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Benefits and Risks of Deferiprone in Iron Overload in Thalassaemia and Other Conditions

Comparison of Epidemiological and Therapeutic Aspects with Deferoxamine

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Contents

Abstract

Deferiprone is the only orally active iron-chelating drug to be used therapeutically in conditions of transfusional iron overload. It is an orphan drug designed and developed primarily by academic initiatives for the treatment of iron overload in thalassaemia, which is endemic in the Mediterranean, Middle East and South East Asia and is considered an orphan disease in the European Union and North

America. Deferiprone has been used in several other iron or other metal imbalance conditions and has prospects of wider clinical applications.

Deferiprone has high affinity for iron and interacts with almost all the iron pools at the molecular, cellular, tissue and organ levels. Doses of 50–120 mg/kg/day appear to be effective in bringing patients to negative iron balance. It increases urinary iron excretion, which mainly depends on the iron load of patients and the dose of the drug. It decreases serum ferritin levels and reduces the liver and heart iron content in the majority of chronically transfused iron loaded patients at doses >80 mg/kg/day. It is metabolised to a glucuronide conjugate and cleared through the urine in the metabolised and a non-metabolised form, usually of a 3 deferiprone **:** 1 iron complex, which gives the characteristic red colour urine. Peak serum levels of deferiprone are observed within 1 hour of its oral administration and clearance from blood is within 6 hours. There is variation among patients in iron excretion, the metabolism and pharmacokinetics of deferiprone.

Deferiprone has been used in more than 7500 patients aged from 2–85 years in >50 countries, in some cases daily for >14 years. All the adverse effects of deferiprone are considered reversible, controllable and manageable. These include agranulocytosis with frequency of about 0.6%, neutropenia 6%, musculoskeletal and joint pains 15%, gastrointestinal complains 6% and zinc deficiency 1%. Discontinuation of the drug is recommended for patients developing agranulocytosis.

Deferiprone is of similar therapeutic index to subcutaneous deferoxamine but is more effective in iron removal from the heart, which is the target organ of iron toxicity and mortality in iron-loaded thalassaemia patients. Deferiprone is much less expensive to produce than deferoxamine. Combination therapy of deferoxamine and deferiprone has been used in patients not complying with subcutaneous deferoxamine or experiencing toxicity or not excreting sufficient amounts of iron with use of either drug alone. New oral iron-chelating drugs are being developed, but even if successful these are likely to be more expensive than deferiprone and are not likely to become available in the next 5–8 years. About 25% of treated thalassaemia patients in Europe and more than 50% in India are using deferiprone. For most thalassaemia patients worldwide who are not at present receiving any form of chelation therapy the choice is between deferiprone and fatal iron toxicity.

Metal ions such as iron, copper and zinc are found in trace amounts in the human body and are essential components of healthy living and normal bodily functions. The presence of homeostatic controls ensures the maintenance of physiological levels of such essential metal ions in all living organisms. The homeostatic controls for metal ions could be affected by genetic, environmental, iatrogenic and other factors.

Iron plays an essential role in the life of humans and all other organisms. Body iron levels and organ distribution are normally governed by the gastrointestinal absorption of dietary iron and the erythropoietic activity of the bone marrow. Iron absorption

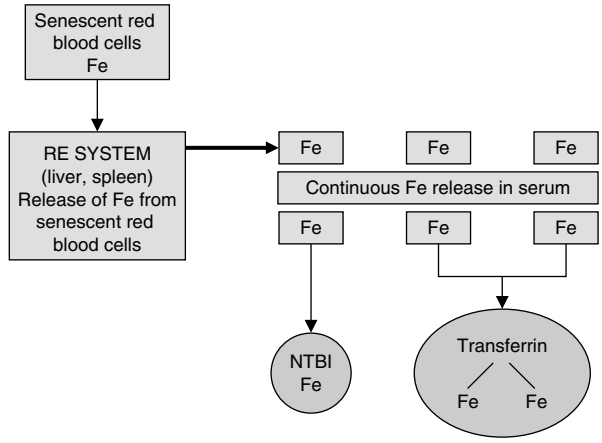

Fig. 1. The recycling of iron (Fe) from senescent red blood cells in thalassaemia and other conditions of transfusional iron overload. Transferrin is fully saturated with iron and NTBI is present in serum. **NTBI** = non-transferrin bound iron; **RE** = reticuloendothelial.

from the intestine depends on the chemical complex form and quantity of iron present in the diet and is regulated by proteins such as hephaestin and ferroportin. In normal conditions the intracellular uptake and storage of iron is regulated by the iron-regulatory proteins (IRPs) through the translational control of the synthesis of the transferrin receptor at the cell surface and of intracellular ferritin.[1,2] The intracellular storage of iron is primarily accomplished by the deposition of iron in the intracellular proteins ferritin and haemosiderin, the latter being in excess in iron-overloading conditions. Iron present in food and absorbed from the gastrointestinal tract or iron released following the breakdown of senescent red blood cells or other cells is transported in blood by transferrin (figure 1). This protein is taken into the cells through the binding of two molecules of mono- or diferric transferrin to a transferrin receptor on the cell surface and subsequent incorporation in the cell within an endosome. The release of iron from transferrin in the endosome intracellularly is accomplished by acidification of the endosome from pH 7.4–5.6. Transferrin in normal individuals is saturated 25–35% with iron, whereas in transfusional iron overload the saturation may exceed 100% and non-transferrin bound iron (NTBI) could be detected in the serum.

Iron overload could be caused by increased gastrointestinal iron absorption or multiple red blood cell transfusions or a combination of these two processes. Iron overload caused by repeated red blood cell transfusions in refractory anaemias is the most common metal toxicity condition with the highest mortality and morbidity rate worldwide. The most seriously affected group of transfused patients are those with thalassaemia, which is the commonest genetic disorder with over 100 million estimated asymptomatic heterozygote thalassaemia gene carriers worldwide.[3-5] In some countries, such as Cyprus, thalassaemia heterozygotes account for 16% of the population, where in India it could account for 1–10% depending on the area.[5,6] Patients with thalassaemia major have to receive red blood cell transfusions every 2–4 weeks for their entire life because of ineffective erythropoiesis, because their haemoglobin and red blood cells are not functioning normally. The iron from senescent red blood cells from transfusions is not excreted but accumulates in the body resulting in iron overload and toxicity, tissue damage and eventually death usually from heart failure, unless iron-chelation therapy is introduced.

A chelator (from the Greek meaning claw of a crab) is a chemical or a drug molecule capable of

forming a heterocyclic ring with a metal ion as the closing member, i.e. is like a crab holding the metal ion in its claw. A chelator has at least two functional groups (ligands) the donor atoms of which can donate a pair of electrons for the formation of a bond with the metal ion. The chelator metal complex has different properties from either the metal or the chelator.

The removal of excess or toxic metal from the body usually requires the use of a specific chelating drug, which in principle should be able to bind and remove this metal through the urinary and or faecal excretion routes, thus maintaining safe levels of metal in the body. This process should be accomplished without causing the removal of other essential metals or other serious adverse effects. Chelating drugs and their metal complexes are currently widely used in medicine for therapeutic or diagnostic purposes. Within this context the role of iron-chelating drugs in thalassaemia and other transfusional iron-loaded patients is to remove sufficient amounts of iron, maintain iron balance and prevent iron accumulation and toxicity in these patients. Iron-chelation therapy is a very complex process, which is affected by various chemical, metabolic and other factors and interactions at the molecular, cellular, tissue and whole body levels.[7-10]

There have been a number of recent reviews covering various aspects of the properties and uses of deferiprone (L1; chemical name: 1,2-dimethyl-3-hydroxypyrid-4-one), which is the only orally active iron-chelating drug available for the treatment of transfusional iron-loading in thalassaemia and other conditions (figure 2).[11-17] This review considers the benefits and risks of the use of deferiprone compared with those of deferoxamine, the only other available iron-chelating drug. Updated information on recent developments in the area of iron-chelation therapy is also provided, including the effects of therapy with a combination of these two drugs.

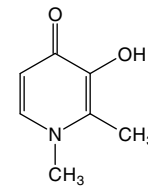

Fig. 2. Chemical structure of deferiprone (L1; 1,2-dimethyl-3-hydroxypyrid-4-one).

1. Epidemiological Considerations

1.1 Epidemiology of Thalassaemia and Estimated Costs of Iron-Chelation Therapy

The treatment of transfusional iron overload in thalassaemia and other refractory anaemia conditions is based on the removal of excess iron by chelating drugs. Thalassaemia is an inherited haemoglobinopathy, which is the most common genetic disease causing the most common chronic metal overloading condition with the highest morbidity and mortality rate worldwide. The prevalence and geographic distribution of thalassaemia is mainly in countries of the Mediterranean area, Middle East and South East Asia.[3-5] Over 80% of the thalassaemia patients live in the Middle East and South East Asia. The geographic distribution of thalassaemia in these areas appears to be related to the high incidence of malaria in past centuries, where there was an increased survival of asymptomatic heterozygote thalassaemia gene carriers compared with normal individuals. However, the flow of immigrants from these areas to Western Europe and North American countries has also increased the incidence of thalassaemia in these countries, where is now considered as an orphan disease because of the small number of patients by comparison to the indigenous population, who are not carriers of the thalassaemia gene.[18]

It is estimated that there are over 100 million of thalassaemia heterozygote asymptomatic carriers

worldwide and that more than 100 000 children with thalassaemia are born annually.[3-5] About 73% of those born could develop iron overload from red blood cell transfusions or increased iron absorption or a combination of both of these two processes.[19] In the absence of red blood cell transfusions, thalassaemia patients die from ineffective erythropoiesis usually by the age of 2–4 years. In the rural areas of developing countries the majority of children with thalassaemia do not receive transfusions mainly because of their poor prognosis, the lack of state healthcare facilities, the difficulties with the treatment and the associated costs, which for most of their families are prohibitive. The life-expectancy of regularly transfused thalassaemia patients could increase to about 10–20 years in areas where transfusion centres are available.[4] Transfused patients could die from iron-overload toxicity, which irreversibly affects major organs such as the heart, unless iron-chelation therapy is introduced within a few years of beginning the transfusions. Most of the patients who have been regularly receiving iron-chelation therapy with deferoxamine since their infancy lived longer than 20 years and many are now over the age of 40 years.[20] In a recent investigation the mean life span of thalassaemia patients in the UK was estimated to be about 35 years, while the major complication and suggested cause of their death was iron overload due to non-compliance with subcutaneous deferoxamine.[21] In Cyprus, Greece, Italy, the UK and most other developed countries the provision of deferoxamine is supported by the state authorities and in most cases provided free of charge to patients. It is estimated that in these developed countries at least one-third of the thalassaemia patients have serum ferritin levels >2.5 mg/L.[22] This is an indication of ineffective or insufficient iron-chelation therapy with deferoxamine, resulting in an increased body iron load and toxicity.

