ELSEVIER

Coordination Chemistry Reviews
184 (1999) 291–310

COORDINATION CHEMISTRY REVIEWS

Iron chelating agents in clinical practice

Gavino Faa [a,*], Guido Crisponi [b]

[a] *Dipartimento di Citomorfologia, Sezione di Anatomia Patologica, Via Porcell 2, I-09124 Cagliari, Italy*
[b] *Dipartimento di Chimica e Tecnologie Inorganiche e Metallorganiche, Via Ospedale 72, I-09124 Cagliari, Italy*

Received 24 November 1998; accepted 16 February 1999

Contents

Abstract . . . 291
1. Introduction . . . 292
2. Iron overload and toxicity . . . 292
3. Desferrioxamine . . . 294
4. Development of non toxic oral iron chelators . . . 298
   4.1 Deferiprone . . . 300
5. New indications for iron-chelating therapy . . . 302
   5.1 Adult Respiratory Distress Syndrome (ARDS) . . . 303
   5.2 Iron chelators and myocardial ischemia . . . 303
   5.3 Iron chelators and cancer . . . 304
   5.4 Iron chelators as antimalarials . . . 304
6. Concluding remarks . . . 306
References . . . 306

Abstract

The relevance of iron chelators in medicine has increased in recent years. Iron is essential for life but it is also potentially more toxic than other trace elements. This is due to the lack of effective means to protect human cells against iron overload and to the role of iron in the generation of free radicals. To protect patients from the consequences of iron toxicity, iron chelating agents have been introduced in clinical practice. Unfortunately, the ideal chelator for treating iron overload in humans has not been identified yet. The aim of this review is to report the experience with desferrioxamine therapy in patients affected by β-thalassemia major according to: bioavailability; mechanism of interactions with hepatocellular iron:

* Corresponding author.
E-mail address: gfaa@vaxca1.unica.it (G. Faa)

PII: S0010-8545(99)00056-9

release of iron chelates and their excretion; impact of iron chelation on survival in thalassemia patients and side effects of prolonged therapy. Problems related to the development of non-toxic oral iron chelators are also discussed, with particular emphasis on the preliminary data on usefulness and safety of deferiprone (L1), recently evaluated in different clinical trials. Iron chelating therapy has been introduced, in recent years, even in the therapy of disorders not characterized by iron overload. Here the following new therapeutic indications are discussed: adult respiratory distress syndrome, myocardial ischemia, cancer and malaria.

Keywords: Iron-chelators; Desferrioxamine; Deferiprone; Thalassemia

1. Introduction

Iron chelators are used in medicine to protect patients from the consequences of iron overload and iron toxicity in organs and tissues. The ideal chelator for treating iron overload in humans should act as a selective depletor of iron, should be efficiently absorbed by the gastrointestinal tract, could not cross the blood–brain and placental barriers and should lack or have a low toxicity. Such a chelating agent has not been identified yet and this goal is at the basis of multiple research projects in this field. In this review, the experience with long-term iron chelating therapy in patients affected by chronic transfusion-dependent anemias will be summarized with particular emphasis on thalassemic patients who are the main target of iron-chelating drugs. The experience in thalassemia patients with the well established chelator desferrioxamine and with the orally active deferiprone will be outlined. This review is also intended to report the most recent development of new non-toxic and orally effective iron chelating agents and their possible application in clinical use. New indications for iron chelating therapy will also be explored, such as the use of iron chelation in oncology to prevent tumor cell growth by the inhibition of iron-dependent enzymes, the application of iron-chelating agents in the therapy of infectious disease with particular emphasis on their action as antimalarials, the experience of iron-chelating drugs in the therapy of adult respiratory distress syndrome and in the therapy of myocardial ischemia. Finally, the relevance of the dialogue among clinicians, pathologists, pharmacologists, biochemists, chemists, molecular biologists and other experts in metal toxicity in order to improve our knowledge on the relationship between the metabolism of iron and other trace elements will be discussed. This implies the final goal of prolonging survival and improving the quality of life of iron-loaded patients.

2. Iron overload and toxicity

Iron is essential for life: all living cells, whether prokaryotic or eukaryotic, need a supply of iron for reduction of oxygen (respiration), reduction of carbon dioxide

(photosynthesis), reduction of dinitrogen or other fundamental biological processes [1]. Excessive amounts of iron may become very toxic to the human body and, eventually, is fatal for vital cell structures [2].

Iron overload may be defined as an excess in total body iron stores. The normal iron concentration in the human body ranges between 40 and 50 mg/kg of body weight [3]. Most of this iron is present in hemoglobin and in myoglobin: all the rest is stored as ferritin or as its less accessible form, hemosiderin. Only a few hundred milligrams of iron are stored in enzymes such as cytochrome *c* oxidase which, however, are essential to human life [1]. Humans have very limited capacity for excretion of excess iron: in particular they lack any effective means to protect cells and tissues against iron overload. As a consequence, any increase in iron intake may cause in a short time an increase in body iron stores [4]. Iron balance is normally regulated by controlling iron absorption in the proximal small intestine [5]. In women, losing iron in the menstrual cycle could protect against excess iron toxicity and it is considered to be, at least in part, responsible for their greater longevity than men [1]. The major regulators of mucosal iron absorption are the amount of body iron stores and the level of erythropoiesis [6]. Iron overload may be caused by two different factors:

1. parenteral administration of iron, as in chronic transfusion therapy;
2. increase in iron absorption from the diet, that may be genetically determined like in hereditary hemochromatosis [7] or caused by dietary iron overload [8].

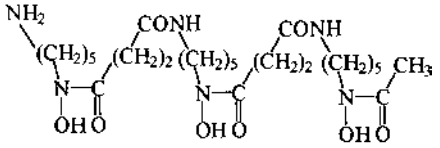
When the accumulation of iron in organs exceeds the body capacity for safe storage, potentially lethal tissue damage results [9]. The severity of iron toxicity seems to be related to the amount of body iron burden. Recent studies in patients with thalassemia major found that the magnitude of the body iron burden was the major determinant of the risk of clinical complications and of early death [10,11]. Target organs for iron-induced injury are the liver, pancreas and heart [12].

In the category of patients affected by chronic anaemia, who need regular blood transfusions in order to sustain their normal growth and development during childhood, β-thalassemia major (BTM) constitutes one of the most serious public health problems in the Mediterranean area [13], in the Middle East, in the Indian subcontinent, in Southeast Asia [14] and, in particular, in the island of Sardinia [15]. BTM is an autosomal recessive disease, characterized by absent or decreased synthesis of the β globin gene: the number of thalassemic children requiring regular blood transfusions and a program of iron chelation has been estimated to be 100 000 world-wide. In patients with thalassemia major, iron contained in transfused red cells inexorably accumulates if a concomitant regular chelation therapy is not programmed [16]. The major pathological manifestations observed in patients with BTM are related to iron overload: chronic liver disease, characterized by hepatocytic and Kupffer cell iron storage, fibrosis and, eventually, cirrhosis [17,18]; dilatative cardiomyopathy with congestive heart failure is nowadays the most common cause of death in patients affected by BTM who reach adolescence and adulthood [19,20]. Even cardiomyopathy is related to cardiac hemosiderosis: a preferential accumulation of iron in the interventricular septum and in the left ventricle wall has been observed [21]. The toxic effects of iron overload has been

demonstrated in cultured heart cells: incubation of rat heart cell cultures with iron concentrations from 20 up to 80 g/ml resulted in a marked decrease in amplitude and rate of contractions and in gross abnormality in rhythmicity [22].

3. Desferrioxamine

Desferrioxamine is the only chelating agent to have been extensively used in clinical practice [23] after its discovery more than 30 years ago. It is a siderophore produced by *Streptomyces pilosus* [24], discovered by the team of Prelog and his co-workers Zahner and Keberle.



Desferrioxamine, earlier recognized as an antagonist of the antibiotic ferrimycin, was successively identified as an iron chelating agent [25]. It is a trihydroxamic acid with three residues of 1-amino-5-*N*-hydroxy aminopentane, two of succinic acid and one of acetic acid organized in a linear array; the free amino group explains its very high water solubility. Although it has been synthesized the use of the natural product is more economic.

