Iron is one of the most common elements in nature and is essential for the functioning of proteins involved in oxidative energy production, oxygen transport, mitochondrial respiration, inactivation of harmful oxygen radicals and DNA synthesis. Because of the poor solubility of iron, living organisms were compelled to develop efficient mechanisms for its transport and storage. However, the subtle balance of normal iron homeostasis is grossly overwhelmed in chronic or hereditary anemias, such as thalassemia major, characterized by abnormal erythropoiesis and a life-long dependence on blood transfusions. Accumulation of iron through transfusions and inappropriate increased erythropoiesis leads to toxic damage to vital organs such as the heart, liver and endocrine organs with severe, life-threatening consequences, the most critical of which is siderotic cardiomyopathy which is responsible for about 70% of premature mortality. This is the background for past and current efforts to develop effective methods for promoting iron excretion using iron chelating drugs. In the current issue of the Journal, Galanello et al. present their experience with the new orally effective iron chelator deferasirox (Exjade, ICL670) in pediatric patients with β-thalassemia major. The paper offers a great deal of new information regarding tolerance, response and pharmacokinetics in the pediatric age group. Hence, it provides useful background information for future studies in children employing higher and more effective doses of deferasirox. Although the goal of a negative iron balance was not achieved in the study because inadequate dosages of the drugs were used, it was a wise safety decision to use low doses of a new compound with unknown toxicity for preliminary studies in children, despite the being inadequate for definitive studies. The study is part of a number of important recent publications introducing new concepts in iron chelation therapy and new dilemmas in the choice of optimal treatment.

Ideally, an iron-chelating drug should have selective affinity for ferric iron, the stable oxidized form of iron found in plasma and stores, should be orally effective, soluble in both water and lipids to allow easy access to tissues, affordable, and reasonably non-toxic. These goals are difficult to achieve. As of today, there are three leading chelators available commercially for which there is sufficient clinical experience in thousands of patients to allow reasonably balanced decisions regarding their suitability: deferoxamine, deferiprone and deferasirox. Table 1 offers comparison of some of the basic properties of these chelators. As a rule, molecules larger than 600 daltons are poorly absorbed and because of its size and poor lipid solubility, deferoxamine requires parenteral administration. By contrast, deferiprone and deferasirox are smaller and very effective orally. Deferoxamine is hexadentate i.e. a single molecule covers all six co-ordination sites of ferric iron, entirely preventing its catalytic action in the production of toxic hydroxyl radicals through Fenton chemistry. By comparison, deferiprone is bidentate and three molecules are required to cover the 6 coordination sites of iron. Deferasirox is tridentate and two molecules are required to offer perfect coverage of the six co-ordination sites. In theory, at suboptimal concentrations, bidentate and tridentate chelators may form incomplete complexes with iron and promote, instead of prevent, iron toxicity, but no indications have so far been found in animal and clinical studies of such a paradoxical effect. The half-life in plasma is an important feature because prolonged presence of the drug in the circulation may offer more effective protection against non-transferrin-bound iron (NTBI), the low-molecular toxic form of iron which is likely responsible for most clinical manifestations of iron toxicity.

Water-soluble complexes of chelated iron are excreted in the urine (deferiprone, deferoxamine). Fecal iron excretion occurs when the chelated iron complex is lipid-soluble (deferasirox) or when iron is chelated in situ in hepatocytes followed by biliary excretion (deferoxamine) (Figure 1A-C). These differences have a practical relevance because iron excretion with deferasirox cannot be monitored by testing the urine and because renal failure or obstructive jaundice will prevent the excretion of one or other chelator.

