

Recent Advances in Iron Metabolism

Chaim Hershko, Abraham M. Konijn, and Gabriela Link

Iron is an essential element for life and, as a transition metal existing in both divalent (ferrous) and trivalent (ferric) forms, plays a key role as a donor and acceptor of electrons in redox reactions. Hence, living organisms were compelled to develop highly efficient mechanisms for the acquisition of iron from the environment on one hand, and for its storage and self-protection from iron-mediated toxicity on the other. In the normal adult, iron exchange with the environment is minimal, and over 97% of iron needs are supplied internally, mainly by recycling of hemoglobin iron from senescent red blood cells. Reticuloendothelial cells are responsible for this internal recycling of catabolic iron. In addition, about 1 mg or 3% of the daily plasma iron turnover is absorbed each day to compensate for iron losses through exfoliation of cells and physiologic blood loss.

Although all mammalian cells are able to acquire and to donate iron, one may distinguish between cells whose main function is iron donation—such as macrophages and intestinal mucosal cells—and cells that are iron receptors, such as the erythroid bone marrow and the placenta. Hepatocytes play an intermediate role by acting as iron donors mobilizing ferritin iron stores in situations of increased iron requirement, or acting as a storage depot for excess iron when plasma iron supply exceeds demand.

Molecular control of iron homeostasis

In recent years, remarkable insight into the control of iron homeostasis has been gained from studies involving the molecular control of mammalian iron metabolism. Transferrin iron uptake from the plasma involves the binding of iron-rich transferrin (FeTf) by the transferrin receptor protein (TfR) on the cell surface, followed by endocytosis of the FeTf-TfR complex with subsequent release of the iron from the endocytotic vesicle to a labile intracellular iron pool (LIP). Iron from the LIP is either utilized for the immediate metabolic needs of the cell (heme proteins, cytochromes, mitochondrial enzymes, etc.) or stored in the iron-storage protein ferritin (Ft). The metabolic needs of an iron-deficient mammalian cell require increased production of TfR to facilitate iron uptake and suppression of ferritin synthesis. Conversely, in iron excess, ferritin synthesis needs to be enhanced to prevent a potentially toxic expansion of the LIP, while at the same time TfR production should be shut off to prevent the uptake of unnecessary iron. Both of these processes are regulated simultaneously by an elegant reciprocal and coordinated translational control involving cytoplasmic mRNA by the iron-responsive element (IRE) and iron-responsive protein (IRP) mechanism (1). When cytoplasmic iron is low, IRP binds to the IRE of both TfR and ferritin mRNA. This binding stabilizes TfR mRNA and re-

sults in increased TfR translation. At the same time IRP binds to the IRE of ferritin mRNA repressing its translation and inhibiting the formation of new apoferritin. Conversely, when cytoplasmic iron is high, IRP dissociates from IRE on both ferritin and TfR mRNA, resulting in de-repression of apoferritin synthesis and destabilization and accelerated degradation of TfR mRNA. This reciprocal control allows an appropriate response to iron deficiency by increasing TfR production and decreasing iron storage in ferritin, whereas in iron overload the same mechanism results in decreased TfR production and increased iron accumulation in newly formed ferritin.

More recently, additional key proteins in the control of cellular iron regulation have been discovered. Ferrous iron uptake from the intestinal lumen is mediated by a transmembrane metal iron transporter DMT1 (formerly designated Nramp2 or DCT1). Similar to TfR, DMT1 mRNA contains an iron responsive element which stabilizes the molecule in iron deficiency resulting in increased DMT1 expression (2). DMT1 is responsible for the transport of ferrous iron through the luminal cytoplasmic membrane of the intestinal mucosal cell. Other proteins are responsible for exporting iron through the basolateral membrane of the intestinal mucosa. Ireg1 encodes a protein with multiple transmembrane domains and its mRNA contains an IRE structure binding specifically to IRP1 and IRP2⁽³⁾. Ireg1 mRNA levels are positively correlated with three independent conditions associated with increased iron absorption: iron deficiency, hypoxia, and atransferrinemia.

Another protein involved in the export of iron from the intestinal cell is Hephaestin, a transmembrane-bound ferroxidase that bears a 50% identity with ceruloplasmin⁽⁴⁾. Hephaestin does not localise to the basolateral membrane, and its relation to Ireg1 is presently unclear. However, its putative ferroxidase activity should facilitate iron egress from the cell by creating a steep transmembrane concentration gradient of ferrous iron. Finally, a ferric reductase has been recently described in the duodenal mucosa⁽⁵⁾ which is a b-type heme-containing cytochrome reductase and is assumed to be responsible for the ferric-reducing activity of duodenal mucosa, required for the transport of luminal iron associated with DMT1. Thus, an overall picture of the regulatory mechanisms associated with iron absorption is starting to emerge in which the IRE-IRP translational mechanism plays a key role determined by the magnitude of the intracellular labile iron pool.

