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EDITORIAL

New developments and controversies in iron metabolism and iron chelation therapy

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Abstract

Iron is essential for all organisms including microbial, cancer and human cells. More than a quarter of the human population is affected by abnormalities of iron metabolism, mainly from iron deficiency and iron overload. Iron also plays an important role in free radical pathology and oxidative damage which is observed in almost all major diseases, cancer and ageing. New developments include the complete treatment of iron overload and reduction of morbidity and mortality in thalassaemia using deferiprone and selected deferiprone/deferoxamine combinations and also the use of the maltol iron complex in the treatment of iron deficiency anaemia. There is also a prospect of using deferiprone as a universal antioxidant in non iron overloaded diseases such as neurodegenerative, cardiovascular, renal, infectious diseases and cancer. New regulatory molecules of iron metabolism such as endogenous and dietary chelating molecules, hepcidin, mitochondrial ferritin and their role in health and disease is under evaluation. Similarly, new mechanisms of iron deposition, removal, distribution and toxicity have been identified using new techniques such as magnetic resonance imaging increasing our understanding of iron metabolic processes and the targeted treatment of related diseases. The uniform distribution of iron in iron overload between organs and within each organ is no longer valid. Several other controversies such as the toxicity impact of non transferrin bound iron *vs* injected iron, the excess levels of iron in tissues causing toxicity and the role of chelation on iron absorption need further investigation. Commercial interests of pharmaceutical companies and connections to leading journals are playing a crucial role in shaping worldwide medical opinion on drug sales and use but also patients' therapeutic outcome and safety. Major controversies include the selection criteria and risk/benefit assessment in the use of deferasirox in thalassaemia and more so in idiopathic haemochromatosis, thalassaemia intermedia and ex-thalassaemia transplanted patients who are safely treated with venesection. Iron chelating drugs can override normal regulatory pathways, correct iron imbalance and minimise iron toxicity. The use of iron chelating drugs as main, alternative or adjuvant therapy

is in progress in many conditions, especially those with non established or effective therapies.

Key words: Iron metabolism; Iron chelation therapy; Deferiprone; Deferoxamine; Deferasirox; Iron diseases; Medical journals; Controversies

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Core tip: Abnormalities of iron metabolism including iron deficiency and overload affect more than a quarter of the world's population. Iron also plays a major role in free radical pathology and associated tissue damage. Iron chelating drugs can override normal regulatory pathways, correct iron imbalance and minimise iron toxicity. Deferiprone and especially its combination with deferoxamine can completely treat iron overload in thalassaemia. Deferiprone can minimise the toxic effects of pathological iron in neurodegenerative, renal and other diseases. Controversies in the risk/benefit assessment for the use of deferasirox in many conditions appear to involve commercial influence on academic journals and physicians.

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INTRODUCTION

Iron is an essential metal found in all living organisms including microbial, cancer and normal human cells. More than a quarter of the human population is affected at some stages in their life by iron deficiency. Similarly, many millions suffer from other abnormalities of iron metabolism, such as iron overload in hereditary haemochromatosis which is caused by increased iron absorption and iron overload in thalassaemia which is a result of chronic transfusions[1,2]. Iron also plays an important catalytic role in free radical pathology and oxidative damage which is observed in almost all major iron loaded and non iron loaded diseases such as cardiovascular, neurodegenerative, hepatic and renal diseases, as well as in cancer and ageing[3].

Most of the diseases related to iron metabolic imbalance can be treated using established and effective therapeutic approaches, *e.g.,* iron supplementation for the treatment of iron deficiency anaemia and venesection in hereditary haemochromatosis. Iron overload in thalassaemia is more difficult to treat using chelation therapy and the same applies for the treatment of the anaemia of chronic disease in many conditions such as cancer, rheumatoid arthritis and haemodialysis, where oral or intravenous iron, with or without erythropoietin combination may be used.

Most of the therapies of abnormal iron metabolism described above are widely applied in developed countries but there are financial constrains for their use by patients in the developing countries. In particular the treatment of thalassaemia using regular transfusions and chelation therapy and also the use of erythropoietin in the anaemia of chronic disease is not affordable for the vast majority of patients in the developing countries[4,5].

The disease with the highest mortality and morbidity rate related to iron metabolic disorders worldwide is thalassaemia, which is found mainly in developing countries of South East Asia, Middle East and Mediterranean. More than 100000 thalassaemia babies are born every year with 9000 in India alone, most dying without treatment[4-6]. Despite that health facilities including blood transfusions are improving in developing countries, the cost of the chelating drugs is still not affordable for most patients living in these countries and therefore life expectancy is low[5]. Usually, non-transfused thalassaemia patients die by the age of 7 years and transfused but not chelated thalassaemia patients die by the age of 20 years, mainly from congestive cardiac failure due to cardiac iron overload toxicity[7,8]. The life expectancy of thalassaemia patients receiving chelation therapy increases substantially and many patients adhering to the chelation protocol with deferiprone (L1) and deferoxamine (DF) are now exceeding the age of 50 years (Figure 1)[9].

However, despite the wide availability of the chelating drugs DF, L1 and deferasirox (DFRA) in developed countries and indications that the use of appropriate effective protocols can lead to the complete treatment of iron overload, their application to thalassaemia patients appears to be influenced by physician decisions associated with literature rivalry and commercial interests[5]. As a result of the commercial interference and influence which is mainly caused by the manufacturers of chelating drugs and their marketing methods the overall treatment outcome, safety and survival of the thalassaemia patients is greatly affected[5].

Clinical trials and preclinical studies suggest that there are increasing prospects of using chelation and in particular L1 as a universal antioxidant in non iron overload diseases such as neurodegenerative, cardiovascular, renal and infectious diseases as well as cancer and ageing[10,11].

The discovery of new regulatory molecules of iron metabolism such as endogenous and dietary chelating molecules as well as the proteins hepcidin, ferroportin, mitochondrial ferritin and their role in normal and iron overload disease states is subject to continuous investigation[1,2]. Similarly, the identification of new mechanisms of iron deposition, removal, distribution and toxicity have increased further our understanding of iron metabolic processes and improved the use of specific targeting treatments in iron metabolic diseases.

In general, the acquisition and distribution of important and significant knowledge for all diseases is becoming a major issue in the treatment outcome and

