron homeostasis by inflammation, established iron recycling from old RBCs, and the intake of iron by enterocytes (Drakesmith and Prentice, 2012).

Generally, the level of sTfR was negatively correlated with hepcidin levels, but to a lesser degree in cases chronic TB infection disrupts with regulatory processes. This is consistent with previous studies on the effect of inflammation on sTfR concentrations (Isanaka et al., 2012). The increased levels of sTfR found in TB cases matched to controls indicate erythropoetic stimulation and requirement for iron in TB patients. The two separate stimuli, increased hepcidin concentrations that cause hypoferremia and erythropoetic stimulation, suggest a sensitive iron balancing act that is needed in chronic infection: both deficient and excessive iron (Drakesmith and Prentice, 2012) that raise the chance of worse TB therapy outcomes (Isanaka et al., 2012).

Anemia caused by chronic inflammation has a complicated pathophysiology based on the underlying mechanism of the disease (Kerkhoff et al., 2016). There are doubts about the clinical value of general iron therapy in anemic TB patients (McDermid et al., 2013) Furthermore, medical studies of iron therapy in TB patients revealed a positive impact on hematological indices after one month, however these results vanished between two and six months (Hella et al., 2018). This means that the disease is cured (and the a mount of hepcidin decreased). Increased iron levels are accessible in the blood of the vast bulk of TB patients through release of confined iron and enhanced iron absorption. Even so, a tiny number of TB patients with established ID may indeed gain from iron therapy, but appropriate ID identification stays an unresolved issue in these patients. Low concentrations of hepcidin can help to distinguish between IDA and ACD patients (Girelli et al., 2016) and those most likely to gain from iron supplements. Further research is needed to clarify the therapeutic use of hepcidin in the form of coinfections (Wang and Babitt, 2016).

Conclusion

Hepcidin is a major regulator of iron homeostasis in TB and, as a consequence, has a potent antimicrobial effect on TB infection and may be a marker for the detection of more serious TB disease and high-risk individuals among those exposed to TB. Anemia is the outcome of the immune response. Even so, the iron supplement should be treated with caution because the functional iron deficiency found in TB patients with anemia is often temporary due to the sequester of iron in the cells and reversible during TB therapy.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

Author declares that they do not have conflict of interest

Research involving human participants and/or animals

No need for ethical approval since the manuscript is review article

Funding Information

This study was not funded by any company or agency.

Acknowledgments

This Publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University.

Conflict of interest

Author declared that there is no conflict of interest in this research.

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