The rate of iron loading in thalassaemia patients depends mainly on the rate of red blood cell transfusions, which is usually aimed at maintaining haemoglobin levels at about 11 g/dL (1–3 units of packed red blood cells per 2–4 weeks). This rate of transfusion causes a net iron deposition in the body of about 15–35 mg/day. Additional iron of up to about 6 mg/day could also be deposited in the body from increased gastrointestinal iron absorption as the patients become progressively anaemic between transfusions. A rate of iron removal of at least 15–40 mg/day by iron-chelating drugs is therefore generally required in order to maintain patients in negative iron balance and also to minimise the onset of organ damage due to iron-overload toxicity.

Daily doses of about 35–70 mg/kg for subcutaneous deferoxamine or 75–120 mg/kg for oral deferiprone are mostly used to achieve negative iron balance.[14,15] This amounts on average to about 2–3g of deferoxamine or 4–6g of deferiprone per day in a 50kg human. We estimated that the annual cost of iron-chelation therapy per patient receiving deferoxamine is $US5000–$US10 000. This cost depends on the amount of deferoxamine used in each patient and the country where is sold. In our view deferiprone could be sold at a price that is 50 times cheaper than the price of deferoxamine; however, it is sold at the same price as deferoxamine in Europe and only eight times cheaper than deferoxamine in India. It should be noted that the starting materials and method of synthesis of deferiprone are inexpensive.

1.2 Epidemiology of Other Conditions Requiring Iron-Chelation Therapy

In addition to transfusional iron overload in thalassaemia major, iron overload and the associated complications are also found in many other conditions where red blood cell transfusions are repeatedly used, such as other forms of thalassaemia, sickle-cell anaemia, myelodysplasia, myelofibrosis, aplastic anaemia, sideroblastic anaemia, Blackfan Diamond anaemia, Fanconi's anaemia, hereditary hy-

Table I. Clinical uses of iron chelators in iron overload and other metal imbalance and toxicity conditions

Iron overload	**Neoplastic and other diseases**
β-Thalassaemia major	Neuroblastoma[24]
β-Thalassaemia intermedia	Various forms of cancer[24-31]
HbE β-Thalassaemia	AIDS[31,32]
HbS β-Thalassaemia	Microbial infections[33,34]
Sickle-cell anaemia	Malaria and other parasitic infections[35-38]
Aplastic anaemia	**Iron imbalance conditions**
Sideroblastic anaemia	Iron deficiency anaemia[39]
Pyruvate-kinase deficiency	Anaemia of chronic disease[40-42]
Blackfan-Diamond anaemia	Friedreich's ataxia[43]
Fanconi's anaemia	Hallevorden-Spatz syndrome[44]
Pernicious anaemias	Free radical and other toxicity
Hereditary spherocytosis	Conditions of free radical damage[45-52]
Congenital dyserythropoietic anaemia	Cyclo-oxygenase and lipoxygenase inhibitors[53,54]
Congenital atransferrinaemia	Drug toxicity e.g. doxorubicin[55-57]
Hereditary hypochromic anaemia	Ischaemia reperfusion injury[58,59]
Haemolytic disease of the newborn	Storage of organs for transplantation[10]
Idiopathic haemochromatosis	Alzheimer's disease[60]
Iron overload in liver disease	Parkinson's disease[10]
Iron overload in haemodialysis	Rheumatoid arthritis[40-42]
Prophyria cutanea tarda	**Other uses**
Intramuscular iron dextran	Metal imbalance conditions[32,61]
Dietary or latrogenic iron intake	Radiopharmaceuticals[62]
Iron poisoning	Diagnostic medicine[63,64]
Other metal toxicity	Magnetic resonance imaging[63]
Aluminium overload[65,66]	
Wilson's disease (copper toxicity)[67]	
Plutonium toxicity[61,68]	
Uranium toxicity[69]	
Indium toxicity[70]	
Gallium toxicity[62]	

Hb = haemoglobin.

pochromic anaemia, haemodialysis, different forms of cancer, etc. (table I). With the exception of thalassaemia and sickle-cell anaemia, all other conditions are distributed worldwide. Sickle-cell anaemia is prevalent in Africa and in countries with immigrant populations that originated from Africa. The treatment of iron overload in these conditions has been carried out using subcutaneous deferoxamine. Deferiprone has also been used in an increasing number of patients in most of these conditions.[23]

In addition to the expansion of the number of conditions, there is also a progressive increase in the number of patients in each condition receiving regular red blood transfusions worldwide. This is mainly due to the increased healthcare options that are becoming available for a number of conditions, such as myelodysplastic syndrome, which mainly affect geriatric patients in developed countries. An increase in the number of transfusion centres has also been observed in developing countries that have large numbers of thalassaemia patients. This is partly because of increases in health spending and also because of the awareness of the possibility of increased survival and improved prognosis.

There are several other metal toxicity conditions in addition to iron overload where chelating drugs

could be used such as aluminium overload in renal dialysis. Patients receiving long-term maintenance haemodialysis could develop aluminium-related bone diseases and encephalopathy due to aluminium accumulation in bones or in the brain. The excess aluminium is incorporated in these organs from the use of aluminium hydroxide-containing drugs to prevent hyperphosphataemia or because of the use of water with high aluminium content for the dialysis.[71] Increased aluminium has also been detected in the bones of infants receiving intravenous therapy and in the brains of patients with Alzheimer's disease.[72,73] Deferoxamine is being widely used in the treatment of aluminium-loaded renal dialysis patients, but has several drawbacks such as the cost, oral inactivity and toxicity such as mucormycosis.[74,75] The removal of aluminium by deferoxamine has been suggested as the cause of the decrease in the progress of dementia in Alzheimer's disease patients.[60] Orally administered deferiprone has been tested in animals and shown to cause similar levels of aluminium excretion to that of deferoxamine.[65] Similarly, deferiprone has been shown in clinical trials to increase serum aluminium levels and aluminium removal in the peritoneal dialysis fluid of renal dialysis patients and may have a use in the treatment of this condition.[66]

1.3 Controversies Surrounding the Use of Iron-Chelation Therapies

There were many controversies among academic groups and individuals as well as regulatory authorities and pharmaceutical companies involved with the development and clinical use of deferiprone, which is an orphan drug designed and developed following mainly academic initiatives. These differences have been cited in the scientific literature and highlighted in the mass media of many countries.[22,76-86] The first country to approve the use of deferiprone in thalassaemia was India in 1994, where most patients were not receiving any chelation therapy because they could not afford the high cost of deferoxamine.[87] The deferiprone capsule formulations of 250 and 500mg are sold at a price at least eight times cheaper than deferoxamine in India.[22] No major adverse effects, other than those previously reported, were identified following a number of clinical trials and general postmarketing surveillance in various parts of India.[22,87] There are more patients receiving deferiprone than deferoxamine in India at present but even with much lower price than deferoxamine the drug has not yet become available to the vast majority of thalassaemia patients. We believe that this is partly because some of the clinicians are reluctant to use it because of the adverse publicity and also because the cost of deferiprone is still not affordable for the average Indian thalassaemia patient.

A 500mg tablet formulation of deferiprone received conditional approval for its use as a second-line chelating drug by the European Union (EU) regulatory authorities in 1999. This formulation is intended for use in thalassaemia patients who have contraindications to, or are unable to tolerate treatment with deferoxamine. Despite the very low cost of production of deferiprone compared with deferoxamine, there is little price difference between these two drugs in the EU.[22] It should be noted, however, that more toxicity tests are required by the EU authorities for general approval of deferiprone, in addition to the monitoring of blood counts for agranulocytosis and general postmarketing surveillance. We estimated that within 3 years following the approval of deferiprone, more than 25% of thalassaemia patients would be using deferiprone in Greece and most other countries of the EU. Approval of deferiprone in the US by the FDA is pending. In the meantime, a number of clinical trials have been carried out with deferiprone in the US confirming previous findings of iron removal efficacy and low toxicity.[88,89] In addition to the patients participating in the trials, deferiprone has so far been

widely used in the US through the health authorities by providing it on a named patient approval basis to clinicians treating patients not tolerating deferoxamine. It would appear that more preclinical and clinical toxicity data are required both in the US and the EU for deferiprone approval as a first-line chelating drug despite the fact that even more toxicity data are lacking in the case of deferoxamine.[12] In both cases the pharmaceutical companies involved are cautious in proceeding to full and long-term toxicity screening because of the costs involved. Almost all the adverse effects and improved therapeutic protocols for both deferiprone and deferoxamine have been identified by academic research, which was not supported by the pharmaceutical companies marketing the drugs.

