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Author manuscript
Free Radic Biol Med. Author manuscript; available in PMC 2016 July 11.

Published in final edited form as:
Free Radic Biol Med. 2014 July ; 72: 23–40. doi:10.1016/j.freeradbiomed.2014.03.039.

Physiology and Pathophysiology of Iron in Hemoglobin-Associated Diseases

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Abstract

Iron overload and iron toxicity, whether because of increased absorption or iron loading from repeated transfusions, can be major causes of morbidity and mortality in a number of chronic anemias. Significant advances have been made in our understanding of iron homeostasis over the past decade. At the same time, advances in magnetic resonance imaging have allowed clinicians to monitor and quantify iron concentrations non-invasively in specific organs. Furthermore, effective iron chelators are now available, including preparations that can be taken orally. This has resulted in substantial improvement in mortality and morbidity for patients with severe chronic iron overload. This paper reviews the key points of iron homeostasis and attempts to place clinical observations in patients with transfusional iron overload in context with the current understanding of iron homeostasis in humans.

Introduction

Toxicity and increased morbidity due to iron overload are common and well-recognized complications associated with various hemoglobin disorders. Chronic iron overload occurs primarily from repeated blood transfusions in a number of hematological disorders. In fact, the most extensive information regarding severe chronic iron overload comes from decades of experience with the management of patients with thalassemia major, a hemoglobinopathy where the primary morbidity stems from iron overload and that is fatal, if untreated. The toxicity due to transfusional iron overload depends upon a number of factors in addition to the degree of tissue iron loading itself. While our experience with thalassemia has been very helpful, it is not entirely applicable to all disorders associated with iron loading, as the patterns of tissue iron distribution and the severity of tissue damage differ among them.

Many advances in our understanding of the treatment of transfusional overload have occurred, particularly in the last 15 years. The ability to noninvasively measure tissue iron in humans by magnetic resonance imaging (MRI), major breakthroughs in our understanding of the molecular physiology of iron regulation, and the availability of new iron chelating

Conflict of interest: Dr Coates is on the Speaker's Bureau or consults for Novartis Pharma, Shire Pharma, Apo Pharma, and Celeron.

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agents have resulted in a dramatic improvement in the survival of patients with severe iron overload [1, 2].

The purpose of this review is to summarize our current understanding of iron homeostasis, briefly introduce the hematological disorders primarily associated with iron overload, and discuss how new knowledge regarding iron homeostasis informs and is validated by observations made in course of clinical monitoring and management of humans with transfusional iron overload.

Iron homeostasis

Biological organisms have evolved to conserve iron and as such, humans have no mechanisms for the excretion of iron. Approximately 1 to 2 mg per day, or about 0.05% of the total body iron, is lost through desquamation of the gastro-intestinal tract lining and skin, and in small amounts, through blood loss [3]. This is balanced through absorption of dietary iron, primarily in the duodenum. Iron balance is maintained entirely through the regulation of absorption and recycling of iron from red cells. Iron absorption can be increased by as much as 20 fold in cases of acute blood loss (reviewed in [4, 5]). Iron absorption can also be pathologically increased in certain genetic disorders of iron transport as well as in hemoglobin disorders associated with ineffective erythropoiesis. Figure 1 summarizes key features of normal and pathologic iron balance.

Patients with hemoglobin disorders have significant differences in iron utilization, erythropoietic drive and iron input from transfusion that result in pathological iron absorption, iron loading and toxicity. In these patients, the relatively small changes in dietary absorption and minimal iron excretion are not sufficient to maintain iron balance.

Regulation of iron proteins

Iron balance is maintained by controlling the levels and function of iron transport proteins. Transferrin is the main plasma iron transporter that binds two molecules of ferric iron (Fe^{3+}). Transferrin is usually between 20 and 30% saturated with iron (see below). At the systemic level, transferrin saturation is the main iron sensor and plays a role in controlling the levels of the iron regulatory peptide, hepcidin. At the cellular level, there are two common mechanisms that apply to most of the proteins involved in iron homeostasis. First, iron regulatory protein 1 (IRP1) and 2 (IRP2) bind to iron response elements (IRE) in untranslated regions (UTR) of mRNA encoding proteins involved in cellular iron uptake, storage and export (transferrin receptor-1, TfR; divalent metal transporter-1, DMT1; ferritin-H/ferritin-L/ferroportin, FPN). IRP1/2 bind to IRE under conditions of low iron, while they dissociate from IRE in high iron states (reviewed in [6]). If the IRE is in the 3'UTR, IRP binding stabilizes the mRNA, prevents degradation, and increases protein production. If the IRE is in the 5'UTR, mRNA translation is inhibited [6–8]. The second general mechanism imparts tissue specific sensitivity to iron balance by modulation of the proportion of iron sensitive and iron-insensitive mRNA. At least for DMT1 and FPN, two different splice variants of mRNA exist, one with IRE, and the other without. This means that one variant responds to iron levels and one does not. The ratio of IRE to non-IRE differs in different tissues, resulting in differences in responsiveness to iron and differences in loading [9, 10].

