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Transfusional iron overload and chelation therapy with deferoxamine and deferiprone (L1)

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Abstract

Iron is essential for all living organisms. Under normal conditions there is no regulatory and rapid iron excretion in humans and body iron levels are mainly regulated from the absorption of iron from the gut. Regular blood transfusions in thalassaemia and other chronic refractory anaemias can result in excessive iron deposition in tissues and organs. This excess iron is toxic, resulting in tissue and organ damage and unless it is removed it can be fatal to those chronically transfused. Iron removal in transfusional iron overload is achieved using chelation therapy with the chelating drugs deferoxamine (DF) and deferiprone (L1). Effective chelation therapy in chronically transfused patients can only be achieved if iron chelators can remove sufficient amounts of iron, equivalent to those accumulated in the body from transfusions, maintaining body iron load at a non-toxic level. In order to maintain a negative iron balance, both chelating drugs have to be administered almost daily and at high doses. This form of administration also requires that a chelator has low toxicity, good compliance and low cost. DF has been a life-saving drug for thousands of patients in the last 40 years. It is mostly administered by subcutaneous infusion (40–60 mg/kg, 8–12 h, 5 days per week), is effective in iron removal and has low toxicity. However, less than 10% of the patients requiring iron chelation therapy worldwide are able to receive DF because of its high cost, low compliance and in some cases toxicity. In the last 10 years we have witnessed the emergence of oral chelation therapy, which could potentially change the prognosis of all transfusional iron-loaded patients. The only clinically available oral iron chelator is L1, which has so far been taken by over 6000 patients worldwide, in some cases daily for over 10 years, with very promising results. L1 was able to bring patients to a negative iron balance at doses of 50–120 mg/kg/day. It increases urinary iron excretion, decreases serum ferritin levels and reduces liver iron in the majority of chronically transfused iron-loaded patients. Despite earlier concerns of possible increased risk of toxicity, all the toxic side effects of L1 are currently considered reversible, controllable and manageable. These include agranulocytosis (0.6%), musculoskeletal and joint pains (15%), gastrointestinal complaints (6%) and zinc deficiency (1%). The incidence of these toxic side effects could in general be reduced by using lower doses of L1 or combination therapy with DF. Combination therapy could also benefit patients experiencing toxicity with DF and those not responding to either chelator alone. The overall efficacy and toxicity of L1 is comparable to that of DF in both animals and humans. Despite the steady progress in iron chelation therapy with DF and L1, further investigations are required for optimising their use in patients by selecting improved dose protocols, by minimising their toxicity and by identifying new applications in other diseases of iron imbalance. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Iron imbalance

Iron is an essential trace element found in many proteins, which play a key role in the metabolic pathways involved in the growth and development of humans and almost all other organisms. Under normal conditions total body iron in adults is about 4.5 g and is uniformly distributed as haemoglobin (2.6 g), myoglobin (0.4 g), non-haem storage iron in tissues (1.4 g), smaller amounts as enzymes and minute amounts in plasma. About 1–4 mg of dietary iron is absorbed daily mainly from the duodenum and equivalent amounts are lost from various parts of the body. Body iron levels are regulated by the gut through the absorption of iron from the diet and by the erythropoietic activity of the bone marrow. Increased iron absorption is observed with increased erythropoietic activity, for example in conditions with ineffective erythropoiesis. The essential need for iron has resulted in the evolution of homeostatic regulatory mechanisms for the preservation, storage and distribution of this metal in humans but not its rapid excretion or detoxification when in vast excess in the body. Under normal conditions the intracellular uptake and storage of iron are regulated by the iron regulatory proteins (IRPs) through the translational control of the synthesis of the transferrin receptor and intracellular ferritin. The intracellular storage of iron is primarily accomplished by the intracellular proteins ferritin and haemosiderin. Following absorption, the transport of iron in blood is accomplished by transferrin and into the cells by 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. Iron release from transferrin in the endosome intracellularly is accomplished by acidification of the endosome from pH 7.4 to 5.6. In normal individuals transferrin in plasma is saturated 25–35% with iron. Iron imbalance in the body may lead to many complications. Iron deficiency is the most common abnormality in relation to iron metabolic disorders affecting almost all populations, certain ages and both sexes. In this condition the amount of iron entering the body is lower than the amount lost from the body.