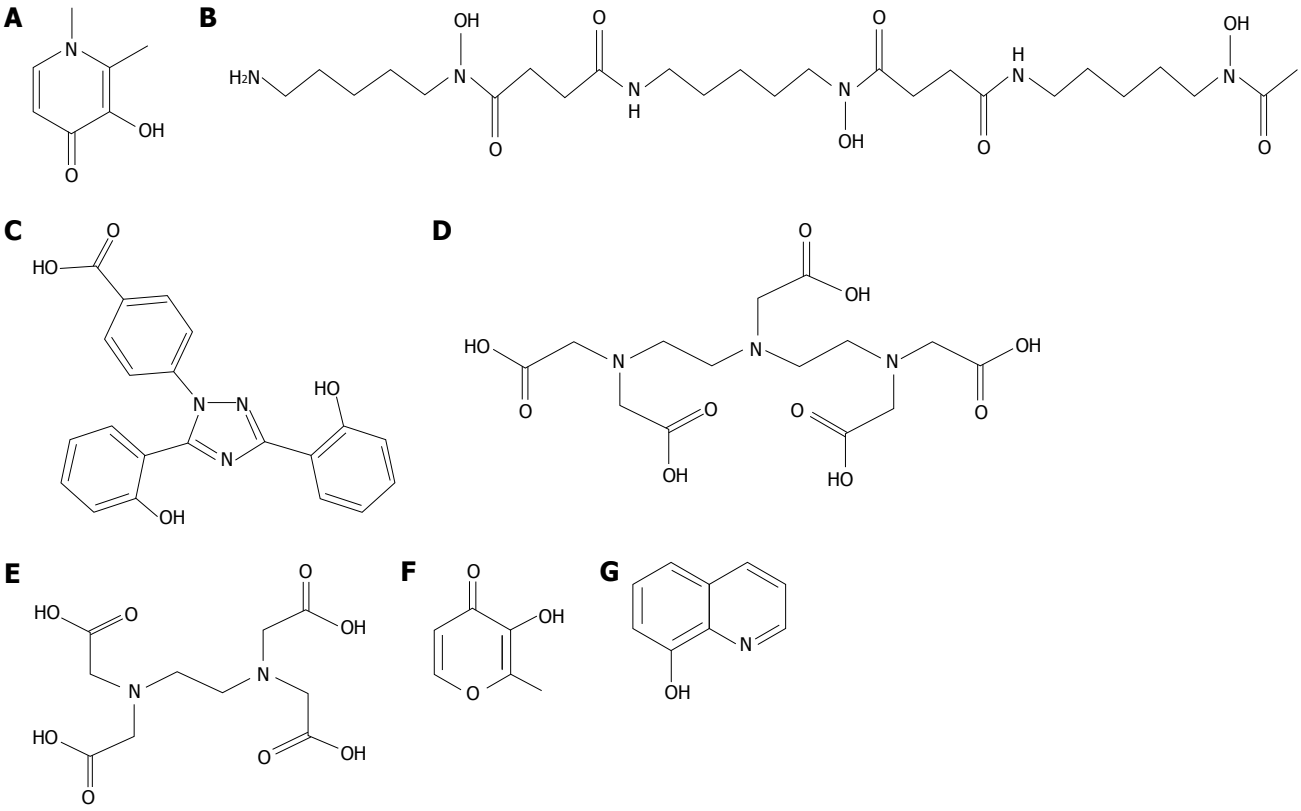

Figure 1 The chemical structure of the iron chelating drugs. L1 (A), DF (B) and DFRA (C) are currently used for the treatment of thalassaemia and other transfusional iron loading conditions. DTPA (D) and EDTA (E) have been previously used for the treatment of iron overload but are now used for the detoxification of toxic metals and in particular EDTA in alternative medicine. The maltol (F) iron complex is used for increasing iron absorption and 8-hydroxyquinoline (G) is a lipophilic chelator used for radiolabeling in diagnostic medicine and for experimental purposes. L1: Deferiprone; DF: Deferoxamine; DFRA: Deferasirox; DTPA: Diethylenetriaminepentaacetic acid; EDTA: Ethylenediaminetetraacetic acid.

safety of patients. Within this context many controversies related to the diagnosis and treatment of iron abnormalities have been identified involving influence by commercial interests, especially by pharmaceutical companies and related support by a section of leading medical journals in the selection and promotion of drug treatments with high risk and low benefit outcomes for patients[5].

The controversies are also extended to research findings in relation to basic mechanisms of chelating drug action and also to iron metabolism and toxicity. These issues include for example the impact of toxicity of non transferrin bound iron (NTBI) found mainly in iron loaded patients *vs* the lack of toxicity of injected iron used in anaemic patients. Similarly, the role and limitations of the function of hepcidin as a universal regulator of normal and abnormal iron metabolism are also questioned.

Controversies associated to basic mechanisms of drug toxicity especially in the case of DFRA, for example in relation to the increased absorption of toxic metals such as Al are still unanswered[12,13]. Furthermore, the promotion of the use of DFRA instead of the safer and more effective use of venesection is also questioned especially in relation to the treatment of idiopathic haemochromatosis, thalassaemia intermedia and in ex-thalassaemia transplanted patients. Another issue in relation to the use of DFRA which are also controversial is its effect in the mortality and morbitidy rate of transfused patients with myelodysplastic, myelofibrosis, sickle cell anaemia and also in non iron loaded conditions.

It appears that in general many controversial issues including the risk/benefit assessment of the use of iron chelating drugs worldwide in different conditions seem to be based on the marketing policies and commercial influence of pharmaceutical companies and not the therapeutic needs and safety of patients[5]. Within this context some recent developments in iron metabolism will be reviewed with emphasis on the topics and issues that affect the treatment of patients with iron metabolic disorders both in developed and developing countries. Similarly, recent developments that affect the treatment outcome and safety of patients including commercial influence and other non medical factors will also be discussed.

MOLECULAR ASPECTS OF IRON METABOLIC DISORDERS

The molecular aspects of iron including its chemical and biochemical properties are important in the understanding of iron metabolism and chelation therapy. Iron is generally found in the ferrous Fe (Ⅱ) and ferric Fe (Ⅲ) oxidation states in the human body. For example, it is transported in the plasma by transferrin and stored in ferritin and haemosiderin in the ferric form, whereas it is

found in the ferrous form in haemoglobin and myoglobin bound to oxygen and also in other proteins involved in redox reactions[14]. It can also sometimes be found in the Fe (Ⅳ) form in haem, which is associated with pathological effects[15].

The solubility of iron under physiological conditions is an important property for its metabolic functions and toxicity. Ferric iron hydrolyses at pH 7.4 forming insoluble oxohydroxy polynuclear complexes which precipitate. The solubility of ferric iron is extremely low and at physiological pH 7.4 is estimated to be less than 10^{-18} mol/L. The amount of soluble iron is negligible compared to the iron turnover needed for the different physiological functions and in particular for the production of haemoglobin. The solubility of iron (Ⅲ) can increase by different methods such as by decreasing the pH, reducing iron (Ⅲ) to iron (Ⅱ) or using chelating agents. Examples of such processes in physiological conditions is the solubilisation of iron in food in the acidic medium of the stomach, reduction of iron (Ⅲ) to iron (Ⅱ) in the duodenum by a cytochrome b-like ferrireductase (Dcytb) and chelation and transport of iron (Ⅲ) by transferrin[1,2,14]. Another method for the solubility of polynuclear iron is achieved intracellularly by ferritin, which encloses the insoluble oxohydroxy polynuclear iron within a soluble protein shell[1,2,16,17].

The redox changes of iron are of biological and toxicological importance. In particular, iron toxicity arises mainly from the catalytic activity of ferrous iron in the formation of free radicals and other reactive oxygen species which have been shown to cause molecular damage to all organic biomolecules including lipids, sugars, proteins and DNA[3,18]. Such biomolecular damage can lead to subcellular, cellular, tissue and organ damage, which can be permanent or reversible[19]. Ferric iron cannot catalyse the production of free radicals and is mostly not toxic unless it is reduced. However, *in vivo* iron (Ⅲ) can be reduced to iron (Ⅱ) by reducing agents such as ascorbic acid and other organic acids and consequently catalyse free radical production[20].

The presence of excess iron is considered a potential source of toxicity which can be expressed at the molecular, subcellular, cellular, tissue and organ level. Such forms of excess iron in polynuclear form include ferritin, haemosiderin and NTBI. Usually the damage to tissues and organs in iron loaded diseases depends on the concentration of excess stored iron mainly in the form of haemosiderin. At low iron concentrations of excess iron such damage is considered reversible due to the effective antioxidant protection mechanisms and antioxidant molecules and also the efficiency of the repair mechanisms[19]. However, at high concentrations excess iron can cause permanent damage and can be fatal, *e.g.*, in cardiac iron overload in thalassaemia[7,8].

Under normal conditions iron is essential to all cells and plays an important role in physiological functions including the growth and development of the body. It is absorbed from ingested food in small quantities of about 1-2 mg/d. The total body iron of normal adult humans is estimated at 3-5 g. Most of the iron is found in blood in the form of haemoglobin (58%) in red blood cells, as myoglobin (9%) in muscle tissue and as intracellular ferritin/haemosiderin (30%) mainly in the liver and spleen[1,2,16].

Iron absorption, transport, storage, utilisation, recycling and excretion are mostly genetically controlled by effective regulatory metabolic pathways, homeostatic mechanisms and related proteins[1,2]. A large number of iron containing proteins play an essential role in physiological functions such as oxygen and electron transport, DNA synthesis, food oxidation, drug detoxification, *etc.*[1,2,14,19]. Genetic changes, iatrogenic, nutritional and other factors can affect all the metabolic pathways and physiological functions related to iron and result in iron metabolic abnormalities.

General aspects of iron metabolism have been previously reviewed[1,2,14,19]. Basically, under normal conditions iron is absorbed from the gut then transferred to transferrin in the blood which distributes and delivers iron to the tissues primarily for storage in the liver and utilisation in the production of haemoglobin in the bone marrow. Different but smaller amounts of iron are distributed to other cells and tissues primarily for storage and utilisation for the synthesis of iron containing proteins. Iron from the catabolism of haemoglobin of effete red blood cells is recycled and redistributed by transferrin.