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In general, the IRP/IRE system protects against iron loss. There are over thirty-five mRNAs including hypoxia inducible factor 2α that have IRE and are responsive to iron [7, 11].

Dietary uptake

Under normal circumstances, dietary ferric iron is reduced by cytochrome B (DcytB) to ferrous iron (Fe^{2+}) at the apical brush border of duodenal enterocytes, and transported into the cell by divalent metal transporter-1 (DMT1). DMT1 expression is highest at the duodenum and decreases toward the colon [12]. Dietary heme iron is absorbed into the enterocyte via the heme carrier protein-1 (HCP1). Inside the enterocyte, heme is degraded by heme oxygenase and iron is released into the cytosol [13–15]. The free iron, referred to as labile cellular iron (LCI), is stored in the cells by ferritin or exported to the plasma by FPN. As enterocytes recycle about every three days, the iron stored in enterocytes is lost in the stool. This and the very small amount of iron excreted in the bile are the only natural mechanisms for iron removal in humans and accounts for a 1–2mg loss per day, as mentioned above [3].

Macrophage phagocytosis of erythrocytes

Recycling of iron from heme is a main component of iron homeostasis. Macrophages in the reticuloendothelial system recycle iron from senescent red cells via erythrophagocytosis [16]. About 90% of senescent endogenous or transfused red cells are eliminated by this mechanism. The internalized heme is degraded by heme oxygenase and the iron is either stored by ferritin, or released into the plasma through FPN by the macrophages, which are the main regulators of plasma iron levels [16–19]. The effects of this regulation are seen clinically in the case of acute inflammation. If iron release into plasma by the macrophages is blocked, as is the case in response to fever, plasma iron levels drop within hours because of the continued requirement for 25 mg/day of iron to make red cells [20, 21]

Free hemoglobin and heme, which may be present in the plasma of patients with hemoglobin disorders because of shortened red blood cell survival and intravascular hemolysis, bind to haptoglobin and hemopexin, respectively, and are taken up by haptoglobin- or hemopexin-mediated binding to the scavenger receptor, CD163 on reticuloendothelial macrophages [22–24]. Like with intact red cells, heme oxygenase releases iron in the macrophage where it is then stored by ferritin in the cytosol or transported back to the plasma via FPN.

DMT1 regulation

In addition to enterocytes, erythroid precursors, hepatocytes, macrophages and other cells also express DMT1, which transports iron released from transferrin in endosomes into the cytosol [25]. Its expression is markedly increased by iron deprivation in the intestine, and less so in the kidney, liver, brain, and heart [12, 26]. DMT1 mRNA has a splice variant with an IRE in the 3'-UTR of the mRNA and one that has no IRE. Hence, when cellular iron levels are low, transcription of DMT1 is favored and more iron is transported into the enterocytes [26]. The ratio of the two variants differs depending on the tissue. Brain has the highest IRE/non-IRE ratio, while spleen, thymus, pancreas have the highest non-IRE/IRE

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ratio. Thus, for example, iron entry into the pancreas would not be expected to decrease in the presence of high iron [5, 10, 12, 26].

Ferritin storage

In the cytosol, labile cellular iron (LCI) binds to ferritin or is exported in the Fe^{2+} state to the plasma via FPN. Ferritin is a multimeric iron storage protein that is found in animal and plant cells as well as in fungi and bacteria, and can bind about 4500 molecules of iron. Iron is incorporated into ferritin as Fe^{2+}, but is quickly oxidized to Fe^{3+} within the ferritin shell by H-ferritin ferroxidase. The main function of ferritin within the cells is to protect them from iron toxicity. Small amounts of ferritin are released in the plasma by macrophages as L-ferritin via a lysosomal secretory pathway [27]. Ferritin mRNA has an IRE, and an increase in intracellular free iron leads to translational increase in ferritin production [28]. Ferritin has been used as an estimate of iron loading, although the correlation between iron load and ferritin is only accurate in patient populations[29].

Transferrin transport of iron

Transferrin (Tf) is the main iron transport protein and binds two molecules of ferric iron. Transferrin-bound iron (TBI) is the primary source of iron available to cells under normal conditions. Holotransferrin binds to the homologous transferrin receptors, TfR1 and TfR2, and is endocytosed. In the acidic lysosomal environment, Fe^{3+} is released from Tf, and exits the lysosomes via DMT1 into the cytosol. In order for the transfer into the cytosol to occur, Fe^{3+} has to be reduced to the ferrous state, Fe^{2+}(reviewed in [5]). Iron can also be transported out of the endosomes by the metal iron transporter, ZRT/IRT-like protein 14 (ZIP14) [30].