Initially used in the therapy of acute iron poisoning [26], desferrioxamine was later introduced in thalassemia treatment, giving a fresh chance to more than 100 000 thalassemic patients requiring iron chelating therapy. The widespread use of desferrioxamine was firmly established, thanks to British investigators such as Berry, Modell, Pippard and Hoffman and to Italian clinicians such as Cao. The relevance of desferrioxamine in clinical practice and its role in the progress of the therapy of thalassemia patients has been underlined on the occasion of the 1991 Pharmaceutical of the Year price, awarded to desferrioxamine by the Munchener Medizinische Wochenschrift [27]. In order to better understand the value of this award, it is useful to observe that it had been previously given to aspirin, cortisone and penicillin. Desferrioxamine is the only iron-chelating agent approved for clinical use [28]. In the following, we shall focus on

- the bioavailability;
- the mechanism whereby it interacts with hepatocellular iron, with iron stored in macrophages and with iron in transit;
- the release of its iron chelates and their subsequent biliary or urinary excretion;
- the impact of iron chelation on survival in thalassemia patients;
- the side effects of prolonged therapy.

Bioavailability is defined by Hider [29] as the percentage of absorbed dose of a drug which reaches the systemic blood circulation. It depends on the absorption of the drug from the gastrointestinal tract and from the extraction by hepatocytes from the portal blood supply. Low or high bioavailability may be requested in iron chelators, depending on the clinical setting and on the main target of chelating

therapy. Thus, when a general systemic action of a chelator is required, a high bioavailability is ideal. On the contrary, when liver is the target of iron chelation, an efficient absorption of the chelator by hepatocytes and, consequently, a low bioavailability is ideal. Two major factors influence the absorption of iron chelators from the gastrointestinal tract: the oil/water distribution coefficient [30] and the molecular weight. To achieve 70% absorption of an iron chelator, its molecular weight [31] needs to be < 300. A 70% absorption of the dose is mandatory for iron chelators: 50% absorption could leave in the lumen such a level of the chelator that might disturb the microbiological flora [29]. Most siderophores, including desferrioxamine, have a molecular weight from 500 to 900 Da which effectively excludes hexadentate ligands, such as desferrioxamine, from consideration as orally active chelators. In contrast deferiprone and 1,2-diethyl-3-hydroxy-4-pyridinone, two ligands of molecular weight 139 and 167 Da, respectively, are both efficiently absorbed in man [32]. Since desferrioxamine given orally is poorly absorbed, to be effective it must be administered subcutaneously [33], intramuscularly [34] or by intravenous infusion with a small portable syringe pump, ideally for 9–12 h each day [35]. This difficult regimen of desferrioxamine parenteral treatment easily explains why only part of thalassemic patients comply with iron chelating therapy, in spite of the knowledge that the advent of treatment with desferrioxamine has changed the gloomy prognosis of thalassemia patients [36]. On entering blood by intravenous injection, desferrioxamine plasma clearance [37] is generally considered rapid, with half life of 5 to 10 min, also if a longer half life (3.05 ± 1.30 h) has been estimated in a recent study [38]. While only a small part of desferrioxamine is inactivated within human plasma, the major part undergoes uptake by hepatocytes. The rapid loss of circulating activity of desferrioxamine after intravenous injection is the main reason why prolonged infusion results in more efficient iron chelation [39]. After injection of desferrioxamine, both fecal and urinary iron excretion are observed. Probably the two excretion pathways reflect two different actions of the drug. Fecal excretion could only arise through iron chelation within the hepatocytes, followed by excretion in the bile of the iron–desferrioxamine complex [39]. The source of urinary iron remains more controversial: urinary excretion could derive from iron chelation within Kupffer cells and within other monophagocytic cells like spleen macrophages [40,41]. A further source to the urine is likely to be any nontransferrin bound iron in plasma of patients with a fully saturated plasma trasferrin, this kind of iron is reduced by desferrioxamine treatment [42]. The picture emerges that the major source of iron excreted through faeces by desferrioxamine is iron within hepatocytes, while urinary excretion could reflect an additional pathway of chelation, predominantly extracellular, regarding plasma nontransferrin bound iron, membrane-related iron of hepatocytes and iron in macrophages [39]. The mechanism by which desferrioxamine is effective in removing intra and extracellular iron is not yet completely understood. To further the understanding of how desferrioxamine, as well other chelators, exert their effects on cells, many animal [43] and cellular models [44] have been proposed. Experiments on hepatocytes have shown that the hepatocyte plasma membrane possesses a number of facilitated transport processes which are particularly efficient for the absorption of

small peptides as well as of desferrioxamine [29]. Once entered into the hepatocytes, the targets of desferrioxamine are the different pools of iron. Ferritin, whose major function is the storage of iron, is a protein of molecular weight 450 000 Da which can contain ca. 4500 iron atoms [45]. Desferrioxamine has been shown in vitro to be able of removing Fe(III) from ferritin: however, iron release requires chelator concentrations much higer than expected [45]. Probably the interaction of desferrioxamine with ferritin-bound iron is indirect. Ferritin is continuosly metabolized within lysosomes; the iron released in lysosomes enters a low molecular weight pool of iron which is the principal source of iron for desferrioxamine [46], whose main target is probably the pool of chelatable free iron in lysosomes. Another possible source of iron for desferrioxamine is trasferrin-bound iron. Transferrin is a glycoprotein, with a molecular weight of about 80 000 Da, which transports iron from the sites of absorption to those of storage and utilization. Desferrioxamine is generally unable to remove iron from transferrin; it may chelate transferrin-bound iron only in the presence of organic pyrophosphates, whose presence facilitates release of iron from transferrin [47]. Once in contact with the cellular membrane of hepatocytes, transferrin attaches to specific receptors and it is internalized by hepatocytes enclosed within endocytic vesicles in which iron is released. Iron released inside endocytic vesicles enters a labile intracellular transit iron pool consisting of low molecular iron complexes which may be chelated by desferrioxamine. A contribution to this intracellular transit iron pool may even derive by degradation of iron rich ferritin. Hepatocytes contain, in their cellular membrane, receptors for ferritin utilized for the uptake of ferritin released by Kupffer cells, following phagocytosis of erythrocytes [48,49]. The ingested ferritin is degraded within hepatocytic lysosomes and the released iron enters the chelatable transit iron pool, sensitive to the action of desferrioxamine. In patients affected by severe iron overload, when complete saturation of transferrin occurs, a low molecular weight iron pool, not associated to transferrin, is always present even in plasma. This plasmatic fraction of nontransferrin-bound iron may be bound to albumin or complexed by amino acids, citrate or sugars. Even this pool is a main target for chelation by desferrioxamine. The iron overload observed in hepatocytes from patients affected by congenital atransferrinemia [50] has demonstrated the existence of systems for the uptake of nontransferrin-bound iron. In vitro studies have shown that desferrioxamine may act even at this level, inhibiting the transferrin receptor-independent iron uptake systems [51]. Desferrioxamine acts even on macrophages and, particularly, on Kupffer cells. An important function of Kupffer cells is phagocytosis of senescent erythrocytes. Iron derived from hemoglobin enters a labile transit pool which is either returned to plasma transferrin or retained in ferritin [45]. Likely desferoxamine competes with apotransferrin for this pool of iron, which is on its way out of Kupffer cells and it is considered the major source of urinary iron excreted during desferrioxamine therapy [40]; fecal iron excretion on the contrary reflects the action of desferrioxamine on the chelatable iron pool present in the cytoplasm of the hepatocyte. The intimate mechanism of action of desferrioxamine is probably similar to that typical of the majority of iron chelators. Desferrioxamine promotes the ferric form of iron and suppresses excellently iron