Many dietary and other factors can affect iron uptake and distribution in the body. For example, vegetarian diet low in iron can in general result in iron deficiency, whereas in conditions such as the anaemia of chronic disease iron is diverted to the reticuloendothelial system. Similarly, some drugs can cause abnormal body distribution of iron.

In iron overload, body iron levels increase above normal because the amount of iron entering the body is higher than the amount leaving the body. Excess amounts of iron can enter the body mainly because of increased intestinal iron absorption, e.g., in hereditary haemochromatosis, or because of red blood cell transfusions. In both cases, the rate of iron loading and total body iron levels are critical for the early manifestation of iron toxicity.

2. Transfusional iron overload in thalassaemia and other conditions

Transfusional iron overload is the most common metal-related toxicity condition with the highest mortality rate worldwide. The risk is higher in those belonging in the category of the most common inherited disorder namely the haemoglobinopathies. It is estimated that 270 million people are heterozygotes of a haemoglobinopathy and at least 200 000 babies are born each year, half with sickle cell disease and the other half with thalassaemia [1–3]. About 73% of those born with thalassaemia could potentially develop iron overload and toxicity from transfusions and/or increased iron absorption. In particular, β -thalassaemia major accounts for 40% of births and has a high mortality rate if regular red blood cell transfusions are carried out but no chelation therapy is available. HbE/ β -thalassaemia which accounts for 30% of births has a lower risk with respect to iron overload and toxicity because of less severe anaemia and a slower rate of iron accumulation from increased iron absorption and/or transfusions. β -Thalassaemia major patients are unable to make normal red blood cells and can only survive if they receive regular red blood cell transfusions every two to six weeks. Without blood transfusions they die within 4 years from

birth. The mean life span of transfused red blood cells is about 60 days. Usually the rate of red blood cell transfusions is designed in each patient so as to maintain haemoglobin levels preferably above 11 g/dl. Each unit of blood transfused contains 200–250 mg iron and the average daily body intake of iron as haemoglobin in adult patients is 25–30 mg. This amount of iron is released daily into the plasma from the breakdown of effete red blood cells by the reticuloendothelial system mainly of the spleen and the liver. The iron released in the plasma from the transfused effete red blood cells cannot be reutilised in iron-overloaded patients. It is transferred by transferrin, or if transferrin is saturated with iron it finds its way and is eventually stored intracellularly in the form of ferritin and mostly as haemosiderin in various organs such as the liver, the pancreas, the endocrine and the heart. In transfused patients serum transferrin iron saturation increases to a variable degree above normal and in most cases exceeds 100% following the transfusion of less than 50 units of blood. At this stage, non-transferrin bound iron (NTBI) is circulating in the plasma and because tissue iron distribution is no longer primarily regulated by transferrin it may result in variable tissue distribution and toxicity.

The provision of regular red blood cell transfusions and iron chelation therapy is able to achieve normal growth and development in β -thalassaemia major patients and some of these patients have now reached their fourth decade of life. Iron chelation therapy in such patients should be introduced by the 2–3rd year of life if permanent organ damage due to iron overload and toxicity is to be avoided. Regular red blood cell transfusions and iron chelation are the main form of therapy for β -thalassaemia patients (over 99%), while other forms of treatment such as bone marrow transplantation account only for less than 0.1%.

The incidence and distribution of thalassaemia are mainly in developing countries, where carriers or heterozygotes account for 1–10% of the population (e.g., India) or in other countries such as Cyprus it is as much as 16% of the population [4]. Due to inadequate health resources, most of the cases of thalassaemia are not diagnosed or treated

in developing countries. However, despite the low prognosis of thalassaemia the number of transfusion centres in such countries is expanding and as a consequence the number of iron-loaded patients and the need for chelation therapy have substantially increased. In contrast to reality, because of the small number of thalassaemia patients in Europe and North America, thalassaemia is regarded as an orphan disease and the drugs for its treatment as orphan drugs.

Transfusional iron overload and the associated complications could also be developed in many other conditions in addition to the thalassaemias, such as in sickle cell anaemia, myelodysplasia, myelofibrosis, aplastic anaemia, sideroblastic anaemia, pyruvate kinase deficiency, Blackfan Diamond anaemia, Fanconi anaemia, hereditary hypochromic anaemia, liver disease, porphyria cutanea tarda, haemodialysis, cancer, etc. [4].

