IRON OVERLOAD: CAUSES, CONSEQUENCES, AND CONTROL

***Douglas P. Bentley**

Department of Pathology, Spire Cardiff Hospital, Cardiff, UK
**Correspondence to dpbentley45@gmail.com*

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ABSTRACT

The endeavours of physicians taxed with the problem of iron deficiency over the last 100 years have successfully eliminated this as an issue of hematological interest. Iron overload, although by no means a new problem, has drawn much more attention over the last few decades. The development of reliable indicative tests of iron status since the early 1970s has sparked a revolution in our understanding of the mechanisms of iron absorption, the processes of internal iron metabolism, and the effects of iron in excess of physiological needs. There continues to be regular reviews of specific issues in iron overload but this article sets out our understanding of the principles of iron metabolism in the context of iron overload, and is intended to point the ways by which this potentially fatal disorder, created by nature or by man, might be overcome.

Keywords: Iron overload, hemochromatosis, iron depletion, iron chelation.

THE BASIC PRINCIPLES OF HUMAN IRON METABOLISM

At our current state of knowledge, iron is essential for all life. It provides the vital functions of electron transport, reversible oxygen binding, enzyme activation, DNA synthesis, and pro-oxidant scavenging, but is required only in amounts necessary for these metabolic functions. Small amounts of iron storage, as reflected by a serum ferritin concentration in the range 20-400 µg/L, merely act as a reserve for when iron demands are increased.

Human iron metabolism is highly conserved: approximately 30 mg of iron are required daily, predominantly for hemoglobin synthesis. This iron is derived mainly from the digested hemoglobin of senescent red cells recycled by the macrophages of the reticuloendothelial (RE) system. Iron exchange with the environment is, by comparison, very slow: plasma iron turns over every 2 hours, whereas total body iron turnover time is 10 years. Only 10-20% of the iron is absorbed from the diet (which on average contains about 10 mg of iron daily) to maintain a total body iron content of about 35-50 mg/kg body weight, and to compensate for the small insensible iron losses incurred by desquamation and minor bleeding. The major quantity of functional iron is in the form of hemoglobin and iron stores (of variable magnitude up to 500 mg) and these are metabolically inactive. Except in active pathological conditions, any iron excess above immediate requirements is retained largely by the RE system in the form of ferritin and the related compound hemosiderin, but some is also found in the hepatic parenchymal cells. These stores are exhausted in uncomplicated iron deficiency prior to the development of anaemia. However, iron depletion without anaemia may afford some protection against microbial infection,[1] and iron loading may increase susceptibility to infection with some specific microorganisms.[2] Other mammals have a completely different scale of iron turnover, with much of the daily erythropoietic iron requirements met from the diet and, consequently, there is a greater rate of iron loss and a high level of protection from iron overload and toxicity. Man has no controlled iron excretory mechanism.

The hepatic antibacterial peptide hepcidin[3] is the central regulator of the iron supply. Its function is to suppress iron release from macrophages and hepatocytes, to limit the iron supply to that required

for erythropoiesis and other metabolic functions, and form enterocytes to maintain an adequate level of iron stores, but to limit these to non-toxic levels. Continuous cellular iron release is affected through the transmembrane (TM) sole iron exporter ferroportin. Ferroportin expression increases in iron deficiency[4] but its iron transport role is restrained by hepcidin binding,[5] which leads to internalisation and degradation of both molecules.[6]