Assessing the severity of siderosis and response to therapy

Important recent technological developments led to improved ability to identify patients at risk and objective assessment of a favorable response to therapy. Traditionally, serum ferritin and chemical measurement of liver iron were the tools available for documenting the severity of iron overload. It has been claimed that serum ferritin is unreliable because it is modified by co-existent inflammation and hepatocellular damage. However, the recent large-scale studies evaluating deferasirox showed very good correlation between liver iron concentrations and serial ferritin measurements (see below). Biopsy measurements of liver iron are still regarded the gold standard. However, these require extensive experience and only a handful of laboratories are qualified to provide reliable results. Non-invasive methods are rapidly evolving. The SQID method is non-invasive but has calibration problems and is available at only four centers worldwide. Finally, a modification of magnetic resonance imaging (MRI), the R2 MRI has recently been validated and standardized as a reliable and convenient method for measuring liver iron concentrations, now approved by health authorities in the US and Europe, and may soon become the preferred method for estimating these concentrations. Threshold values of
15 mg/g dry liver weight and 2500 mg/L serum ferritin have been established in the past as indicators of increased risk of cardiac complications. However, symptomatic heart disease and cardiac mortality may still occur in patients compliant with chelation therapy with liver iron and serum ferritins within the recommended range of values. Hence, direct assessment of myocardial iron may offer a valuable additional parameter, as symptomatic heart disease is unlikely to develop in patients with normal myocardial iron. The gradient-echo imaging for calculating T2\* used by Anderson et al. for documenting myocardial iron overload, involves a short imaging time allowing completion of the procedure in one breath-hold, and decreasing movement artifacts. Among 109 thalassemic patients, all subjects with significant ventricular dysfunction had a myocardial T2* of less than 20 ms and, as myocardial iron increased, there was a progressive decline in ejection fraction. In this study, no significant correlation could be shown between heart T2* and liver iron concentration or with serum ferritin. Studies of myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous deferoxamine using T2* measurements of cardiac iron showed that clearance of iron from the heart was considerably slower than from the liver. Thus, the poor correlation between cardiac T2* and liver iron may be explained by partially effective treatment that may have been sufficient to whitewash the liver, but not enough to clear iron from the heart for effective cardioprotection.

**Impact of iron chelation**

Armed with these powerful tools for assessing the severity of transfusional siderosis, we may now examine the beneficial effects of iron chelators.

**Deferoxamine**

The introduction of deferoxamine for the treatment of transfusional siderosis has changed the life expectancy and life quality of patients with thalassemia major. The long-term efficacy of this drug has been extensively documented in large multicenter trials in Italy and elsewhere. These studies have shown a cohort-of-birth related improvement in survival, reflected in an inverse, mirror-like decrease in
cardiac mortality, supporting the claim that prevention of cardiac mortality is the most important beneficial effect of chelation therapy. The strongest direct evidence for the beneficial effect of deferoxamine is the reversal of established myocardial pathology in some far-advanced cases. In former years, the course of established myocardial disease in transfusional hemosiderosis was uniformly fatal. More recent experience indicates that most such patients may be salvaged by intensified chelating treatment. Employing continuous 24-hour i.v. deferoxamine infusion via indwelling catheters, reversal of cardiac arrhythmias and congestive heart failure has been achieved and none of the compliant patients died. Reversal of cardiac arrhythmias, previously unresponsive to medical treatment, was achieved in some cases within a few days of starting treatment and therefore cannot be attributed to normalization of iron stores but to the depletion of a putative limited toxic labile iron pool. Although it has been suggested that continuous i.v. treatment may be essential for improving cardiac outcome due to the uninterrupted chelation of circulating NTBI, intermittent high dose deferoxamine for 8-10 hours per day was equally effective. Hence, it is quite likely that the improved response to i.v. deferoxamine treatment using central lines is simply due to better compliance with a device controlled by nursing and medical staff instead of the patient and family. Improved compliance has been maintained even after discontinuation of i.v. therapy, contributing to the improved long-term survival of these high-risk patients.

Unfortunately, compliance with the rigorous requirements of daily subcutaneous infusions is still a serious limiting factor in treatment outcome. In the United Kingdom, survival among thalassemic patients by the age of 35 years is only 50% and mortality is largely attributed to poor compliance. Symptoms of siderotic heart disease was encountered in 25% of North American thalassemic patients over 25 years old and all such patients who are non-compliant with intensified deferoxamine treatment remain at risk of fatal cardiac complications. This is the rationale behind the intensive efforts to identify alternative, orally effective iron chelators which would be more convenient for use and could improve compliance.