Iron deficiency

In spite of the subtle and efficient regulation of normal iron balance, iron deficiency anemia is still one of the most com-

mon problems in the human population of the world. Largely, its causes are iron loss by physiologic or pathologic bleeding and dietary iron deficiency. These two causes of iron deficiency involve different age groups. In adult males and post-menopausal females, iron deficiency is almost always the result of pathologic blood loss and a thorough gastrointestinal workup to identify the source of bleeding is necessary. By contrast, in infants and pregnant women, in whom iron requirements greatly exceed the amount of dietary iron normally available, nutritional iron deficiency is responsible for most cases diagnosed with iron deficiency, and in most cases there is no need for gastrointestinal studies. An intermediate group is young females at the reproductive period of life. In such patients excessive menstrual bleeding is a major cause of anemia, and other causes are rare but should not be overlooked. A careful history is mandatory to exclude gastrointestinal complaints and an occasional background of familial gastrointestinal malignancies. If these are negative, laboratory workup can be limited to repeated negative occult blood tests in the stool.

The laboratory diagnosis of iron deficiency is based on several tests. Not all of these are necessary for establishing the diagnosis of iron deficiency in daily practice. Automated blood counts are helpful in indicating the presence of microcytic anemia. Typically, iron deficiency is characterized by microcytosis, which is proportional to the severity of anemia, and an increased RDW, whereas in thalassemia microcytosis is often out of proportion to the degree of anemia and the RDW is near normal. Other diagnostic tools include measurements of serum iron (SI), total iron binding capacity (TIBC, an indirect measure of transferrin protein), percentage of saturation of transferrin derived from the former two measurements, free erythrocyte protoporphyrin (FEP), serum ferritin, and soluble serum transferrin receptors. Each one has its own advantages, pitfalls, and cost-benefit considerations⁽⁶⁾. Most diagnostic laboratories readily provide SI, TIBC, and ferritin. SI and TIBC are extremely useful in diagnosing advanced iron deficiency anemia, but their sensitivity for early iron depletion is limited. The same is true for FEP. By contrast, serum ferritin is very useful in the diagnosis of early iron depletion, closely correlated with the magnitude of iron stores as determined by bone marrow non-heme iron or quantitative phlebotomy. An important limitation of serum ferritin is a non-specific increase in other disease states such as inflammation (ferritin is an acute phase reacting protein) and hepatocellular damage. A useful rule of thumb in inflammation is that iron deficiency may be excluded if ferritin exceeds 100 $\mu\text{g/L}$.

Serum transferrin receptors (TfR) have been introduced relatively recently and are used increasingly for clinical diagnosis. Their serum concentration increases steeply in the presence of iron deficiency anemia of increasing severity. Although they are useful in diagnosing iron deficiency anemia, they largely reflect the total body mass of TfR and are therefore an excellent indicator of plasma iron turnover. Indeed, ferrokinetic measurements of plasma iron turnover and erythron iron uptake have been largely abandoned in

favour of TfR measurements⁽⁷⁾.

In the management of iron deficiency anemia several guidelines should be kept in mind:

(a) The rate of response is slow, about 1 to 1.5 gm increase in hemoglobin per week, and there is no advantage in parenteral vs. oral iron therapy. (b) The difference between various oral iron medications is mainly in price, not in quality of response. Some slow-release tablets are poorly absorbed, which may explain failure of response. (c) The duration of treatment should be prolonged, between 3 to 6 months. In most cases the cause of anemia cannot be easily eradicated, such as in angiodysplasia, menorrhagia, and in all patients with a negative GI workup. Such patients should be under permanent surveillance, and iron medication renewed at the earliest indication of relapse. The long-term management of iron deficiency anemia is mainly the domain of the family physician. (d) Response to iron is the ultimate diagnostic test of iron deficiency anemia. If there is no response, compliance is good, and continued significant blood loss is excluded, the diagnosis might be questioned. Finally (e), compliance in maintaining oral iron therapy is an important test of patient-doctor relations. If the possible minor side effects are explained in advance, and if compromises are made by decreasing dosage or altering the timing of administration, very few patients will fail to adhere to iron therapy because of undesirable side effects.