A major ethical dilemma is the use of deferiprone in the developing countries of the Middle East and South East Asia, where no serious attempts have been made to introduce iron-chelation therapy to help to treat thousands of patients who are currently receiving red blood cell transfusions but no iron-chelation therapy. A major part of the problem is the prohibitive cost of the chelating drugs provided by the multinational and local pharmaceutical companies.[6,22] In most of these countries thalassaemia patients have only two choices, deferiprone and fatal iron toxicity, with the latter still prevailing.[90]

In the last 30 years there have been many investigations into the replacement of deferoxamine with an orally effective, inexpensive and non-toxic iron-chelating drug. Hundreds of iron chelators have been tested in animals and 17 in humans for the treatment of iron overload and other conditions.[12] Deferiprone is the only alternative drug that successfully passed the screening process and is used at present in competition with deferoxamine. In general, the development of the most promising iron-chelating agents is based on financial, toxicological and efficacy considerations. Deferoxamine is considered to be a very successful commercial drug with annual sales exceeding $500 million.[90] It is now a generic drug and is produced by at least three pharmaceutical companies either chemically or from *Streptomyces pilosus*. With regards to deferiprone, one of the patents filed mainly in the EU and the US is due to expire in 2008, including the 5-year extension. This patent is used for the chemical preparation and marketing of a deferiprone product derived from a 3-step synthetic route.[91] Another patent in Greece describing the simple 1-step method of preparation of deferiprone is due to expire in 2017.[92] There are at least five companies producing and marketing deferiprone at present, mainly in Europe and India.

The design, development and expanding use of deferiprone, as well as the possible application of iron-chelating drugs in many clinical conditions has increased the commercial interest in this group of drugs and their future development. At least four new oral iron-chelating agents are being tested and are at different phases of drug development by four different companies. Even if successful, the new chelating agents are unlikely to become available for general clinical use in the next 5–8 years. These are unlikely to be less expensive than deferoxamine or deferiprone and their introduction is related to the lucrative market being developed mainly by the companies already marketing deferoxamine and deferiprone.

2. Aetiopathogenesis and Assessment of Iron-Overload Toxicity

2.1 Mechanisms of Iron Toxicity

Under normal conditions, iron toxicity is controlled mainly by the iron-protein transferring that controls iron transport and the iron-protein ferritin that controls iron storage. These iron-protein complexes have the capacity to mobilise iron extracellularly and store it intracellularly, respectively. Iron in these protein-bound forms causes no detectable

damage. In iron overload caused by red blood cell transfusions or increased gastrointestinal iron absorption, the concentration of ferritin and particularly its aggregate form, haemosiderin, increases substantially in most organs such as the liver, spleen, heart and pancreas. Under these conditions, iron toxicity may arise mainly from the incapacity of cells in these organs to store iron in a safe storage form. This results in lysosomal rupture and release of proteolytic and other enzymes, which could potentially damage the cells, tissue and the organs involved in the storage of excess iron.

Iron toxicity in iron overload may also arise from the presence of labile, toxic iron pools found intracellularly, for example, in a low molecular weight transit iron pool or low molecular weight iron released from haemosiderin. Under these conditions, transferrin in the serum is fully saturated with iron and low molecular weight NTBI is formed, which could also be a source of toxicity. In all these cases low molecular weight iron has the potential to catalyse the production of toxic free radicals and the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA, etc.[58,93] A combination of all these factors, such as the deposition of excess iron in cells and organs coupled together with the breakdown of antioxidant controls and of other controls related to iron regulatory mechanisms, could also result in molecular, cellular and tissue damage. As this damage is continuous and not controlled at any of these levels, it causes a further release of toxic forms of iron from damaged cells and subsequently a vicious circle of increased toxicity.

Clinical abnormalities in transfusional iron overload are generally manifested following the transfusion of 50–100 units of red blood cells, which is equivalent to 12.5–25g of excess storage iron in various organs of the body. In the absence of chelation therapy, this level of iron load and the onset of organ damage could be reached within 5–9 years in children who are regularly transfused. Most transfused patients receive the equivalent of 1–3 units of red blood cells every 2–4 weeks. The abnormalities in these patients include liver and spleen enlargement associated with excessive red blood cell destruction and iron deposition in these and other organs. Many patients with splenomegaly caused by iron overload undergo splenectomy resulting in reduction of the transfusion requirements of red blood cells. Excess iron could also damage the pancreas resulting in diabetes mellitus, which is treated with insulin injections. It could also damage the endocrine organs resulting in growth failure and delayed or absent puberty, which is treated using hormone replacement therapy. Other adverse effects of iron overload include joint pains and infections. Cardiac damage is the most serious adverse effect of transfusional iron overload, which may lead to cardiac arrhythmias and congestive heart failure. The latter is the most common cause of death in thalassaemia major patients, occurring in the second and third decade of life especially in those patients not receiving sufficient or effective chelation therapy.[20]

The use of antioxidants such as tocopherol (vitamin E) and ascorbic acid (vitamin C) in combination with chelation therapy has not so far conclusively been shown to minimise the adverse effects of iron overload or to increase survival in these or other patients.[94] Despite that, a combination of deferoxamine with vitamin C has been shown to increase iron excretion, the same has not so far been confirmed using the combination of vitamin C with deferiprone.[95]

2.2 Assessing Iron Load and Progress of Chelation Therapy

The assessment methods used for the determination of iron overload are essential tools for monitoring and preventing iron toxicity, for adjusting chelation therapy protocols and improving the prognosis of iron-loaded patients. The organ distribution of

iron is not uniform in such patients and iron-chelating drugs have variable effects. The aim of the treatment with iron-chelating drugs is to bring patients to a negative iron balance by mobilising and removing sufficient amounts of iron and maintaining non-toxic iron levels in the body, particularly in organs like the heart.

The efficacy of chelating drugs is assessed by using iron metabolic balance studies, where the intake of iron from red blood cell transfusions and iron absorption is compared with the amount of iron excreted in the urine and faeces.[95-98] The reduction of the excess iron deposited in the organs of iron-loaded patients is a very slow process.[99,100] For example, if a patient has been transfused with 50 units of red blood cells before initiating chelation therapy and on average the chelating drug causes a net iron removal of 10 mg/day (iron removed – iron intake), then normal iron stores could theoretically be reached following approximately 3 years of chelation therapy. Iron chelation therapy in thalassaemia patients and others receiving regular transfusions should start as early as possible in order to prevent irreversible organ damage. Chelation therapy in thalassaemia using deferiprone or deferoxamine is usually initiated from the age of 2 years.

The progress of iron chelation therapy is assessed by using a number of different diagnostic methods, which are related to the estimation of iron in the body as a whole or in the various organs or tissues.[101-105] The monitoring of parameters assessing organ damage due to iron overload such as liver enzyme levels are also important indicators of the overall progress of the chelation therapy.[106] All the diagnostic methods have limitations and none can precisely predict either the total body iron load or the extent of iron toxicity. The limitations are caused by several factors such as variations in the body and organ distribution of iron, the accuracy of the detection methods and the estimation techniques used for iron. Some of the diagnostic assessments of iron overload could also be influenced by a number of factors or conditions such as dietary components, infection, inflammation, erythropoietic activity, etc. Under these conditions measurement of serum ferritin, for example, could give rise to wide errors in the estimations of iron overload and organ damage.

An initial assessment of the iron load of patients is carried out by measuring serum ferritin, serum iron and transferrin iron saturation. All three parameters are at much higher levels in iron-loaded transfused patients than individuals with normal iron stores. Another indirect method for estimating the body iron status is the measurement of urinary iron excretion in response to chelating drugs such as deferiprone or deferoxamine. A more accurate method for assessing liver iron and total body iron is the estimation of iron concentration and histochemistry iron grading of a sample of a liver biopsy.[101]

A number of new techniques have recently become available for the non-invasive measurement of iron in the liver and other organs. One of those is superconducting quantum interference device (SQUID)-biosusceptometry, which is used for the estimation of iron in the liver and correlates well with results from liver biopsies.[102] Similarly, magnetic resonance imaging (MRI) techniques are also used for assessing differential organ iron deposition and in particular iron load of the heart.[103,104] Low molecular weight NTBI, which is usually present in excess when transferrin is saturated, is another indirect parameter for assessing iron overload and potential toxicity.[105] Regular clinical and biochemical monitoring of the parameters associated with the body iron load and organ function provides an indication of the general progress of the chelation therapy. Such indications may prove unreliable if are assessed unilaterally. Serum ferritin levels in particular are unreliable with regards to the estimation of iron deposits and extent of damage to the myocardium. Similarly, serum ferritin levels may not be

directly related to liver iron and liver iron to heart iron, etc.