3. Iron toxicity mechanisms

In all the conditions of iron overload, iron toxicity may arise mainly from the incapacity of cells to store iron in a safe storage form, resulting in lysosomal rupture and subsequent cellular and tissue damage. Potentially toxic forms of iron in iron overload are the low molecular weight labile iron pool and haemosiderin found intracellularly and the low molecular weight non-transferrin bound iron (NTBI) found extracellularly. Iron could catalyse the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA etc. [5]. 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 can also result in molecular, cellular and tissue damages.

Clinical abnormalities are usually manifested following the transfusion of 50–100 units of red blood cells, which is equivalent to 12.5–25 g of storage iron in the body. Such abnormalities include liver and spleen enlargement associated with excessive red cell destruction and iron deposition, damage to pancreas resulting in diabetes mellitus, damage to the endocrine organs, resulting in growth failure and delayed or absent puberty, joint

pains, infections and finally cardiac damage, which may lead to cardiac arrhythmias and congestive heart failure. The last abnormality is the most common cause of death in β -thalassaemia major patients, which occurs by the second decade of life especially in those not receiving chelation therapy [2].

4. Methods for the assessment of iron overload

Estimation of the body iron status and the progress of iron chelation therapy in iron-loaded patients could be achieved by a variety of methods, all of which have limitations and none of which could precisely predict total body iron and extent of iron toxicity. This is mainly because of variability in body and organ iron distribution, iron detection methods, iron estimation and extrapolation to all other body iron pools and other factors such as the diet, infection, inflammation, erythropoietic activity, etc. The most common and indirect methods for estimating body iron status are serum ferritin, transferrin iron saturation and urinary iron excretion in response to chelating agents such as DF, L1 and DTPA. The most accurate but invasive method of body iron determination is iron estimation and histochemistry of a liver biopsy, which could identify the non-haem iron concentration and the level as well as the pattern of iron deposition in hepatocytes and Kupffer cells, thus reflecting total body iron load. A non-invasive method of liver iron estimation which is gaining ground in the diagnosis of iron overload is SQUID-biosusceptometry, which correlates well with results from liver biopsies. Magnetic resonance imaging (MRI) techniques are also becoming useful tools for assessing differential organ iron deposition and in particular heart iron load which is critical for the prognosis of iron-loaded patients. Another indirect parameter for assessing potential iron toxicity rather than iron overload is NTBI, which usually appears when transferrin is fully or nearly fully saturated with iron. All the above methods could be used periodically for assessing the prognosis of the patients, which is directly related to their body iron load and organ function as well as for the adjustment of

the dose protocol during iron chelation therapy. In addition to the regular clinical and biochemical monitoring, serum ferritin or urinary iron estimations every three months and liver iron estimation annually from liver biopsies or by using SQUID-biosusceptometry or by other methods could be sufficient for monitoring the iron loading and chelation therapy progress of patients. Usually, serum ferritin levels above 2.5 mg/l, transferrin iron saturation over 100% and liver iron concentration above 7 mg iron per g liver dry weight suggest the presence of toxic levels of iron in the tissues and the need for the use of a more aggressive chelation therapy regime. It is considered that above these iron levels the damage to organs and especially the heart could be irreversible.

If the chelation therapy protocol proves very successful and serum ferritin is reduced to levels about 0.5 mg/l, then the chelator dose should be reduced progressively in order to maintain this low iron load and avoid at the same time possible toxicity arising from excess drug administration.

5. Chelating drugs and their mode of action

A chelator ($\chi\eta\lambda\eta$, Greek claw of a crab) is a naturally occurring or chemically designed molecule which has high specificity and affinity for a metal ion, forming a complex with it. An ideal chelator designed for the decorporation of a particular metal from the body should be able to bind, carry and remove the metal out of the body without causing any toxicity. The chelator dose protocol should be selected for each patient and aimed to achieve maximum efficacy in iron removal and minimum toxicity. Within this context the concentration of the drug and its metabolites in plasma and tissues as well as their clearance should be considered. Iron chelating drugs could in principle reduce iron overload by causing a negative iron balance, which means an increase in iron excretion at levels higher than the amount of iron taken into the body from transfusions and iron absorption. The site of action of chelating drugs in vivo is not fully understood. At the molecular level, almost all the chelating agents form complexes with low molecular weight iron in