Homeostatic hepcidin regulation is determined by the degree of plasma transferrin saturation and liver iron status.[7] Diferric transferrin displaces human hemochromatosis protein (high-Fe; HFE), the gene product of HFE, from the widely expressed cell surface membrane transferrin receptor 1 (TfR1). HFE then forms complexes with hepatic parenchymal cell-derived[8] hemojuvelin (HJV), and in turn, bonds with TfR2, expressed almost exclusively on the hepatocyte surface and stabilised by holotransferrin. HJV, amongst other functions, is the key regulator of hepcidin expression and, via its co-receptor, the uniquely iron-sensitive[9] bone morphogenetic protein 6 (BMP6),[10,11] which together with the essential binding of neogenin,[12] initiates a complex signalling process and by phosphorylation of some proteins of the intracellular SMAD family upregulates the nuclear HAMP coding for hepcidin.[13] The mature protein is a cleavage-induced single product of furin action.[14] Digestive activity of the TM serine protease matriptase-2, also liver–derived, and whose expression is increased in iron depletion,[15] cleaves HJV into multiple inactive sub-components[16] to decrease membrane HJV expression and competitively block intact HJV-induced hepcidin synthesis. Enterocytes are less sensitive to hepcidin than macrophages,[17] which reflects the different order of their respective volumes of iron exported to the plasma. By lowering the plasma iron concentration, hepcidin may increase host resistance to infection.

A well-mixed diet contains three chemically different forms of iron. Heme iron, once liberated from myoglobin and hemoglobin, is absorbed by the heme carrier transport protein HCT1,[18] located on the apical aspect of the duodenal enterocyte. It also functions as a folate transporter and its regulatory role in iron metabolism has not been elucidated. Inorganic iron is reduced to the Fe2+ form by duodenal cytochromes prior to absorption by the divalent metal transporter 1 (DMT1), the expression of which on the duodenal enterocyte is increased in iron deficiency.[19,20] The degree of transferrin saturation is conveyed to the replicated duodenal crypt cell and in low iron states stabilises the iron responsive protein (IRP) binding to the iron responsive element (IRE) on the 5'-untranslated DMT1 mRNA to signal increased expression. In the iron replete state mRNA translation is inhibited, and in addition, DMT1 can become saturated and iron absorption becomes limited – the so-called 'mucosal block'.[21] Iron nanocages, largely of non-animal origin,[22] are absorbed through a specific receptor, and release from internalised endosomes is controlled by protonation. Once intracellular iron enters a common intracellular pool it is released into the plasma by ferroportin[23] and bound to transferrin through the oxidative action of ceruloplasmin and its homologue hephaestin.[24]

IRON OVERLOAD

Definition

There is no precise definition of iron overload. The presence of excess iron may be determined by a gross elevation in serum ferritin concentration or expansion of iron stores demonstrated histologically by bone marrow or liver biopsy. Magnetic resonance imaging will detect ferric iron in the liver, heart, and the brain which has largely replaced chemical measurements made on liver biopsy samples.[25] Elevated serum ferritin concentrations may be found in non-iron deficiency anaemias, indicating the displacement of iron unused for hemoglobin synthesis into stores. Increases in serum ferritin concentration are seen in acute liver disease,[25] acute leukaemia,[26] inflammatory disease,[27] hyperthyroidism,[28] and the rare hereditary hyperferritinaemia-cataract syndrome,[29] and these do not indicate total body iron excess. Fully saturated transferrin is also a feature of iron overload. By exception in untreated megaloblastic anaemia elevated transferrin saturation, non-transferrin bound iron (NTBI), and hyperferritinaemia may arise through the severe anaemia and massively increased (up to 9-fold[30]) ineffective erythropoiesis and rapid shunting of recycled hemoglobin iron through macrophages to the plasma. NTBI disappears and transferrin saturation precipitously falls to normal within the first 2 days,[31] before there is any increase in the hemoglobin concentration, after effective replacement therapy. The serum ferritin concentration is usually raised due to the severe anaemia and with effective treatment returns to normal or occasionally levels indicative of iron deficiency; iron overload is not a feature.

