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IRON HOMEOSTASIS AND ITS DISORDERS IN MICE AND MEN: POTENTIAL LESSONS FOR RHINOS

Tomas Ganz, Ph.D., M.D. and Elizabeta Nemeth, Ph.D.

Abstract: During the last decade, there have been remarkable advances in the understanding of iron homeostasis and its disorders. This review summarizes our presentation at the International Workshop on Iron Storage Disease in Black Rhinos that was held in Orlando, Florida, USA, from 23 to 26 February 2011, and it is directed to veterinarians and nutritional experts who treat rhinoceroses. This review summarizes the current knowledge in humans and mice regarding the physiology and molecular basis of iron overload, and it explores how it can be applied to the problem of iron overload in captive rhino populations.

Key words: Hereditary hemochromatosis, iron loading anemias, iron absorption, hepcidin, ferroportin.

INTRODUCTION

The last decade witnessed remarkable advances in the understanding of iron homeostasis and its disorders.[1] Many of these breakthroughs resulted from studies of naturally occurring genetic diseases in humans or from genetic manipulation of mice and zebrafish, the two model vertebrate species that have dominated medical genetics research. Directed to veterinarians and nutritional experts who treat rhinoceroses, this review summarizes what has been learned, and it explores how it can be applied to the problem of iron overload in captive rhino populations.

IRON BALANCE

The average healthy adult human contains ca. 4 g of iron, most of which is in hemoglobin of erythrocytes (ca. 1 mg/ml in packed erythrocytes), and ca. 1 g is in storage in the liver and the spleen. Reflecting the essential role of iron in oxygen transport and key metabolic processes, and the poor iron availability in most natural settings, iron is strictly conserved. Unless there is bleeding, only ca. 1–2 mg/day of iron (<0.1% of the total body iron) is lost from the body, predominantly through the shedding of iron-containing cells from the gastrointestinal tract and the skin. In comparison, healthy human urine contains negligible amounts of iron. Although during massive iron overload, the total amount of iron lost from the body may increase somewhat, the incremental iron loss is relatively minor. Iron balance must therefore be maintained by the physiological regulation of dietary iron absorption.[12] In humans, iron overload is most often caused by genetic diseases that cause disruptions in the regulation of iron absorption or through blood transfusions that bypass normal regulatory mechanisms.

The maintenance of iron balance in the mouse[27] follows the same principles: provision of appropriate amounts of iron for metabolic needs and storage through regulated dietary absorption and strict iron conservation achieved by very low excretion and other losses.

ABSORPTION OF DIETARY IRON

Although in humans and other carnivores most bioavailable dietary iron[15] is present as heme, the absorption of inorganic iron has been best studied and may be more relevant for plant-eating species. At the apical surface of absorptive enterocytes in the duodenum, ferric iron is reduced by duodenal cytochrome B and other reductases to ferrous iron that is then transported across the apical membrane by the divalent metal transporter-1 (DMT1)[17] in a process that requires acid conditions (protons) on the apical surface (Fig. 1). It is not yet clear whether the requirement for reduction of ferric to ferrous iron significantly limits absorption or whether the well-documented lower absorption of ferric compared with ferrous salts[5] is limited by the low solubility of ferric iron. Depending on the need of the organism for iron, intracellular iron is stored in cytoplasmic ferritin or exported on the basolateral surface to plasma, via the iron exporter ferroportin. In the basolateral membrane, ferrous iron exported by ferroportin undergoes oxidation to the ferric form, catalyzed by the copper-containing ferroxidase hephaestin[30] with a smaller contribution from a related copper-containing ferroxidase, plasma

From the Departments of Medicine (Ganz) and Pathology (Nemeth), David Geffen School of Medicine, 10833 Le Conte Avenue, Los Angeles, California 90095-1690, USA. Correspondence should be directed to Dr. Ganz (tganz@mednet.ucla.edu).

