[Frontiers In Bioscience, Landmark, 23, 1082-1098, January 1, 2018]

Chelation protocols for the elimination and prevention of iron overload in thalassaemia

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1. ABSTRACT

Iron overload toxicity is the main cause of mortality and morbidity in thalassaemia patients. The complete elimination and prevention of iron overload is the main aim of chelation therapy, which can be achieved by chelation protocols that can effectively remove excess iron load and maintain body iron at normal levels. Deferiprone and selected combinations with deferoxamine can be designed, adjusted and used effectively for removing all excess stored iron and for maintaining normal iron stores (NIS) in different categories of thalassaemia patients. High doses of deferiprone (75-100 mg/kg/day) and deferoxamine (50-60 mg/kg, 1-7 days/week) combinations can be used for achieving and maintaining NIS in heavily iron loaded transfused patients. In contrast, deferiprone (75-100 mg/kg/day) can be used effectively and sometimes intermittently for maintaining NIS in non heavily transfused patients. Deferasirox can in particular be used in patients not tolerating deferoxamine and deferiprone. The design of tailored made personalised protocols using deferiprone and selected combinations with deferoxamine should be considered as optimum chelation therapies for the complete treatment and the prevention of iron overload in thalassaemia.

2. INTRODUCTION

All forms of excess iron are potentially toxic mainly because of the ability of iron to catalyse free radical reactions which can cause molecular, cellular and tissue damage (1-4). In transfusional iron overload in thalassaemia and related conditions excess iron is deposited in many organs and can progressively cause cardiac, liver, endocrine and other damage leading to congestive cardiac failure, liver fibrosis and cirrhosis, stunted growth in children, diabetes etc. (5-7).

Iron chelation therapy can decrease the mortality and morbidity associated with iron overload toxicity in thalassaemia and other regularly transfused patients (8,9). This can be achieved using chelating drug protocols that can cause negative iron balance, in which case the amount of iron removed by the chelating drugs is higher than the amount of iron accumulating in the body mainly from the intake of iron from red blood cell transfusions and also from dietary iron absorption (10,11).

The main aim of iron chelation therapy in transfusional iron overload in thalassaemia and other refractory anaemias is the removal of all excess toxic iron and the maintenance of normal iron stores (NIS) (12,13). Normal body iron store levels in thalassaemia patients are associated with an overall decrease in morbidity and mortality due to a decrease in cardiac, liver, endocrinological or other organ damage, which are caused by iron overload toxicity (14,15). In general, NIS can be characterised by normal physiological range of serum ferritin (350 μg/L >), cardiac (> 20 ms) and liver (> 6.3. ms) magnetic resonance imaging (MRI) T2* relaxation time levels (16-19).

It has previously been shown in a number of studies, that NIS in thalassaemia patients can be achieved using specifically effective chelation therapy protocols and in particular the International Committee on Chelation (ICOC) combination protocol