Both TfR1 and TfR2 have IREs in the 3'UTR and are post-transcriptionally regulated by IRP. TfR1 is expressed in most tissues, but at much higher levels in erythroid precursors and liver. TfR1 is also expressed in the heart at about the same level as in the liver, but 7.5 times less than in the spleen, and by implication, in splenic macrophages [31]. TfR2 is exclusively expressed in the liver and intestine, and at levels 5.8 times higher in the liver than the intestine. Levels of TfR2 are much higher than those of TfR1 in human liver [32]. Both receptors preferentially bind diferric Tf, but the affinity of TfR1 for iron is 25 times higher than that of TfR2. TBI is taken up exclusively by TfR1 in erythroid precursors, but is taken up by both TfR1 and TfR2 in the liver [5, 33]. Unlike TfR1, TfR2 does not have IRE and its expression does not respond to iron levels [34, 35].

Ferroportin export of cellular iron

FPN is the only known cellular iron exporter. It is expressed at very low levels in the membranes of most cells, but is abundant in macrophages, liver, syncytiotrophoblasts in placenta, the basolateral membranes of enterocytes [36, 37], and in erythroid precursors [38]. FPN gene expression in the heart is about 3 fold less than in the liver, and does not change with iron deficiency [31]. However, FPN mRNA and protein levels do increase in the heart about 2 fold with iron loading, which is sufficient to cause a Tf saturation of 70% [39]. Like DMT1, FPN has two mRNA splice variants, one that contains an IRE and one that does not, allowing for tissue iron export variability in response to cellular iron based of the

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relative proportion of the two forms of mRNA [38, 40]. FPN exports Fe^{2+}, which must then be oxidized to Fe^{3+} in order to bind Tf. Though the exact mechanism is still unclear, an oxidase must be at play in order for iron to be exported. Ceruloplasmin, a multi-copper oxidase in plasma, facilitates release of iron and oxidizes Fe^{2+} into Fe^{3+} for binding to Tf. Low levels of ceruloplasmin in copper deficiency or congenital aceruloplasminemia lead to intracellular iron accumulation. The resulting high intracellular iron causes mitochondrial damage and can trigger progenitor apoptosis [41–43]. An analogous but membrane-bound multi-copper oxidase called hephaestin is present in the basolateral membrane of enterocytes and facilitates iron transport from the gut into the plasma [4, 5].

FPN is the target of the iron regulator peptide, hepcidin [44, 45].

Hepcidin

Hepcidin is a 25 amino acid, defensin-like peptide that was discovered in the course of purifying β-defensin 1 from urine [46]. This peptide hormone is made in the liver and regulates the flow of iron from enterocytes and macrophages into the plasma by binding to FPN, thereby causing its internalization and degradation by the ubiquitin pathway [44, 45]. It is the primary regulator of the movement of iron into the plasma. Hepcidin is expressed almost exclusively in the liver, with 31 and 15 fold lower levels detectable in intestine and heart, respectively [31].

Hepcidin levels are very low or absent in iron deficiency, leading to increased transport of iron via FPN from enterocytes and macrophages into the plasma. Conversely, hepcidin is elevated in iron overload and inflammatory states [4, 20, 47, 48]. This results in decreased iron absorption, decreased release of iron into the plasma, and sequestration of iron in tissue macrophages.

Iron-mediated regulation of hepcidin levels is through bone morphogenetic protein-6 (BMP-6) and its receptor on hepatocytes (Figure 2). The regulation is complex, and in humans, involves several proteins in addition to the BMP-6 receptor, i.e., the co-receptor hemojuvelin (HJV), the hereditary hemochromatosis protein (HFE), TfR1, TfR2, and matriptase-2 (coded by TMPRSS6). BMP-6, produced by sinusoidal endothelial cells in the liver, binds to the BMP-6 receptor complex on the hepatocyte, which in turn activates hepcidin transcription through a SMAD1/5/8 pathway. HJV, which is responsible for juvenile hemochromatosis, acts as a co-receptor and increases the sensitivity of the BMP receptor to BMP-6. Neogenin, a ubiquitous membrane protein, may also act as part of the BMP-6 receptor complex to enhance hepcidin production. High levels of holotransferrin stabilize TfR2 and displace HFE from TfR1, allowing it to interact with TfR2. The TfR2-HFE complex then associates with the BMP receptor complex, ultimately increasing hepcidin production. Thus, TfR2 is acting as an iron sensor that shuts down release of iron from enterocytes or reticuloendothelial macrophages into the plasma when iron is high and Tf is saturated (reviewed in [49, 50]). Finally, matriptase-2 (TMPRSS6) is a metalloproteinase on the hepatocyte membrane that is stabilized by iron deficiency and cleaves HJV, leading to decreased activation of the BMP-6/SMAD pathway, and hence decreased production of hepcidin. Mutations in TMPRSS6 lead to loss of inhibition of

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