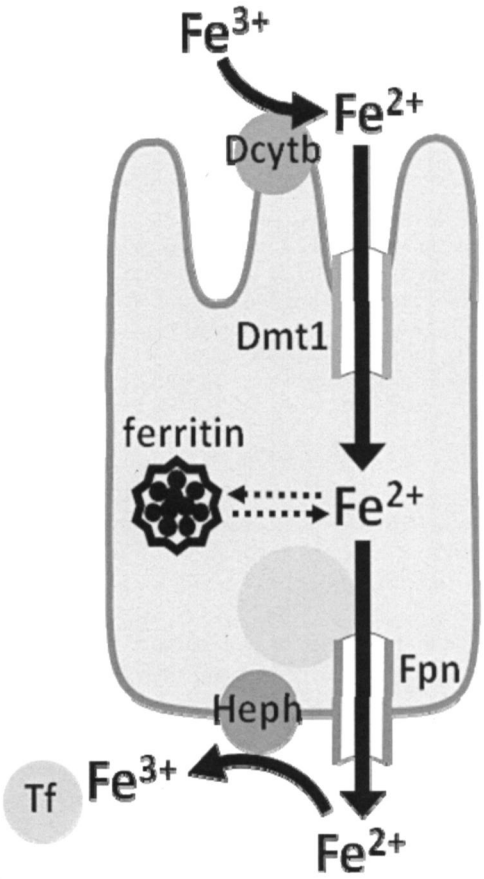

Figure 1. Transport of inorganic iron across the duodenal epithelium. Duodenal cytochrome b (Dcytb) reductase (or another reductase) converts Fe^{3+} into Fe^{2+} that is then transported across the apical surface by Dmt1. Inside the cell, iron can either be stored in ferritin or exported to blood through ferroportin (Fpn), the cellular iron exporter. Fe^{2+} is oxidized by the copper-containing ferroxidase hephaestin (Heph) into Fe^{3+} before loading onto transferrin (Tf). Iron transport by Fpn apparently controls the overall rate of iron absorption.

ceruloplasmin.[8] Ferric iron is then taken up by plasma transferrin for distribution to iron-consuming organs, chiefly the erythropoietic compartment in the bone marrow. In humans and in mice, enterocytes are short-lived cells (days) and are shed into the fecal stream with any residual ferritin-bound iron they may contain.

Although the mechanism of iron absorption in rhinos is probably going to be the same as in mouse and human, molecular studies have not been performed, and most of the relevant genes in the rhino have not been sequenced yet.

SYSTEMIC CONTROL OF IRON ABSORPTION BY HEPCIDIN

The liver is the central regulator of iron absorption and distribution, through its secretion of the iron-regulatory hormone hepcidin[13] (Fig. 2). Circulating hepcidin regulates iron absorption by binding to ferroportin on the basolateral surfaces of enterocytes and inducing the endocytosis and degradation of ferroportin. When hepcidin concentration is high, little ferroportin is available to export iron from enterocytes to plasma, iron is retained in enterocyte ferritin, and iron delivery to plasma is inhibited. Conversely, low hepcidin concentrations allow iron absorption and delivery to plasma transferrin. This mechanism is conserved in all the vertebrates where it has been examined (human, mouse, rat, and zebrafish), but the presence of hepcidin in avians has been questioned,[16] and hepcidin is not found in invertebrates. In contrast, ferroportin is widely expressed not only in vertebrates but also in plants and invertebrates. Hepcidin-independent modes of ferroportin regulation may be important in species that lack hepcidin.

In humans and mice, alterations in hepcidin and ferroportin expression or interaction lead to the development of several iron disorders ranging from iron-restricted anemias to iron overload.

HEPCIDIN IS REGULATED BY IRON AND ERYTHROPOIETIC DEMAND

Hepcidin mRNA in the liver and hepcidin concentrations in plasma are increased in response to iron loading, as reflected by increased plasma iron concentrations or increased hepatic iron stores. Conversely, hepcidin is dramatically decreased by iron deficiency. Increased erythropoietic activity after bleeding, hemolysis, or erythropoietin administration also suppresses hepcidin production,[21,22] in anticipation of the requirement for iron for increased hemoglobin synthesis. Although some experiments suggest that erythropoietin could directly regulate hepcidin production by hepatocytes, this seems unlikely at erythropoietin concentrations involved in physiologic regulation or therapy.