The transport and distribution of iron is tightly controlled. Under normal conditions transferrin is saturated 25%-35% with iron. The intracellular uptake of iron from transferrin and its storage or utilisation in the cells is regulated by the iron regulatory proteins through the translational control of the synthesis of the transferrin receptors at the cell surface and also that of intracellular ferritin. The amount of iron delivered to cells is mainly determined by the number of transferrin receptors and also the iron saturation of transferrin[1,2,14,19].

Cellular iron export is controlled by ferroportin and hepcidin. The latter is an iron-regulatory 25 amino acid peptide hormone produced by the liver. Serum hepcidin concentrations appear to correlate with liver hepcidin mRNA expression, transferrin saturation and nonheme liver iron[1,2,21]. It also appears that hepcidin controls iron export by binding to the protein ferroportin and causing its internalization from the cell surface and subsequent degradation. In general, it is thought that increased liver hepcidin expression decreases the activity of the cellular iron exporter ferroportin. For example in hereditary hemochromatosis, decreased activity of hepcidin in the enterocyte will increase basolateral iron transfer into plasma and consequently cause an increase in dietary iron absorption[22,23]. Hereditary hemochromatosis is mainly caused by a mutation in the *HFE* gene that involves the HFE protein which is predominant in the expression of hepcidin[24]. In contrast, in the anaemia of chronic disease the opposite action, *i.e.,* increased activity of hepcidin in the reticuloendothelial macrophages would decrease iron transfer to plasma and consequently cause

a decrease in the transport of iron to the bone marrow and reduction in haemoglobin production.

In general iron balance in normal individuals is achieved when the rate of dietary iron absorption is equivalent to the rate of iron utilisation and excretion[25]. Iron imbalance can occur due to genetic, regulatory, environmental, iatrogenic and dietary factors. The imbalance is usually related to changes in the rate of iron absorption, utilisation, distribution, excretion, blood loss and intake from transfusions. Iron deficiency for example can occur if the rate of iron absorption is lower than the rate of iron excretion, *e.g.*, nutritional iron deficiency in vegetarians[25]. Similarly, iron deficiency can occur if the rate of the iron utilised, *e.g.*, by the foetus and the mother in pregnancy is higher than the rate of iron absorbed. Another example is the anaemia of chronic disease where iron is diverted and accumulated in the reticuloendothelial system instead of the erythropoietic tissues resulting in anaemia. Blood loss from trauma, haemorrhage and blood donation can also result in iron deficiency anaemia.

In contrast, in iron overload the rate of iron absorption is higher than the rate of iron excretion, *e.g.,* in hereditary haemochromatosis[26,27]. Iron overload can also be caused by regular red blood cell transfusions in conditions such as in thalassaemia, myelodyspasia and sickle cell diseases[7,8,28]. In contrast to the tissue damage observed in hereditary haemochromatosis and thalassaemia, which proceeds progressively for several years, the tissue damage observed in iron poisoning from the accidental ingestion of oral iron preparations is an acute form of iron toxicity and can be fatal in most cases within hours or days unless emergency treatment is provided[29,30].

Overall, many abnormalities exist in relation to body iron balance and distribution, the iron containing proteins and their function and the regulation of the iron metabolic pathways. Many of these iron abnormalities can lead to a number of serious diseases. Within this context, our understanding of the molecular aspects and metabolic pathways related to iron and chelation therapy, as well as other therapeutic interventions can improve therapeutic targeting in diseases of iron metabolism. At the same time misinformation on the iron metabolic pathways may lead to the development of ineffective or potentially toxic therapeutic interventions.

The spectrum of therapeutic interventions in relation to iron metabolism is not limited only to abnormalities of iron metabolism but is extended to many other pathological conditions since iron is playing an important role in the growth and development of all type of cells including normal, microbial and cancer cells. Furthermore iron plays an important role in free radical metabolism and pathology, which is a key factor in tissue damage in almost all pathological conditions[10,11,19].

Simple and inexpensive therapeutic procedures such as iron supplements to treat iron deficiency anaemia and red blood cell transfusions to treat refractory anaemias are widely used. In contrast, venesection is widely used in blood donation and to treat hereditary hemochromatosis[27]. Similarly, erythropoietin in combination with iron is used in the treatment of the anaemia of chronic disease. The therapeutic targeting and interventions can involve many other aspects of the iron metabolic pathways including genetic manipulation, biological therapies using antibodies against regulators, *e.g.*, hepcidin and erythropoietin or antibodies against receptors, *e.g.*, transferrin receptors, *etc*[31,32].

A major role in the development of therapeutic strategies in the treatment of abnormalities of iron metabolism is the design of targeted therapies using iron chelators. Within this context, although the primary therapeutic role of iron chelating drugs is the treatment of transfusional iron overload, many other possible applications of chelators involving all metabolic aspects of iron could be developed. For example the iron chelating drugs DF and L1 could be used in the detoxification of other toxic metals such as aluminium overload, as antioxidants or as antimicrobial agents, *etc*[19,33-35].

THERAPEUTIC APPLICATIONS AND CONTROVERSIES IN THE USE OF CHELATING DRUGS IN IRON METABOLIC DISORDERS

Chelating drugs and chelators could in principle affect and target all the metabolic pathways and proteins involved in iron metabolism either directly through iron binding or indirectly through the intracellular iron pools. They can also affect other metabolic pathways indirectly which are related or influenced by chelation of other metals or related to other aspects of the chelator molecular structure not related to iron[14].

In principle iron chelators can remove, donate and exchange iron, form ternary iron complexes with proteins, other chelators or ligands. They can also be involved in redox reactions mainly with iron and copper and proteins carrying these metals. The chemical, biological, pharmacological and toxicological properties of the chelators are different to those of their iron complex or their metabolites. Chelators have to compete for iron at all the stages of iron absorption, storage, utilisation and excretion with endogenous natural low molecular weight chelators such as citrate, glutathione, ATP, ADP, *etc.*, and also with protein chelators such as transferrin, lactoferrin, haem containing proteins *etc*[14,18,36]. Similarly, the presence of other metals may interfere with chelator iron binding and chelators may affect the metabolic pathways of other metals[37,38]. Overall many interactions can affect the efficacy and toxicity of the chelating drugs *in vivo*[14,36,37].

The mode of action, efficacy and toxicity of the iron chelating drugs DF, L1, DFRA and of other iron chelators are directly related to their physicochemical, pharmacological, toxicological, iron binding and other

Table 1 Property differences and mode of action of chelating drugs

Recommended doses for the chelating drugs in thalassaemia patients
DF subcutaneously 40-60 mg/kg per day; Oral L1 75-100 mg/kg per day; Oral DFRA 20-40 mg/kg per day
Transfusional iron loaded patient compliance with chelating drugs
Low compliance with DF in comparison to oral L1 and oral DFRA
Increase in iron excretion and route of elimination in iron loaded patients
L1: Urinary iron; DFRA: Faecal iron; DF: Urinary and faecal iron
Effect of chelating drugs on iron absorption
Increase of iron absorption by the lipophilc maltol, 8-hydroxyquinoline and DFRA. Decrease of iron absorption by the hydrophilic DF, EDTA, DTPA and L1
Iron removal from diferric transferrin in iron loaded patients
About 40% at L1 concentrations > 0.1 mmol/L, but not by DF or DFRA
Differential iron removal from various organs of iron loaded patients
L1 preferential iron removal from the heart and DFRA from the liver
DF from the liver or heart. (Efficacy is related to dose for all chelators)
Iron redistribution in diseases of iron metabolism by chelating drugs
L1 and to a lesser extent DF can cause iron redistribution from the reticuloendothelial system to the erythron in anaemic rheumatoid arthritis patients. DFRA may cause redistribution of iron from the liver to other organs in thalassaemia and other iron loaded patients. Enterohepatic circulation by DFRA and metabolites
Increase excretion of metals other than iron, *e.g.*, Zn and Al
Order of increased Zn excretion in iron loaded patients: DTPA > L1 > DF
DF and L1 cause increase Al excretion in renal dialysis patients
DFRA causes an increase in Ca excretion and Al absorption (?)
Iron mobilisation and excretion of chelator metabolite iron complexes
Several DF metabolites have iron chelation potential and increase iron excretion but not L1 glucuronide
Chelating drugs minimising other drug toxicity
L1 but not DFRA, inhibit doxorubicin induced cardiotoxicity
Combination chelation therapy
L1, DF and DFRA combinations are more effective in iron excretion than monotherapy. The ICOC L1 and DF combination causes normalisation of the iron stores in thalassaemia patients
Chelating drug synergism with reducing agents
Ascorbate act synergistically with DF but not L1 for increasing iron excretion
Chelating drug antioxidant effects
L1 and DF have shown antioxidant action in *in vitro*, *in vivo* and clinical settings. The antioxidant effects of DFRA are under evaluation

L1: Deferiprone; DF: Deferoxamine; DFRA: Deferasirox; ICOC: International Committee on Chelation; DTPA: Diethylenetriaminepentaacetic acid; EDTA: Ethylenediaminetetraacetic acid.

properties (Figure 1 and Table 1). Within this context the property differences and mode of interactions with different molecular targets are the most important and critical parameters determining the specificity of the iron chelating drugs and also their targeting profile for the treatment of iron overload and other diseases (Table 1)[14,36].