There is wide variation in the response of patients treated with iron-chelating drugs. The level of iron excretion per given dose of a chelating drug such as deferiprone is different for each patient and an assessment of various dose protocols may be required to achieve optimum therapy. Accordingly, the selection of the appropriate dose protocol is critical for achieving negative iron balance and maintaining non-toxic body iron load in each patient. Monitoring of urinary iron excretions at the beginning of the chelation therapy and thereafter monthly, together with serum iron and transferrin iron saturation, as well as serum ferritin estimation every few months could give an initial indication of the effectiveness of the chelation therapy. Liver iron estimations using liver biopsies or SQUID-biosusceptometry and MRI T2 or T2* monitoring of the iron content of the heart and other organs every year or 6 months could give an indication of the long-term efficacy of the chelating drug and help in determining the use of an effective dose protocol for each patient. Serum ferritin levels above 2.5 mg/L, transferrin iron saturation over 100%, the presence of NTBI and liver iron concentration above 7mg iron per gram of liver dry weight suggest the presence of toxic levels of iron in the tissues and blood. Under these conditions there is a need for the use of higher doses of the same chelating drug or a combination of chelating drugs in order to increase iron excretion and prevent irreversible organ damage especially to the heart.

In contrast to the above observations there are many patients who respond exceptionally well with either deferiprone or deferoxamine even at lower doses resulting in low iron levels in the organs and serum ferritin levels similar to normal levels. In such cases a new dose protocol is usually designed and the chelating drug dose is progressively reduced in order to maintain the low iron body load and, at the same time, avoid possible toxicity arising from the administration of excess chelating drug.

3. Mechanisms of Action of Iron-Chelating Agents

3.1 Molecular Aspects of Chelation Therapy

A chemical compound with chelating properties usually possesses at least two ligands with electron donor atoms such as nitrogen, oxygen and sulphur, which have affinity for metal ions and bond with the metal forming a chelator-metal ion complex. The complex formed has different physicochemical, pharmacological and toxicological properties by comparison with the chelator or the metal involved in the chelator-metal ion complex. The electron donor atoms of the ligands involved in the complex could be present in acidic groups such as -COOH, -OH, -SH, -NOH, where the proton could be displaced by the metal ion or in Lewis bases such as -C = O, -NH_2, -O-R, -OH, -S-R. These functional groups with chelating potential could have affinity not only for iron but for several other metal ions such as copper, zinc and aluminium. There are many drugs and biomolecules, such as proteins, fatty acids, sugars, DNA and RNA, with chelating potential for iron and other metals.[107]

Transferrin is a specific protein for iron transport. It has metal binding sites with high affinity for iron but also affinity for some other metal ions such as aluminium, indium and gallium. Chelating drugs have to compete with transferrin and other such endogenous chelators for iron and other metals at all the stages of absorption, metabolism and excretion of the chelating drug and its metal complexes. The competition between transferrin, deferiprone and deferoxamine for iron as well as the interaction between the chelating drugs and other biomolecules for iron is governed by thermodynamic and kinetic parameters. Deferiprone can exchange iron with transferrin under certain conditions, which mainly

depend on the concentration of deferiprone and transferrin as well as their iron saturation. Iron exchange between transferrin and deferoxamine is very slow and almost negligible at physiological conditions due to kinetic restrictions imposed by the chemical structure of deferoxamine and its iron complex. Iron exchange interactions have also been shown between deferiprone or deferoxamine and their iron complexes with other drugs possessing iron-binding properties.[108]

Chelating drugs vary in size, charge lipid/water partition and bind metal ions with different affinity both *in vitro* and *in vivo*. At pH 7 the deferiprone iron complex is composed of one molecule of iron bound by three molecules of deferiprone and in the case of deferoxamine one molecule of iron by one molecule of deferoxamine. One method of assessment of the chelator affinity for various metal ions is the determination of the metal stability constants (log β) such as those shown for deferiprone, deferoxamine and diethylene triamine penta-acetic acid (DTPA) in table II. Deferiprone appears to have the highest stability constant for iron in comparison with the other two chelating drugs. In addition to iron, the second and third most competing metal ions for deferiprone appear to be copper and aluminium, respectively.[108] DTPA is less specific for iron than deferoxamine and deferiprone. It was observed during clinical trials with DTPA that in addition to an increase in iron excretion, the excretion of zinc, copper and magnesium also appeared to increase.[96] Increased zinc excretion resulting in symptoms of zinc deficiency was one of the major adverse effects in patients treated with DTPA. Treatment of these patients with zinc supplements was mandatory. Minor increases in zinc excretion were also observed in iron-loaded diabetic patients receiving intravenous deferoxamine or oral deferiprone.[109,110]

The affinity of chelating drugs for iron, as measured by various chemical parameters such as the stability constants, cannot reflect the ability of the drug to remove iron *in vivo*.[111] The pharmacokinetic and metabolic properties of the chelating drug may not allow sufficient time and concentration of the active chelating molecule to bind and remove the necessary quantity of iron needed for reaching a negative iron balance in patients. Other factors affecting the efficacy of chelating drugs is the number and chelating properties of its metabolites as well as the size, charge, lipid/water partition and clearance of their iron complexes, which may overall influence the efficacy of the drug in iron removal and also its toxicity (table III).

3.2 Iron Mobilisation and Displacement from Iron Pools

There are various iron pools and forms of iron available for binding, exchange and removal during chelation therapy. In humans and biological systems in general iron is found bound to ligands present in proteins and other biomolecules or stored in forms, which are similar to inorganic precipitates within proteins such as ferritin. With regard to molecular size, iron could be found in complexes of a single ion (mononuclear) [e.g. in transferrin], of several ions bound together (oligonuclear) [e.g. NTBI] or many ions bound together (polynuclear) [e.g. ferritin and haemosiderin]. Both the chelator and its iron complex interact with the various intracellular or extracellular iron pools and endogenous chelators or

Table II. Metal stability constants (log β), charge and molecular weight (MWt) of iron-chelating drugs

Agent	Fe^{3+}	Cu^{2+}	Co^{3+}	Zn^{2+}	Charge (pH 7)	MWt
DTPA	28.6	21.0	19.0	18.4	Negative	393
Deferoxamine	30.6	14.0	11.0	11.1	Positive	561
Deferiprone	35.0	19.6	11.7	13.5	Neutral	139

DTPA = diethylene triamine penta-acetic acid.

Table III. Comparison of the properties of deferiprone (L1) and deferoxamine

Properties	Deferiprone	Deferoxamine
Molecular weight	139	561
Charge	Neutral	Positive
Iron complex charge	Neutral	Positive
Lipid/water partition (Kpar)	0.18	0.02
Ratio of chelator : iron complex at pH 7	3 : 1	1 : 1
Iron binding constant (log β)	36	31
Iron removal from transferrin and lactoferrin	Yes	No
Ferritin, haemosiderin, NTBI	Yes	Yes
Binding of other metals in humans	Al > Zn > Cu	Al >> Zn > Cu
Effective dose (mg/kg)	50–120 PO	40–60 SC
Stability/storage	Very stable	Unstable (4°C)
Metabolites	L1-glucuronide	Metabolite C and others
Site of elimination	Urine (not faeces)	Urine >> Faeces
Elimination half-life (min)	47–134	5–10 IV; 60 IM
Peak serum levels (μmol/L)	100–450	5–20[a]
Cost per gram ($US)	3–10 Europe; 0.4 India	5–10 worldwide

a Concentrations of more than 300 μmol/L could be achieved at doses of 80 mg/kg.[112]

IM = intramuscularly; **IV** = intravenous; **NTBI** = non-transferrin bound iron; **PO** = orally; **SC** = subcutaneous.

other biomolecules containing iron-binding ligands. The same interactions apply to metal ions other than iron.

Thermodynamic and kinetic parameters govern these interactions. Similar interactions could be observed with chelating metabolites of the chelating drug. The overall result on iron exchange or excretion will depend on all the above parameters as well as the pharmacokinetic properties of the chelating species and their complexes and a number of other factors such organ function, transfusion effects, the influence of dietary components and other drugs, etc.

Within this context, the rate of iron removal from the various iron pools and its clearance out of the body is crucial in establishing the level of efficacy of any chelating drug intended for the treatment of iron overload. Each of the iron pools has different characteristics and interactions with the chelating drug. This could be demonstrated from the ability of the chelating drugs to remove iron from different iron pools *in vitro* at variable rates. Low molecular weight soluble forms of iron such as aqueous iron, NTBI and intracellular low molecular weight iron are rapidly mobilised, usually within minutes, by most chelators, including deferiprone and deferoxamine.[113] Iron removal from transferrin and lactoferrin could take up to 2–3 hours to reach completion, but only in the case of deferiprone (figure 3, table III).[114,115] The mobilisation of iron

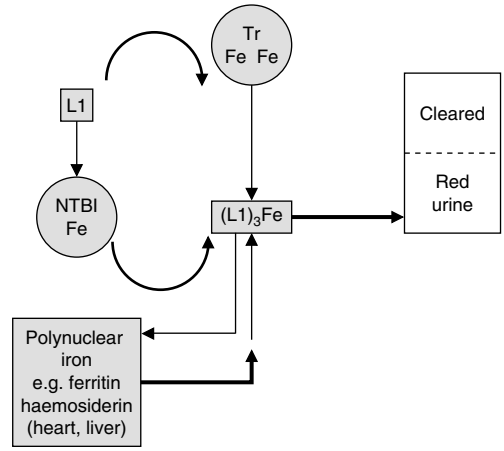

Fig. 3. Iron (Fe) pools mobilised by deferiprone (L1) include polynuclear iron deposits present intracellularly in ferritin and haemosiderin and iron in NTBI and transferrin present in serum. **NTBI** = non-transferrin bound iron; **Tr** = transferrin.

from these two proteins is negligible with deferoxamine because of the kinetic restrictions imposed by the structures of both deferoxamine and the proteins. Iron from these two proteins could not become easily accessible to deferoxamine unless a mediator molecule such as other chelators such as deferiprone or ascorbic acid is present. Polynuclear forms of iron such as those found in ferritin and haemosiderin are accessible to most chelators including deferiprone and deferoxamine but iron mobilisation is very slow and the process may take several days to reach completion with only a portion of the iron stored in the protein being removed (figure 3).[116] Decrease in the quantity of iron mobilised from polynuclear iron forms including ferritin was observed *in vitro* following repeated chelator treatments, suggesting that the lower the concentration of iron stored in ferritin the smaller the amount of iron that could be removed.[117] Mobilisation of iron from heme has not been shown by any of the chelating drugs examined. Overall, at any given time iron mobilisation from most of the iron pools may become possible *in vivo* depending on the chelating drug, its concentration at the site of the iron pool, the rate of iron binding and the clearance of the iron complex.