MECHANISM OF HEPCIDIN REGULATION

In humans and in mice, hepcidin is produced and secreted by hepatocytes, under transcriptional control by a molecular complex centered on a

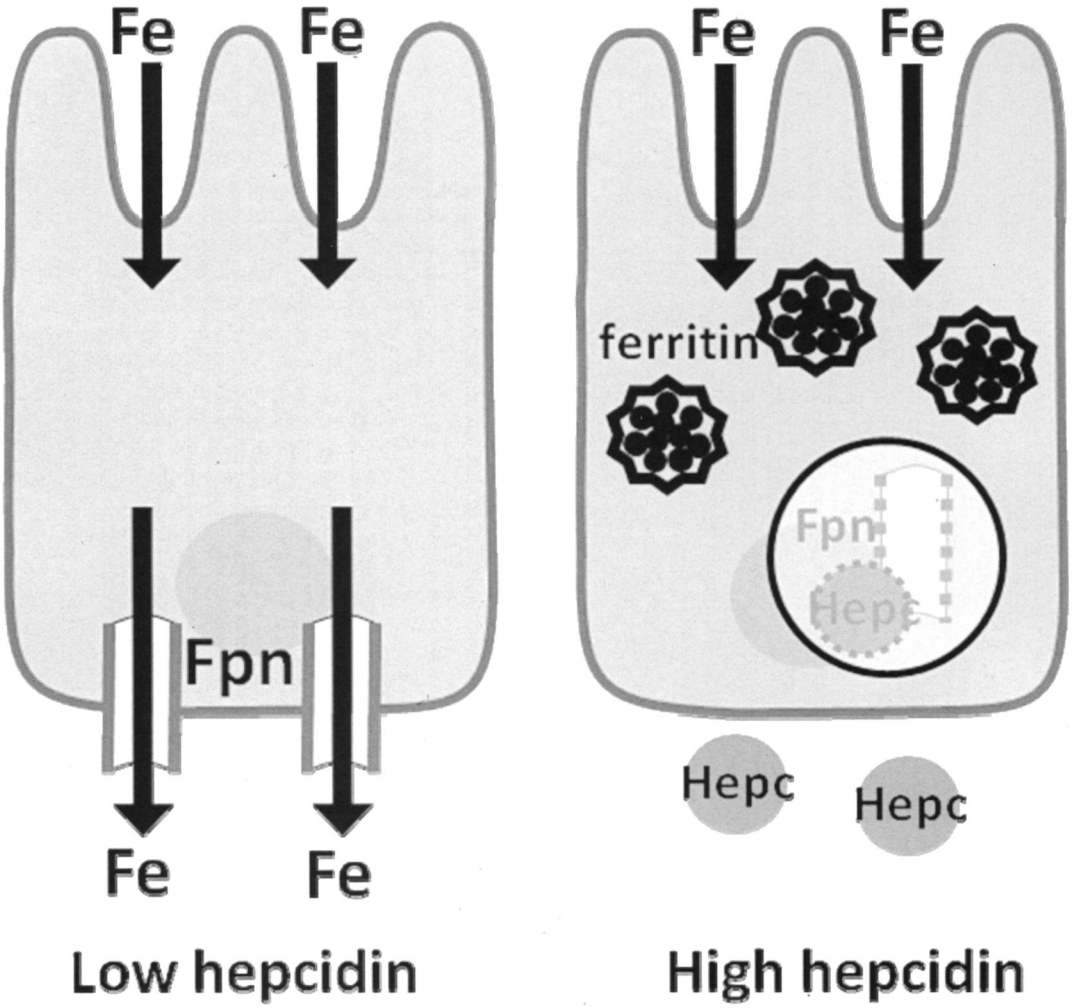

Figure 2. Hepcidin controls the absorption of dietary iron by regulating the transfer of iron from duodenal enterocytes via ferroportin to plasma. When hepcidin is low (left) ferroportin exports cellular iron to plasma. When hepcidin is high (right), it binds to ferroportin and causes its internalization and degradation. Iron export to plasma ceases and iron is retained in cellular ferritin. At the end of their life cycle (a few days), enterocytes are shed into the fecal stream, and their residual iron is largely lost from the body.

bone morphogenetic protein receptor (BMPR) and its signaling components.[3] The intensity of signaling through this pathway is modulated by several iron-specific proteins (Fig. 3), including transferrin receptor (TfR) 2, hemojuvelin (HJV), and the hemochromatosis protein (HFE).[9,25] The obligate ligand of the BMPR in the mouse is bone morphogenetic protein-6 (BMP6),[2,18] but it is not certain that the same protein is required in humans or other mammals. Hepcidin production is stimulated by both plasma iron and iron stored in hepatocytes.[9,25]