solution and remove iron from ferritin and haemosiderin *in vitro* [6]. L1 but not DF could also remove iron from transferrin both *in vitro* and *in vivo* [7]. Since transferrin is in equilibrium with all the iron pools, iron removal by a chelator could result in iron exchange with these pools through transferrin. The variation in the mode of action by DF and L1 could result in differences in iron decorporation from various organs. Similarly, because of variation in the lipid/water partition coefficient of the chelators and their chelating metabolites, there could be differences in accessing and removing intracellular iron resulting in variations between chelators in iron decorporation from various intracellular compartments, tissues and organs. Within this context, combination of chelators and complementary therapy may be more appropriate for targeting specific organs, tissues and chelatable iron pools. Chelating drugs on their own or in combination could in addition to removing iron offer protection against iron toxicity by inhibiting the catalytic formation of toxic oxygen-activated species such as free radicals [5]. As in the case of all other drugs, the risk to benefit ratio should be considered as the primary factor to warrant the use of iron chelating drugs or their combination. However, additional factors such as the cost, compliance and marketing promotion could also influence the use of such drugs in each country and worldwide.

6. Deferoxamine

DF has been the mainstay of iron chelation therapy in transfusional iron loaded patients in the last forty years. It is a generic drug and could be produced chemically or isolated from the fungus *streptomyces pilosus*. The development of chelation therapy with DF involved different stages of various application techniques and experimentation protocols over the last 40-year period, including various methods of administration in order to improve its overall efficacy. The prolonged subcutaneous and intravenous routes of administration proved more successful in iron removal than all other routes. In most patients DF is injected subcutaneously 8–12 h per day at 40–60

mg/kg, with the aid of a portable electronic pump at least five times a week and in some DF is administered intravenously with the aid of a port-cath catheter [8]. The intramuscular route and its administration as a suppository had some limited success but the oral route was ineffective [9,10]. Recently, the administration of a bolus subcutaneous infusion of DF has been shown to be as effective as the prolonged 8–12 h subcutaneous infusion but further investigations are needed to confirm these observations [11]. In general, DF is regarded as a safe and effective drug when used correctly and when patients comply with its subcutaneous administration. Its overall efficacy has never been questioned because no other competing drug has been shown to be as effective. It is estimated that only a maximum of 66% of the patients using DF receive it regularly without complications and achieve body iron levels within the non-toxic range, achieving serum ferritin levels below 2.5 mg/l. Patients below this limit are known to have a lower morbidity and mortality rate by comparison to more heavily iron-loaded patients. However, despite its efficacy DF treatment is available to less than 10% of the transfusional iron-loaded patients worldwide mainly because of the high cost (£4000/patient/year) and the low compliance associated with its subcutaneous administration. In India for example, less than 3% of thalassaemia patients could receive DF [12] while in Cyprus 45% of the health budget for drugs are used for making DF available to all the patients who need it [13].

The toxic side effects of DF are many but the most common are swelling and soreness at the side of the injection. This is the major cause for the low compliance observed in patients receiving DF and one of the major reasons for searching new iron chelators.

The introduction of an orally effective, non-toxic and inexpensive iron chelating drug would decrease the morbidity and mortality rate of the vast majority of transfusional iron-loaded patients worldwide. The financial incentives for the development of such oral chelating drugs present a major dilemma to pharmaceutical companies and health organisations worldwide. Iron chelators have been classified as orphan drugs in Europe and

North America because they are primarily intended for the treatment of an orphan disease namely thalassaemia [14].

7. The α -ketoxyhydroxyridines

Hundreds of potentially orally active chelators have been tested in vitro and in animals and only 16 reached the stage of clinical evaluation, with mostly disappointing results because of either ineffectiveness or toxicity in humans [15–17]. The α -ketoxyhydroxyridines (KHP) is the most promising group of chelators [18]. They were designed to mimic the naturally occurring chelators mimosine, tropolone and maltol which were previously shown to be orally active and effective in binding iron in animals [19].