toxicity, inhibiting the formation of hydroxyl radicals and lipid peroxidation [52] by its ferroxidase activity [53]. A strong ferroxidase activity is also exerted by transferrin and by ceruloplasmin, a 132 kDa α-2-glycoprotein containing a trinuclear copper cluster responsible for its oxidase activity and radical scavenging [54]. The report that patients affected by dietary or genetic deficiency of ceruloplasmin show severe accumulation of iron in liver [55] as well in other organs [56] is at the basis of recent studies on the relevance of ceruloplasmin in iron metabolism, both in physiologic and in iron overloaded patients. One of the multiple functions of ceruloplasmin is to aid the release of iron from the hepatocyte to plasma transferrin. Ceruloplasmin is probably localized inside the endoplasmic reticulum, where it acts as a ferroxidase on Fe(II) mobilised from intracellular ferritins which may initiate free radical reactions [57]. This is a crucial step in iron release; in fact, transferrin may only bind Fe(III) [58]. The relevance of ceruloplasmin in iron excretion has been confirmed by the demonstration that infusion of ceruloplasmin may rapidly enhance iron release from liver in patients with congenital aceruloplasminemia [55]. In Wilson's desease, characterized by low plasma levels of ceruloplasmin, we have reported high iron levels in the liver in an autoptic case [59] and in a percentage of liver biopsies (G.Faa et al., unpublished data). A severe iron deposition has been moreover reported in the liver of LEC rats [60], an animal model in Wilson's desease. To our knowledge, the correlation between ceruloplasmin status and desferrioxamine has not been extensively studied. On the basis of the crucial role of ceruloplasmin in iron release from hepatocytes, a deficiency in ceruloplasmin could explain why some thalassemic patients, in spite of their good compliance in desferrioxamine therapy, show massive iron storage in the liver and in the heart.

An interesting property of desferrioxamine is that it is not only an excellent iron chelator, thereby decreasing the free-radical generating reactions [61], but can also directly scavenge some radical species [62].

Although desferrioxamine has been demonstrated to be a safe drug when administered in the presence of an elevated body iron burden [63], serious complications may arise as a consequence of long term chelation, mainly in young patients with low body iron stores. At the basis of desferrioxamine toxicity is the fact that, like other chelators, desferrioxamine is not completely iron-selective. The most frequent complications of long-term desferrioxamine treatment are growth retardation, described in patients with thalassemia major [64,65]. Other complications described include bone changes [66], visual and auditory neurotoxicity [67]. Severe limb and metaphyseal deformities have been reported in patients who began chelation in the first year of life [64]. Other adverse effects of desferrioxamine, noted in patients receiving continuous 24-h infusions, are impairment of vision, with defective dark adaption and peripheral field loss [68]. Hearing and vision abnormalities may occur more readily in patients with low iron stores, but the relationship is still not entirely clear and other factors may play a role [69]. Different hypotheses have been postulated to explain the adverse effects of desferrioxamine treatment:

1. depletion of other trace elements, such as zinc, copper, manganese, cobalt;
2. a direct toxic effect of free desferrioxamine;

3. depletion of iron from critical iron-dependent enzymes, such as cytochrome *c* oxidase [39].

A pulmonary syndrome, characterized by diffuse lung infiltration and restrictive dysfunction, has been rarely observed in patients with thalassemia major receiving intravenous high doses of desferrioxamine [70]. Local skin reaction at the site of subcutaneous injection of desferrioxamine is also common, in the form of acute inflammation or of subcutaneous nodules persisting for several days [23]. Another complication occurring during chronic treatment with desferrioxamine is opportunistic *Yersinia* infection [71]. This complication of desferrioxamine treatment probably occurs since the virulence of *Yersinia* is enhanced by the presence of a membrane receptor which binds desferrioxamine [72]. Desferrioxamine toxicity may be controlled or prevented: the majority of all the reported adverse effects may be reduced or prevented by avoiding high doses and over-early commencement, and by long-term clinical monitoring once the overload is significantly reduced [23].

In conclusion, there is no doubt that desferrioxamine, with all its limitations, has changed the quality of life and life expectancy of many patients affected by thalassemia [13], by preventing the complications of iron overload [73].

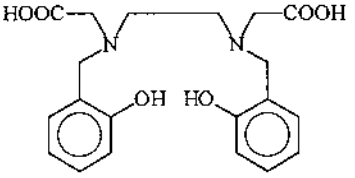
4. Development of non toxic oral iron chelators

Despite the well-acknowledged successes of desferrioxamine in the medical treatment of iron overload in thalassemic patients, long-term compliance with a regimen of prolonged nightly infusion may be problematic for many patients in the developed world [63], while in developing countries such a regimen is impractical and unaffordable [28]. On this basis, the search for an orally-active iron chelator as an alternative to desferrioxamine in the treatment and prevention of chronic iron overload has continued in recent years. To this end, chemists have used their knowledge to synthesize a wide variety of chelators often borrowing from examples of nature [44]. Desirable properties for an oral iron chelator are:

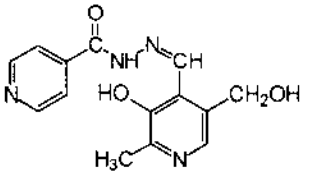
1. specificy and affinity for iron;
2. molecular weight lower than 400 Da, for gastrointestinal absorption;
3. sufficient lipophilicity for gastrointestinal absorption and intracellular chelation;
4. sufficient hydrophilicity, to limit liver absorption.

In the past 20 years a large variety of ligands with oxygen binding groups, above all those containing hydroxamates, catechols and hydroxypyridinones has been systematically tested as potential orally active drugs. Some of these do not present proper pM values for iron chelation (pM was defined by Harris et al. [74] as the negative logarithm of free iron(III) concentration in a solution that is 10 μM in ligand and 1 μM in metal at pH 7.4. The pM concept is now of common use to evaluate the strength of chelates taking into account proton competition). Other ligands, while promising from a chemical point of view later proved toxic and could not be introduced in clinical practice. Many authors first focused on catecholate sequestering agents, which resulted in stronger iron chelators than desferrioxamine [75]. Unfortunately, no catechol ligands were found to be orally active and some of

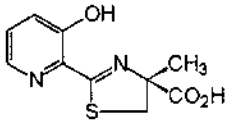
them strongly promoted the growth of pathogenic microorganisms, precluding designs based on these ligands [76]. Among the molecules showing the more interesting properties as oral chelators are HBED [77] ant its derivative dimethyl-HBED [78], pyridoxal isonicotinoyl hydrazone [79], desferrithiocin [80] and hydroxypyridinones [81].



HBED, an analogue of EDTA with two phenolate replacing two carboxylate groups shows a log K value much higher than EDTA (36.7 with respect to 25.1); nevertheless this value is mitigated by its strong affinity for hydrogen ion resulting in a pM 26.74 with respect to 22.3 for EDTA. While it is well absorbed orally and it is effective in rodent assays its response in primates and in patient trials was remarkably less effective [82]; its dimethyl derivative is now under clinical trials [83].



Due to the high activity of PIH [79] and of its analogues in mobilising iron, their chelating properties were extensively studied by Vitolo et al. [84]: the pM values for these ligands indicate that they are thermodynamically able to mobilise transferrin-bound iron, but a kinetic barrier however inhibits their exchange properties. At any rate the response in clinical tests was discouraging under different points of view [82].

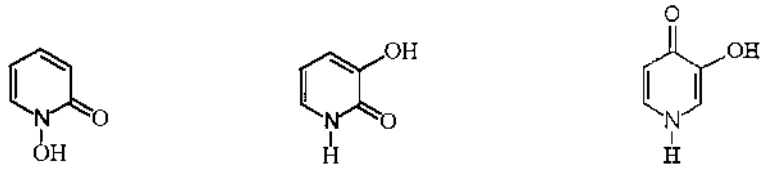


Desferrithiocin, isolated from *S. antibioticus*, is one of the few siderophores not belonging to hydroxamate or catecholate classes and it forms a stable 2:1 complex [85] with iron ($K = 4 \times 10^{29}$ M^{-1}). This compound proved effective in iron mobilisation both in rat and in primate models but animals exposed to it presented with nephrotoxicity [86]. In any case because of the strong chelating properties of desferrithiocin investigation is yet performed on its analogues [87].