Iron overload

In the Western, Caucasian population, the most common cause of chronic iron overload in hereditary hemochromatosis (HH) and the carrier rate in the population is about 1:12. In over 85% of homozygous HH the disease is caused by a C282Y point mutation of the HLA-linked HFE gene on chromosome 6⁽⁸⁾. In non-Caucasians HH is believed to be rare and is not HLA-linked. The most common cause of iron overload in non-Europeans, and in Southeast Asia in particular, is β -thalassemia major and β -thalassemia/HbE-disease. In the following discussion, therefore, I shall focus on iron overload in thalassemia.

The primary abnormality in thalassemia major is a wasteful, ineffective erythropoiesis resulting in a 10- to 15-fold expansion of the erythroid bone marrow and a drastic increase in hemoglobin catabolism. Iron accumulation is the consequence of blood transfusions as well as of increased iron absorption caused by erythropoietic activity. The combination of iron overload and increased outpouring of catabolic iron from the reticuloendothelial system overwhelms the iron-carrying capacity of transferrin, resulting in the emergence of toxic non-transferrin bound plasma iron⁽⁹⁾. Recognition of NTPI as a potentially toxic component of plasma iron in thalassaemic siderosis has important practical implications for designing better strategies for the effective administration of DF and other iron chelating drugs.

In thalassaemic patients who are not receiving iron chelation therapy, the accumulation of iron will progress relentlessly, and when about 20 grams of iron have been acquired, severe clinical manifestations of iron toxicity may

be anticipated. The most important complications of transfusional siderosis are cardiac, hepatic, and endocrine disease⁽¹⁰⁾. Pathologic findings in the *heart* include dilated, thickened ventricular walls with particularly heavy iron deposits in the ventricles, epicardium, and papillary muscles. Advanced cardiac siderosis results in heart failure and life-threatening arrhythmias. Myocardial siderosis is the single most important cause of mortality in inadequately treated thalassemic patients.

Cirrhosis is a common complication of thalassemia and, similar to cardiac problems, its incidence is age-related. However, the coexistence of chronic hepatitis B or C with an incidence ranging from 9 to 70% of thalassemic patients in various geographic areas underlines the complexity of this problem. Iron overload per se is responsible for the development of cirrhosis in many cases.

Endocrine problems caused by direct accumulation of iron in endocrine glands or indirectly through the hypothalamic-pituitary axis are common. Stunted growth, delayed puberty, hypothyroidism, hypoparathyroidism, and diabetes mellitus are all well-established complications of transfusional siderosis. Because diabetes and hypothyroidism appear when most endocrine cells are destroyed and replaced by fibrosis, these complications are rarely reversible.

Results of deferoxamine therapy

Although the impact of DF therapy on the survival and well-being of thalassemic patients has never been proven by prospective, randomized clinical studies, the beneficial effects of long-term deferoxamine treatment are clearly demonstrated by comparison of treatment outcome with historical controls. Iron chelating treatment should be started when serum ferritin levels reach about 1000 mg/L which usually occurs after the first 10 or 20 transfusions. DF is infused via a thin needle inserted s.c. to the arm or abdomen nightly, connected to a portable pump over 8-12 h, 5 to 7 times per week at a daily dose of 20-60 mg/kg. A urinary iron excretion of 0.5 mg/kg/d is usually sufficient to ensure negative iron balance. A new delivery system for continuous DF infusion has been introduced by Baxter allowing continuous 48 h s.c. or continuous 24 h i.v. delivery for 7 days each week. This technology allows effective removal of toxic free iron (NTPI) from the plasma, resulted in a significant decrease in serum ferritin within 4 weeks, and improves patient compliance compared to conventional s.c. DF pumps.

The impact of deferoxamine treatment on life expectancy is convincingly demonstrated by comparison of survival in well-chelated versus poorly chelated patients. In a major study of 1127 thalassemic patients at seven Italian teaching hospitals, it was shown that 70% of patients born before 1970 (hence prior to the modern era of chelation) survived to the age of 20 years compared to 88% of patients born after 1970 who received effective chelation from an early age⁽¹⁰⁾. Most of the improvement in survival was attributed to decreased cardiac mortality. This cohort of birth-related improvement in survival is reflected in a mir-

ror-like inverse decrease in cardiac mortality, supporting the assumption that prevention of cardiac mortality is the most important beneficial effect of DF therapy. Improved survival in well-chelated thalassemic patients has been reported in several other major studies from the UK and North America.