The primary use of the chelating drugs is the treatment of iron overload in thalassaemia and other transfusional iron loaded conditions. Iron overload toxicity from chronic transfusions involves multi-organ damage and low life expectancy. In the absence of chelation therapy thalassaemia patients die by the age of 20 years, mainly from congestive cardiac failure caused by cardiac iron overload toxicity[5,7,8].

There are big differences in the efficacy, tolerance, site of action, toxicity profile and the cost of the chelating drugs, which affects the morbidity and mortality of thalassaemia patients both in developed and developing countries (Table 1)[5,7,8].

There are also general variations among patients in response to each chelating drug, which is related to their differences in the absorption, distribution, metabolism, elimination and toxicity[5,9,39-41].

The recommended doses for the chelating drugs in thalassaemia are 40-60 mg/kg per day for subcutaneous DF, 75-100 mg/kg per day for oral L1 and 20-40 mg/kg per day for oral DFRA. Compliance is low with subcutaneous DF in comparison to oral L1 and DFRA. The site and level of iron removal is different among the chelators with L1 being the most effective in iron removal from the heart resulting in an increase in life expectancy in thalassaemia patients that have been using it in the last two decades[9,42]. In contrast, high morbidity and mortality have been reported in different categories of patients that have been treated with DFRA[43,44]. The efficacy of iron removal from thalassaemia patients by DFRA is lower than DF or L1, especially regarding iron removal from the heart[45]. The most effective treatment of cardiac iron overload are selected combinations of L1 and DF[46].

Many of the controversies in the use of chelating drugs arise from the different influences and priorities for use by the regulatory authorities, clinicians and patients[45]. For example, there is no consensus in the ultimate goal or aim of the chelation therapy in thalassaemia and other transfusional iron loaded conditions or the selective use of each of the chelating drugs for optimal therapy. There is also no consensus in the evaluation criteria and risk/benefit assessment for the use of each of the chelating drugs in personalised medicine[47]. In most countries the selection of the chelating drug for the treatment of iron loaded patients depends on the commercial influence of pharmaceutical

companies[5]. The situation regarding the use of the chelating drugs in the developing countries where most patients live is not only concerning issues related to the risk/benefit assessment but mainly issues regarding their availability and cost. Such issues have been recently highlighted within the broad context of the use of orphan drugs in orphan and rare diseases which includes thalassaemia and other transfusional iron loaded conditions[5].

Recent developments involving mainly clinical findings and the application of new diagnostic techniques such as magnetic resonance imaging (MRI) T2 and T2* has increased our understanding of iron metabolic and chelation pathways of iron removal and resulted in improved drug targeting therapies of iron toxicity[48-50]. These developments increased the prospects of the introduction of personalised medicine in thalassaemia and other iron metabolic disorders. Based on these findings the complete treatment of iron overload and reduction of morbidity and mortality in thalassaemia using L1 or the L1/DF combination has been recently achieved[9].

Similarly, recent developments involving the prospect of wider use of chelating drugs and in particular of L1 as a universal antioxidant in non iron overload diseases such as neurodegenerative, cardiovascular, renal, infectious diseases as well as other diseases including cancer and ageing has been investigated in clinical trials and within the broad context of the risk/benefit assessment because of the absence of other effective therapeutic approaches and developments in many of these conditions[9,36,51].

The introduction of L1 for the treatment of non iron loaded patients by targeting focal toxic iron deposits, *e.g.*, in Friedreich ataxia and toxic labile iron, *e.g.*, in diabetic and non-diabetic glomerular disease is a reflection of the antioxidant and safety potential of this drug[10,11,19]. The safety of L1 in many categories of non iron loaded diseases has also been confirmed in clinical trials involving patients with the anaemia of chronic disease, renal dialysis, infections, Parkinson's and other neurodegenerative diseases, *etc*[10,11,19]. As in many other cases of drug development the introduction prospects of L1 in these diseases is based on commercial and not ethical criteria[5].

CONTROVERSIES REGARDING MOLECULAR ASPECTS OF IRON METABOLIC DISORDERS AND CHELATOR INTERVENTION

Normal iron metabolism is generally characterised by the normal function, pathways and activity of iron containing proteins including physiological levels of haemoglobin, serum ferritin, serum iron, serum transferrin saturation, liver and other organ iron store levels, *e.g.*, those estimated by MRI, *etc*[48-50]. These physiological levels are the main regular parameters measured in clinical laboratories and MRI units for the identification of iron overload and other metabolic abnormalities.

Many of the disease models related to iron metabolic abnormalities appear in general to be affected by genetic, regulatory and iatrogenic factors. However, like in all other diseases there are different levels of pathological and compensatory mechanisms working in parallel with the main disease pathways and mechanisms. Similarly, there are also many other factors such as dietary, pharmacological and environmental factors that can influence or supersede the normal pathways and affect the levels of iron, as well as the prognosis and treatment of patients with iron abnormalities.

Some compensatory mechanisms of limited impact observed in beta thalassaemia are related to the variation of the age range of survival of non transfused patients. In these cases despite the absence of the production of normal haemoglobin (HbA) the survival is not uniform and can range from 1 to 7 years. The difference in the survival age among this group of patients appears to be related to a number of factors. For example beta thalassaemia patients producing higher levels of foetal haemoglobin (HbF) have increased survival prospects and agents inducing the production of HbF are the subject of clinical investigations and development for the treatment of beta thalassaemia[52,53].

Another compensatory mechanism in iron metabolism is observed during venesection in hereditary haemochromatosis and blood donation where stored iron mainly originating from the liver is steadily transported to the bone marrow for restoring iron balance and the normal production of haemoglobin.

Variations in the progression of neurodegeneration, cardiomyopathy and other toxic side effects observed among Friedreich Ataxia patients is thought to be related to the production of the protein frataxin and many other factors influencing the rate of accumulation and toxicity of iron in mitochondria[54,55].

Many other iron regulatory and compensatory mechanisms operate under normal conditions and iron metabolic disorders. One major intervention mechanism or pathway that can supersede many regular pathways and can affect many diseases of abnormal iron metabolism is targeted chelation therapy. Within this context, most physiological process related to iron can be affected including iron absorption, excretion and delocalisation[14,36].

MECHANISMS OF IRON ABSORPTION AND THE INFLUENCE OF CHELATORS

Body iron intake under normal conditions is mainly controlled by the rate of iron absorption and the rate of iron turnover in the bone marrow for the production of haemoglobin and red blood cells. In considering the iron absorption mechanisms the main classical pathway is thought to involve the iron uptake from the gut lumen