Despite their favourable iron binding properties none of the molecules presented above has proven completely satisfactory.

Considering both their binding properties and the results of biological trials hydroxypyridinones appear to be the most promising oral chelators; their functional group was isolated in siderophores from a culture of *Pseudomonas alcaligenes* in 1979 by Barker et al. [88]. Among 1-hydroxy-2-pyridinone, 3-hydroxy-2-pyridi-

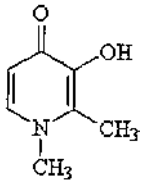
none and 3-hydroxy-4-pyridinone studied by Scarrow [81] with pM values 22.3, 21.2 and 25.7, respectively,



the third presents the most interesting properties and its 1,2-dimethyl derivative, known as deferiprone (L1 or CP20) is at the present the orally active iron chelating agent with the broadest clinical experience [63].

4.1. Deferiprone

Deferiprone [29] is a member of the family of hydroxypyridinones, of molecular weight 139 Da. By virtue of its low molecular weight, deferiprone possesses high absorption efficiency and it is efficiently absorbed from the human intestinal tract [32].



Deferiprone was originally synthesized by Robert Hider and his colleagues at Essex University, patented in 1982 as an alternative to desferioxamine in the treatment of iron overload [89] and the early biological assessments were performed at University College Hospital in London [36]. Deferiprone, in common with all hydroxypyridinones, forms 5-membered chelate rings in which iron is bound by two oxygen atoms [76]. Little is known about mobilization of iron stored in hepatocytes and in Kupffer cells by deferiprone. Deferiprone, in common with other low molecular weight iron chelators, likely removes Fe(III) from ferritin by penetration of the protein shell [90]. In other studies, it has been shown that deferiprone may mobilize iron even from hemosiderin, lactoferrin and from transferrin [91]. Deferiprone, like other bidentate hydroxypyridones, forms a 3:1 complex with iron; its efficiency in chelating iron, determined in iron-loaded monkeys, is significantly less than that observed in the same animals with parenteral administration of desferrioxamine [82]. Deferiprone is metabolized in the liver, where it undergoes rapid conversion to nonchelating metabolites. The major metabolite of deferiprone in man is the glucuronide which, as a result of conjugation of the 3-hydroxyl function, is unable to bind iron [92]. The majority of deferiprone–iron complex is excreted in the urine (70%), while little iron is excreted in the feces [93]. Although deferiprone, such as other bidentate ligands, has a clear advantage over desferrioxamine with respect to oral viability, it is potentially more toxic. In fact, by virtue of its relatively low molecular weight, it may easily penetrate most cell membranes,

blood–brain barrier and placental barrier. The efficacy of deferiprone in inducing and maintaining a negative iron balance in iron loaded transfused patients has been demonstrated by the first long-term trials. Iron chelation therapy with deferiprone in a large series of subjects affected by thalassemia intermedia showed a reduction of liver iron stores and a normalization of serum ferritin levels [94]. This observation was followed by the report of a significant decline in liver iron concentration in patients affected by thalassemia major, during a 5-year treatment with deferiprone [95]. In β-thalassemic patients who reach adolescence and adulthood, the heart has become the core of medical interest, since the main cause of death in adult β-thalassemia patients is dilatative cardiomyopathy with congestive heart failure [96], myocarditis [19] and sudden cardiac tamponade [97]. On this basis, the ability to prevent and reverse cardiac iron loading has become the most critical requirement of any iron-chelating drug [63]. The ability of deferiprone to remove excess iron not only from liver cells but also from heart cells has been recently demonstrated in vitro [98]. This observation was followed by evidence of reduction in cardiac iron stores, evaluated by magnetic resonance imaging, in patients with β-thalassemia major during long-term treatment with deferiprone [99].

The initial evaluations of the efficacy of deferiprone in the short term treatment of iron overload in thalassemia patients was completely favorable [95], based on the evaluation of the ability of L1 to maintain iron stores at hepatic and cardiac levels associated with a low risk of sudden death. Recently, the same authors have raised concern that long-term therapy with deferiprone may not provide adequate control of body iron burden in a substantial proportion of patients affected by thalassemia major [100]. This assumption was based on the observation that the hepatic iron concentration was in the optimal range only in 7% of patients with thalassemia major after 2 years of therapy with deferiprone. In another comunication at the same meeting [101], Olivieri reported the results of a long term follow-up of 18 patients receiving deferiprone for 2–6 years. In six of these patients, the hepatic iron concentration was found above the threshold associated with increased risk of heart disease and early death in thalassemia major (>15 mg/g of dry tissue). Ten of the same patients had a serum ferritin concentration higher than 2500 μg/l, the treshold identified for development of cardiac disease in thalassemia major. On the basis of these results, the authors suggested that long term therapy with deferiprone should be carefully investigated before the introduction of the drug in routine clinical practice. In the debate on the efficacy of deferiprone in the treatment of iron overload, an important role has been played by a study on the effect of 1,2-diethyl-3-hydroxypyridin-4-one (CP94), closely related to L1, in an animal model of iron overload. In this study, the treatment was associated with the initial efficacy of the drug in reducing hepatic iron content, followed by worsening of hepatic fibrosis and increased iron storage in heart, with the development of cardiac fibrosis [102]. The concern that hydroxypyridinones could exacerbate iron-related tissue damage had been raised theoretically by Halliwell [103] in 1994. Since three molecules of L1 are needed for complete iron coordination, one may speculate that, at very low deferiprone concentration, also iron complexes of 1:2 stoichiometry may appear at physiological pH, in which the unoccupied coordination sites are bound to water

(see the distribution curves for the system L1–Fe(III) at mM and μM concentrations presented by Motekaitis and Martell [104]). These unsaturated forms of iron catalyze the formation of hydroxyl radical and of other reactive oxygen species, causing free radical-mediated lipid peroxidation. This process could be, theoretically, more frequently associated with L1 therapy than during desferrioxamine treatment for two main reasons:

1. deferiprone, because its low molecular weight, easily enters most cells so that the hypothetical formation of free radical by unsatured iron could cause severe damage inside hepatocytes, Kupffer cells and myocytes;
2. by contrast, only one molecule of desferrioxamine is necessary for iron coordination.

Recently, new data have been published on the long-term effectiveness of deferiprone and on safety of iron chelating therapy with this drug [105]. In this study, five out of 14 deferiprone treated patients for >1 year (mean: 4.6 years) showed progression of fibrosis. Seven patients also had an increase in liver iron content, with values associated with an increased risk of cardiac disease and early death. On the basis of these data, deferiprone could not adequately control body iron burden and could worsen hepatic fibrosis. In the same number of The New England Journal of Medicine, an editorial raises some question on this study, in particular on the methods used for the evaluation of hepatic fibrosis. The debate on safety of therapy with deferiprone for thalassemia major remains open: new long-term trials are necessary to obtain sure conclusions on this subject.

5. New indications for iron-chelating therapy

New insights on the relevance of iron in the generation of free radicals from reaction with activated oxygen species (Fenton reaction) and the knowledge of the role of iron in the metabolism of tumor cells and parasites is at the basis of the introduction of iron chelation in the therapy of disorders not characterized by iron overload. In recent years, it became evident that iron may be responsible for tissue damage even in subjects with normal iron stores and that iron chelators may have a relevant therapeutic role even in non-iron-overload conditions [106]. An impressive body of evidence supports the role of iron in the pathophysiology of oxygen-radical-mediated tissue damage [107]. Oxygen radicals are constantly formed in all living cells, in particular during activation of phagocytes or during reoxygenation following ischaemia [108]. Damage, however, only occurs in the presence of catalytic transition metals, among which iron is the most important in human pathology. H_2O_2, a relatively stable molecule by itself non-toxic, has an essential role in the oxygen-radical-generating system as a precursor of $HO^{\bullet}$, which oxidizes all substances close to it. The generation of $HO^{\bullet}$ from H_2O_2 is fully dependent upon the availability of iron, thanks to the favourable redox potential of the Fe(II)/Fe(III) couple and because iron is always present in tissues [108]. The catalytic activity of iron is mainly expressed in its ferrous form, leading to the generation of toxic free radicals, the final common pathway of a wide variety of disease states.