Several other factors could also influence the efficacy of chelating drugs in iron removal *in vivo* such as the rate of biotransformation of the chelating drugs and the ability by their metabolites to bind and clear iron from the body. The metabolite of deferiprone, which is the glucuronide conjugate, has very low affinity for iron and no major effect on iron excretion. In contrast, deferoxamine forms several metabolites with chelating potential, which contribute to the overall iron excretion observed during chelation therapy.[118,119]

In addition to the iron removal effects, chelators could also be involved in other forms of interaction with the iron pools and other metal ion pools. Such interactions may involve the donation and redistribution of iron and of other metal ions to proteins involved in metal metabolism, cells and organs. Ternary complex between chelators, iron or other metals and a protein could also be formed. All these forms of interaction could influence the overall efficacy and toxicity of chelating drugs. In the competition between deferiprone and deferoxamine, iron could be exchanged between the two depending on their concentration.[108] These forms of interaction known as the 'shuttle effect' may explain some of the findings in the variation in iron excretion observed during combination therapy involving the coadministration of deferiprone and deferoxamine (figure 4). Iron bound to deferiprone could be taken up by deferoxamine when the latter is present at equal or higher concentration than deferiprone *in vitro* and *in vivo*. Deferiprone could also remove iron from deferoxamine under similar conditions but only if is present at much higher concentration than deferoxamine.

In transfusional iron overload, the most labile form of iron available during chelation therapy with deferiprone and deferoxamine is NTBI, which is in a low molecular weight form and rapidly mobilised. NTBI is formed mainly from iron continuously released by the reticuloendothelial system in serum

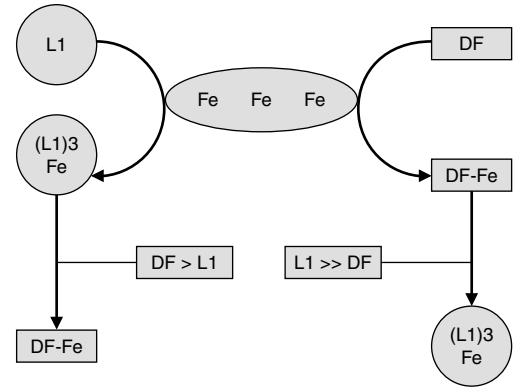

Fig. 4. The 'shuttle' effect works both ways? Exchange of iron (Fe) can take place when both chelating drugs are present. Iron bound by one chelating drug can be removed by the other chelating drug when the latter is present in excess. Higher concentrations of deferiprone (L1) are needed to remove iron from deferoxamine (DF) than the other way round.[108]

following the breakdown of senescent red blood cells (figure 1). NTBI is usually formed when transferrin is saturated with iron. Another source of chelatable iron in iron-loaded thalassaemia patients is that present in iron saturated transferrin, which is partly depleted by deferiprone at serum concentrations of 0.1–0.4 mmol/L and then replenished with iron when the chelating drug is cleared from blood.[120] The situation is reversed in normal individuals, where deferiprone removes iron from the tissues and donates it to the partly iron saturated transferrin, thus transiently increasing transferrin saturation for up to 6 hours, which then reverts back to normal when deferiprone is cleared from the blood.[120]

The removal of iron from the intracellular low molecular weight iron pool and polynuclear iron deposits (ferritin and haemosiderin) requires the transport of the chelating drug across the cell membrane and its exit as a chelator iron complex. The mechanism of iron removal from tissues or cells (e.g. hepatocytes by deferiprone and deferoxamine) is thought to involve the stepwise mobilisation of ferritin and haemosiderin iron, resulting in the gradual formation of a large intracellular low molecular weight chelator iron complex pool, which then diffuses out of the cells.[121] The iron complex could then be cleared through the urinary or biliary route depending on the chelating drug used.

The mechanisms of the removal of iron from different cell types by chelating drugs may also vary. Their effects on hepatocytes and myocardial cells are particularly important in transfusional iron overload as these are the primary cell types involved in iron storage and toxicity, respectively. Iron removal by deferiprone, deferoxamine and other chelators has been shown in hepatocytes, myocardial cells and many other cell types. These effects may be relevant to the applications of chelating drugs for the treatment of many other iron-depended diseases, as iron is essential for the growth of all cell types.[121-123]

In conditions of abnormal iron metabolism, such as transfusional iron overload, idiopathic haemochromatosis and the anaemia of chronic disease the distribution of iron in the body varies. Similarly, the effects of chelating drugs may also result in variable patterns of iron organ deposition and distribution due to variable iron depletion patterns by the chelating drugs. During intensive phlebotomies in idiopathic haemochromatosis, the progressive depletion of all the iron pools and organs might be steadily and slowly achieved through the iron transport and distribution properties of transferrin. In the anaemia of chronic disease, however, iron is deposited in the reticuloendothelial system, reducing its availability to the erythron for the production of haemoglobin. Both deferoxamine and deferiprone have been shown to increase the production of haemoglobin in this condition by the displacement of iron and its diversion from the reticuloendothelial system to the erythron.[40,41] Deferiprone and other specifically designed chelating drugs may prove beneficial in the treatment of the anaemia of chronic disease and of other conditions of abnormal iron metabolism. This approach could benefit tens of thousands of patients currently using erythropoietin in combination with iron. Many of these patients are not, however, responding to iron, which in many cases is only partly effective at increasing the production of haemoglobin. Deferiprone could be used in combination with erythropoietin and iron thus facilitating the donation of iron to transferrin, which in turn could transport iron to the erythron.[120]

The variation in the mode of action of deferiprone, deferoxamine and other chelating drugs may have advantages in the design of improved therapeutic strategies for the treatment of transfusional iron overload and other conditions of abnormal iron metabolism. Selective chelating drugs or their combination may prove to be beneficial for specific organ

iron depletion or displacement, provided that these could be appropriately targeted and selected doses are used for optimum therapeutic activity.

4. Comparative Pharmacology and Toxicology of Deferiprone and Deferoxamine

4.1 Properties of Deferoxamine

A summary of the general properties of deferoxamine and deferiprone is shown in table III. The formulation used in patients contains deferoxamine as a mesylate salt in a lyophilised form in a vial. It is white in colour and is dissolved in distilled water forming a clear solution prior to its injection. There are two different sources of deferoxamine, one that is a fungal product isolated from *S. pilosus* and another that is chemically produced. There have been no reports of comparative studies on efficacy and toxicity between the two different sources of deferoxamine.

Deferoxamine is the most widely used drug for the treatment of transfusional iron overload in developed countries. The mortality and morbidity in thalassaemia patients has been reduced since its introduction in the mid-1960s. Numerous studies have been performed to identify its optimal activity via various routes of administration and dose protocols.[124] Deferoxamine is effective in maintaining negative iron balance in most thalassaemia patients if it is injected subcutaneously for 8–24 hours daily at 35–70 mg/kg with the aid of an electronic or elastomeric pump at least 5 days per week. Compliance with the subcutaneous administration using the electronic pump is <50%, but there is an improvement using the elastomeric pump. The intravenous administration using an intravenous line has been proved more effective in iron removal than the subcutaneous route but with increased risk of toxicity in the former.

The animal and human toxicity of deferoxamine has been previously reviewed.[12] There are many adverse effects associated with deferoxamine therapy as shown in table IV. Discomfort due to hardness, swelling and soreness at the side of the injection is observed in over 80% of the thalassaemia patients treated with deferoxamine.[12] Adverse effects that have been reported include events with fatal outcomes that were related mainly to pulmonary complications, mucormycosis, yersiniosis and pancytopenia.[12,125-129] The adverse effects of defer-

Table IV. Comparative toxicity of deferiprone (L1) and deferoxamine

Deferiprone toxicity	Deferoxamine toxicity
Agranulocytosis in 0.6%[130-134]	Skin rashes/local reactions in 80%
Neutropenia in 6%[88,134-136]	Ophthalmic toxicity[12,125,137-140]
Musculoskeletal/joint pains in 15%[131,136,141,142]	Auditory toxicity[143-145]
Gastric intolerance in 6%[12,77,134,141]	Neurotoxic abnormalities[146,147]
Zinc deficiency in 1%[12,110]	Pulmonary toxicity[126,148]
Liver fibrosis (?)[84,85,149,150]	Growth failure and bone abnormalities in children[129,151,152]
Fatal systemic lupus erythematosus in one patient (?)[76,78]	Yersiniasis[153-156]
Increase in ANA and rheumatoid factors (5–10%)[77,157,158]	Mucormycosis[159]
	Thrombocytopenia[160]
	Leukopenia[161]
	Aplastic anaemia[127]
	Allergic and anaphylactic reactions[162-165]
	Interactions with other drugs[147]
	Renal toxicity[166,167]

ANA = antineuclear antibody.

oxamine reported in animals have not been shown in humans and vice-versa, mainly because of differences in the level of iron overload, the duration, dose and mode of administration of the drug between the different animal species as well as many other factors.[80,82]

On the molecular level, deferoxamine has been shown to cause oxidation of haemoglobin, to inhibit ribonucleotide reductase, which is involved in DNA synthesis and to form toxic oxygen-free radical species on binding iron (II).[168-170] Deferoxamine could also inhibit the growth and proliferation of many cell types in a concentration-dependent process.[169,171]

There is limited information on the pharmacokinetic properties of deferoxamine. Its clearance from blood has been shown to be faster (elimination half-life [$t_{1/2}$] = 5–10 minutes) than its iron complex ($t_{1/2}$ = 90 minutes).[172] Deferoxamine forms several metabolites, some of which have chelating properties.[118,119] There have been no reports as to which metabolite molecules cause the various adverse effects or contribute to iron mobilisation and increased iron excretion. Overall, the most serious adverse effects of deferoxamine are observed in non-heavily iron loaded patients receiving high doses of the drug.