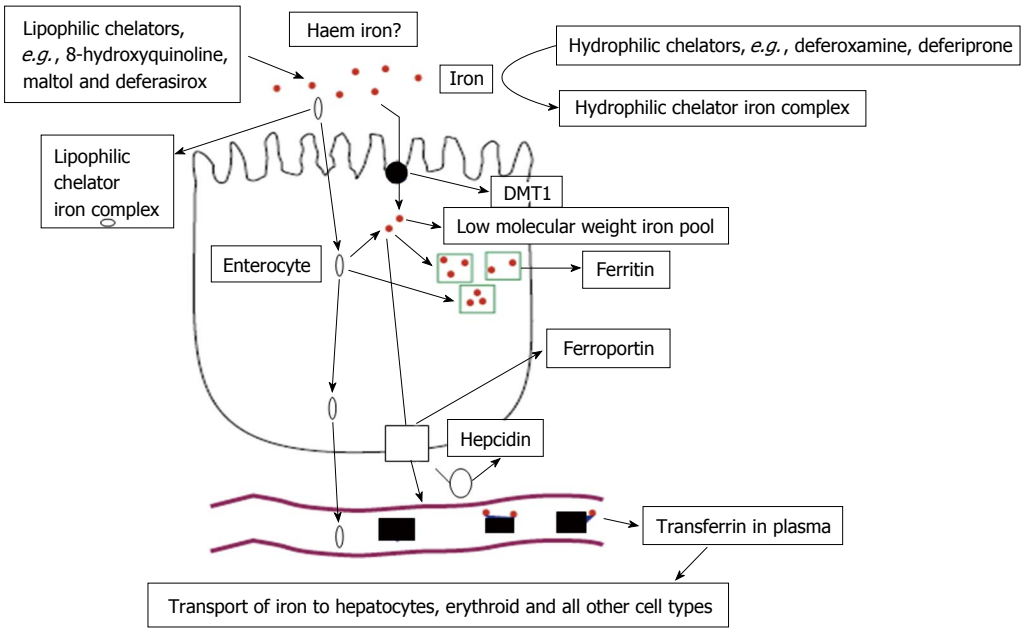

Figure 2 Iron absorption mechanisms at the enterocyte. Under normal conditions the regulatory pathway of iron absorption at the enterocyte involves the regulatory molecules DMT1, hepcidin, ferroportin and then iron transfer and uptake by transferrin in plasma. A parallel pathway of iron absorption may involve lipophilic dietary chelating molecules like maltol. Different pathway of iron uptake by the enterocyte also exists for haem iron. Adapted from ref. [22]. DMT1: Divalent metal transporter 1.

by the enterocytes using the Dcytb and divalent metal transporter 1 pathway, incorporation intracellularly into the low molecular weight iron pool and ferritin. Iron within the enterocyte is then thought to be partially exported *via* the regularly controlled ferroportin/hepcidin pathway, oxidation of iron by hephaestin and lastly uptake by transferrin in plasma for distribution to all cell types of the body and in particular the hepatocytes for storage and the erythroid cells for the production of haemoglobin (Figure 2)[1,2].

Despite that this may appear to be the main iron absorption pathway under normal conditions there are clinical and laboratory evidence of alternative independent mechanisms operating at different levels (Figure 2)[22]. Clinical evidence for the operation of alternative pathways of increased iron absorption which supersedes the main mechanism is observed in the use of iron supplements and food fortification, also in Bandu siderosis where excess iron is absorbed from iron pots used for cooking and lastly in acute iron poisoning from the accidental ingestion of tablets or other oral iron formulations[25,29,30]. In all the above cases the presence of increased quantities of iron in the gut results in excess iron absorption, transport and deposition in the body[22,25]. It appears from these and also other cases that the rate of iron absorption partly depends on the quantity of iron present in the gut[25,26].

In addition to the quantity, the quality of iron presented in the gut lumen is another determining factor affecting iron absorption with ferrous and haem iron being more readily absorbed than ferric iron (Figure 2)[22,25,26]. Another, more effective pathway that supersedes the main pathway and causes substantially higher amounts of iron absorption is lipophilic iron chelator complexes including different haem compounds, which may have a use in the treatment of iron deficiency anaemia (Figure 2)[22]. For example the long term oral administration of the lipophilic chelator 8-hydroxyquinoline caused iron overload in animals and also oral administration of several lipophilic iron complexes such as those of 8-hydroxyquinoline, 2-hydroxy-4-methoxypyridine-1-oxide and maltol caused several fold increases of iron absorption in comparison to animals used as controls (Figures 1 and 2)[56,57]. Maltol in particular, was originally identified as a chelator intended for clinical use in iron deficieny at the same time that L1 was identified for the treatment of iron overload[58]. Maltol also caused increased iron absorption in several clinical trials and in particular it reached phase Ⅲ clinical trial stage in patients with iron deficiency anaemia with inflammatory bowel disease[59,60].

In contrast to lipophilic chelator iron complexes, chelators forming charged hydrophilic iron complexes such as DF and L1 or chelators causing iron precipitation such as phytates and tannins appear to decrease iron absorption and may have a use in the treatment of thalassaemia intermedia and hereditary haemochromatosis[22,57]. Similarly, chelators inhibiting iron absorption and the prevention of iron uptake by the cancer cells of the colon may have a preventative and therapeutic use in the iron induced colorectal cancer[61]. It is envisaged that overall many naturally occuring dietary compounds and medicinal drugs with chelating properties will affect iron absorption in a manner similar to that observed by lipophilic and hydrophilic chelators[18,25].

Another controversial issue in the mechanism of iron absorption which is also promoted in textbooks for cellular iron export is the suggestion of the presence or

need of an oxidation pathway for iron by hephaestin or caeruloplasmin before iron chelation by transferrin. This process and suggested pathway is questioned since transferrin has strong ferroxidase activity similar to the chelating drugs L1 and DF, oxidising Fe (Ⅱ) to Fe (Ⅲ) before chelation and ferric complex formation. In fact, the ferroxidase and iron binding activity of transferrin is one of the most effective and efficient antioxidant systems operating in blood plasma and no mediator protein is required or envisaged to participate in this process[62,63].

In addition to chelator iron uptake and transfer pathway by transferrin, many other pathways and mechanisms are thought to operate in parallel with the main proposed mechanisms. It should be noted for example that even in the case of the rare disease atransferrinaemia, iron is absorbed and finds its way to the liver and the erythropoietic tissues, suggesting that a compensatory mechanism is in operation in addition to transferrin for iron transport in blood and supply to the tissues[64]. Although this secondary pathway is not as efficient and leads in the long term to iron toxicity, the mechanism operating is not clear but resembles or is related to another controversial issue of iron metabolism namely NTBI.

The formation and potential toxicity of NTBI has been previously discussed and reviewed with different opinions on the impact on iron overload and other diseases[62,63,65,66]. Almost all thalassaemia patients with serum ferritin greater than 500 μg/L appear to have fully saturated transferrin and different amounts of NTBI[62,63,65]. Despite that there is evidence of oxidative stress toxicity caused by NTBI in iron overloaded thalassaemia, hereditary haemochromatosis and other categories of patients, there is no evidence that the level of toxicity by NTBI is sufficient to cause tissue damage. In contrast, the level of excess deposited iron and especially of haemosiderin iron is considered the main cause of tissue damage and organ toxicity (*e.g.*, heart, liver, pancreas, *etc*.) in iron overload in thalassaemia and other conditions[48-50,67].

Another controversy in relation to the NTBI toxicity in clinical practice is the regulatory health authorities approved administration of intravenous iron which is widely and routinely used in renal dialysis, inflammatory bowel disease and many other categories of anaemic patients. The amount of NTBI formed during intravenous iron is much higher than thalassaemia or other iron loading conditions but no permanent or serious iron related toxicity has generally been reported[68,69].

MECHANISMS OF IRON EXCRETION AND THE INFLUENCE OF CHELATORS

Iron excretion is a major area of iron metabolism, which however is generally neglected in comparison to iron absorption and other pathways of iron physiology. The mechanisms and pathways of iron excretion and iron loss as well as their implication on the body iron status have been previously reviewed[25,70]. Despite the fact that the presence of a regulatory iron excretion model has not yet been fully explored, such a pathway plays an important role in iron balance. For example, iron deficiency anaemia in adults under normal conditions can only be manifested if the rate of iron excretion or loss is higher than the rate of iron absorption[25,70].

In general several factors such as the body iron load, plasma iron concentration, physical activity, infections, pathological conditions and dietary habits affect the level of iron excretion[25]. The presence of regulatory iron excretion is also supported by other clinical findings such as the slow but steady reduction in the iron load of transplanted ex-thalassaemia patients in the absence of chelation or venesection[71,72].

The concept of iron excretion is mostly highlighted in studies involving iron chelation therapy in conditions of iron overload and also in iron balance studies of non iron loaded conditions. In the latter cases there have been reports of decrease in haemoglobin levels following treatment using L1 for several months, *e.g.*, in Friedreich ataxia patients[73]. In iron overload the level of iron excretion generally depends on the chelating drug and the dose used and also the body iron load of the patients[74]. The iron pools affected during the iron mobilisation and the routes of excretion (faecal and or urinary) vary among the chelating drugs and other chelators[14,25,47]. In the case of L1 iron is excreted almost exclusively in the urine, DFRA is almost exclusively in the faeces and DF mostly in the urine and some in the faeces (Table 1)[14,25].