Here, the following new therapeutic indications for iron chelation will be treated: adult respiratory distress syndrome, myocardial ischemia, cancer and malaria.

5.1. Adult Respiratory Distress Syndrome (ARDS)

ARDS is a clinical entity, characterized by an high mortality, which can result from a variety of unrelated insults such as sepsis, aspiration, polytrauma, hyperoxia and poisoning with drugs and chemicals [109]. The lung injury seen in ARDS is the result of an inflammatory process which leads to hypoxaemia, hypercapnia, acidosis, increased permeability of the alveolar barrier membrane and lung oedema. Oxygen radicals, produced by activated polymorphonuclear leukocytes (PMNs), are always present in high number in the diseased lung and may represent 90% of total cells in broncho–alveolar lavage of patients with ARDS [110]; these radicals play an essential role in the development of lung injury, by destroying the integrity of the alveolar cell membrane. The involvement of free radicals in the pathogenesis of ARDS is also demonstrated by the increased concentration of H_2O_2 in the expired breath of affected subjects [111]. The production of oxygen radicals by activated PMNs may be enhanced by the release of platelet-derived growth factors [112]. The beneficial effect of iron chelators has been demonstrated in multiple experimental models of ARDS. The administration of desferrioxamine, as a single intratracheal bolus, prolonged survival of rats in pure oxygen [113]. Desferrioxamine administered to vitamin-E-deficient rats significantly reduced mortality by paraquat, a widely used herbicide, able to generate oxygen radicals, and toxic for humans in whom it may cause ARDS and respiratory failure [114]. These experiments have credibly shown that iron chelators can prevent free radical-mediated tissue damage leading to the development of ARDS. The experimental achievements should be now transferred to clinical practice, in order to benefit patients affected by oxygen radical-mediated injury.

5.2. Iron chelators and myocardial ischemia

In myocardial tissue exposed to ischemia, there is much evidence for the generation of free radical species [115]. Almost all open heart operations, like cardiopulmonary bypass, involve limited periods af aortic cross-clamping which initiates an ischemia–reperfusion sequence, a situation that promotes free radical generation [116,61]. The release of iron, the reductive generation of Fe(II) and the subsequent free radical generation from reaction with activate oxygen are now thought to represent the major cause of tissue damage following myocardial infarction [117]. Treatment with desferrioxamine during experimental heart ischemia has been shown to improve functional and metabolic recovery in rabbits [118] and in dogs [119]. These studies my be considered the experimental basis to assess the effects of desferrioxamine in the setting of clinical myocardial ischemia.

5.3. Iron chelators and cancer

In recent years, many studies provided evidence of a potential utility of iron deprivation in the treatment of multiple neoplasias. The first studies on the role of the transferrin receptor (TR) in cell growth, indicated that monoclonal antibodies against the TR could inhibit iron uptake by tumor cells in vitro, as much as 80% but that even this degree of injury was insufficient to inhibit tumor growth [120]. When desferrioxamine was associated with anti-TR antibodies in the same in vitro model, a marked inhibition of tumor cell growth was observed [121]. These data were subsequently confirmed in vivo: using the high molecular weight hydroxyethyl-starch conjugate of desferrioxamine combined with a single dose of IgA anti-TR antibodies, a synergistic and nearly complete inhibition of tumor cell growth was obtained [122]. The usefulness of desferrioxamine has been also demonstrated in the treatment of neuroblastoma, a solid tumor which predominantly occurs in childhood. In fact, carriers of neuroblastoma frequently show high plasma ferritin levels which correlate with a poor outcome [123]. The antitumor activity of desferrioxamine against neuroblastoma cells was first reported in vitro [124] and subsequently confirmed in man [125]. A trial based on desferrioxamine employed in a multi agent regimen with cyclophosphamide, etoposide, carboplatin and thiotepa is now being planned in the USA [120] and in Italy by the Italian Cooperative Study Group for Neuroblastoma [125].

Desferrioxamine has recently been proposed in conjunction with recombinant α-2-interferon in the treatment of hepatocallular carcinoma [126]. This evidence that desferrioxamine may be employed against certain solid tumors has been paralleled by reports on the use of desferrioxamine, in combination with ARA-C, in the treatment of acute lymphoblastic leukemia [127]. The intimate mechanism of action of desferrioxamine as an antineoplastic agent has not yet been identified. Many studies have shown that acute iron deprivation caused by desferrioxamine is cytotoxic for some tumor cells resulting in increased levels of apoptosis in the neoplasm [128]. By reducing the amount of iron inside tumor cells, desferrioxamine could also interfere with electron transport function in mitochondria [129]. In recent years, other more complex effects of desferrioxamine have been revealed: desferrioxamine may inhibit cell proliferation by inactivation of the iron dependent enzyme ribonucleotide reductase, a rate-limiting enzyme in DNA synthesis [130,131]; it may interfere on the post-transcriptional regulation of the transferrin receptor and ferritin genes [132] and it may modulate the transcription of the erythropoietin gene [133]. In conclusion, further clinical and experimental studies with desferrioxamine alone or in combination or in sequence with other antineoplastic drugs are required, in order to establish the exact role of iron deprivation in the treatment of cancer in man.

5.4. Iron chelators as antimalarials

Malaria is a haemolytic, febrile disease which is considered the world's most major health problem. In fact, the World Health Organization considers malaria as

causing more mortality than any other known human disease. It affects over 200 million persons and kills more than 1 million people per year. Tropical and subtropical areas are mainly affected, especially tropical Africa, South and Central America, India and Southeast Asia. Malaria is caused by four species of plasmodium: plasmodium falciparum, vivax, ovale and malariae. Malaria is transmitted from person to person by the bite of the female anopheles mosquito. Plasmodia are obligatory parasites which grow inside human erythrocytes. The life cycle of the plasmodium species requires both human and mosquito hosts. The parasite needs iron for its life: the putative source of iron is probably obtained by endocytosis and protelysis of haemoglobin [134]. Despite growing inside red blood cells, i.e. in an environment rich with iron, plasmodia display a marked susceptibility to iron chelators [135] which only act as antimalarials on intracellular parasites. On this basis the treatment of malaria with iron chelators has been introduced in clinical practice [136].

The introduction of the use of iron chelators for curbing the proliferation of plasmodium was based on the assumption that drug-induced iron deprivation could be less tolerated by invading organisms than by humans [137]. In fact, iron is required for many parasite enzymes necessary for the explosive proliferation of *Plasmodium falciparum*. Desferrioxamine was shown to be able to arrest the growth of *P. falciparum* in vitro [138]. Subsequently, the effect of desferrioxamine was tested in mouse and in monkeys, in which it suppressed *P. falciparum* [139]. In recent years desferrioxamine has been introduced in clinical practice [140]: iron chelation with desferrioxamine was effective in adults with asymptomatic *P. falciparum* parasitemia. The treatment with desferrioxamine proved to be efficient even in children with cerebral malaria who recovered from deep coma [141]. In a clinical study in Zambia and Thailand, a 3-day desferrioxamine infusion (100 mg/kg per day) enhanced the clearance of periferal blood parasites, but recrudescence occurred in most patients [142].

As for the mechanism of desferrioxamine as antimalarial, in addition to withholding iron from the parasite, it could also enhance the host immune response and it could protect against iron-mediated preoxydant damage in tissues. When used as an antimalarial desferrioxamine was well tolerated: no side effects or toxicity could be attributed in some studies [140], while in other clinical trials, some of the subjects reported transient visual blurring [143]. All these data provide evidence that desferrioxamine, even at low doses, may enhance clearance of *plasmodia* from blood in humans. Unfortunately, only rarely does desferrioxamine lead to a radical cure of malaria and recrudescence of the disease occurred in most subjects treated. Further clinical trials are necessary to decide whether extending the duration of therapy or increasing the dosage of desferrioxamine would be more effective to eradicate malaria from infected patients. Recently, the results of some clinical trials of the oral iron chelator deferiprone as an antimalarial agent have been reported [144]. In this study, deferiprone was administered daily for 3 or 4 days at doses of 75–100 mg/kg of body weight, and showed so modest an antimalarial effect that additional trials of deferiprone as an antimalarial agent would not seem to be justified.

