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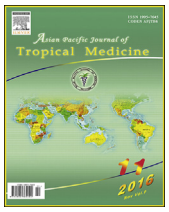

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Iron dysregulation in beta-thalassemia

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ABSTRACT

Iron deficiency anemia and iron overload conditions affect more than one billion people worldwide. Iron homeostasis involves the regulation of cells that export iron into the plasma and cells that utilize or store iron. The cellular iron balance in humans is primarily mediated by the hepcidin–ferroportin axis. Ferroportin is the sole cellular iron export protein, and its expression is regulated transcriptionally, post-transcriptionally and post-translationally. Hepcidin, a hormone produced by liver cells, post-translationally regulates ferroportin expression on iron exporting cells by binding with ferroportin and promoting its internalization by endocytosis and subsequent degradation by lysosomes. Dysregulation of iron homeostasis leading to iron deposition in vital organs is the main cause of death in beta-thalassemia patients. Beta-thalassemia patients show marked hepcidin suppression, ineffective erythropoiesis, anemia and iron overload. Beta-thalassemia is common in the Mediterranean region, Southeast Asia and the Indian subcontinent, and the focus of this review is to provide an update on the factors mediating hepcidin related iron dysregulation in beta-thalassemia disease. Understanding this process may pave the way for new treatments to ameliorate iron overloading and improve the long term prognosis of these patients.

1. Introduction

With the exception of a few species of bacteria, all living things need iron as an absolute requirement for viability. The ability of iron to act as both an electron donor and an electron acceptor makes it a critical component of many cellular oxidation/reduction reactions, and in addition iron is the substrate for heme, the critical component of hemoglobin, the essential oxygen carrying molecule of all vertebrates [1]. However, free iron is potentially extremely toxic to cells. Iron can donate electrons to oxygen resulting in the formation of the reactive superoxide radical (O^{2-}) or to hydrogen peroxide generating the hydroxyl (•OH) radical [1], and these molecules can oxidize biological macromolecules including lipids, proteins and DNA with extremely damaging consequences to the cell [2].

Humans contain approximately 3–4 g of iron in various forms [3]. Although iron is extremely plentiful in the environment, much of it is present in insoluble, non-bioavailable forms, and so humans have evolved to be highly efficient in conserving iron. Indeed, humans have no mechanism for excretion of excess iron under conditions of iron overload. Bioavailable iron in the diet serves mainly to replace iron lost from the body through processes such as the shedding of cells from the surface of the skin and lumen of the gut as part of the normal process of epithelial cell turnover. Additional loss of iron

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from the body may occur through minor bleeding events. In general it is believed that only some.

1–2 mg of iron (less than 0.1% of the total iron in a body) are lost from the body each day that require replacement though dietary sources [3].

The majority of the iron in humans is in the form of hemoglobin in red blood cells, and red blood cells combined contain between 2 g and 2.5 g of iron (out of 3–4 g in total). Red blood cells have a life span of some 120 days under normal conditions [2], after which they are degraded by macrophages and the iron returned to the plasma. Plasma contains some 2–3 mg of iron bound to a protein called transferrin, which is the primary molecule that transports iron for use in erythropoiesis in the bone marrow and by other iron requiring cells. Macrophages return some 20–25 mg of iron daily, ensuring a rapid turnover of iron in the plasma. When the binding capacity of serum transferrin is exceeded, iron starts to make complexes with other plasma proteins and molecules such as citrate. This iron is generally termed non-transferrin bound iron (NTBI) [4]. NTBI is easily taken up by hepatocytes and other parenchymal cells, and the intracellular accumulation of iron in these cells rapidly causes damage through oxidation reactions [2].

Within cells, iron is normally stored as the ferric (Fe^{3+} form) in association with a globular protein complex called ferritin. Ferritin is essentially a hollow sphere in which can sequester up to 4500 iron atoms. The ferritin complex consists of 24 subunits of heavy (H) and light (L) chains the exact composition of which can vary between tissues. The H chain has ferroxidase activity which converts Fe^{2+} to Fe^{3+} for storage inside the shell, while the L chain primarily stabilizes the structure and facilitates transport of iron ions to the inside of the structure [1]. This formation is the main storage system of iron (outside of the iron in hemoglobin). Under conditions of iron deficiency, iron is released from the complex to the plasma, while under conditions of mild iron excess the system can provide some buffering against the increased iron levels. In the average male, one gram of iron is held in storage mostly in hepatocytes and macrophages in the liver but also in spleen red pulp macrophages. Women of reproductive age tend to have significantly lower stored iron as a consequence of menstruation and childbearing [5].