Causes of Iron Overload

It is evident that iron overload occurs only when intake is increased beyond normal daily requirements. The stringent control of iron absorption prevents large quantities of dietary and inappropriate therapeutic iron given by the mouth leading to iron overload, but prolonged iron medication has been identified as a possible cause in a small number of patients.[32,33] There are usually contributing factors such as a genetic mutation causing increased iron absorption in these recorded instances. Patients with chronic kidney disease are at risk of iron overload if intravenous iron therapy, given to correct the increased blood lost during hemodialysis, is not monitored closely.[34] Although it is possible that two-way trafficking of iron in enterocytes[35] may provide a mechanism for increased iron loss, there are no data available on iron losses in iron overload patients. Primary or secondary increase in iron absorption and the administration of iron parenterally, usually as a blood transfusion, are the responsible causes of iron overload. In inherited states of increased iron absorption penetrance is always highly variable. Gender,[36] iron-limiting disorders such as coeliac disease,[37] hepatic dysfunction,[38] the effects of diet, alcohol consumption,[39] obesity,[40,41] and, of course, blood donation, will alter the penetrance of inherited disorders leading to iron overload.

Primary Increase in Iron Absorption

In hereditary hemochromatosis (HH) a genetic defect in the iron absorption pathway leads to progressive iron accumulation and is a result of insensitivity or loss of the ferroportin/hepcidin iron regulatory mechanism. In the classical form, mutation of the HFE gene[42] prevents expression of the HFE protein by substitution of the cysteine residue with tyrosine (C282Y), thereby losing a disulphide bond. Defective post-translation processing of HFE causes its intracellular retention and subsequent degradation and failure of cell surface expression, where bonding with β-2 microglobulin (β-2M)[43] is essential for its function. Suppression of hepcidin production leads to failure of ferroportin degradation and uncontrolled iron export from enterocytes. Transferrin saturation thereby rises and iron is deposited in vulnerable organs.

The C282Y mutation is very common in the people of Northern and Western Europe, and therefore, frequently alters the phenotype of the less common inherited disorders of iron absorption. The less critical H63D mutation allows cell membrane HFE expression and binding to β-2M on the cell surface. It therefore produces a milder phenotype as there is a lower suppression of hepcidin expression. In compound heterozygotes, clinical disease is often associated with comorbid factors.[44] Many further mutations of HFE, 18 to date, have been identified but are very rare. Generally the iron accumulation in classical hemochromatosis is insidious, and organ damage becomes apparent only in later life, also, females are affected less severely than males.

Juvenile hemochromatosis (JH) is phenotypically similar, and the autosomal recessive form of HH arises as a result of HJV mutation.[45] This produces a more severe degree of iron overload with organ damage evident in the first two decades of life. Over 30 mutations in HJV have been identified, but G320V is the most common and has been reported in several populations worldwide. Hypogonadism and cardiac involvement are typical features. Mutation of HAMP, the coding gene for hepcidin causes a much more severe form of JH[46] because of a total lack of hepcidin. Mutation of TfR2[47] is an exceedingly rare cause of HH although >30 single nucleotide polymorphisms have been identified. The condition is less severe than classical HH, but with generally earlier development of iron overload. Co-inheritance of C282Y is commonly found in affected patients.

Ferroportin mutations,[48] although rare, are the most common causes of dominant HH in East Asia, where the C282Y mutation is rare. The unusual phenotype of RE iron loading in the face of low transferrin saturation is found in the typical form. The failure of hepcidin to denature mutated ferroportin and consequent loss of iron export from macrophages and enterocytes explains these features. In the atypical form, inheritance of ferroportin retains its iron export function but is insensitive to the effects of hepcidin to produce a phenotype similar to classical HH, but without enterocyte iron accumulation.[49] Homozygous ferroportin disease has not been described and is probably incompatible with life.

Neonatal hemochromatosis is the most common cause of rapidly fatal liver disease in the neonate and is caused by intrauterine iron loading. It does not have a known genetic basis.[50] However, the discovery of maternal anti-liver antibodies, of unknown antigen, indicates a complement-fixing immunoglobulin G alloantibody attack. Hepcidin

expression is lowered secondary to liver dysfunction and leads to increased foetal iron uptake from the ferroportin-rich placenta. The elevated serum ferritin is typical of inheritance from the mother, and a high recurrence rate in siblings suggests a bigenic origin of a mitochondrial disorder.