6. Concluding remarks

The relevance of iron chelators in medicine has increased in recent years. New insights on the role of iron in free radical generation and in the metabolism of tumor cells and parasites led to the introduction of iron chelators in the therapy of a variety of diseases not characterized by iron overload.

This increase of new indications for the use of iron chelating agents contrasts with the inadequacy of drugs available for this purpose. In fact, a drug which could act as a selective depletor of iron and should be efficiently absorbed by the gastrointestinal tract, with absence of toxicity, has not yet been identified. Desferrioxamine, the only iron-chelator extensively used in clinical practice, is not absorbed by the gastrointestinal tract and, to be effective, requires prolonged parenteral infusion. Such a regimen makes compliance difficult in the developed world and unaffordable in developing countries.

In spite of the considerable effort devoted by the scientific community to finding alternative oral-active iron chelators, none of the multiple candidates succeeded, until now, to be recognized as an ideal iron chelator. Deferiprone, the last evaluated in different trials, did not adequately control body iron burden in patients with thalassemia.

The failure to find the ideal iron chelator can be ascribed, in our opinion, to difficulties inherent in the problem due to the biological and clinical restraints. This is true despite being a project able to unify researchers in basic sciences and medical doctors involved in clinical practice. Chemists have used their knowledge to synthesize a large variety of iron chelators according to the structural requisites for their introduction in clinical practice; nevertheless the clinical results were not satisfactory.

We hope that the dialogue among chemists and clinicians will be able to achieve the common target of prolonging survival and ameliorating the quality of life of iron-loaded patients.

References

[1] J.B. Neilands, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 152.
[2] P. Ponka, Blood 89 (1997) 25.
[3] G.M. Brittenham, E.H. Danish, J.W. Harris, Semin. Hematol. 18 (1981) 194.
[4] R. Green, R. Charlton, H. Seftel, T. Bothwell, F. Mayet, B. Adams, C. Finch, M. Layrisse, Am. J. Med. 45 (1968) 336.
[5] I.N. Kuhn, E.R. Monsen, J.D. Cook, C.A. Fincg, J. Lab. Clin. Med. 71 (1968) 715.
[6] P. Pootrakul, K. Kitcharoen, P. Yansukon, P. Wai, S. Fucharoen, P. Charoenlarp, G. Brittenham, M.J. Pippard, C.A Finch, Blood 71 (1988) 1124.
[7] J.M. Deugnier, O. Loreal, B. Turlin, D. Guyader, H. Jouanolle, R. Moirand, C. Jacquelinet, P. Brissot, Gastroenterology 102 (1992) 20.
[8] E. Mandishona, A.P. MacPhail, V.R. Gordeuk, M.A. Kedda, A.C. Paterson, T.A. Rouault, M.C. KeW, Hepatology 27 (1998) 1563.

[9] G.M. Brittenham Jr., in: R. Hoffman, E.J. Benzo Jr., S.J. Fhattil, B. Furie, H.J. Cohen, L.E. Silberstein (Eds.), Haematology: Basic Principles and Practice, Churchill and Livingstone, New York, 1995, p. 492.
[10] E. Angelucci, A. Giovagnoni, G. Valeri, E. Paci, P. Muretto, C. McLaren, G.M. Brittenham, G. Lucarelli, Blood 90 (1997) 4736.
[11] G.M. Brittenham, P.M. Griffith, A.W. Nienhuis, C.E. McLaren, N.S. Young, E.E. Tucker, C.J. Allen, D.E. Farrel, J.W. Harris, New Engl. J. Med. 331 (1994) 567.
[12] L. Lerner, F. Blei, F. Bierman, L. Johnson, S. Piomelli, Am. J. Pediatr. Haematol. Oncol. 12 (1990) 56.
[13] A. Cao, Am. J. Hum. Genet. 54 (1994) 397.
[14] Group WW, Bull. WHO 61 (1983) 63.
[15] A. Cao, L. Pinto, U. Lecca, G. Olla, P. Cossu, C. Rosatelli, R. Galanello, Clin. Genet. 26 (1984) 12.
[16] A.R. Cohen, Hematol. Oncol. Clin. North Am. 1 (1987) 521.
[17] G. Masera, G. Jean, V. Conter, S. Terzoli, R.A. Mauri, M. Cazzaniga, Arch. Dis. Child. 55 (1980) 800.
[18] A.S. Tavill, B.R. Bacon, in: D. Zakim, T.D. Boyer (Eds.), Hepatology, A Textbook of Liver Disease, 2nd ed., W.B. Sanders, Philadelphia, PA, 1991, p. 1273.
[19] D.T. Kremastinos, G. Tiniakos, G.N. Theodorakis, D.G. Katristis, P.K. Toutouzas, Circulation 91 (1995) 66.
[20] K.H. Ehlers, A.R. Levin, A.L. Markenson, J.R. Marcus, A.A. Klein, M.W. Hilgartner, M.A. Engle, Ann. N.Y. Acad. Sci. 344 (1980) 397.
[21] G. Faa, P.F. Todde, G. Cattani, F. Sau, P. Abbruzzese, R. Ambu, G. Parodo, R. Pinna, R. Silvagni, V.M. Nurchi, G. Crisponi, Cardiovasc. Pathobiol. 1 (1996) 1.
[22] G. Link, A. Pinson, C. Hershko, J. Lab. Clin. Med. 106 (1985) 147.
[23] V. Gabutti, A. Piga, Acta Haematol. 95 (1996) 26.
[24] H. Keberle, Ann. N.Y. Acad. Sci. 119 (1964) 758.
[25] C. Hershko, G. Link, M. Tzahor, A. Pinson, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 75.
[26] S. Moeschlin, U. Schnider, New Engl. J. Med. 269 (1963) 57.
[27] K. Gross, J. Aumiller, J. Gelzer (Eds.), Desferrioxamine: Desferal; MMW Pharmaceutical Award 91; History, Clinical Value, Perspectives, Symposium on the Occasion of the Award Presentation at the Swiss Federal Institute of Technology, Zurich, MMW Medizin Verlag GmbH, Munich, 1992.
[28] G.M. Brittenham, Blood 80 (1992) 569.
[29] R.C. Hider, R. Chourdhry, B.L. Rai, L.S. Dehkordi, S. Singh, Acta Haematol. 95 (1996) 6.
[30] A. Lea, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525.
[31] D.G. Maxton, I. Bjarnson, A.P. Reynolds, S.D. Catt, T.J. Paters, I.S. Menzies, Clin. Sci. 71 (1986) 71.
[32] G.J. Kontoghiorghes, J.G. Goddard, A.N. Bartlett, L. Sheppard, Clin. Pharmacol. Ther. 48 (1990) 255.
[33] R.D. Propper, S.B. Hurin, R.R. Rufo, New Engl. J. Med. 297 (1977) 418.
[34] M. Barry, D.M. Flynn, E.A. Letsky, A. Risdon, Br. Med. J. 2 (1974) 16.
[35] R.D. Propper, S.B. Shurin, D.G. Nathan, New Engl. J. Med. 294 (1976) 1421.
[36] D.G. Nathan, New Engl. J. Med. (Editorial) 332 (1995) 953.
[37] M.R. Summers, A. Jacobs, D. Tudway, P. Perera, C. Ricketts, Br. J. Haematol 42 (1979) 547.
[38] P. Lee, N. Mohammed, L. Marshall, R.D. Abeysinghe, R.C. Hider, J.B. Porter, S. Singh, Drug Metab. Dispos. 21 (1993) 640.
[39] M.J. Pippard, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 57.
[40] C. Hershko, Blood 51 (1978) 415.
[41] C. Hershko, E.A. Rachmilewitz, Br. J. Haematol 42 (1979) 125.
[42] C. Hershko, T.E.A. Peto, Br. J. Haematol 66 (1987) 149.
[43] E.J. Gralla, D.H. Burgess, Exp. Clin. Pharmacol. (1982) 4.