The strongest direct evidence supporting the beneficial effect of DF on hemosiderotic heart disease is the reversal of established cardiomyopathy in some far-advanced cases. Earlier experience in hereditary hemochromatosis has shown that the cardiomyopathy of iron overload is potentially curable by effective iron mobilization through phlebotomy. However, in transfusional hemosiderosis, the course of established myocardial disease was uniformly fatal and, until recently, believed to be non-responsive to iron chelating therapy. Such patients may still be responsive to aggressive chelating treatment. Continuous 24-hour ambulatory intravenous infusion of DF through central venous ports, using standard portable infusion pumps or the new Baxter delivery system, is a very effective method for the rapid reversal of established hemosiderotic heart disease. In addition, it is an excellent tool for improving patient compliance, allowing uninterrupted delivery of 6 to 12 grams DF per day and the effective depletion of very large iron stores.

Deferiprone (L1)

The family of 3-hydroxypyrid-4-one bidentate chelators, designed by Hider and Kontoghiorghes⁽¹¹⁾ binds to iron in a 3:1 ratio with a stability constant of 37, about six orders of magnitude higher than DF. The most important compound of this family is 1,2-dimethyl-3-hydroxypyrid-4-one, (deferiprone or L1). The results of long-term iron chelating therapy with L1 in thalassaemic patients have been summarized in recent years in several reviews^(12,13), and the combined experience of the four major European and Canadian groups pioneering the clinical use of L1 up to June 1994 has been described in a report of the International Study Group for Oral Iron Chelators (ISGOIC)⁽¹⁴⁾. The study involved 84 patients, 74 with thalassemia major or intermedia, representing a total of 167 patient-years of L1 treatment. Compliance was rated as excellent in 48%, intermediate in 36% and poor in 16% of patients. On an L1 dose of 73 to 81 mg/kg/d, urinary iron excretion was stable, at around 0.5 mg/kg/d with no indication of a diminishing response with time. Serum ferritin showed a very steady decrease with time from an initial mean \pm 1SD of 4207 \pm 3118 to 1779 \pm 1154 after 48 months ($p < 0.001$). Seventeen patients abandoned L1 therapy. Major complications of L1 requiring permanent discontinuation of treatment included agranulocytosis (3), severe nausea (4), arthritis (2), and persistent liver dysfunction (1). The remaining patients abandoned treatment because of low compliance (3) and conditions unrelated to L1 toxicity. Lesser complications permitting continued L1 treatment included transient mild neutropenia (4), zinc deficiency (12) transient increase in liver enzymes (37), moderate nausea (3), and arthropathy (16). There was no treatment-associated mortality. Two patients

died, both while off treatment: one of hemosiderotic heart disease and the other of *Pneumocystis carinii* pneumonia with AIDS. This study demonstrates the efficacy of L1 in long-term use for the treatment of transfusional iron overload, but also underlines the complications associated with such treatment.

In a recent major multicenter study employing the Apotex formulation of L1, involving 187 patients from Cagliari, Torino, Ferrara, Philadelphia and Toronto (the LA-2 study), subjects were closely monitored for L1 side effects by weekly clinical and laboratory assessment (15). The dose of L1 in this study as well as in those of three recent reports by Olivieri (16), Hoffbrand and Wonke (17), and Tondury (18) was 75 mg/kg/day. The mean duration of treatment was 1.61 years to 7.14, with the rest ranging from a mean of 2.75 to 3.28 years. Comparing these data with the ISGOIC study terminated in mid-1994 one can see that, with the exception of the Tondury study, there was no evidence of a consistent decrease in mean serum ferritins or liver iron concentrations comparing pre-treatment values with subsequent measurements. The percentage of patients in whom liver iron concentrations remained above 15 mg/g dry weight (identified in previous studies as the limit above which a significant risk of cardiac complications continues to exist) was 18 to 59%. Agranulocytosis developed in 6 patients and transient neutropenia in 19. Of a total of 92 patients, 36 (39%) discontinued L1 therapy. Six patients died. Of particular concern is the observation that four of the patients died with congestive heart failure due to iron overload, a complication that was shown previously to be prevented by effective deferoxamine therapy. Other important causes of L1 discontinuation were agranulocytosis or neutropenia (6 patients), arthropathy (5 patients), nausea (5 patients), or unsatisfactory response to L1 (8 patients) defined as low compliance (2), rising serum ferritins (4), request to resume DF (1), and change of residence (1). Collectively, these data imply that oral L1 treatment alone will not ensure sufficient protection in all patients and that close monitoring is required to identify patients in whom additional conventional chelating treatment with DF is indicated. Indeed, the combined use of oral L1 and DF infusions given 2 to 6 times weekly to patients with an unsatisfactory response to L1 alone has been advocated in a recent report by Wonke et al⁽¹⁹⁾.

