Therapeutic Potential of Iron Chelating Drugs

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The primary abnormality in thalassemia major is a wasteful, ineffective erythropoiesis resulting in a 10 to 15-fold expansion of the erythroid bone marrow (1) 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 (NTPI, 2-4).

Recent studies by Porter et al (5) have shown that plasma NTPI is removed by intravenous deferoxamine (DF) therapy in a biphasic manner and that upon cessation of DF infusion it reappears rapidly, lending support to the continuous, rather than intermittent, use of DF in high risk patients. The rate of low molecular weight iron uptake by cultured rat heart cells is over 300-times that of transferrin iron (6). Moreover, unlike transferrin-iron uptake which is inhibited at high tissue iron concentrations by down-regulation of transferrin receptor production, non-transferrin iron uptake is increased by high tissue iron (7). Such uptake results in increased myocardial lipid peroxidation and abnormal contractility, and these effects are reversed by *in vitro* treatment with DF (8). Recognition of NTPI as a potentially toxic component of plasma iron in thalassemic siderosis has important practical implications for designing better strategies for the effective administration of DF and other iron chelating drugs.

In thalassemic 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 (9) . The most important complications of transfusional siderosis are cardiac, hepatic and endocrine disease. Pathologic findings in the *heart* include dilated, thickened ventricular walls with particularly heavy iron deposits in the ventricles, epicardium and papillary muscles. These cellular deposits induce increased membrane lipid peroxidation in the sarcolemma resulting in impaired Na,K,ATPase activity (10), increased lysosomal fragility (11) and, in particular, impaired mitochondrial inner-membrane respiratory chain activity (12). It is possible to demonstrate early myocardial dysfunction in asymptomatic patients using MUGA scan (13) or dobutamine stress echocardiography (14). 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 (9) underlines the complexity of this problem. Iron overload per se is responsible for the development of cirrhosis in many cases. It has been proposed, that patients free of hepatitic virus infection, with liver iron concentrations below 15 mg/gm dry weight may have a prolonged survival free of the clinical complications of iron overload (15). *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 (16). 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. Experience with long-term DF therapy in thalassemic patients has been summarized in several extensive recent reviews (9,17,18)

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 (17). DF is infused via a thin s.c. needle inserted 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 to 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 (19). This technology allows effective removal of toxic free iron (NTPI) from the plasma, a significant decrease in serum ferritins within 4 weeks, and improves patient compliance compared to conventional s.c. DF pumps.

Recently, a depot preparation of DF has been developed by Novartis which, by delivering a smaller dose over a longer period of time, makes it more efficient and reduces the proportion of non-chelated wasted DF which is up to 90% of the drug delivered by current methods (20). Preliminary studies have shown that the duration of action of the depot formulation is over 30 hours, and cumulative iron excretion is more than 3 times that of standard *s.c.* bolus DF injections. If local torelability of the new depot preparation in current clinical studies proves to be acceptable, this new technology may permit once-daily or alternate day s.c. injections, obviating the need for portable pumps and improving patient compliance.

Response to treatment may be assessed by serum ferritin measurements, liver biopsies, computed tomography, or magnetic susceptibility (SQUID) (21). Serum ferritins are disproportionately low in patients with coexistent ascorbate deficiency (22) and high in active liver disease or inflammation. Nevertheless, serum ferritin is the most accessible and inexpensive tool for the long-term monitoring of chelating efficiency and protection from cardiac complications may be achieved when ferritin levels are kept below 2,500 mg/L (23).

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 7 Italian teaching hospitals, it was shown that 70% of patients born before 1970 and hence prior to the modern era of chelation survived to the age 20 y. compared to 88% in patients born after 1970 and therefore receiving effective chelation from an early age (9). Most of the improvement in survival was attributed to decreased cardiac mortality. This cohort-of-birth related improvement in survival is reflected in a mirror-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 U.K. (24) and North America (15,23,25).

The strongest direct evidence supporting the beneficial effect of DF on hemosiderotic heart disease is the reversal of established myocardiopathy in some far-advanced cases. Earlier experience in hereditary hemochromatosis has shown that the myocardiopathy 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. Several reports indicate that such patients may still be responsive to aggressive chelating treatment. Marcus et al (26) described first the reversal of established symptomatic myocardial disease in 3 of 5 patients by *continuous high-dose* (85-200 mg/kg/d) *i.v.* DF therapy at the cost of severe reversible retinal toxicity. Reversal of symptomatic myocardiopathy has been reported by others, without significant drug toxicity (27,28). 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.

CONDITIONS UNRELATED TO IRON OVERLOAD

Because iron plays a central role in many important biological reactions such as the formation of toxic oxygen species, 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 limit 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. However, this hypothesis is not universally accepted, as other studies have been unable to show an adverse effect of iron administration on human malaria (29), and severe iron deficiency may interfere with the normal immune response thus aggravating, rather than inhibiting infection.

In view of the possible beneficial effects of iron depletion, DF has been studied as a potential antimalarial agent. DF inhibits the growth of *P. falciparum* cultures at concentrations above 20 mM (30). *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 (31,32), 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 (33) 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 6 patients receiving DF and chloroquine than in the 3 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 (34) 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 9 patients. Recrudescence was observed within the subsequent 3 weeks in all but 2 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 (35,36). A detailed description of these important clinical studies can be found in a subsequent chapter of this volume.

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 (37), additional large-scale carefully controlled studies are needed, with particular emphasis on mortality and neurological sequelae, before DF could be recommended for the treatment of cerebral malaria.

In order to explore the role of lipophilicity in antimalarial activity, we have examined the antimalarial effects of 3-hydroxypyrid-4-ones (38), a family of bidentate orally effective iron chelators. All 3-hydroxypyrid-4-ones have an identical stability constant for iron(III), but they may be made more, or less lipophilic by increasing or reducing the length of the R_2 substituent on the ring nitrogen. Of the hydroxypyridin-4-ones investigated in our studies), those with the highest lipid solubility proved to be the most efficient antimalarial compounds. Subsequent studies by Shanzer et al (39) employing a series of synthetic iron chelators have confirmed our conclusions that the antimalarial effect of iron chelators is determined by their lipophilicity as well as their affinity to iron.

Other studies have shown that DF is able to inhibit the proliferation *in vitro* and *in vivo* of *Leishmania donovani* (40), *Trypanosoma cruzi* (41), *Pneumocystis carinii* (42), and *Legionella pneumophila* (43). These intriguing observations on the antimicrobial effects of DF and other iron chelators lend new meaning to the term «Nutritional Immunity» (44) 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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