Iron excretion by deferoxamine is mainly through the urine and to a lesser extent through the faecal route, depending on the dose, iron load of the patient and state of erythropoiesis.[95,97] The major sites of iron removal in iron-loaded patients by subcutaneous or intravenous deferoxamine is thought to be NTBI iron from the serum and iron from the liver. Other organs such as the heart are also gradually depleted of iron during deferoxamine therapy provided the patients can tolerate higher doses and continuous administration.[173,174] Attempts to improve the compliance of patients using oral or suppository formulations of deferoxamine have not been effective.[175,176] Similarly, attempts to modify its structure or produce formulations that could facilitate its oral activity have also been unsuccessful.

4.2 Properties of Deferiprone

The general properties of deferiprone are shown in table III. Deferiprone is a member of the α-ketohydroxypyridine class of iron chelators, which were originally designed and tested for the treatment of iron overload in 1981.[11,91] Deferiprone is a white crystalline solid, which has a very bitter taste and is stable at room temperature for more than 5 years. It is sparingly soluble in water at pH 7.4 (about 20 mg/mL, at 37°C) and stable in solutions of physiological and acidic pH. It is more soluble in acid, for example the stomach acidity than in alkaline or neutral pH. It forms red colour complexes with iron, similar to the red colour of the urine of iron loaded patients treated with deferiprone. Its affinity for iron is higher than its affinity for copper, aluminium and zinc at pH 7.4. It is a hydrophilic chelator (Kpar = 0.18) forming hydrophilic iron complexes (Kpar = 0.01) at physiological pH thus ensuring rapid excretion and not accumulation in lipids.[177,178]

Pharmacokinetic studies of orally administered deferiprone have shown that in most patients it is rapidly absorbed from the stomach and appears in blood within minutes.[98,179] A lag period of 1–3 hours in the appearance of deferiprone in blood was observed in a few cases, which may be related to food and other gastric factors slowing its absorption from the gastrointestinal tract. The half-life of absorption of deferiprone to the stage of peak serum concentration was shown to range from 1–32 minutes. The clearance of deferiprone from blood was estimated to have a half-life of 47–134 minutes.[98,179-181] Deferiprone is metabolised to a glucuronide conjugate, which is formed at the 3-OH position and blocks the iron-binding site and chelating properties of the drug (figure 2). Deferiprone, its metabolite, and its iron and other metal complexes

are all excreted in the urine to almost 100% recovery.[106] In metabolic balance studies, no deferiprone, deferiprone-glucuronide or increased iron excretion was detectable in the faeces of patients treated with deferiprone.[180] Similar results were obtained in clinical studies using ^{59}Fe labelling, where ^{59}Fe increased excretion caused by deferiprone was only apparent in the urine but not faeces.[182] There are wide variations in the metabolism and clearance of deferiprone among patients, which may be related to a number of factors such as idiosyncratic, dietary, age or organ function variation factors.[183] Similar variations have been shown with other drugs where in some cases rapid metabolism could result in partial or total loss of the efficacy of a drug. In the case of deferiprone, it would appear that in most patients iron chelation precedes glucuronidation and that the overall rate of iron excretion depends mainly on the availability of chelatable iron rather than the extent of glucuronidation of the drug.

Urinary iron excretion caused by deferiprone at effective doses is similar to that caused by deferoxamine both in animal models and various categories of patients in short- and long-term clinical studies.[88,95,135,184-195]

The overall amount of iron excretion caused by deferiprone depends mainly on the dose, frequency of administration and the iron load of patients. However, there is wide variation with regards to dose protocols and iron excretion results in patients taking deferiprone. Total daily doses of 50–120 mg/kg subdivided into 15–50 mg/kg doses have been widely used. In moderately iron-loaded patients doses as low as 10 mg/kg could increase iron excretion. In contrast, higher doses of as much as 50 mg/kg could cause much lower iron excretion in normal individuals (1–2mg iron/day).[95,184-187] The highest level of iron excretion ever recorded by deferiprone was in an iron-loaded thalassaemia patient who excreted 325mg of iron following the administration of six divided doses to a total of 16g within 24 hours. The amount of iron excreted in this patient was equivalent to about 13 days' intake of iron from transfusions.[98] This high dose was well tolerated and urinary iron excretion was continuous with no signs of levelling out. Deferiprone has been shown to cause negative iron balance in many groups of patients who have been taking effective doses (>75 mg/kg) for periods of 0.5–1 year.[141] A decrease in serum ferritin and liver iron to near normal levels has also been observed in many other groups of patients.[99,149] In recent studies after 3 years of therapy, deferiprone has been shown to be more effective than deferoxamine in reducing myocardial iron and to improve ventricular function in thalassaemia patients.[100]

The iron pools and major iron-containing organ sites as well as the quantity of iron removed from each of these has not yet been fully determined *in vivo*. Monitoring of serum transferrin saturation of thalassaemia patients has shown that both NTBI iron and transferrin-bound iron are mobilised after deferiprone administration (figure 3).[179] The depletion of iron deposited in the liver and the myocardium requires the daily use of effective doses of deferiprone for months or years.[99,100,149] It is expected that the depletion of iron from other organs could also be progressively achieved because of the equilibrium of the intracellular iron pools with transferrin, which is continuously depleted of its iron by deferiprone. No increase in iron absorption has been shown in animals or humans taking deferiprone and like deferoxamine it may have a use in the treatment of iron poisoning caused by accidental overdose of iron tablets, which mainly occurs in children.[39,196]

4.2.1 Toxicity and Safety of Deferiprone

The adverse effects of deferiprone in animals have been previously reported.[12] The lethal dose $(LD)_{50}$ of oral deferiprone was estimated to be between 1–2 g/kg in rats.[197] No deferiprone overdose toxicity has yet been reported in patients and the

maximum dose ever used in 24 hours was 250 mg/kg subdivided into six doses.[198] With regard to long-term safety, there are patients who have been taking deferiprone daily at 75–120 mg/kg for over 14 years with no reports of major toxicity.[22] Similarly, a Swiss patient was reported to have taken deferiprone 150 mg/kg/day of for 2 years without any apparent toxicity.[14] Maternal, embryonic and teratogenic toxicity has been reported in animals treated with deferiprone with some adverse effects similar to deferoxamine.[12,79,199] Deferiprone appears to enter most organs and has been detected in the saliva of patients.[200] The administration of deferiprone during pregnancy and lactation is not recommended. Caution should also be used when young children are treated with deferiprone. Despite that, so far there have not been any reports of adverse effects during treatment of thalassaemia in children as young as 2 years of age. Geriatric patients as old as 85 years with myelodysplastic syndrome have also been treated with deferiprone with no reports of adverse effects.

The adverse effects of deferiprone have been reported sporadically from various centres worldwide and with the exception of some long-term monitoring studies no data have become available from the postmarketing surveillance by companies supplying the drug.[89,130-134,136,142,150,157,158,201-206]

The major adverse effects reported so far in over 7500 patients receiving deferiprone for periods of up to 14 years and at doses of 50–150 mg/kg/day are as follows: (i) transient agranulocytosis in 0.6% of patients;[130-134] (ii) neutropenia in about 6% of patients;[88,134-136] (iii) transient musculoskeletal and joint pains in about 15% of patients;[131,136,141,142] (iv) gastric intolerance in about 6% of patients;[12,77,134,141] and (v) zinc deficiency in about 1%[12,110] (table IV). All the adverse effects of deferiprone are considered reversible, controllable and manageable. The cause of deferiprone-induced toxicities are not known but some may be idiosyncratic.[207] In some cases the use of lower effective doses of deferiprone from 100 mg/kg/day to 75 mg/kg/day resulted in significant reduction in the incidence of some of these adverse effects.[141]

In most patients treated with deferiprone, the incidence of agranulocytosis is monitored using mandatory weekly or fortnightly blood counts. The recovery of patients following deferiprone-induced agranulocytosis usually takes 1–7 weeks and treatment may involve the use of growth colony stimulating factors (G-CSF).[208,209] Patients who have developed or are susceptible to agranulocytosis are not usually allowed to continue with the deferiprone treatment. Similarly, prolonged neutropenia may also require the withdrawal of deferiprone treatment in some patients. In most patients with musculoskeletal and joint toxicity, the pains may subside despite the continuation of deferiprone therapy, but in other cases reduction of the dose or its short-term withdrawal may be required. If the pains persist patients could be treated using a nonsteroidal anti-inflammatory drugs such as indomethacin.[131] Gastrointestinal symptoms could also be treated using metoclopramide or antiacids.[141] Zinc deficiency caused by deferiprone is more common in diabetic thalassaemia patients. This could be easily corrected using zinc supplements.[12,110]