[44] J.B. Porter, Acta Haematol. 95 (1996) 13.
[45] P. Ponka, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 2.
[46] A. Jacobs, Blood 50 (1977) 433.
[47] S. Pollack, P. Aisen, F.O. Laskey, G. Vanderhoff, Br. J. Haematol. 34 (1976) 231.
[48] H. Kondo, K. Saito, J.P. Grasso, P. Aisen, Hepatology 8 (1988) 32.
[49] J.C. Sibille, H. Kondo, P. Aisen, Hepatology 8 (1988) 296.
[50] R.L. Hamill, J.C. Woods, B.A. Cook, Am. J. Clin. Pathol. 96 (1991) 215.
[51] R.Y. Chan, P. Ponka, H.M. Schulman, Exp. Cell. Res. 202 (1992) 326.
[52] J.M.C. Gutteridge, R. Richmond, B. Halliwell, Biochem. J. 184 (1979) 469.
[53] J.F. Goodwin, C.F. Whitten, Nature 205 (1965) 281.
[54] M.C. Linder, M. Hazegh-Azam, Am. J. Clin. Nutr. 63 (1996) 797S.
[55] K. Joshida, K. Furihata, S. Takeda, A. Nakamura, K. Yamamoto, H. Motita, S. Hiyamuta, S. Ikeda, N. Shimizu, N. Yanagisawa, Nat. Genet. 9 (1995) 267.
[56] Z.L. Harris, L.W.J. Klomp, J.D. Gitlin, Am. J. Clin. Nutr. 67 (1998) S972.
[57] M.J. O'Connell, B. Halliwell, C.P. Moorhouse, O.I. Aruoma, H. Baum, T.J. Peters, Biochem. J. 229 (1985) 135.
[58] E. Friend, in: H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 13, Marcel Dekker, New York, 1981, p. 117.
[59] M.C. Aragoni, G. Crisponi, V.M. Nurchi, R. Silvagni, R. Sciot, R. Ambu, V. Costa, G. Faa, J. Trace Elements Med. Biol. 9 (1995) 215.
[60] J. Kato, Y. Kohgo, N. Sugawara, S. Katsuki, N. Shintani, K. Fujikawa, E. Miyazaki, M. Kobune, N. Takeichi, Y. Niitsu, Jpn. J. Cancer Res. 84 (1993) 219.
[61] A. Bel, E. Martinod, P. Menasché, Acta Haematol. 95 (1996) 63.
[62] J. Sinaceur, C. Ribière, J. Normann, R. Normann, Biochem. Pharmacol. 33 (1984) 1693.
[63] N.F. Olivieri, Acta Haematol. 95 (1996) 37.
[64] S. DeVirgiliis, M. Congia, F. Frau, F. Argiolu, G. Diana, F. Cucca, A. Varsi, G. Sanna, G. Podda, M. Fodde, G.M. Pirastu, A. Cao, J. Pediatr. 113 (1988) 661.
[65] A. Piga, L. Luzzato, P. Capalbo, S. Gambotto, F. Tricta, V. Gabutti, Eur. J. Haematol. 40 (1988) 380.
[66] N.F. Olivieri, G. Koren, J. Harris, S. Khattak, M.H. Freedman, D.M. Templeton, J.D. Bailey, B.J. Reilly, Am. J. Pediatr. Haematol. Oncol. 14 (1992) 48.
[67] N.F. Olivieri, R.J. Buncic, E. Chew, T. Gallanti, R.V. Harrison, N. Keenan, N. Logan, D. Mitchell, G. Ricci, B. Skarf, M. Taylor, M.H. Freedman, New Engl. J. Med. 314 (1986) 869.
[68] S.C. Davies, R.E. Marcus, J.L. Hungerford, M.H. Miller, G.B. Arden, E.R. Huehns, Lancet ii (1983) 181.
[69] A. Cohen, M. Martin, J. Mizanin, D.F. Konkle, E. Schwartz, J. Pediatr. 117 (1990) 326.
[70] M.H. Freedman, D. Grisaru, N.F. Olivieri, I. MacLusky, P.S. Thorner, Am. J. Dis. Child. 144 (1990) 565.
[71] T. Gallant, M.H. Freedman, H. Vellend, W.H. Francombe, New Engl. J. Med. 314 (1986) 1643.
[72] R.M. Robin-Browne, J.K. Prpic, J.K. Stuard, Contrib. Microbiol. Immunol. 9 (1987) 254.
[73] N.F. Olivieri, D.G. Nathan, J.H. MacMillan, A.D. Wayne, M. Martin, A. McGee, G. Koren, P.P. Liu, A.R. Cohen, New Engl. J. Med. 331 (1994) 574.
[74] W.R. Harris, K.N. Raymond, F.L. Weitl, J. Am. Chem. Soc. 103 (1981) 2667.
[75] K.N. Raymond, V.L. Pecoraro, F.L. Weitl, in: A.E. Martell, W.F. Anderson, D.G. Badman (Eds.), Development of Iron Chelators for Clinical Use, Elsevier, New York, 1981, p. 165.
[76] K.N. Raymond, J. Xu, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 308.
[77] G.N. Long, Stability Constants of HBED with Various Metal Ions, Master's Thesis, Texas A&M University, College Station, 1990.
[78] C. Hershko, R.W. Grady, G. Link, Haematologia (Budap.) 17 (1984) 25.
[79] P. Ponka, J. Borova, J. Neuwirt, O. Fuchs, FEBS Lett. 97 (1979) 317.
[80] H.U. Naegeli, H. Zaehner, Helv. Chim. Acta 63 (1980) 1400.
[81] R.C. Scarrow, P.E. Riley, K. Abu-Dari, D.L. White, K.N. Raymond, Inorg. Chem. 24 (1985) 954.