Iron removal in animals by orally administered KHP varies, with L1NAlI being the most effective and L1 and other derivatives being equally or slightly more effective than parenterally administered DF at selected doses [20]. The reverse is true at lower doses, where DF is much more effective than the KHP. Several in vitro and in vivo studies using KHP have suggested that lipophilic chelators and especially EL1NEt was particularly promising for iron removal [21,22]. However, this was not confirmed from animal studies by other investigators [23,24]. Apart from the oral activity of the KHP, one of the major reasons for the interest in their development is the low cost and very easy procedure of preparation. L1 could be prepared from the natural product maltol and methylamine within a few hours and its cost of production could be as low as 50 times cheaper than the sale price of DF [25]. Hundreds of studies have been performed and published since the original work with these chelators and, in particular, following the publication of the promising clinical results with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1 or INN: Deferiprone) [18]. More than a hundred KHP derivatives have been synthesised and tested in vitro and in vivo and only a few have reached the stage of clinical evaluation in humans. In addition to L1, the ethyl derivative of L1 namely L1NEt [26] and its more lipophilic diethyl derivative

(EL1NEt) have also been tested in a few patients but later abandoned as there were no advantages over L1 [27]. It is interesting that a number of investigators have shown in the last 10 years that many other KHP derivatives and also other groups of chelators could be superior to L1 in certain in vitro and animal models and at selected doses. Despite such results they were later abandoned, mainly because any new chelator has to be shown to have a better therapeutic index than L1, to be effective in prolonged administration over many years, and also not to be associated with major toxicity over the same period.

8. Deferiprone

L1 is the first oral iron chelating drug to be used in thalassaemia and other iron-loaded patients [28,29]. Progress in its development has been very slow because it was mostly undertaken through research-orientated projects and supported by non-profit establishments. Pharmaceutical companies only showed an interest on L1 after it became clear that it would be a profitable financial venture.

It is estimated that more than 6000 patients in 40 countries have received L1 since the initiation of clinical trials [30]. There are groups of patients in India, Switzerland and Cyprus who have been taking L1 daily since 1989. India was the first country where L1 has been registered in 1994 and now there are more patients on L1 than DF. L1 has also been registered in Europe in 1999, following the recommendation by the EC CPMP that its use be restricted to patients not tolerating or using DF and also that it is closely monitored for toxicity. Registration of L1 is pending in many other countries.

L1 is a white crystalline solid offered to patients as capsules or as tablets. It is very stable at room temperature and in solutions of physiological or acidic pH and forms red colour complexes with iron, similar to the red colour of the urine of patients treated with it. Its affinity for iron is higher than for Cu, Al, Zn and other metals. Three molecules of L1 are needed to bind one molecule of iron at physiological pH in contrast to DF

which binds iron at one:one molar ratio [31,32]. L1 is a neutral molecule forming a neutral complex with iron, whereas DF is positively charged and forms a positively charged complex with iron at physiological pH [32]. At low concentrations (10^{-6} M) the L1 iron complex is less stable than that of DF and the labile complexes of L1 with iron or copper may promote the formation of toxic oxygen-activated species [33]. Both L1 and DF are hydrophilic and unlike lipophilic chelators they do not accumulate in lipids for example in cell membranes or in the brain. L1 is about 10 times more lipophilic than DF and may be easier to diffuse through cell membranes than DF, unless other forms of membrane transport are involved.

The interaction of L1 and DF with the iron pools varies. Soluble forms of mononuclear or oligonuclear iron such as NTBI and low molecular weight intracellular iron could be rapidly mobilised and form iron complexes within minutes. In contrast, the mobilisation of iron from ferritin and haemosiderin is very slow, taking days to reach completion both with L1 and DF, whereas that from transferrin and lactoferrin is completed within hours only with L1 but is not effective with DF [34,35].

Both L1 and DF are unable to mobilise iron from haemoglobin and other haem containing proteins but DF can cause oxidation of haemoglobin. Ribonucleotide reductase which is an iron containing enzyme involved in DNA synthesis is also inhibited by both L1 and DF at high concentrations (mM) to about the same degree. Finally both chelators are known to inhibit the formation of toxic oxygen-activated species (H_2O_2 , superoxide and hydroxyl radical) in a concentration depended process [36].

L1 and DF have variable effects on cells. Red cells are not affected, whereas the growth of white cell progenitors and PHA stimulated lymphocytes is inhibited. White blood cell progenitors have been shown to be inhibited by both L1 and DF at 4 days incubations and at concentrations lower than those used for the inhibition of growth of other cells. The inhibition of cell growth was reversed when iron was added to the incubation mixtures or when the chelators were washed out of the incubation medium [37].

Despite its antiproliferative effect on mammalian cells, DF could enhance the growth of microbes through iron delivery and spark potentially lethal infections namely yersiniosis and mucormycosis [38–40]. L1 does not appear to act as a siderophore for either of the two microbes [39,41]. Iron, chelators, microbes and disease is of great relevance to thalassaemia and other iron-loaded or non-iron-loaded patients [42].