Nutritional Iron Overload

An iron loading syndrome similar to HH is seen in some native Africans. In the past this has been attributed to the effects of an acid beer and alcohol-induced hepcidin suppression, before its possible genetic basis was identified. The Q248H ferroportin mutation is unique to Africans and is associated with increased serum ferritin concentrations in adults[51,52] and children.[53]

Secondary Increase in Iron Absorption

Although iron absorption is controlled mainly by body iron status, the rate of erythropoiesis also plays a role. Conditions in which erythropoiesis is increased, irrespective of its effectiveness, will elevate iron absorption non-specifically. Congenital hemolytic anaemias, such as hereditary spherocytosis and pyruvate kinase deficiency leads to iron overload, only if they are co-inherited with an additional mutation such as the C282Y of classical hemochromatosis.[54] The massive increase in ineffective erythropoiesis also occurs in β-thalassaemia, which majorly contributes to the iron loading caused by the life-maintaining blood transfusions. Non-syndromic inherited sideroblastic anaemia (SA), usually caused by congenital primary δ-aminolevulinic acid synthetase deficiency, or GLRX5-dependent ALAS2 deficiency are associated with systemic tissue iron loading.[55] Rarer disorders of effective red cell production leading to iron overload include xerocytosis[56] and congenital dyserythropoietic anaemias.[57] Iron overload is also found without blood transfusion in mild SA58 and other myelodysplastic syndromes.[59] In erythroid hyperplasia iron absorption is increased as a result of low hepcidin secretion. There may be additional marrow signalling pathways which are not fully understood. Erythropoietin stabilised duodenal ferroportin,[60] growth derived factor 15 (GDF-15),[61] and the twisted gastrulation protein homologue 1[62] have been associated with low hepcidin secretion, but other candidates continue to be sought.

METABOLIC DISORDERS CAUSING INAPPROPRIATE IRON DEPOSITION

Acaerulplasminaemia

Mutation in the ceruloplasmin gene inhibits transferrin iron uptake because of a lack of ferroxidase activity. This is dependent on the trinuclear copper clusters which stabilise this circulating protein.[63] At least 40 mutations have been identified and the majority lead to premature stops in protein synthesis. The truncated ceruloplasmin is degraded and the oxidation of Fe2+, essential for binding to transferrin, fails. The autosomal recessive syndromes of hepatic iron overload, cerebellar ataxia, retinal degeneration, and diabetes mellitus result[64,65] together with growth retardation. The unusual iron accumulation in the brain is thought to be due to the inability of neuroglial cells to donate iron to plasma transferrin.[66]

DTM1 mutation

The rare non-functional DTM1 mutations[67] inevitably lead to defective duodenal inorganic iron absorption and low red cell iron incorporation. Liver iron overload is a usual feature and presumably results from excess iron absorption through the alternative pathways. The serum ferritin concentrations, however, are less markedly raised[68,69] for unknown reasons. Both homozygotes and compound heterozygotes have been identified.

Atransferrinaemia

There are many acquired causes of hypotransferrinaemia but the very rare autosomal recessive inheritance of atransferrinaemia causes a microcytic anaemia with absent marrow iron but widespread siderosis. Homozygous mutations and compound heterozygotes have been described.[70] Excessive iron absorption results from lowered hepcidin secretion, and iron loading in the liver results from the absence of transferrin binding.

Transfusional Iron Overload

Patients with β-thalassaemia constitute the majority of those transfusion-dependent from an early age. Young sickle-cell anaemia patients with cerebrovascular occlusion are becoming increasingly common. However, the number of adults requiring maintenance transfusion is increasing with the ageing population in whom refractory anaemias are very common. Each 250 mL red cell transfusion delivers variably up to 250

mg of iron, which would add as much as 13 g of iron annually in a transfusion-dependent adult. Iron from senescent red cells accumulates in the RE cells, and although these iron deposits are relatively non-toxic, the iron load will saturate transferrin from an early stage while RE iron loading continues. Whenever plasma transferrin iron binding sites are saturated, variable amounts of labile (chelatable) iron can be detected in developing erythroid cells and this increases β-thalassaemia[71] propensity for toxic NTBI to appear in the plasma. The greatest morbidity from chronic transfusions is due to cardiac failure and this is usually apparent after two decades of transfusion-dependency without iron chelation.