**Deferiprone**

Deferiprone (L1), a 3-hydroxy-4-phenyl-1-benzoate chelator, designed by Hider and Kontoghiorghes, has been used in long-term clinical trials over the last 20 years. Major studies involving hundreds of patients have shown, that oral L1 treatment alone will ensure a negative iron balance in some, but not all patients and, that close monitoring is required to identify patients in whom additional chelating treatment is indicated. Because of an increased risk of neutropenia/agranulocytosis, weekly monitoring of blood counts is required. In the following, the discussion is limited to some of the most important recent studies related to survival and a possible cardioprotective effect in patients receiving deferiprone. In a non-randomized retrospective study from a single institute, 54 deferiprone-treated patients were compared with 75 deferoxamine-treated patients for cardiac complications and survival. After 6 years of follow-up, three patients had died, all in the deferoxamine group. Worsening of pre-existing cardiac disease or new onset of cardiac abnormalities was observed in 4% of the deferiprone group and in 20% of the deferoxamine-treated patients. The authors concluded that these findings suggest long-term deferiprone treatment provides greater protection than does deferoxamine against the cardiotoxicity of iron. However, the three patients on deferoxamine who died were 6 to 8 years older than the mean age of the deferiprone group. Moreover, five patients in the deferoxamine group had NYHA class II-IV cardiac disease at outset as compared to only one in the deferiprone group.

Employing their newly developed T2* method, Anderson et al.22 showed significantly lower cardiac iron concentrations in patients treated with deferiprone than in patients treated with deferoxamine. The authors concluded that conventional treatment with deferoxamine did not prevent excess cardiac iron accumulation in more than half the patients with thalassaemia major and that oral deferiprone was more effective at removing cardiac iron. This was a retrospective non-randomized study and, although great efforts were made to match the two groups properly, only 15 patients were treated by deferiprone whereas the 30 deferoxamine-treated controls had to be selected from a large group of 160 patients receiving deferoxamine. In a subsequent study performed by the same group, 61 thalassemic patients with moderate cardiac siderosis (T2* 8 to 20 ms) were randomized to continue on deferoxamine 45 mg/kg/day or deferiprone 92 mg/kg/day. After 1 year of treatment, the improvement in T2* and increase in left ventricular ejection fraction were significantly greater in patients treated with deferiprone than in those receiving deferoxamine. The authors concluded that deferiprone monotherapy was significantly more effective than deferoxamine in improving asymptomatic myocardial siderosis in β-thalassaemia major.