9. Clinical use of L1

Many centres have reported the results of clinical trials since the first reports of the iron removal effects of L1 in transfusional iron loaded patients in 1987, all confirming the original findings that L1 is an effective, non-toxic, orally active chelator [28,29,43–56]. Overall, L1 has so far been assessed in three different therapeutic areas of medicine, mainly for iron removal from iron-loaded patients, aluminium removal from renal dialysis patients and in rheumatoid arthritis patients where it has been used for reducing inflammation and for correcting the anaemia of chronic disease [57,58].

Iron excretion by L1 in humans depends on the dose and frequency of administration of the drug and the iron load and rate of iron loading of the individual [19]. Doses of a total of 50–120 mg/kg/day are currently being used which are administered in 2–4 divided doses of 25–50 mg/kg. Doses as low as 10 mg/kg are sufficient at increasing urinary iron excretion in patients with relatively low serum ferritin levels (0.9–1.7 mg/l) [28]. Typical doses of 50–100 mg/kg of L1 can result in the excretion of 10–120 mg iron in patients with serum ferritin of 3–12 mg/l. By comparison, a dose of about 50 mg/kg in normal individuals could cause much lower iron excretion (1–2 mg). Intensive chelation using escalating doses of L1 further increases iron excretion. In one thalassaemia patient of 67 kg with serum ferritin of 8 mg/l repeated administration of 5×2 g and 2×3 g within 24 h resulted in the excretion of 325 mg, which is equivalent to a 13-day intake of iron from transfusions [47]. In general, there is variation amongst patients in iron excretion and sometimes the same patients respond differently given the same dose.

Some patients can excrete more iron when given high single doses over 24 h, whereas others excrete more iron when smaller doses are administered more frequently over the same period. The factors causing such variations are unknown but the same is observed during DF therapy. Dietary components, vitamin C status, erythropoietic activity, rate and extent of chelator biotransformation and the presence of labile low molecular weight iron could be few of many such factors influencing iron excretion by L1 [59,60].

L1 has been shown in many studies to cause negative iron balance and to deplete iron from the liver and other organs of iron-loaded animals and patients [30,61,62]. Thalassaemia intermedia or HbE/ β -thalassaemia patients are more rapidly depleted of iron by comparison to β -thalassaemia major patients [61]. Iron excretion by L1 appears to be variable. In about 70% of thalassaemia patients treated over 2 years with a daily 75–120 mg/kg dose of L1, serum ferritin could be reduced and remain below 2.5 mg/l. One patient in Switzerland was reported to have taken L1 at 150 mg/kg/day for 2 years and had a liver fibrosis score of 0, while in other patients the dose was reduced to 50 mg/kg/day because serum ferritin was approaching normal values [30]. In the remaining 30% of patients who have been treated with L1 at a dose of 75 mg/kg/day or less and who do not excrete sufficient amounts of iron to reach negative iron balance or are experiencing toxic side effects with L1, a combination therapy with DF appears to be more effective and less toxic than using L1 alone. Combination therapy of DF and L1 could also be used for patients not responding well to DF or experiencing toxic side effects with DF [30,37]. It should be noted that there are many approaches to the combination therapy of L1 and DF, all aiming at minimising the toxicity of one of the drugs or improving the overall iron excretion, or for both reasons. Despite that studies of combination of these two chelators have been going on arbitrarily, specific dose protocols are needed since each of the drugs mobilises iron from different iron pools and has different ferrokinetic, metabolic and toxicity profiles. The timing of administration of these two drugs is also significant because it could cause variable effects in the iron pools and on toxicity.

For example, L1 is known to donate iron to DF and to exchange iron with transferrin depending on its concentration.

10. Pharmacology of deferoxamine and deferiprone

DF is supplied in a vial as a freeze-dried solid of its mesylate salt, which is dissolved in water and administered with the aid of an electronic pump over an 8–12 h period. The rate of elimination of DF in blood is faster than L1 and depends on the route of administration. The half-life of elimination of i.v. DF is 5–10 min and im DF is 60 min. It is estimated that during the 8–12 h long s.c. DF administration, the elimination of DF is very fast and a plateau level in the plasma is achieved after about 4 h [37,42].

