Iron, Free Radicals, and Oxidative Injury[1]

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EXPANDED ABSTRACT

KEY WORDS: • *iron* • *free radicals* • *superoxide* • *hemochromatosis* • *hepcidin*

The ability of transition metal ions to undergo facile 1-electron oxidation or reduction makes them obvious potential chemical partners for reactions involving biological free radicals. It is not coincidental that superoxide dismutases, the enzymes that catalytically destroy the superoxide radical (O_2) by alternately oxidizing and reducing it, have been found containing 3 different transition metals (Cu, Mn, or Fe) at their active sites. Iron is by far the most abundant transition metal in the human body because of its roles in oxygen binding and transport and electron transport. Because of the central and essential roles of iron in the metabolisms of all aerobic organisms, humans have evolved some peculiar ways of dealing with it. These peculiarities provide opportunities for the cause of diseases related to iron absorption, transport, and metabolism, as well as for the exacerbation of general mechanisms of disease involving free radical injury.

Peculiarity 1: Iron must be handled very carefully due to its ability to catalyze potentially destructive redox chemistry

In healthy cells, iron ions are never found in a naked state, but are always tightly chelated, usually by proteins. If iron is being transported or stored it must be chelated in very specific ways that discourage redox cycling (e.g., by transferrin or ferritin). When iron is allowed to redox cycle (e.g., as in cytochromes or peroxidases), it is tightly held in the context of the protein's active site. Free iron is a loose cannon, chemically (1). One of the most devastating actions of free redox-active iron within the cell is the initiation of lipid peroxidation. Lipid peroxidation is a free radical chain reaction between polyunsaturated fatty acyl groups in cell membranes and molecular oxygen. It leaves in its wake dysfunctional membranes and cell death. Perhaps the most interesting iron-containing proteins are those that may release free redox-active iron when a free radical–mediated 1-electron reduction occurs (ferritin, aconitase) or that may take on new biologic activity, such as the transformation of cytosolic aconitase into iron-responsive protein-1, a protein that can modulate the efficiency of translation of mRNA encoding the iron-storage protein ferritin. Thus, on a small scale the free radical-induced liberation of iron from an iron-binding protein may reflect an evolved signaling pathway; on a larger scale, however, it may result in the wholesale destruction of the organism.

Peculiarity 2: Humans have evolved an iron-storage system to ensure adequate iron in case of blood loss

Most iron in humans is in the blood, a liquid tissue that may rather abruptly be lost in significant amounts. The rate at which the human body can resynthesize lost blood cells exceeds by ~20-fold the rate at which it can absorb the necessary amount of dietary iron. Therefore, the ability to store some excess iron became an evolutionary advantage, allowing for rapid recovery from an unexpected loss of blood. The cost of maintaining this advantage is not insignificant; carrying excess iron puts the organism at elevated risk in the face of excessive free radical production associated with disease states. What was once a valuable survival mechanism has become an anachronism. In today's civilized societies, lost blood is quickly replaced by transfusion. The iron stores are no longer required. Only the associated liability of maintaining iron stores remains (2).

Peculiarity 3: They who control the availability of iron control the playing field when organisms battle for dominance

As multicellular organisms evolved, they faced the problem of dealing with microbial infection. One of the earliest defense mechanisms to evolve was the ability to produce antimicrobial peptides such as the β-defensins (3). One such antimicrobial peptide, liver-expressed antimcrobial peptide 1 or hepcidin, appears to have evolved additional talents—the ability to regulate iron uptake and transport (4,5). For microbial growth, iron is often the rate-limiting nutrient. Plasma is a nutritious growth medium as long as iron-containing proteins such as

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transferrin are plentiful. When a microbial infection occurs, hepcidin synthesis is strongly upregulated in the liver, and hepcidin is secreted into the circulation. This hepcidin is directly microbicidal (6) but serves, in addition, to sharply downregulate iron uptake by enterocytes (7) and, presumably, to shut down iron mobilization and utilization within the body for as long as the infection persists. This sudden restriction of iron availability in plasma is then thought to substantially impair microbial growth until the infection can be cleared by the immune system.

Nearly all Caucasians with iron-overload disease (hemochromatosis) have a C282Y mutation in the *HFE* gene (8). *HFE*-knockout mice produce a model of hemochromatosis much like the human disease (9), and these mice are especially sensitive to ischemia and reperfusion injury of the heart (10). Until recently, however, the mechanism of iron regulation by HFE was not understood. $HFE^{-/-}$-knockout mice produce little hepcidin and quickly develop iron overload, but when they are crossed with transgenic mice that constitutively produce hepcidin, the iron overloading is completely suppressed (11). Thus, it appears that HFE protein may serve as a transcriptional factor that regulates the hepcidin gene, *HAMP*. When HFE function is absent or impaired, little hepcidin is produced and iron absorption runs at full throttle.

Regulation of the system is now thought to be controlled by the circulating level of iron-loaded transferrin. When this level is high, iron-loaded transferrin binds to its receptor, transferrin receptor 2 (Tfr2), on the hepatocyte. On the cytosolic side, this receptor can bind HFE and β_2-microglobulin. On binding iron-loaded transferrin it releases HFE, which translocates to the nucleus and upregulates hepcidin production. This hepcidin is secreted into the circulation, signaling intestinal enterocytes to stop absorbing iron from the gut. Conversely, when the plasma iron-loaded transferrin level drops, the Tfr2 receptor is not occupied and resumes binding of HFE at the plasma membrane. The absence of HFE from the nucleus causes hepcidin production to drop; its secretion ceases, and iron uptake at the enterocyte resumes.

Transgenic mice that overexpress hepcidin develop severe iron-deficiency anemia and die shortly after birth (5). This suggests that inappropriate hepcidin regulation may account not only for iron-overload disease, but also for the iron-deficiency anemia often associated with chronic inflammatory disease, cancer, and infections such as HIV (12).

The study of iron metabolism is nearly as old as medicine itself, yet the intricacies of its regulation have remained mysteries until very recently. Our new understanding of the roles of HFE and hepcidin promise rapid progress for the development of effective therapies for diseases involving misregulation of iron metabolism. Recombinant hepcidin, or the development of an agonist that mimics its activity, may correct the defect causing hemochromatosis in millions of people. Just as important, the development of a hepcidin antagonist may permit correction of the iron-deficiency anemia associated with chronic diseases. New diagnostic tests may make routine the assessment of hepcidin levels as part of normal clinical screening procedures. Perhaps most important of all, this new understanding of iron metabolism may help to dispel finally the long-standing beliefs in the medical profession that "iron is difficult to absorb" and "more is better." Neither is true. This understanding may lead to new definitions of iron deficiency, iron adequacy, and iron overload.

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