Concerns related to the accelerated development of hepatic fibrosis have been expressed based on observations made on patients on long-term L1 therapy at the Toronto Hospital for Sick Children⁽¹⁶⁾. However, none of the 17 HcV-negative patients reported by Tondury and by Hoffbrand developed this complication. This important issue is likely to determine the future of L1 in clinical medicine and needs urgent clarification by reviewing the hepatic status of all other patients on long-term L1 treatment.

Iron chelation in conditions unrelated to iron overload

Because iron plays a central role in many important biological reactions such as the formation of toxic oxygen spe-

cies, mitochondrial inner membrane respiratory complex activity, and the activity of ribonucleotide reductase, a rate-limiting enzyme in cell replication, iron chelators have a potential role as therapeutic agents in conditions wherein interference with the above functions may modify the pathogenetic process. Because of limitations of space, I shall confine this part of my review to the effect of iron chelators on intracellular parasites.

The antimalarial effect of iron chelators: A number of experimental and clinical studies indicate that iron deficiency may have an important inhibitory effect on the progression of malarial infection and, conversely, that iron repletion may result in the exacerbation of malaria. In view of the possible beneficial effects of iron depletion, DF has been studied as a potential antimalarial agent. DF inhibits the growth of *Plasmodium falciparum* cultures at concentrations above 20 mM⁽²⁰⁾. In vivo studies in rats infected with *P. berghei*, mice with *P. vinckei* and monkeys with *P. falciparum* have shown that DF is able to suppress malaria if a continuous supply of the chelator is assured by frequent (8 hourly) subcutaneous injections^(21,22) or by osmotic pumps.

Encouraged by these studies in experimental animals, several investigators have tested the antimalarial effect of DF in humans. Traore et al⁽²³⁾ have studied the effect of DF 0.5 g i.m. given twice daily for 3 days on the rate of clearance of parasitemia in patients with *P. falciparum* malaria who were also receiving chloroquine. Although parasitemia appeared to decrease more rapidly in the six patients receiving DF and chloroquine than in the three controls treated by chloroquine only, the small number of patients, and the inclusion of chloroquine-resistant cases with resurgent malaria limit the value of this preliminary report. In another clinical study by Bunnag et al⁽²⁴⁾ 14 patients with symptomatic *P. vivax* and 14 with uncomplicated *P. falciparum* malaria received continuous i.v. DF 100 mg/kg for 72 hours. No other antimalarial treatment was given. In both groups DF reduced the parasitemia to zero within 57 to 106 hours. There was significant drug toxicity with transient visual blurring in nine patients. Recrudescence was observed within the subsequent 3 weeks in all but two patients. A major weakness of this study was the absence of a control group. Two controlled studies of DF in human malaria have been conducted by Gordeuk et al. In the first of these, the effect of DF therapy in partially immune adults with asymptomatic *P. falciparum* parasitemia has been tested^(25,26).

Collectively, these studies leave no doubt as to the ability of DF to hasten recovery from malaria, presumably by inhibiting parasite growth in a similar fashion to its effect in experimental in vitro and in vivo systems. In cerebral malaria, an additional beneficial effect could be inhibition of oxidative brain damage by preventing the formation of toxic free radicals through the iron-driven Fenton reaction. However, as emphasized in several recent editorials⁽²⁷⁾, additional large-scale carefully controlled studies are needed, with particular emphasis on mortality and neurological sequelae, before DF can be recommended for the treatment of cerebral malaria.

Other studies have shown that DF is able to inhibit the proliferation in vitro and in vivo of *Leishmania donovani*, *Trypanosoma cruzi*, *Pneumocystis carinii*, and *Legionella pneumophila*. These intriguing observations on the antimicrobial effects of DF and other iron chelators lend new meaning to the term “nutritional immunity” and open new channels for exploring the possibility of controlling infection by means of selective intracellular iron deprivation. Experimental models for studying the effect of iron chelators on other intracellular pathogens such as *Toxoplasma gondii*, *Chlamidia psittaci*, or *Mycobacterium tuberculosis* should be established. Packaging the chelator in liposomes or red cell ghosts, or manipulating their lipid solubility to improve their delivery to appropriate target organs such as the macrophage system may greatly improve their efficiency. In view of the short half-life and poor oral effectiveness of DF, it is unlikely that this drug will be suitable for clinical use as a practical antimicrobial agent. However, with the introduction of simple, orally effective new chelators, it is reasonable to expect that future research may lead to the identification of iron chelators with considerable usefulness in the control of infectious disease.

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