L1 has a very bitter taste, is sparingly soluble in water at neutral pH but is highly soluble in the acidic environment of the stomach. L1 is rapidly absorbed from the stomach and appears in blood within minutes [63,64]. Several factors such as food and drugs could delay the absorption of L1 and in some cases a lag period is observed before its appearance in blood, which suggests that L1 could also be absorbed from the intestine as it was suggested from studies in animals [43]. The half-life of the rate of its absorption from the stomach or the intestine and appearance in blood was estimated to be 1–32 min. L1 is metabolised primarily in the liver into its glucuronide conjugate which has no iron chelating properties. It is cleared from the blood with a half-life of 47–134 min, while its glucuronide conjugate takes much longer time to be cleared. L1 is excreted in the urine in various forms, mainly as a glucuronide conjugate, in an unchanged form, bound to iron and to a lesser extent bound to other metals. There are variations amongst patients in relation to the rates of absorption and clearance of all the forms of L1. However, it would appear that iron chelation precedes glucuronidation and that the major factor influencing iron mobilisation is the availability of the chelatable iron pools and not the extent of glucuronidation of L1 [59]. L1 and its metabolites are excreted in the urine and neither L1 nor its glucuronide metabolite have yet been identified in

the faeces of patients, nor was there an increase in faecal iron or ^{59}Fe excretion of patients taking L1 [65,66]. In further clinical studies L1 has been shown to inhibit iron absorption and to be secreted in the saliva of patients [67,68]. Iron mobilisation by L1 appears to be orientated primarily from NTBI. Removal of iron from saturated transferrin is also a major site of iron mobilisation by L1 provided that L1 exceeds 100–200 μM in plasma [69]. Liver iron is another major site which was identified in animals and humans to be depleted during long-term chelation with L1. Depletion of iron from the liver of patients taking L1 at doses of 75 mg/kg/day or more has been confirmed repeatedly by liver biopsies and SQUID-biosusceptometry monitoring. Similarly, it would appear from MRI monitoring that L1 is more effective than DF in the removal of iron from the heart [30,61,62].

In general, it is believed that all the compartments of the body containing excess iron will be depleted if negative iron balance could be achieved by chelation because of the equilibrium between the various iron pools with unsaturated transferrin. The effect of L1 in iron removal and decrease in serum ferritin was clearly demonstrated by Agarwal et al. [70,71] and Tondury et al. [72]. Reduction of serum ferritin to normal or near normal levels takes usually months or even years to be completed. Doses of 75–120 mg/kg/day of L1 should in most cases be sufficient to cause negative iron balance. Similarly, i.v. DF appears to be much more effective than s.c. DF at 40–60 mg/kg for decreasing serum ferritin levels and maintaining low iron stores.

11. Toxicity of deferiprone and deferoxamine

Most drugs have toxic side effects which may or may not be related to their pharmacological activity or toxicity findings in animals. A major aspect of toxicity in relation to iron chelation therapy is the removal or displacement of other essential metals. Two other iron chelating drugs namely DTPA and EDTA have been previously shown in iron-loaded patients to increase the excretion of Zn, Cu, Mn in addition to Fe resulting

in toxic side effects. Increased excretion of Zn and Cu has also been observed in a few cases during treatment with DF and also with L1, especially increased Zn excretion in diabetic thalassaemia patients [37]. The incidence of Zn deficiency in patients treated with L1 is estimated to be about 1%. Zn removal by L1 and rarely by DF could easily be corrected using Zn supplements. In addition to Zn deficiency the other major toxic side effects of L1, which have been identified in the last 10 years are neutropenia and agranulocytosis, joint/musculoskeletal pains and gastric intolerance. No new toxic side effects of L1 have been identified or confirmed since then and all those known are controllable and manageable. Agranulocytosis which is estimated to be less than 0.6% and is monitored with weekly or fortnightly blood counts still remains the most serious toxic side effect, while transient joint/musculoskeletal pains are effecting up to 15% and gastrointestinal complaints up to 6% [30]. Reports of significant increases in hepatic fibrosis or liver cirrhosis by a Toronto group have not been confirmed by any other group from any other country using L1 and by comparison to patients taking DF [30,62,73,74]. In particular, this was emphasised by those in charge of the groups of patients who have been taking L1 daily for over 10 years in India and Switzerland [30].