The efficacy in iron mobilisation of excess stored iron from the organs of iron loaded thalassaemia patients is different among the chelators used with L1 being the most effective in the mobilisation of excess iron from the heart, DF less effective and DFRA the least effective. In contrast DF and DFRA appear to be more effective in the mobilisation of iron from the liver than the heart[45,46,50,74,75]. In most clinical trials studying the efficacy and effects of iron removal by chelating drugs in iron loaded thalassaemia patients the results are inconclusive because of the use of different dose or range of doses[74].

The most effective chelation treatment leading to the complete normalisation of the iron stores in iron loaded thalassaemia patients is the combination of L1 and DF (Figure 3)[76-80]. Specific dose protocols have to be used for this purpose, for example the International Committee on Chelation (ICOC) protocol which consists of daily oral L1 at 75-100 mg/kg per day and subcutaneous DF at 40-60 mg/kg at least 3 d/wk[77]. Thereafter monotherapy of L1 at 50-100 mg/kg per day is sufficient in most cases for maintaining normal range body iron store levels[78,80].

Many naturally occuring iron chelators present in food, usually of plant origin are expected to affect the rate of iron absorption and excretion in a mode of action

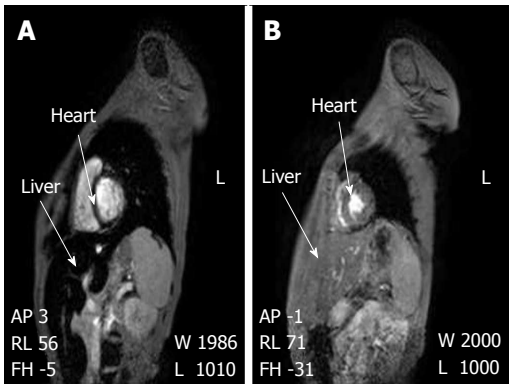

Figure 3 Clearance of iron overload of the liver and heart of a thalassaemia patient using the deferiprone deferoxamine combination. The MR image changes before (A) and after (B) the L1/DF combination therapy. Short axis view of liver and heart of a thalassaemia patient at 4 mo before the L1/DF combination (A: Cardiac T2* was estimated as 9.3 ms and liver T2* as 3.8 ms. The serum ferritin was 727 μg/L, 2.5 mo before the MRI scan) and 9 mo after the combination (B: Cardiac T2* was estimated as 23.0 ms and liver T2* 26.2 ms. The serum ferritin was 166 μg/L, 0.5 mo after the MRI scan). Arrows indicate the liver and interventricular septum of the heart, respectively. Adapted from ref. [74]. MRI: Magnetic resonance imaging; L1: Deferiprone; DF: Deferoxamine.

similar to that described by lipophilic and hydrophilic chelators. Within this context under normal conditions naturally occuring chelators with similar properties to the chelating drugs L1, DFRA and DF are expected to increase iron excretion and affect the overall body iron balance[25]. The chelating efficacy of naturally occuring chelators is concentration dependent and in most cases low and may act synergistically with other chelators or the chelating drugs in iron mobilisation.

On the molecular level iron mobilisation by chelators is thought to proceed at different rates from the available chelatable pools with NTBI to be readily and instantly available by comparison to transferrin iron which is only available to L1 and can take about 1 h to reach completion *in vitro*[58,81]. The reaction is L1 concentration dependent and partial transit de-ironing from transferrin is observed in the serum of iron loaded thalassaemia patients[58,62,63,81-84].

In the intracellular iron mobilisation by chelators, the transit low molecular weight iron pool is readily available followed by haemosiderin and then ferritin iron[85]. The reaction is chelator concentration dependent and takes 2-3 d to reach completion[86]. In the iron mobilisation from ferritin the first in last out principle of iron removal operates. Less iron removal is observed by L1 and other chelators with ferritin molecules containing smaller iron cores in comparison to ferritin molecules containing larger iron cores[87]. It appears that there is lower exposure of the surface iron core to chelators by comparison to larger iron cores[87]. It was also observed that the solubility and mobilisation of iron by chelators increases in ferritin and haemosiderin with newly formed more hydrated oxohydroxy iron cores in comparison to ferritin and haemosiderin with less hydrated older cores of iron oxohydroxy bridges[85,87].

Mobilisation of iron by L1, DF and other chelators from other iron containing proteins, *e.g.*, haemoglobin has not been shown[88]. Exception was lactoferrin where iron removal by chelating drugs has only been shown in the case of L1[89].

CONDITIONS WITH ABNORMAL IRON DEPOSITION AND THE RELOCATION OF IRON BY CHELATORS

Under normal conditions iron is considered to be uniformly distributed in the various organs. In hereditary haemochromatosis the storage of excess iron is primarily in the parenchyma cells of the liver. The storage of excess iron in transfusional iron conditions is mostly in the parenchyma and Kuppfer cells cells of the liver, spleen and cardiocytes.

Until recently it was believed that in transfusional iron overload in thalassaemia, iron was uniformly distributed in the various organs and also that serum ferritin and liver iron reflected body iron store levels. However many clinical findings and iron load estimations using MRI T2 and T2* suggests that serum ferritin is in most cases only related to liver iron stores but not to spleen, heart and pancreas iron load[50,90-93]. It was also observed using MRI that in many thalassaemia patients the liver is overloaded with iron but the heart has normal iron range levels. In contrast, in some thalassaemia patients the reverse is true, i.e., the heart is overloaded with iron but the liver has normal iron range levels (Figure 4)[14,90,93]. This last finding provides an explanation for many of the fatal cases of thalassaemia patients prior to the introduction of MRI, who died from congestive cardiac failure despite very low serum ferritin and liver iron concentration. Within this context, the prophylactic use of L1 is essential for preventing cardiac damage[94,95].

The role of spleen as a major iron storage organ, sometimes of equal importance to liver iron storage and also in the ferrikinetics of iron overload in thalassaemia patients was highlighted in a number of studies (Figure 5)[96,97]. Despite that an increase in haemoglobin was expected following splenectomy in thalassaemia patients the substantial increase in serum ferritin provided further evidence that serum ferritin is not related to total body iron load but mostly to the concentration of stored iron in the liver[95]. Furthermore, following splenectomy excess iron may be diverted to the heart causing myocardiac iron loading and cardiomyopathy[97].

In general, it appears that serum ferritin and liver iron estimations are misleading regarding cardiac and other organ iron load as well as total body iron load in thalassaemia patients[93]. MRI T2 and T2* findings also appear to suggest that in many cases of iron loaded thalassaemia patients the deposition of iron in the liver, spleen and heart is not uniformly distributed within each organ[50]. These mosaic iron distribution of dense and light iron deposits in the liver and heart was particularly evident during the normalisation of the iron stores of

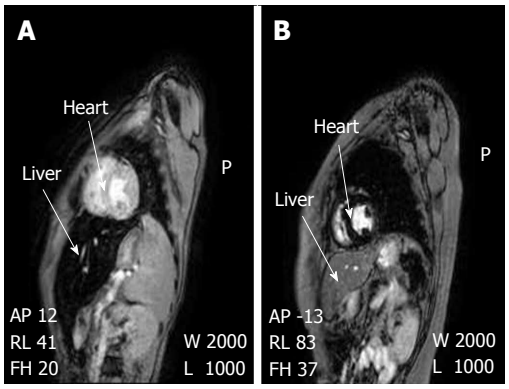

Figure 4 Non homogeneous iron distribution among the organs of iron loaded thalassaemia patients. Differential iron loading of the heart and liver of two iron loaded thalassaemia patients using MRI and T2* estimation. A: Heavy haemosiderosis of the liver [T2* = 1.2 ms (normal T2* ⩾ 6.3)] and normal T2* of the heart (T2* = 20.6). The top arrow shows the interventricular septum of the heart of the patient with no iron deposition (normal) where the bottom arrow shows the heavy iron loading within the liver parenchyma, demonstrated as low signal intensity (dark); B: Heavy haemosiderosis of the heart (T2* = 6.32 ms) and normal T2* of the liver (T2* = 19.2 ms). The top arrow shows the abnormal iron deposition in the interventricular septum of the heart of the patient, which is shown with low signal intensity (dark). The bottom arrow shows the liver of the patient with no iron deposition (normal). Adapted from ref. [14]. MRI: Magnetic resonance imaging.

thalassaemia patients treated with the L1/DF ICOC combination protocol[50]. Similar findings of non uniform iron distribution are observed in liver and spleen biopsies (Figure 5). These findings provide an explanation for the high level of error of liver biopsies for estimating iron load which was previously observed in many studies with thalassaemia patients.