EFFECTS OF IRON OVERLOAD

In excess, iron is damaging to biological macromolecules by the very same properties that make it essential to life. However, the formation of free radicals through its interaction with oxygen and water is inevitable in the neutral pH and oxygen-rich and humid conditions of mammalian existence. Intra-cytoplasmic cell membranes and nucleic acids are particularly vulnerable, and iron toxicity is manifested by irreversible tissue damage and the risk of malignant disease. Iron in the Fe2+ state will induce free radical formation by donating electrons to oxygen to generate superoxide or hydrogen peroxide to give highly reactive ($OH^{\cdot}$) oxygen species. However, iron in this reduced state is essential for participating in reversible oxygen binding to heme. A complex system of binders, chaperones, and reductases is therefore essential to protect the organism while maintaining these metabolic processes. The transport protein transferrin and the storage protein ferritin (i.e. that are not required for metabolic purposes), hold the iron in the less catalytic Fe3+ form.

The location of the increasing iron deposits during positive iron balance is determined through the route by which the iron is acquired. Increased iron absorption initially elevates plasma transferrin saturation and increases iron uptake in any cells expressing a transferrin receptor. Iron loading in RE cells is derived from senescent red cells acquired by blood transfusion or from ingested parenchymal cells damaged by iron toxicity, such as is seen in the hepatic Küpffer cells. RE dysfunction, however, is not a notable feature of iron overload, although in HH there is an increased susceptibility to infection by iron-dependent micro-organisms.[2]

There is, however, a wide margin of safety in pathological iron loading. From the optimal iron status (a normal hemoglobin concentration and adequate iron reserves) iron stores may be harmlessly increased by several grams, but no threshold of toxicity can be defined for an individual. RE cells have the largest, but a still-limited capacity, for iron storage. At some stage additional iron progressively saturates transferrin and loads parenchymal cells indiscriminately, but the skin, heart, liver, pancreas, and other ductless glands are particularly vulnerable. NTBI appears in the plasma when all transferrin iron binding sites are occupied and are thought to be the direct cause of iron toxicity. The associated labile plasma iron (LPI) pool, by definition, may have the greater propensity to cause organ damage by the production of free radicals but also may be more readily chelated when an iron binding site is available. Indeed, a common observation is that chelators may eliminate the LPI pool but elevate the NTBI.[72] It has been suggested that iron forms the LPI pool, which may be internalised by DMT1 expressed on hepatocyte parenchymal cells, but the metal transporter ZIP 14 is also a likely candidate.[73]

MANAGEMENT OF IRON OVERLOAD

Venesection

In patients with excessive iron absorption the most effective means of iron depletion is by venesection, which is highly effective, controlled, and predictable in its effect. Furthermore, there should be no adverse systemic effects, although with progressive iron depletion iron absorption will inevitably increase in cases of primary iron hyperabsorption.[74] Most HH patients will require many months of weekly or twice-weekly venesection. The volume of iron to be removed is difficult to predict and lifelong treatment will be required. A target serum ferritin of 50 µg/L is recommended but depletion of NTBI and LPI pools are probably more relevant; liver damage will misleadingly elevate the serum ferritin concentration. Cardiac involvement, liver cirrhosis, and arthropathy are the complications most refractory to the effects of iron depletion in HH. Devoid of these complications patients should have a normal life expectancy.[75] Many patients are nowadays identified prior to the development of iron toxicity by family studies or unexpectedly by routine testing intended to detect iron deficiency. The exception to response by venesection is typical

ferroportin disease where anaemia will result without iron depletion. Iron depletion, however, may not be so critical because of the low transferrin saturation, which would not lead to excessive NTBI, and therefore, little risk of tissue damage.