The claims by a group of investigators that deferiprone initiates or increases the progression of liver fibrosis have not been confirmed by any other groups of investigators monitoring the long-term therapy of deferiprone. The incidence of this toxicity has not been shown to be statistically different from groups of patients treated with deferoxamine.[84,85,149,150] Similarly, a report that deferiprone may cause systemic lupus erythematosus has not yet been confirmed by any other groups of investigators (table IV).[76,78]

5. New Therapeutic Strategies with Iron-Chelating Drugs

5.1 Benefits and Risks of Combination Therapy with Deferiprone and Deferoxamine

The concept of combination therapy with deferiprone and deferoxamine originated from earlier observations that patients have variable response in terms of iron excretion and toxicity to these two chelating drugs and that their combination could increase the overall iron excretion and reduce the toxicity in susceptible individuals.[210] The combination has been used previously for comparative metabolic iron balance studies in animals and patients without apparent toxicity or reduction in the efficacy of either drug.[95,211,212] The major benefit of this combination is that there is an improvement in the compliance with subcutaneous deferoxamine and subsequent total iron excretion because of the increase of the overall dose of the chelating drugs.[158,213] A drawback of the combination therapy may be the reduction of iron removal from the myocardium because of the overall decrease of the dose of deferiprone, which appears to be more effective in myocardial iron removal than deferoxamine administered at the regular doses.[214]

The greater efficacy of oral deferiprone administered to a group of patients using a mean total dose of 80.5 mg/kg/day, in three divided doses over subcutaneous deferoxamine administered to a mean total dose of 37.4 mg/kg, 5.1 days/week, on myocardial iron removal and improved ventricular function in thalassaemia patients was shown in a recent comparative study following at least 3 years' treatment.[100] Iron deposition in the myocardium was estimated using the MRI T2* technique.[104] However, there was no such apparent difference in an 1-year study between the efficacy of the two chelating drugs using a slightly different dose protocol of lower deferiprone dose of 75/mg/kg/day and higher deferoxamine dose of 50 mg/kg/day.[215] The assessment of patients in the 1-year study was based on serum ferritin measurements and nuclear magnetic resonance of liver and heart iron. Although different diagnostic methods have been used, it would appear that differences between deferiprone and deferoxamine in iron removal from the myocardium become apparent following long-term therapy and using appropriate dose protocols.

Identification of the difference in the mechanisms of iron removal between these two chelating drugs may help in the design of new generations of chelating drugs with different organ targeting potential and new more effective and less toxic chelation strategies. It appears that doses of oral deferiprone >80 mg/kg/day for a minimum of 3 years are sufficient for substantially reducing myocardial iron in most patients.[100,214] Similar results were also obtained using higher doses of subcutaneous or intravenous deferoxamine than are currently used, provided that these higher doses can be tolerated in the long-term.[173] An additional complication of high doses of parenteral deferoxamine therapy is the toxicity of the mesylate salt, which is coadministered with deferoxamine.

Iron removal by chelating drugs depends on the dose used and the concentration reached at the site from where iron could be mobilised (figure 4).[214] Deferiprone (75–120 mg/kg/day, twice or three times daily) could reach much higher concentrations in blood (100–450 μmol/L) than subcutaneous deferoxamine (5–20 μmol/L) infused over 8–24 hours (35–70 mg/kg, 5 days/week). Similarly, the concentration of deferiprone entering most tissues and cells is also many times higher than deferoxamine, resulting in higher iron mobilisation. Deferiprone at the above concentrations also removes iron from transferrin, thus decreasing the deposition of iron by transferrin to the myocardium and other tissues. A further advantage of deferiprone is that is a neutral molecule and more lipophilic than deferox-

amine thus allowing higher cell penetration than deferoxamine, which is charged. Similarly, the iron complex of deferiprone is also neutral and more hydrophilic than deferiprone, facilitating extracellular iron flow and rapid clearance from the body.

The liver is the major site of active uptake and metabolism of drugs such as deferiprone and deferoxamine. Both of these chelating drugs have been shown to be effective in removing iron from the liver.[99,174] The reduction of 'free' deferoxamine concentration in blood due to prior mobilisation of iron from the liver and NTBI may contribute to a further decrease in the iron removal capacity of deferoxamine from the myocardium.

The variation among patients in response to the iron removal efficiency and toxicity of chelating drugs, may be a reflection of the differences in the absorption, metabolism and excretion of the chelating drugs, their metabolites and their iron complexes. Dietary and other factors such as exercise may also contribute to this variation. Similar variations among patients have also been observed in response to other drugs.[216] The dose, metabolism and mode of administration of deferiprone and deferoxamine appear to be the main factors causing variation in the iron removal efficacy from the liver and myocardium. Overall, both chelating drugs can produce a reduction in myocardium iron but at different levels, with deferiprone being more effective because of its molecular properties and mode of action (figure 4, table III).[214]

Simultaneous administration of both chelating drugs at lower doses but overall higher chelating capacity dose may increase the total excretion of iron, which is mainly mobilised from the liver and serum, but at the same time it may reduce the iron removal efficacy of deferiprone from the myocardium. Similar results could also be obtained by giving the two drugs on different days because of the overall reduction of the dose of deferiprone, which is necessary for mobilising excess iron from the myocardium (figure 5).[214] In contrast, if the therapeutic target is the removal of iron from the liver then this could be easily accomplished, either by deferoxamine or deferiprone used at high effective doses or using their combination. Since it is established that most fatal incidences in thalassaemia are related to iron-overload toxicity of the myocardium, chelating strategies should be designed primarily involving tolerable high doses of oral deferiprone or intravenous deferoxamine or their combination in order to reduce mortality. It would appear that therapeutic protocols using at least 80 mg/kg/day of deferiprone or in combination with additional doses of deferoxamine would be sufficient in reducing myocardial iron and overall mortality in most thalassaemia patients.[100,214]

The mechanism of action of the combination therapy depends on the timing of administration, the dose, frequency and route of administration of each of the two chelating drugs. The difference in the pharmacological properties between these two chelating drugs requires the selection of appropriate dose protocols that could either maximise overall iron excretion, deplete iron from a specific organ, minimise toxicity or combination of all these processes.

5.2 Other Uses of Iron-Chelating Drugs

The main clinical uses of iron-chelating drugs is the treatment of iron overload caused by red blood cell transfusions and aluminium overload in renal dialysis (table I, table V). The removal by defer-

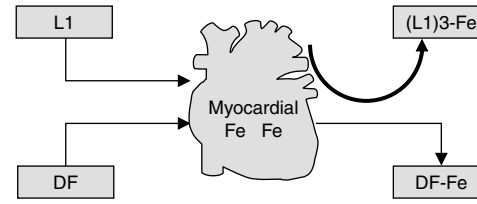

Fig. 5. Higher efficacy of deferiprone (L1) over deferoxamine (DF) in the removal of iron (Fe) from the heart. This effect is related to the physicochemical and pharmacological properties of the two chelating drugs (table III).[214]

Table V. Categories of patients treated with deferiprone (L1)

Conditions with iron overload[10,88,95,99,100,134-136,141,149,150,157,158,184-195,201-206]
β-Thalassaemia major
β-Thalassaemia intermedia
HbE β-Thalassaemia
HbS β-Thalassaemia
Sickle cell anaemia
Myelodysplastic syndrome
Aplastic anaemia
Fanconi's anaemia
Blackfan-Diamond anaemia
Pyruvate kinase deficiency
Idiopathic haemochromatosis
Iron overload in haemodialysis
Conditions with normal iron stores
Normal volunteers[10,179,217]
Renal dialysis[66,218]
Rheumatoid arthritis[40,42]
Malaria[38,219]

Hb = haemoglobin.

iprone and other chelators of toxic metals such as the heavy metals lead, mercury and arsenic, which are environmental pollutants, and radioactive metals such as plutonium and uranium, which are used in the nuclear industry, have been the subject of preclinical investigations with scope for clinical development.[61,68,69] There is an advantage in the clinical use of deferiprone over other experimental chelators in the decorporation of these toxic metals as it is already an established drug with long-term clinical experience in humans, whereas other chelators are still in the preclinical stage of development. A unique advantage of deferiprone in this field is its oral activity, whereas all other established chelating drugs including deferoxamine and DTPA are only active parenterally.

A combination of chelators with metal ions could have a use in the treatment of metal deficiency conditions such as iron deficiency anaemia.[39] Therapeutic metal complexes such as those of platinum and gold have also been widely used in cancer chemotherapy and rheumatoid arthritis, respectively. Similarly, combinations of chelators with radioisotopes such as indium, gallium and technetium could have a use as radiopharmaceuticals, whereas metals such as gadolinium could have a use in clinical diagnosis techniques such as MRI.[62-64]

Chelating drugs such as deferiprone may have a use in the correction of the anaemia observed in many chronic diseases, which are currently treated using erythropoietin and iron.[75] The combination of erythropoietin and selective chelators such as deferiprone, which could facilitate the transport of iron from the reticuloendothelial system to the erythron directly or via transferrin, could improve the treatment of the chronic anaemia in these conditions.