[82] H.H. Peter, R.J. Bergeron, R.R. Streiff, J. Wiegand, in: R.J. Bergeron, G.M. Brittenham Jr. (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 373.
[83] C. Hershko, A.M. Konijn, G. Link, Br. J. Haematol. 101 (1998) 399.
[84] L.M.W. Vitolo, G.T. Hefter, B.W. Clare, J. Webb, Inorg. Chim. Acta 170 (1990) 171.
[85] G. Anderegg, M. Raeber, J. Chem. Soc. Chem. Commun. (1990) 1194.
[86] R.J. Bergeron, R.R. Streiff, E.A. Creary, R.D. Daniels Jr., W. King, G. Luchetta, J. Wiegand, T. Moerker, H.H. Peter, Blood 81 (1993) 2166.
[87] R.J. Bergeron, J. Wiegand, M. Wollenweber, J.S. McManis, S.E. Algee, K. Ratliff-Thompson, J. Med. Chem. 39 (1996) 1575.
[88] W.R. Barker, C. Callaghan, L. Hill, D. Nobll, P. Acred, P.B. Harper, M.A. Sowa, R.A. Fletton, J. Antibiot. 32 (1979) 1096.
[89] R.C. Hider, G.J. Kontoghiorghes, J. Silver, UK Patent GB-2118176, 1982.
[90] G.J. Kontoghiorghes, Biochem. J. 233 (1986) 299.
[91] G.J. Kontoghiorghes, Biochim. Biophys. Acta 869 (1986) 141.
[92] R.C. Hider, J.B. Porter, S. Singh, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 354.
[93] N.F. Olivieri, G. Koren, C. Hermann, Y. Bentur, D. Chung, J. Klein, P. St. Louis, M.H. Freedman, R.A. McClelland, D.M. Templeton, Lancet 336 (1990) 1275.
[94] N.F. Olivieri, G. Koren, D. Matsui, P.P. Liu, L. Blendis, R. Cameron, R.A. McClelland, D.M. Templeton, Blood 79 (1992) 2741.
[95] N.F. Olivieri, G.M. Brittenham, D. Matsui, M. Berkovitch, L.M. Blendis, R.G. Cameron, R.A. McClelland, P.P. Liu, D.M. Templeton, G. Koren, New Engl. J. Med. 332 (1995) 918.
[96] M. Jessup, C.S. Manno, Ann. N.Y. Acad. Sci. 850 (1998) 242.
[97] E. Angelucci, E. Mariotti, G. Lucarelli, D. Baronciani, P. Cesaroni, S.M.T. Durazzi, M. Galimberti, C. Giardini, P. Muretto, P. Polchi, E. Sgarbi, Lancet 339 (1992) 287.
[98] J. Link, A. Pinson, C. Hershko, Blood 83 (1994) 2692.
[99] N.F. Olivieri, N. Beluzzo, M. Muraca, C.C. Mac Kenzie, K. Polsinelli, G. Korean, P.P. Liu, G.M. Brittenham, Blood 84 (Suppl. 1) (1994) 109a.
[100] N.F. Olivieri for the Iron Chelation Research Group, Randomized trial of deferiprone (L1) and desferrioxamine (DFO) in thalassemia major, 38th Annual Meeting of the American Society of Hematology, Orlando, FL, December 6–10, 1996.
[101] N.F. Olivieri for the Iron Chelation Research Group, Long term followup of body iron in patients with thalassemia major during therapy with the orally active iron chelator deferiprone, 38th Annual Meeting of the American Society of Hematology, Orlando, FL, December 6–10, 1996.
[102] P. Carthew, A.G. Smith, R.C. Hider, B. Dorman, R.E. Edwards, J.E. Francis, BioMetals 7 (1994) 267.
[103] B. Halliwell, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, Boca Raton, FL, 1994, p. 33.
[104] R.J. Motekaitis, A.E. Martell, Inorg. Chim. Acta 183 (1991) 71.
[105] N.F. Olivieri, G.M. Brittenham, C.E. McLaren, D.M. Templeton, R.G. Cameron, R.A. McClelland, A.D. Burt, K.A. Flemming, New Engl. J. Med. 339 (1998) 417.
[106] E.E. Voest, G. Vreugdenhil, J.J.M. Marx, Ann. Intern. Med. 120 (1994) 490.
[107] C.E. Cross, B. Halliwell, E.T. Borish, W.A. Pryor, B.N. Ames, R.L. Saul, J.M. McCord, D. Harman, Ann. Intern. Med. 107 (1987) 526.
[108] J.J.M. Marx, B.S. van Asbeck, Acta Haematol. 95 (1996) 49.
[109] R. Balk, R.C. Bone, Med. Clin. North Am. 67 (1983) 685.
[110] J.E. Weiland, W.B. Davis, J.F. Holter, J.R. Mohammed, P.M. Dorinsky, J.E. Gadek, Ann. Rev. Respir. Dis. 133 (1986) 218.
[111] S.R. Baldwin, C.M. Grum, L.A. Boxer, R.H. Simon, L.H. Ketal, L.J. Devall, Lancet i (1986) 11.
[112] T.F. Deuel, J.S. Huang, J. Clin. Invest. 74 (1984) 669.
[113] W.A.A. van der Wal, J.F. van Oirschot, C.J. Brandt, B.S. van Asbeck, Clin. Res. 36 (1988) 511A.
[114] L.L. Smith, Hum. Toxicol. 6 (1987) 31.
[115] J.L. Zweier, J.T. Flaherty, M.L. Weisfeldt, Proc. Natl. Acad. Sci. USA 84 (1987) 1404.

[116] N.C. Cavarocchi, M.D. England, H.V. Schaff, P. Russo, T.A. Orszulak, W.A. Schnall, J.F. O'Brien, J.R. Pluth, Circulation 74 (Suppl. 3) (1986) 130.
[117] L. Wolfe, N.F. Olivieri, D. Sallan, S. Colan, V. Rose, R. Propper, R.H. Freedman, D.G. Nathan, New Engl. J. Med. 312 (1985) 1600.
[118] R.E. Williams, J.L. Zweier, J.J. Flaherty, Circulation 83 (1991) 1006.
[119] M. Maruyama, G.M. Pieper, B. Kalyanaramen, P.E. Hallaway, B.E. Hedlund, G.J. Gross, J. Cardiovasc. Pharmacol. 17 (1991) 166.
[120] J.D. Kemp, Histol. Histopathol. 12 (1997) 291.
[121] J.D. Kemp, K.M. Smith, L.J. Kanner, F. Gomez, J.A. Thorson, P.W. Naumann, Blood 76 (1990) 991.
[122] J.D. Kemp, T. Cardillo, B.C. Stewart, E. Kehrberg, G. Weiner, B. Hedlung, P.W. Naumann, Cancer Res. 55 (1995) 3817.
[123] T.C. Iancu, H. Shiloh, A. Kedar, Cancer 61 (1988) 2497.
[124] J. Blatt, S. Stitely, Cancer Res. 47 (1987) 1749.
[125] A. Donfrancesco, G. Deb, A. Angioni, C. Maurizio, R. Cozza, A. Jenkner, A. Landolfo, C. Boglino, L. Helson, Anti Cancer Drug 4 (1993) 317.
[126] J. Kontouras, P. Boura, A. Karolides, E. Zaharioudaki, G. Isapas, Hepatogastroenterology 42 (1995) 31.
[127] Z. Estrov, A. Tawa, X-H. Wang, I.D. Pube, A. Cohen, E.W. Gelfand, M.H. Freedman, Blood 69 (1987) 757.
[128] D. Hileti, P. Panayiotidis, A.V. Hoffbrand, Br. J. Haematol. 89 (1995) 181.
[129] H. Wharton, D.L. Granger, D.T. Durack, J. Immunol. 141 (1988) 1311.
[130] C. Hershko, Baillieres Clin. Haematol. 7 (1994) 965.
[131] S. Nyholm, G.J. Mann, A.G. Johansson, R.J. Bergeron, A. Graslund, L. Thelandler, J. Biol. Chem. 268 (1993) 26200.
[132] R.D. Klausner, T.A. Roualt, J.B. Hardford, Cell 72 (1993) 19.
[133] J.M. Gleadle, B.L. Ebert, J.D. Firth, P.J. Ratcliffe, Am. J. Physiol. Cell Physiol. 268 (1995) C1362.
[134] B.E. Goldberg, A.F.G. Slater, A. Cerami, G.B. Henderson, Proc. Natl. Acad. Sci. USA. 87 (1990) 2931.
[135] D.G. Heppner, P.E. Hallaway, G.J. Kontoghiorghes, J.W. Eaton, Blood 72 (1988) 358.
[136] R.C. Hider, Z Liu, J. Pharm. Pharmacol. 49 (1997) 59.
[137] C. Hershko, Z.I. Cabantchik, Isr. J. Med. Sci. 30 (1994) 840.
[138] T.E. Peto, J.L Thompson, Br. J. Haematol. 63 (1986) 273.
[139] S. Pollack, R.N. Rossan, D.E. Davidson, A. Escajadillo, Proc. Soc. Exp. Biol. Med. 184 (1987) 162.
[140] V.R. Gordeuk, P.E. Thuma, G.M. Brittenham, S. Zulu, P. Simwanza, A. M'hango, G. Fleisch, D. Parry, Blood 79 (1992) 308.
[141] V.R. Gordeuk, P.E. Thuma, G.M. Brittenham, C.E. McLaren, D. Parry, A. Backenstose, G. Biemba, R. Msiska, L. Holmes, E. McKinley, L. Vargas, R. Gilkeson, A.A. Poltera, New Engl. J. Med. 327 (1992) 1473.
[142] G.F. Mazeba, G. Biemba, V.R. Gordeuk, Acta Haematol. 95 (1996) 78.
[143] D. Bunnag, A.A. Poltera, C. Viravan, S. Looareesuwan, K.T. Harinasuta, C. Schindlery, Acta Trop. 52 (1992) 59.
[144] P.E. Thuma, N.F. Olivieri, G.F. Mabeza, G. Biemba, D. Parry, S. Zulu, F.F. Fassos, R.A. McClelland, G. Koren, G.M. Brittenham, V.R. Gordeuk, Am. J. Trop. Med. Hyg. 58 (1998) 358.