GENETIC HEPCIDIN DEFICIENCY CAUSES PARENCHYMAL IRON OVERLOAD

Homozygous disruption of HFE, TfR2, HJV, or hepcidin genes in humans or in mice causes disruption of regulated hepcidin production and hepcidin deficiency, mild with HFE mutations and progressively more severe with the other mutations.[7,25] Ablation of BMP6 in mice causes relatively severe hepcidin deficiency, but the human equivalent of this defect has not been identified yet. Hepcidin deficiency results in

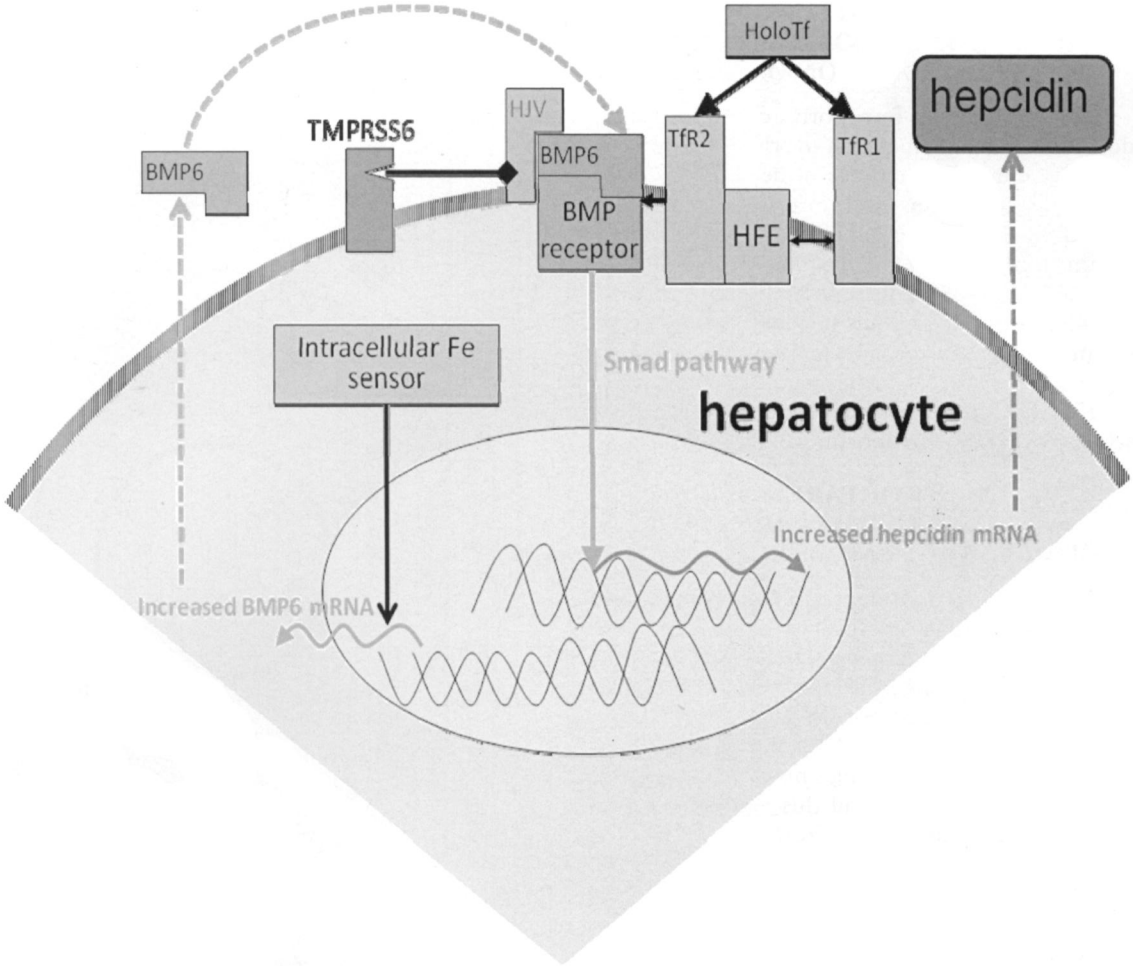

Figure 3. Current model of regulation of hepcidin secretion from hepatocytes by iron. Iron is sensed extracellularly in association with holotransferrin (HoloTf), the iron-loaded form of transferrin. HoloTf concentrations are detected by TfR1 and TfR2 aided by the hemochromatosis-associated molecule HFE (gray). The complex acts to modulate signaling through the BMP (blue) receptor that regulates hepcidin transcription via the Smad (homologs of *Caenorhabditis elegans* SMA genes and *Drosophila* MAD genes) pathway (blue). Two other accessory proteins modulate signaling through the BMP receptor: HJV (pink) that acts as a strong agonist of the receptor and TMPRSS6 (purple), a membrane serine protease that degrades hemojuvelin and inhibits signaling and hepcidin secretion. BMP6 (green) is an obligate ligand of the iron-related BMP receptor in the mouse, but its role in humans has not yet been documented. The production of BMP6 in the liver is increased in response to intracellular iron, constituting one of the pathways by which intracellular iron regulates hepcidin.