Another prospective study focusing on the cardioprotective effects of deferiprone involved 79 non-compliant thalassemic patients with severe iron overload (mean ferritin over 5000 ng/mL). Treatment consisted of deferiprone 70-80 mg/kg/day 7 days/week combined with deferoxamine 40 mg/kg/day subcutaneously 10-24 h/day 2 to 6 days per week. Sixty-four patients continued treatment for at least 12 months. The drop-out rate after 1 to 38 months of treatment was quite high (34 patients); the causes included agranulocytosis in three patients and neutropenia in seven. In 20 patients receiving cardiac medications at the initiation of combined chelation therapy, left ventricular ejection fraction increased from 49 to 57% without modification of cardiac treatment. This study implies that it is possible to salvage non-compliant patients with severe iron overload.
and reduce cardiac complications by adding deferiprone to conventional subcutaneous deferoxamine without resorting to continuous i.v. therapy. However, this report also underlines the significant toxicity and high drop-out rate of such a program. Two major studies attempted to evaluate the effect of deferiprone on cardiac mortality. The first of these involved all thalassemic patients treated at seven Italian hospitals born between 1970 to 1993 who did not experience cardiac complications prior to 1995. There were 359 patients treated by deferoxamine only, and 157 receiving deferiprone for a median duration of 4.3 years between 1995 and the end of 2003. There were 52 cardiac events including ten cardiac deaths among patients treated with deferoxamine. By contrast, no cardiac event occurred in any of the patients during deferiprone treatment or within 18 months after deferiprone. This study, although retrospective, was subjected to exceptionally detailed and meticulous statistical analysis. The estimated hazard of a cardiac event on deferiprone was less than one-tenth that in patients on deferoxamine. As in the previous study, the drop-out rate on deferiprone was high (46 patients, 31%) including 21 patients with increasing ferritin or liver iron, eight with neutropenia and one with agranulocytosis. The second major study on survival and specific causes of mortality originates from Cyprus and involved 539 thalassemic patients born after 1960 for whom accurate clinical data were available. These patients were followed over the period 1980 to 2004. There were 58 deaths, 53% of which were due to cardiac causes. There was a significant trend of increasing cardiac mortality between 1980 and 2000, and a decline after 2000, following the introduction of a national protocol of combined deferiprone-deferoxamine therapy identical to that reported by Origa et al. In this study, as in the study reported from Italy, there were no deaths among patients on combined deferiprone-deferoxamine therapy.

The role of combined deferiprone-deferoxamine treatment has been reviewed previously and will not be discussed in detail in the present review. Combined chelation treatment is a serious option for patients failing monotherapy with either one of the three leading drugs. It is gaining increasing popularity, in particular in Mediterranean countries. Although at inception its rationale was a shuttle-and-sink hypothesis in which iron is changing hands between a small chelator entering cells and a second and more powerful extracellular chelator, there is no compelling evidence that the simultaneous presence of both chelators is necessary. It is quite possible that this is simply an additive effect of two drugs with different modes of action. Alternating chelation in which deferiprone is given for 5 days only and deferoxamine on the other 2 days of the week is identical in efficacy to conventional deferoxamine for 5 to 7 days a week, convenient, and is not associated with increased toxicity.

**Deferasirox (bis-hydroxyphenyl-triazole, ICL670, Exjade®)**

This compound is a member of a new class of tridentate iron-selective synthetic chelators, the bis-hydroxyphenyl-triazoles developed by Novartis. Deferasirox has emerged from the screening of over 700 candidate drugs as the most promising compound, combining oral effectiveness with low toxicity. It has an affinity for ferric iron which is 8 log units higher than that for deferiprone and the ligand is stable both in vivo and in vitro. Preclinical studies in animal models have shown effective and selective mobilization of tissue iron with greater efficiency for deferasirox than for deferoxamine. Studies in cell cultures and in hypertransfused rats with selective radioiron labeling of cellular stores demonstrated the ability of deferasirox to enter and remove iron from cells. The magnitude of efforts invested in the clinical development of deferasirox is unprecedented in the history of chelator research. Within a few years, over one thousand patients have been treated in prospective well controlled trials involving more than 100 medical centers in four continents. Most of these trials were designed to last 1 year but by now, many have been extended to 3 years or more. Phase I clinical trials showed that deferasirox is well tolerated at single oral doses up to 80 mg/kg. Iron excretion was dose-dependent and was almost entirely fecal. Metabolic balance studies showed that excretion averaged 0.13, 0.34 and 0.56 mg/kg/day at deferasirox doses of 10, 20 and 40 mg/kg/day, respectively predicting equilibrium or negative iron balance at daily doses of 20 mg and above. A plasma half-life of 11 to 19 hours supported the use of the once-daily oral dosing employed in subsequent trials. Table 2 describes the leading phase I, II and III clinical trials involving deferasirox published in the current year. The most extensive and informative of these was a 1-year randomized trial comparing deferoxamine (290 patients) and deferasirox (296 patients) in 586 thalassemic patients aged 2 to 53 years. About half the patients were younger than 16 years of age. Discontinuations were rare (5.7% for deferasirox) but the initial selection excluded subjects with a history of non-compliance. A conservative dosing system was adopted, adapted to initial liver iron concentrations (LIC); patients with a LIC below 7 mg Fe/g dry weight received deferasirox 5 to 10 mg/kg/day and those with a LIC above 7 mg Fe/g dry weight received 20 to 30 mg/kg/d. By contrast, patients randomized to deferoxamine were allowed to continue doses closer to those received at baseline, prior to randomization (Table 2). The disproportionately low dosing of patients with deferasirox at 5 and 10 mg/kg/d relative to deferoxamine, and the maintenance of high pre-study deferoxamine doses resulted in the failure of deferasirox to elicit a response equal to that in the corresponding deferoxamine control groups. By contrast, at dose ranges of 20 and 30 mg deferasirox, when a dose relationship of deferasirox to deferoxamine of 1:2 was maintained, both drugs were equally effective as judged by LIC and ferritin measurements. These results, indicating deferasirox efficacy at 20 and 30 mg/kg/day agree very well with the results of previous studies described in Table 2. In general,
Deferasirox is well tolerated. Adverse events were generally mild and included transient gastrointestinal events in 15% of patients, skin rash in 11% and mild, dose-dependent increases in serum creatinine which generally remained within the normal range and never exceeded twice the upper limit of normal. An increase of liver enzymes judged to be related to deferasirox was observed in only two patients. Importantly, no drug-related agranulocytosis has been observed. Evaluation of pediatric patients has shown that growth and development proceeded normally while on deferasirox, lending support to its use in very young patients. Understandably, it is too early yet to allow any statements on the impact of deferasirox on survival.