Chelating drugs could also be used in order to minimise the toxicity of iron in conditions such as Friedreich's ataxia, where excess iron is abnormally accumulated in mitochondria increasing the oxidative stress and cellular damage.[43] The use of deferiprone may be more appropriate than antioxidants for the treatment of the cardiomyopathy observed in this condition because of the inhibition of the oxidative catalytic activity of iron by deferiprone, thus minimising free radical cascades and also because of the ability of deferiprone to remove excess iron from the heart.[100,220]

The potential use of chelators in the inhibition of 'free radical' toxicity involves many therapeutic areas of molecular, cellular and tissue damage. This form of toxicity has been implicated in atherosclerosis, ischaemia/reperfusion injury, cancer and many other conditions.[58,59] The generation of free radicals may also be related to the toxic activity of some drugs. Dexrazoxane or ICRF-187 is a chelating drug that has been designed to protect through iron chelation the cardiotoxicity of doxorubicin.[55,56] Similar protection has also been shown with deferiprone.[57,221] Iron chelators could in principle inhibit the production of toxic free radicals and other oxygen-activated species by binding and removing the 'free' nonprotein-bound iron and/or by inhibiting

iron-containing proteins such as cyclo-oxygenase and lipoxygenase, which also generate toxic free radicals. Iron removal by deferiprone has been established in short- and long-term clinical trials carried out worldwide.[89,130-134,136,142,150,157,158,201-206] Similarly, inhibition of free radical production has been previously shown to occur with deferiprone and other chelators both *in vitro* and *in vivo*.[45-54,107,220]

The design of selective chelators for use in the inhibition of free radical toxicity in tissue damage and other free radical-induced toxicity conditions need further investigations. The targeting of particular iron-containing proteins and other iron pools by specifically designed chelators, as well as combination therapy with antioxidants and other drugs could improve the treatment of such conditions. Similarly, the targeting of drugs to specific sites such as the brain could also be achieved through ternary complexes of cytotoxic and other drugs possessing iron-binding ligands with proteins such as transferrin and lactoferrin. Hydroxyurea (hydroxycarbamide), which is an anticancer drug with iron-chelating properties and used mainly in haematological malignancies, inhibits the iron-containing enzyme ribonucleotide reductase. The inhibition of ribonucleotide reductase and the cytotoxic effects of the iron-chelating drugs deferiprone, deferoxamine and of other chelators have been previously shown on several cancer cell lines and other cells.[24-28,222] Deferiprone and other chelators have also been shown to inhibit the iron-containing enzyme hydroxylase and may have a use in the treatment of fibroproliferative disorders.[29] The treatment of malaria and other infections that are resistant to conventional drugs provide another challenging area where chelators could be used. Inhibition of microbial growth could in principle be accomplished by withholding iron from the microbe as previously shown to occur with deferiprone, and other chelators in many studies involving *Plasmodium falciparum*, *Yersinia enterocolitica* and other pathogenic organisms.[30,31,33-38]

A number of experimental *in vitro*, animal and clinical models have been used to identify possible other uses of chelators, their metal complexes and combinations with other drugs. It is hoped that some of these experimental approaches could soon be developed and become new therapeutic applications of the chelating drugs.[42,223-227] The design of new improved chelators for the treatment of iron, aluminium and other metal overloading conditions is also in progress. Structure/metal binding activity considerations using improved structural, electronic and metabolic features could in principle result in the design of chelators with lower toxicity and higher efficacy for each of the above conditions.

Improvements in chelation therapy could also involve the identification, prevention and elimination of the factors causing the adverse effects of chelating drugs. Within this context the design of new methods of administration, dose protocols and combinations with other drugs could be beneficial.[228] One possibility is the use of copper supplements for reducing the incidence of neutropenia and agranulocytosis caused by deferiprone. Copper deficiency was previously shown to be associated with neutropenia and deferiprone may cause the same effect by redistributing copper and inhibiting copper-related enzymes.[12,15,229-232]

Oral iron chelation therapy with deferiprone is considered as a major breakthrough in the area of development of new chelating drugs intended for clinical use as well as the possible application of such drugs in new therapies (table V).

5.3 New Iron-Chelating Drugs

Ideally any new chelator intended for clinical use worldwide should be inexpensive, orally active and non-toxic at doses that can bring patients to negative iron balance. Several hundred iron chelators have been designed and tested in animals and 17 in clin-

ical trials in humans mainly for the treatment of iron overload[12,113] (table VI). Almost all of the experimental chelators used in animals and most of those used in clinical trials have been abandoned either because of toxicity or ineffectiveness in iron removal. Details of the properties and the clinical trial results of these chelators have been previously reported.[12,113]

At least four chelating agents are currently investigated and are at different stages of development by different pharmaceutical companies. Two of these are α-ketohydroxypyridine analogues, similar to deferiprone, namely 1-allyl-2-methyl-3-hydroxy pyrid-4-one (L1NAll) and 1-methyl-2-methyl-methoxy-6-methyl-3-hydroxypyrid-4-one (CP502), which are in phase I clinical trials and preclinical stage of development, respectively.[233-236] Two other chelators, namely deferasirox or ICL670 (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid) and GT-56252 have reached phase II and phase I clinical trials, respectively.[237-240] The results so far from all these four chelators do not appear to be significantly better, both with respect to efficacy and toxicity, when compared with deferiprone and deferoxamine. The mode of action, metabolism and elimination of deferasirox appears to be different from that of deferiprone and deferoxamine as in humans is excreted almost exclusively in the faeces.[238]

Table VI. Chelators tested in man for the treatment of transfusional iron overload and other conditions. For more details see Kontoghiorghes.[12]

Ethylene diamine tetra acetic acid (EDTA)
Diethylene triamine penta acetic acid (DTPA)
2,3-Dihydroxybenzoic acid (2,3-DHB)
5-Hydroxy-2-formylpyridinethiosemicarbazone (5-HP)
Cholylhydroxamic acid (CHA)
Rhodotorulic acid (RA)
Ethylene diamine hydroxy phenyl acetic acid (EDHPA)
N, N^1-bis (o-hydroxybenzyl) ethylenediamine-N,N^1-diacetic acid (HBED)
[+] 1,2-bis-(3,5-dioxopiperazinyl-1-yl) propane (ICRF-187)
Pyridoxalisonicotinoylhydrazone (PIH)
Salicylhydroxamic acid (SHAM)
1-Ethyl-2-methyl-3-hydroxypyrid-4-one (L1NEt)
1,2-Diethyl-3-hydroxypyrid-4-one (EL1NEt)
1-Allyl-2-methyl-3-hydroxypyrid-4-one (L1NAll)
4-[3,5-Bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid (ICL670)
GT56-252
1-Methyl-2-methylmethoxy-6-methyl-3-hydroxypyrid-4-one (CP502)
Deferoxamine (DF)
Deferiprone (1,2-Dimethyl-3-hydroxypyrid-4-one or L1)

In addition to the successful completion of all the stages of drug development, the new chelators intended for clinical use will also have to be proven to be effective and non-toxic long-term and to have advantages over deferiprone and deferoxamine. This may require comparative studies with these two established chelating drugs. It is conceivable that the sites of iron removal activity of the new chelating drugs will be different from that of deferoxamine and deferiprone. Future therapies may involve combination of new chelators for targeting different organs in addition to the heart and the liver, from where deferiprone and deferoxamine appear to be effective in iron removal, respectively. New therapeutic strategies are currently needed in order to overcome the problems associated with the high cost of chelating drugs as a result of which only a small number of thalassaemia patients are receiving chelation therapy worldwide. Since it is unlikely that in the next 5 years any new chelating drug in developing countries will be less expensive than deferiprone, the only conceivable solution is the provision of a low-cost formulation of deferiprone in these countries.[87,90] This prospect is feasible because of the inexpensive, simple, 1-step synthesis of deferiprone.[241]

6. Conclusions

The history of the discovery and development of deferiprone for clinical use is unique and unparalleled to any other drug. The primary aim of its development was the treatment of an orphan dis-

ease. Prior to the introduction of deferiprone, the development of inexpensive, orally active iron-chelating drugs was urgently needed to save the lives of thalassaemia patients worldwide. In the developing countries 90% of thalassaemia patients were not treated effectively due to the cost of deferoxamine. For at least 50% of iron-loaded patients in developed countries treatment with deferoxamine was not satisfactory because of noncompliance with the need for prolonged (8–12 hours/day) subcutaneous infusion.

From the initial stages of the discovery of deferiprone 22 years ago, there has been a continuous campaign by different individuals and groups in academic, commercial and other related organisations to delay the progress and availability of deferiprone with the hope that a better chelating drug could be developed or deferoxamine could be improved. Despite earlier criticisms and the involvement of commercial and other interests, as well as the benefit-risk assessment for the use of deferiprone, the present state of knowledge supports the wider use of deferiprone because it does not only fulfil the basic criteria of an effective non-toxic iron-chelating drug, but it also has important advantages over deferoxamine. These are better compliance resulting in more effective therapeutic protocols, lower cost that could help thousands of patients who are not currently treated in developing countries and higher efficacy in the removal of iron from the heart, which is the target organ of toxicity and mortality in thalassaemia patients. A further consideration for supporting the wider application of deferiprone is that is unlikely to have an alternative drug for clinical use with the same or better properties in the next 5–8 years.

It should be noted that very few drugs, if any, are used daily at the effective high doses of deferiprone (>80 mg/kg/day) and have so low toxicity. Because of its low toxicity and pharmacological properties, deferiprone has already been used in clinical trials of conditions unrelated to iron overload and in many experimental models with a prospect of wider applications in many other clinical conditions (table V).[223]

Despite the many controversies, for most patients using deferiprone, oral iron-chelation therapy is here to stay.[242] The clinical use of deferiprone has already saved the lives of thousands of thalassaemia and other patients. Our better understanding of the mechanisms of diseases involving iron-containing enzymes and of the mode of action of deferiprone will help in its wider clinical application and the design of more specific chelators for other related diseases. The optimistic preclinical and clinical results of deferiprone reported in the last 20 years and its establishment as a therapeutic agent is a new approach that could lead in the design and development of new drugs, especially orphan drugs that are urgently needed worldwide.

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