hyperabsorption of dietary iron and "parenchymal" iron overload, i.e., iron overload that affects functional cells in multiple tissues, with tissue distribution dependent on the rate of iron accumulation. Typically, macrophages and intestinal epithelial cells are spared. In mild forms of human hepcidin deficiency, the hepatocytes are the predominant target of iron loading, and variably severe disease manifestations in late adulthood (common forms of hereditary hemochromatosis). Severe hepcidin deficiency causes prominent iron loading of endocrine organs and cardiac myocytes, as well as hepatocytes, with severe disease manifestations already in young adults (juvenile hemochromatosis). Although a similar pattern of iron loading is observed in the mouse models of hereditary hemochromatosis, mice seem to be resistant to iron toxicity and the resulting organ injury, for reasons that are not well understood.

FERROPORTIN RESISTANCE TO HEPCIDIN ALSO CAN CAUSE PARENCHYMAL IRON OVERLOAD

Rare mutations in ferroportin cause autosomal dominant parenchymal iron overload that phenotypically mimics the effects of hepcidin deficiency.[31] The mutations fall into two categories: mutations that ablate hepcidin binding to ferroportin (mutations of the cysteine-326 thiol in an extracellular loop of ferroportin[11] and mutations in which ferroportin binds hepcidin normally, but the mutations prevent internalization after hepcidin binding. Both categories would be expected to have high serum hepcidin levels, as was the case in a family with the ferroportin C326S mutation.[26]

TREATMENT OF PARENCHYMAL IRON OVERLOAD IN HEREDITARY HEMOCHROMATOSIS

The standard treatment of iron overload in hereditary hemochromatosis is phlebotomy, initially intensive to remove excess iron.[29] When patients are "deironed," as defined by decrease of serum ferritin into the normal range, they continue maintenance phlebotomy to prevent iron reaccumulation. Deironing, however, decreases hepcidin levels further, and this condition would be expected to accelerate iron absorption and increase the need for phlebotomies. The optimal level of deironing that would prevent tissue injury without excessive phlebotomy is not well established. Experimental treatment with synthetic hepcidin analogs can prevent iron accumulation in hemochromatotic mice, but the therapeutic value of this approach still remains to be demonstrated (Ganz and Nemeth, unpubl. data).

IRON RECYCLING BY MACROPHAGES AND ITS REGULATION BY HEPCIDIN

Human and mouse erythrocytes have a life span of ca. 120 and 45 days, respectively. At the end of their life, the erythrocytes are ingested by macrophages, and their iron content is recycled into the plasma transferrin compartment.[4] Although the iron content of other cell types is much lower, their iron is presumably handled in a similar manner. In the human, the daily turnover of iron is ca. 20–25 mg, substantially exceeding the amount of iron absorbed from the diet. Like duodenal enterocytes, macrophages export iron through ferroportin, and the retention or release of iron is regulated by hepcidin in the same manner (Fig. 4). When hepcidin levels are high, iron is retained in macrophages, and this is characteristic of inflammatory conditions. In contrast, when hepcidin is low, such as in iron deficiency, macrophages release their iron stores. In hereditary hemochromatosis, hepcidin deficiency results in paradoxically low iron retention in macrophages despite systemic iron overload. Because the spleen is a very macrophage-rich organ, the loss of iron is manifested in mouse models of hemochromatosis as relatively low splenic iron content, contrasting with the often massive accumulation of iron in hepatocytes.

IRON-LOADING ANEMIAS

The intimate relationship between iron homeostasis and erythrocyte production is highlighted by disorders in which iron overload develops in the setting of hereditary or acquired anemias. In humans, iron overload develops most commonly in chronic anemias when patients receive multiple erythrocyte transfusions, as each typical unit of blood contains 200 ml of packed erythrocytes or ca. 200 mg of iron. In this setting, iron accumulates initially in splenic or hepatic macrophages that phagocytose erythrocytes, but iron eventually also accumulates in other cell types and tissues in which it causes cell injury and organ dysfunction. Cumulative transfusion of 50 units of erythrocytes will deliver 10 g of iron, an amount that is likely to cause toxicity unless the iron is removed.