As noted above, only a very small fraction of the total iron content is lost daily, and this is replaced through bioavailable iron sourced from the diet. Iron from the diet can be obtained from heme based sources (found in meat) and from non-heme iron sources (iron in cereals, vegetables, pulses etc). Iron absorption takes place in the gut duodenum and upper jejunum and occurs by transport across the apical membrane of enterocytes, which appears to occur through two independent pathways [6], one for heme iron and one for non-heme iron [3]. While absorption of non-heme iron is fairly well understood, the absorption of heme iron and ferritin iron is rather less well understood. Dietary non-heme iron is normally in the form of ferric iron (Fe^{3+}) which is reduced in the gut to the ferrous (Fe^{2+}) form by ferric reductase activity provided by duodenal cytochrome B and possibly Steap2 [3]. The ferrous iron is then transported across the apical (gut lumen) side of enterocytes by the ferrous iron transporter divalent metal ion transporter 1 (DMT-1), also known as Nramp-2 (natural resistance-associated macrophage protein) [7]. Some evidence suggests that heme iron may be taken up by receptor mediated endocytosis, although no high-affinity heme receptor has been identified to date [3]. There is some evidence that dietary ferritin is also taken up by endocytosis [8]. Once inside the enterocyte heme is broken down by heme oxygenase and dietary ferritin iron is released from ferritin. It is currently believed that iron from the various sources enters a common iron pool within the enterocyte. Some of the iron may be stored directly within the enterocyte as ferritin, while other iron will be released from the cell to end up bound to blood transferrin [8].

2. Intracellular iron trafficking and transportation

There is only one known cellular iron exporter, namely ferroportin [9–11]. This protein is found on the basolateral membrane of enterocytes as well as on other cells such as reticuloendothelial macrophages that export recycled iron, hepatocytes that release storage iron and on differentiating erythrocytes. Ferroportin exports iron in the ferrous (Fe^{2+}) form, but transferrin binds iron in the ferric (Fe^{3+}) form, so ferroxidases are believed to play a role in iron export. In intestinal enterocytes it is believed that hephaestin is the active ferroxidase, while in other cells this action is performed by the either circulating or GPI-linked multicopper ferroxidase ceruloplasmin [3,5]. Once bound to transferrin the iron is delivered to peripheral tissues by the transferrin–transferrin receptor system. After binding to the transferrin receptor, transferrin is internalized by receptor mediated endocytosis and upon acidification of the endosome iron is released from transferrin and converted to the ferrous form (Fe^{2+}) by the ferrireductase Steap family proteins [12,13] and transported across the membrane of the endosome into the cytoplasm by the action of the ferrous iron transporter divalent metal ion transporter 1 (DMT-1) protein [3,5].

Ferroportin has been shown to be regulated transcriptionally in enterocytes and macrophages [11,14] and to be translationally regulated by the iron responsive element (IRE) present in the 5′-UTR of the ferroportin mRNA through the action of iron regulatory proteins (IRP). The IRE–IRPs system is controlled by intracellular iron levels [9,11,15,16]. IRPs are activated during low iron condition under which they bind to the IRE of ferroportin mRNA resulting in translational suppression. Restrained ferroportin expression leads to reduced iron export, maintaining iron for cellular requirements. In addition, ferroportin is regulated at a post-translational step by the master iron homeostasis hormone, hepcidin. In erythroid precursor cells (and in enterocytes) a second mRNA encoding for ferroportin has been reported [17,18]. This mRNA is produced by the use of an alternate, upstream gene promoter and has an identical open reading frame in the mRNA, and as such the protein produced is identical. Critically, this second mRNA (termed FPN1B) does not contain an IRE in the 5′-UTR, and as such is not regulated by iron deficit [18]. It is currently believed that during erythropoiesis the relative expression of these two messages is coordinated to ensure that iron is exported from the cells during early differentiation, but kept in the cells during late differentiation when heme synthesis begins and iron demand is at its highest [18].

2.1. Iron regulation by hepcidin

The absorption of iron by enterocytes, the efflux of recycled iron from macrophages and the efflux of stored iron by