The mechanisms involved in the toxic side effects reported by L1 are not yet known. Transient musculoskeletal and joint pains were reported in the first stages of the clinical trials [75]. Further studies reported the same findings especially in heavily iron-loaded patients. In most patients the pains stopped despite continuation of the L1 therapy, in some after short-term withdrawal of L1 and in a few others after administration of anti-inflammatory drugs. The incidence of this toxicity was reduced from 20% to 10% in a study where the dose was reduced from 100 to 75 mg/kg/day [37].

The cause of agranulocytosis in patients taking L1 is still unknown but in almost all the cases this toxic side effect was transient and all the patients recovered following treatment with CSGF. The time of the recovery of the neutrophil count in these patients could vary and be up to seven weeks [76]. An L1-related immune response against white

cell progenitors is also suspected because rechallenging with L1 results in another episode of agranulocytosis [37]. Several drugs are also known to cause this toxic side effect such as the copper chelator penicillamine, clozapine, hydralazine hydrochloride and many other [77,78]. The cause of agranulocytosis by these drugs is also unknown but it may be related to a combination of factors such as the formation of toxic metabolite(s), abnormal sensitivity of the myeloid precursors of the susceptible individual, direct toxicity of the drug and drug-induced immune responses [37,77,78]. Other possible toxic species or processes responsible for agranulocytosis and musculoskeletal/joint pains may be the formation of free radicals by labile L1 metal complexes, inhibition of the turnover of important metalloenzymes, reduced bioavailability of essential metals, etc.

Until now no serious efforts have been made to identify the causes and mechanisms which are related to the toxic side effects of L1, or the individuals who may be susceptible to its toxicity. Prevention of the toxicity may be possible by introducing dose protocols which minimise exposure to continuous high serum levels of the drug or its metabolites. Weekly monitoring of the white cell count and of indices such as antinuclear (ANA), antihistone (AHA), double stranded DNA (dsDNA) and rheumatoid factor should all be used as prophylactic measures [37].

The toxic side effects of L1 mentioned above are not typical of those observed during the treatment with DF. For example, in addition to yersiniosis and mucormycosis which are thought to be caused by the DF iron complex, other fatal or potentially fatal side effects of DF include pulmonary complications and pancytopenia [37]. Also, permanent or transient toxic side effects caused by DF such as auditory, ocular and neurologic abnormalities, growth retardation and bone abnormalities have not yet been encountered during L1 therapy. Other toxic side effects not found with L1 but related to the subcutaneous administration of DF are local reactions such as hardness, swelling, redness and soreness which in one study were shown to occur in up to 80% of the thalassaemia patients [37]. However, despite the differences between the toxic side effects of L1 and s.c. DF there are reports that

i.v. DF may also cause neutropenia, thrombocytopenia and pancytopenia [37]. The low incidence of the last three toxic side effects of DF may be attributed to the rare use of i.v. DF which unlike s.c. DF could cause the presence of higher, more toxic concentrations of DF and its metabolites in the plasma. In addition to toxicity several other factors such as cost/availability, compliance, frequency of use (7/7 days for L1, 5/7 for DF) etc., will be the important determinants in the future use of either of these two drugs or other future drugs. The survival rate of patients using chelation therapy with DF or L1 and of other patients who have chosen bone marrow transplantation will also influence treatment strategies of thalassaemia in the future.

The use of oral chelators in other areas of medicine may also influence subsequent developments in the area of iron chelation. Such examples are the use of DF and L1 in aluminium overload, in the correction of the anaemia of chronic disease where iron is blocked in the reticuloendothelial system and is not released to the erythron for normal Hb production, in the prevention of “free radical toxicity”, drug resistance malaria etc. [37,48].

In conclusion, DF is a life saving drug for those who are using it correctly and can afford it. In the last 10 years, over 6000 patients have been using L1 and this number is continuously expanding. Several other oral chelators, mainly KHP derivatives are being screened for clinical use because the patent covering DF has run out and that of L1 is running out in 2003. These oral chelators are still in the preclinical stage of development and if successful will not be available at least in the next 5 years. New protocols of high efficacy and low toxicity of L1 as well as complementary therapy are also being developed. Combination therapy of L1 with DF is also under development. Overall, L1 remains the drug of choice for those patients who cannot afford the high cost of DF, cannot comply with its 5-day per week, 8–12 h subcutaneous infusion and cannot overcome its toxic side effects. On balance and considering the present stage of knowledge, the prognosis of patients taking L1 is much better in general than those experiencing iron overload toxicity because of lack of chelation

therapy or ineffective or insufficient chelation therapy with DF [79].

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