Perhaps more surprisingly, iron overload can also develop in the absence of transfusions, as a consequence of ineffective erythropoiesis seen in β-thalassemias and congenital dyserythropoietic anemias.[19] Here, the excess iron comes from the hyperabsorption of dietary iron, similarly to hereditary hemochromatosis. The initiating process is the defective maturation of erythrocyte precursors followed by apoptosis in the bone marrow. The resulting anemia induces high levels of erythropoietin, and these stimulate the hyperexpansion of the erythrocyte precursor pool. Through mechanisms that are not completely understood, the expanded precursor population or its increased apoptosis leads to the suppression of hepcidin production in the liver. The BMP family proteins GDF15 and TWSG1 are overproduced in the marrow of thalassemic patients and thalassemic mice, respectively, suppress hepcidin production in isolated primary hepatocytes, and they are reasonable candidates for hepcidin-suppressive mediators in iron-loading anemias.[28] Other contributors may include the as yet unknown physiologic mediators that suppress hepcidin in response to bleeding or erythropoietin administration.

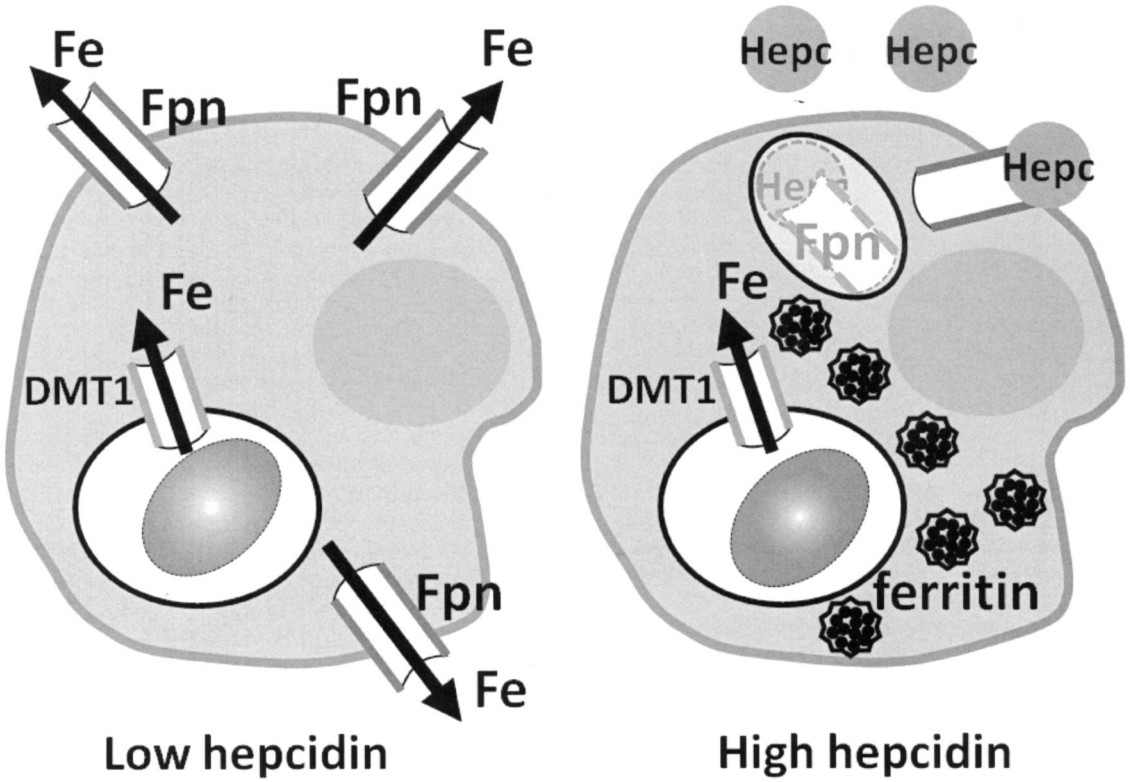

Figure 4. Regulation of the release of stored and recycled iron from macrophages. For splenic and other macrophages involved in iron recycling, red blood cell hemoglobin represents the predominant source of iron. Senescent erythrocytes are phagocytized, their hemoglobin is degraded, and the iron is transported from phagosomes via DMT1 into the cytoplasm where it is associated with chaperone proteins. When hepcidin is low (left), ferroportin then exports cellular iron to plasma. When hepcidin is high (right), it binds to ferroportin and causes its internalization and degradation. Iron export to plasma ceases, and iron is retained in cellular ferritin.