There are many acquired and hereditary conditions with abnormal iron distribution leading to body iron imbalance and in many cases specific tissue iron localisation and anaemia. In the anaemia of chronic disease iron is mostly stored in the cytoplasm of reticuloendothelial macrophages. This form of anaemia is observed in many chronic inflammatory and other conditions such as rheumatoid arthritis, chronic kidney disease and cancer[98,99]. It is believed that in these and other conditions there is an increased production of hepcidin and decrease in the ferroportin activity of the reticuloendothelial macrophages. These changes cause a decrease in iron transfer from the reticuloendothelial macrophages into plasma and subsequently reduction of iron availability to the bone marrow, reduction in haemoglobin production and consequently anaemia[1,2,98,99].

A similar mechanism of increased hepcidin production leading to plasma iron reduction is thought to operate in the hypoferremia of infectious diseases. This mechanism appears to reduce transferrin bound iron and iron bioavailability to the siderophores of microbes restricting their growth[100,101]. This mechanism is important for iron loaded patients who are more susceptible to siderophilic bacteria infections and have increased incidence of morbidity and mortality associated with infections[35,102]. A hepcidin independent pathway for the hypoferraemia in infections has also been identified[103]. Within this context pharmacologic modulation of iron metabolism and chelation therapy may be potential strategies to control infection[35,63].

There are many other diseases of abnormal iron deposition which originate from inherited, environmental, iatrogenic and metabolic factors with different health implications. For example increased iron accumulation and deposition is observed in mitochondria in sideroblastic anaemia and Friedreich Ataxia but not in the mitochondria of iron overloaded thalassaemia or hereditary haemochromatosis patients[54,55,95,104-106]. Furthermore, despite that iron is also diverted and causes mitochondrial iron deposition and anaemia in sideroblastic anaemia patients, in general no anaemia or abnormal serum iron or serum ferritin levels are observed in Friedreich Ataxia patients[9,107-109].

The localisation of focal deposited iron in the brain has been recently identified by MRI in many neurodegenerative and other diseases such as Friedreich Ataxia, Parkinson's and Alzheimer's diseases and Hallevorden-Spatz syndrome[110-115]. However, a major difference between the above conditions and iron overloaded thalassaemia patients is that in the latter group of patients there is no iron accumulation in the brain or related toxic side effects involving the nervous system.

Chelation therapy could be introduced in many of the abnormally localised deposited iron conditions described above by bypassing the related mechanisms and may lead to the correction of the abnormality. Such intervention may restore iron balance, eliminate the associated iron toxicity or reduce the anaemia. Within this context a number of clinical trials were carried out using chelating drugs in different categories of patients where iron was not normally distributed.

In one study the effect of L1 chelation therapy was investigated in the anaemia of chronic disease using a group of anaemic rheumatoid arthritis patients including some not responding to erythropoietin. The patients were treated with L1 up to 2 × 2 g/d for a week. A substantial increase in haemoglobin levels were observed at the end of the study[116,117]. The mechanism operating in this group of patients treated with L1 was thought to involve several stages. In the initial stage, the mobilisation of stored iron by L1 from different sites including the reticuloendothelial macrophages was anticipated as previously shown with *in vitro* macrophage cell studies[118]. In the subsequent stage, the iron mobilised by L1 was thought to be partly donated to unsaturated transferrin increasing transferrin iron saturation as previously shown with *in vitro* studies and the 7 h progressive increase in transferrin iron saturation of up to 80% in normal volunteers treated with L1[83,119]. In the last stage iron saturated transferrin increases the transfer of iron to bone marrow and other erythropoietic tissues causing an overall increase in the production of haemoglobin[116,117].

These studies suggest that the chelation pathway may compete and override the hepcidin and erythropoietin

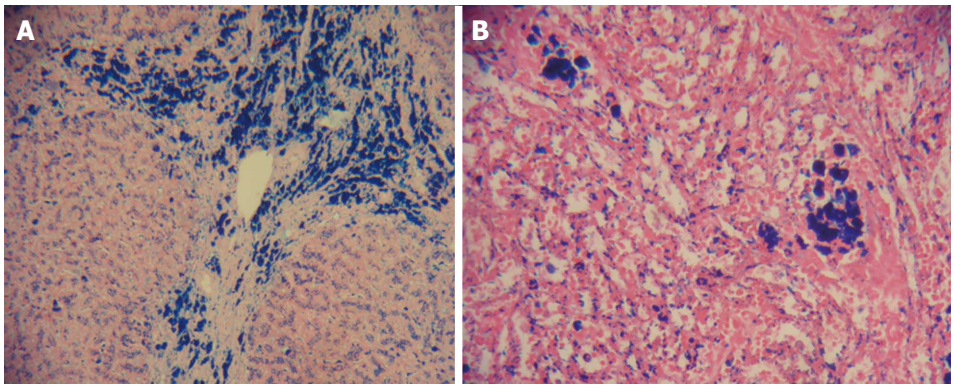

Figure 5 Non homogeneous iron distribution in the liver and spleen of an iron loaded thalassaemia patient. Liver and spleen biopsy photographs (× 20) of a 29-year-old, 55 kg male thalassaemia patient. The liver biopsy was obtained during splenectomy. A: Liver section showing non unifom iron deposition stained with Pearl's Prussian blue. There are hemosiderin deposits in hepatocytes and Kupffer cells and especially within bile ducts; B: Spleen section where iron deposits were stained with Pearl's Prussian blue. There are non uniform hemosiderin deposits within cytoplasma and nucleus of macrophages. Four months before the splenectomy the patient had an MRI T2* (ms) of heart 4.1, liver 0.0, spleen 2.9, and serum ferritin of 3850 μg/L. Adapted from ref. [96]. MRI: Magnetic resonance imaging.

pathways in the anaemia of chronic disease.

Similar results of focal iron deposit removal and relocation was observed in other diseases involving different organs. Iron removal from focal iron deposits in the brain has been shown using L1 in a number of clinical trials involving Friedreich Ataxia patients. In one study nine Friedreich ataxia patients were treated with 20-30 mg/kg per day of L1 for 6 mo. Substantial reduction of the stored toxic iron in the brain was diagnosed using MRI T2* following L1 treatment, which coincided with a reduction in ataxic gait and neuropathy[120]. Similarly, neurological and heart function benefits were identified in further L1 trials in Friedreich Ataxia and other patients[121-124].

Iron toxicity derived from focal or labile iron deposits has also been implicated in the tissue damage of many other diseases. Targeted chelation therapy was also used to prevent or minimize such toxicity. For example encouraging therapeutic results were observed in clinical studies involving about 50 non iron loaded patients with acute kidney disease using L1 at doses of 50-75 mg/kg per day for up to 9 mo[125]. No serious toxic side effects were reported during the studies in this category of patients and L1 was shown to improve kidney function and to cause a decrease in proteinuria[125].

The use of iron chelating drugs in many other conditions such as infections, inflammation, cytotoxic therapies, detoxification of other metals, drug toxicity as well as many other conditions involving proteins and pathways of iron metabolism is currently in progress[9,14,19,22,63]. However, many therapeutic developments are almost exclusively based on commercial and not ethical considerations[5,126-130]. Furthermore the impact and significance of academic findings in relation to therapeutic developments and their applications in medicine is the subject of selective promotion by editorial boards of medical journals most of which are commercial organisations, with commercial connections and interests.

THE ROLE AND CONTROVERSIES OF MEDICAL JOURNALS IN SHAPING MEDICAL OPINION IN IRON METABOLISM AND CHELATION THERAPY

The lucrative revenues of pharmaceuticals which only for the world's twelve richest pharmaceutical companies based in the United States and Western Europe are estimated at 0.5 trillion United States dollars annually, depend on marketing policies and "lobbying" procedures involving physicians, journals, regulatory authorities, patient organisations and other groups[5,131-134]. Within this context there are many grey areas and conflicts of interests regarding the role of pharmaceutical companies and their influence on government, academia, medical journals and many other organisations or institutions[5,131-135].