Iron Chelation

The most powerful iron chelators of necessity are those naturally occurring. Although iron is readily released by metabolic processes, the iron in heme, transferrin, and ferritin is resistant to currently available therapeutic chelating agents. The size of the chelatable iron pool limits the amount of iron available at any one time. Pharmacological iron chelators are therefore very inefficient. However the amount of chelatable iron increases with progressive iron loading but remains only a fraction of the total iron needed to be removed in order to achieve a beneficial degree of iron depletion. NBTI, incorporating the LPI pool and any transitional intracellular iron pool of a low molecular weight (e.g. iron-citrate), have been proposed as the source of chelated iron.[76] Chelation of this pool might temporarily remove the toxic iron,[77] but equilibrium with the large iron deposits in storage organs is rapidly re-established.

The approaches to iron chelation therapy have recently been extensively reviewed.[78,79] Desferrioxamine has been in use for >60 years but suffers the disadvantages of being ineffective by mouth, having a short plasma half-life requiring continuous subcutaneous infusion, and ocular and oto-toxicities. In an emergency situation, as in cardiac failure due to siderosis, continuous intravenous infusion of Desferal may be considered. Newer orally effective iron chelators such as deferiprone and deferasirox, being more lipophilic, may achieve better tissue penetration, giving access to a larger chelatable iron pool. In order to achieve maximum effect, however, trials of combined oral and parental chelators are showing some possibilities of greater effectiveness, but negate the value of avoiding the use of desferrioxamine. Erythropoiesis can be improved in some SA patients by iron depletion with deferasirox.[80] It has been postulated[81] that chelation removes reactive oxygen species and shifts iron from the mitochondria to the cytosol to decrease mitochondrial damage and limit the ineffective erythropoiesis. Chelators have also been used in the treatment of iron overload in acaeruloplasminaemia.[82] In neonatal hemochromatosis the antenatal administration of high-dose immunoglobulin infusions is highly effective[50] and rather unexpected for an alloantibody reaction. Treatment of these new-borns, however, with immunoglobulin and iron chelators is now no longer required.

Modulation of Iron Absorption Control

As a general rule it is more straightforward to achieve a therapeutic benefit by suppression of a biological process than to promote it, and iron metabolism is no exception. Inhibition of the expression of TMPRSS6 would lower matriptase activity and preserve HJV. It has been shown in murine hemochromatosis and β-thalassaemia models that targeted antisense oligonucleotides lower transferrin saturation and liver iron accumulation.[83] In the anaemic β-thalassaemic mice there was a shift towards more effective erythropoiesis. For transfused patients this may suppress the additional burden of increased iron absorption but would not lead to iron depletion to the required degree.

Minihepcidins

Hepcidin therapy would be the most obvious approach to patients with hepcidin defects and has been shown to be effective in mice.[84] Hepcidin is a peptide with a complicated structure, and is difficult to extract or synthesise. Furthermore, it is unlikely to be effective by mouth. Truncated hepcidin analogue structures administered parenterally or by mouth have been effective in lowering the serum iron concentration in normal mice and liver iron content in hepcidin knockout mice.[85]

Transferrin

Transferrin therapy has been shown[86] to clear the toxic NTBI, to lower the serum iron concentration, and apparently decrease the iron overload in β-thalassaemic mice. There still remains the need for total body iron depletion in the highly transfused affected patients. In inherited atransferrinaemia NTBI accumulation and low hepcidin secretion are corrected by apotransferrin infusions[87] but these do not lower the accumulated iron stores, which, however, could be depleted by subsequent venesection or chelation.

CONCLUSION

The last two decades have seen a vast improvement in our understanding of human iron metabolism. Iron overload continues to present great clinical challenges, and new therapeutic

strategies are required to overcome the obstacles presented by nature. Modulation of iron absorption and development of more powerful iron chelators hold the key to improving the lives of the large numbers of patients yet to benefit from the progress made.

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