Another question of vital importance is the effect of deferasirox on cardiac siderosis and the prevention of cardiac complications. Preliminary data on improved cardiac T2* following deferasirox treatment are encouraging and more definitive information would be most desirable.

### Practical implications

(i) The ability of deferoxamine to prevent damage to the heart and other vital organs, and to increase life expectancy in transfusional siderosis is well established. A major limitation of deferoxamine is the inconvenience of parenteral administration, resulting in limited compliance;

(ii) The emergence of a number of orally effective chelators is a very favorable development offering further improvement in quality of life and longevity;

(iii) The current dilemma is related to the following issues: (a) are oral chelators suitable for first-line therapy? (b) should they be only offered as second-line treatment for patients with unsatisfactory response to previous therapy, significant drug-related complications or failure of compliance? (c) should they be used as monotherapy or in combination?

(iv) To answer these questions categorically, solid evidence is needed for the impact of each therapeutic option on disease-related morbidity and mortality as well as tolerability. At present, such evidence is still incomplete and thus further well-controlled clinical trials are necessary;

(v) For the practicing clinician, a minimalistic approach would be to adhere to the treatment plan already in use and change treatment only if careful clinical assessment implies unsatisfactory results;

(vi) At the other end of the spectrum, all patients could be switched to oral chelation. This option offers convenience, improved likelihood of compliance, and may be favored by most patients. To take this option, strong evidence is needed to indicate that the efficacy of upfront treatment with deferiprone or deferasirox monotherapy is equal to, or exceeds that of the other options.

(vii) A reasonable compromise could be oral chelation combined with a limited deferoxamine program. The major advantage of this is to avoid abandoning the old and trusted drug deferoxamine, but limiting its inconvenience by adding an oral drug of reasonable efficacy. Combined chelation adds great flexibility to the treatment program which can be adapted to the individual needs of each patient. Combined treatment is widely accepted in many other fields of medicine and makes a lot of sense in chelation therapy too. In practice, it is quite likely that combined chelation therapy has already been adopted as the preferred chelating strategy by most clinicians taking care of thalassemic patients.

CH is a member of the Deferiprone Drug Safety Committee of Apotex Research Inc. Toronto, Canada, and of the ICL670 Program Safety Board of Novartis Pharma AG Basel, Switzerland.

He received travel support to allow participation at yearly drug safety meetings.
References