OTHER GENETIC DEFECTS THAT CAUSE PARENCHYMAL IRON OVERLOAD

Loss of function mutations in some proteins involved in the transport of iron in the intestine or in other tissues also can cause iron overload probably by affecting hepcidin regulation directly or through interference with erythrocyte development. Hyperabsorption of iron has been associated with severely decreased or absent transferrin, ceruloplasmin, heme oxygenase, or DMT1.[6] These disorders are extremely rare in humans, but the phenotype of anemia and iron overload has been mostly confirmed in mice, with the exception of DMT1 deficiency in which the mouse was not iron overloaded and ceruloplasmin deficiency in which the corresponding mouse was not anemic.

MACROPHAGE IRON LOADING IN "FERROPORTIN DISEASE"

Ferroportin disease is a group of autosomal dominant genetic disorders manifested by iron accumulation in macrophages. In these disorders, missense mutations in ferroportin cause diminished capacity for iron export.[23] Patients with this disorder have highly elevated serum ferritin concentrations, but they are otherwise clinically unaffected. If they are treated with phlebotomy, they can rapidly become anemic, presumably because macrophage iron is not readily mobilized because increased ferroportin activity is needed for export of macrophage iron for augmented erythropoiesis. This outcome contrasts with the effect of phlebotomy in hereditary hemochromatosis in which low hepcidin, and thus high ferroportin activity, favors iron mobilization.

IRON RETENTION IN MACROPHAGES DURING INFLAMMATION

Inflammation induced by infections or autoimmune disorders stimulates the production of hepcidin, at least in part by increasing the concentration of interleukin-6, a known stimulus for hepcidin production.[20] Under the influence of increased hepcidin, macrophages decrease their membrane ferroportin and their ability to export iron, but they continue to take up senescent erythrocytes, most prominently in the spleen. As a result, macrophages will appear iron-overloaded despite low serum iron, and iron delivery to the bone marrow will be restricted. In chronic inflammation, the hepcidin-mediated suppression of intestinal iron absorption may eventually deplete iron reserves and result in frank iron deficiency, but this outcome is mostly seen in children for which substantial amounts of iron are needed for growth.

IRON HOMEOSTASIS: FROM MICE AND HUMANS TO RHINOS

Accumulation of large concentrations of iron in the organs of captive but not wild browser rhino species represents a failure of homeostasis when these animals are fed a diet with higher bioavailable iron. In contrast, homeostasis seems to be maintained in grazing rhino species where the captive diet is more similar to their natural diet. These well-established observations suggest that browser rhinos adapted to an iron-restricted environment by selecting one or more mutations that allow them to absorb more dietary iron when this is transiently available. The accumulated iron would increase their fitness (survival or fertility) in the more common natural situation of iron scarcity. The same argument has been used for explaining the high prevalence of a specific hemochromatosis mutation among northern Europeans.[10] Based on the similarity of humans and mice with respect to iron absorption and its regulation, we would expect that iron absorption in rhinos is regulated in a similar manner, through the interaction of hepcidin and ferroportin, and their regulation by plasma and hepatic iron concentrations. If so, the comparison of hepcidin concentrations in rhino species susceptible and resistant to iron overload in captivity, and the analysis of the expression and gene sequences of hepcidin, ferroportin, and their regulators, would reveal the genetic lesion(s) responsible.

Less likely in our view, iron overload could be a result of excessive or ineffective erythropoietic activity that suppresses hepcidin and therefore leads to the hyperabsorption of iron. This situation would be analogous to iron overload in human β-thalassemia intermedia[14] and its mouse models. Excessive erythropoietic activity would have to be triggered in captivity and only in the browser species, perhaps as a result of the loss of an essential nutrient or protective substance required for efficient erythropoiesis and readily accessible only in the wild.

Finally, the differences in iron toxicity between mice (resistant) and humans (susceptible) point to the need to carefully document the pathologic consequences of iron accumulation on tissue and organ function in browser rhinos.

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