Medical journals are major contributors in the dissemination of basic and clinical science information which is used to guide physicians and health professionals in the selection of therapeutics, which are important for the patients' treatment, safety, morbidity and mortality. Most of the clinical trials on the effects of therapeutics published by medical journals are authored by academics founded or sponsored by pharmaceutical companies[135]. Similarly, despite that most members of editorial boards and referees of medical journals are affiliated to academic institutions, the commercial influence on academia and in particular the medical journals are increasing. Most publications related to new patented drugs are usually biased in relation to efficacy and safety and are controlled by medical writers affiliated to the pharmaceutical companies[5,131-135]. Such information is recycled with repeated publications and citations of only positive results, which are attributed to only authors collaborating with the pharmaceutical companies.

The role of leading medical journals which are based

in Western Europe and North America in providing unbiased information on new patented drugs is also questioned, since almost all such journals are businesses and dependent on income from the pharmaceutical industry including advertisements, page charges, reprints, conferences, *etc*[5,131-135]. Such journals are leading in the marketing promotion efforts of multinational pharmaceutical industry of new expensive patented products which sometimes are less safe or efficacious than generic drugs. Such promotions are considered to serve also the national interest of both the pharmaceutical industry and medical journals since the lucrative income from new patented drug sales are major contributors to the economy of the developed countries involved. However, these efforts in many cases undermine the safety and therapeutic outcome of many categories of patients because of inaccurate risk/benefit assessments and questionable clinical benefits made by physicians, *e.g.*, in the use of chelating drugs[5].

Within this context some controversial cases of risk/benefit assessments have been previously identified and reported during the marketing drive and promotion of the use of chelating drugs in relation to the treatment of thalassaemia and other conditions[5,43,44,136-140]. In particular the promotion and use of DFRA in hereditary haemochromatosis and ex-thalassaemia transplanted patients instead of venesection raises major ethical questions. Similar questions have been raised in the risk/benefit assesment of the use of DFRA in thalassaemia intermedia instead of L1 or DF[22,138]. Furthermore, many clinical investigators have also questioned the therapeutic benefits from use of DFRA or of other chelating drugs in myelodyspasia and sickle cell anaemia patients[141,142].

One major controversial issue that led to exchanges between the pharmaceutical company marketing DFRA and an author questioning the safety of the use of DFRA in non iron loaded patients was highlighted in the journal *Lancet* and *Expert Opinion in Drug Safety*[43,44,143,144]. While the exchanges were published in the last journal only the pharmaceutical company's view were published in the *Lancet*, overturning the Journal's rules of submission of correspondence including the length and timing of submission. The issue was raised in a letter to the *Lancet* editors asking among other for the declaration of the commercial links of the journal but the letter was not published. Furthermore the same issue and the favouritism for the company marketing DFRA was raised with the Lancet ombudsman, who indicated that he will investigate the issue but for more than two years is still under investigation and no reply was provided nor the *Lancet's* commercial links declared.

Similar issues in relation to chelating drug development were raised with the journal *Annals of Neurology* regarding the use of L1 in Friedreich ataxia patients where the lack of crucial diagnostic and therapeutic outcome procedures in relation to focal iron levels and lack of iron balance studies were questioned[73]. The need for personalised medicine was also raised since there is wide variation in the severity of the disease and level of focal iron deposits in the heart and brain of Friedreich ataxia patients. In this case the editors of the journal referred to "expensive studies to track iron scores" and "the company developing the drug spends millions of dollars". It should be noted that the original proposal for the use of L1 in Friedreich ataxia patients was suggested many years ago and L1 was developed following academic initiatives[5,36].

Commercial and academic conflicts in relation to L1 development are widely published in the medical literature since its discovery[5]. Most of the academics involved in such conflicts were financed directly or indirectly by competing pharmaceutical companies and not related to independent assessment on drug safety and efficacy[5]. Similarly, the implications of drug costs and drug availability to patients especially in developing countries, including that of the iron chelating drugs or other orphan drugs is rarely discussed or highlighted in medical journals[5].

There are many other issues in relation to the role played by medical journals in shaping medical opinion on drug use and development including that of iron chelating drugs. Such issues are many and vary. For example in most publications the ultimate aim of iron chelation therapy, which is the normalization of the iron stores of regularly transfused patients is avoided or not specified[5]. Similarly, the background history and information regarding drug assessment is not thoroughly investigated by the journal editors or specified in future publications even in the same journal. In one case a clinician reported liver toxicity in thalassaemia patients treated with L1 which was not confirmed by any other investigator[5,145,146]. This case reached the mass media and delayed the development of L1 but it may have caused the life of thousands of patients from cardiac failure[5,145,146].

Several other controversies are overlooked in publications related to chelating drug efficacy and development which affect patient safety and therapeutic outcomes. In many cases comparative therapeutic assessments are carried out in clinical trials using different dose protocols of the iron chelating drugs[5]. Similarly some journals overemphasize the importance of diagnostic techniques such as liver iron estimations or of NTBI, which are not critical for the prognosis of thalassaemia patients and other iron overloaded conditions in comparison to cardiac MRI T2* and T2[45,46,65,67]. This issue partly diverts attention from the difference in the ability of chelating drugs in the mobilisation of iron from the heart[12]. Within this context even the assessment of cardiac iron using MRI T2* was questioned when L1 was shown to be superior to DF in the removal of iron from the heart[147-149].

Many medical journals express their medical preferences for selecting articles based only on the opinion of clinical and other investigators associated with pharmaceutical companies, while ignoring any other authors opinion and any new developments for example in the area of chelation[150-152]. The influence of medical journals

is also highlighted by the submission of publications of clinical investigations to the regulatory authorities. For example this resulted in the difference of timing in the regulatory approval of L1 first in India in 1994, then the European Union in 1999 and lastly in the United States in 2011[153]. Another example is the generic chelating drug EDTA which despite its approval about 50 years ago for metal detoxification it has been used ever since by millions of patients as alternative medicine for many conditions (Figure 1)[153-155]. It is only recently that the health authorities in the United States took an interest on its therapeutic properties in cardiovascular and other conditions[155,156].

Many future studies could be performed to elucidate further and improve the role of chelating drugs in iron metabolism and generally in health and disease. For example, the antioxidant role of chelating drugs used as monotherapy or in combination therapies with other antioxidants could be envisaged in different inflammatory conditions[19,157]. Similarly, the use of iron metabolism indices and algorithms could be introduced in different clinical conditions in order to best evaluate iron deficiency or overload and accordingly adapt iron chelation or iron supplementation and other related therapies[158].

CONCLUSION

Iron metabolic disorders affect more than a quarter of the world's population with a different range of health implications and rates of morbidity and mortality. Iron deficiency anaemia is a major health hazard found mainly in developing countries but can be relatively easily treated using iron supplements or lipophilic chelator iron complexes. Similarly, hereditary haemochromatosis can be easily treated using venesection. In contrast, iron overload in transfusional iron overload for example in thalassaemia is fatal unless chelation therapy is introduced. In most cases L1 in combination with DF and L1 monotherapy can completely treat iron overload in thalassaemia. Deferiprone has also been shown to minimise the toxic effects of pathological iron found in neurodegenerative, renal and other diseases. Deferasirox is more toxic than L1 and DF and can mainly be used in patients not tolerating L1, DF or their combination. Controversies in the risk/benefit assessment for the use of DFRA in thalassaemia, other iron overloaded and non iron overloaded conditions appear to involve commercial interests, and influence of academic medical journals and physicians.

In addition to iron overload many other abnormalities related to iron metabolism and toxicity can be treated using chelators. In particular, iron toxicity is a major factor in free radical pathology and tissue damage in many diseases. Iron chelating drugs can correct iron imbalance for example in the anaemia of chronic diseases and can also minimise iron toxicity related to proteins or pathways of iron metabolism.

The role of medical journals in shaping medical opinion and updating biochemical and clinical findings including issues relating to the risk/benefit assessment of drugs as well as drug safety and efficacy are crucial for patient survival, morbidity and mortality. Many controversies in relation to drug development and use with emphasis the iron chelating drugs are widely reported in the medical literature. Within this context commercial influence and contacts of the medical journals with the pharmaceutical industry and other commercial or government organisations should be declared.

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