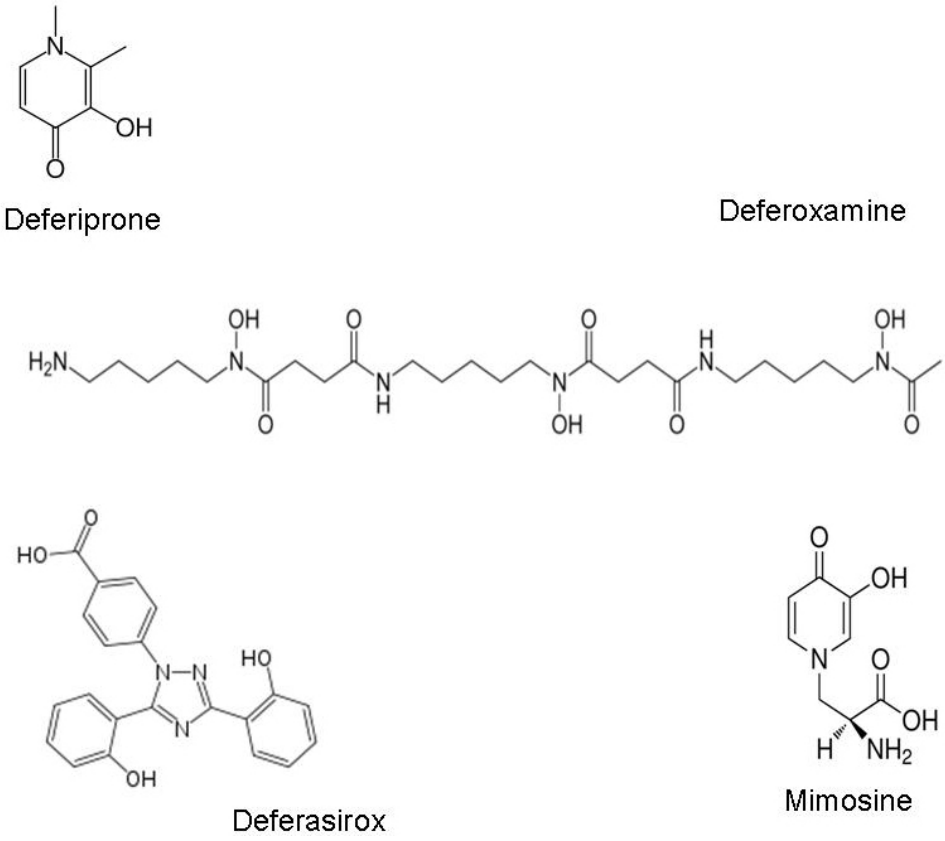

Figure 1. The chemical structure of the iron chelating drugs deferiprone (L1), deferoxamine (DF) and deferasirox (DFRA), which are currently used for the removal of iron in the treatment of thalassaemia and other transfusional iron loading conditions. Mimosine is a natural plant amino acid with iron chelating properties and a potential for clinical use.

of oral deferiprone (L1) (75-100 mg/kg/day) and subcutaneous deferoxamine (DF) (40-60 mg/kg at least 3 days per week), (Figure 1) (11-20). The rate of iron mobilisation and iron excretion in thalassaemia and other refractory anaemia patients using the ICOC or other similar combination protocols depends mainly on the total chelating drug dose, as well as the initial iron overload and the rate of body iron intake, which mostly originates from red blood cell transfusions (11, 13,14, 20, 21).

Many patients with thalassaemia and other refractory anaemias are also using oral deferasirox (DFRA) (Figure 1) (22, 23). Deferasirox can be eso pecially useful for patients not tolerating L1 and DF. Furthermore DFRA's oral administration is generally more convenient for transfusional iron loaded patients especially in comparison to the daily prolonged subcutaneous injections of DF.

Higher chelating drug doses are used for achieving negative iron balance in heavily iron loaded transfused patients in comparison to patients with reduced iron load (13,24). Similarly, overall much lower chelating drug doses are used for maintaining negative iron balance in thalassaemia and other refractory anaemia transfused patients who have already achieved NIS (13,24). Within this context individualised chelating drug protocols can be designed for different categories of iron loaded patients for achieving and maintaining NIS in thalassaemia and other regularly transfused patients (13,24).

3. SELECTION OF EFFECTIVE AND SAFE CHELATING DRUG PROTOCOLS

There is worldwide variation and no consensus in the use of iron chelation therapy protocols for the removal of the excess toxic deposits of iron in thalassaemia and other refractory anaemia patients including the use of the three chelating drugs (L1, DF, DFRA) and their combinations (20-26). The selection of chelating drug protocols for the treatment of regularly transfused patients in most countries is mainly based on many different factors and not necessarily on the efficacy or safety criteria in relation to the chelating drug potential for the complete removal of all excess toxic iron (20-26). Furthermore, the aim of the chelating drug protocol and the targeting methods are not sufficiently specified for achieving non toxic body iron load levels and also most importantly NIS. In this context, chelation therapy protocols for personalised

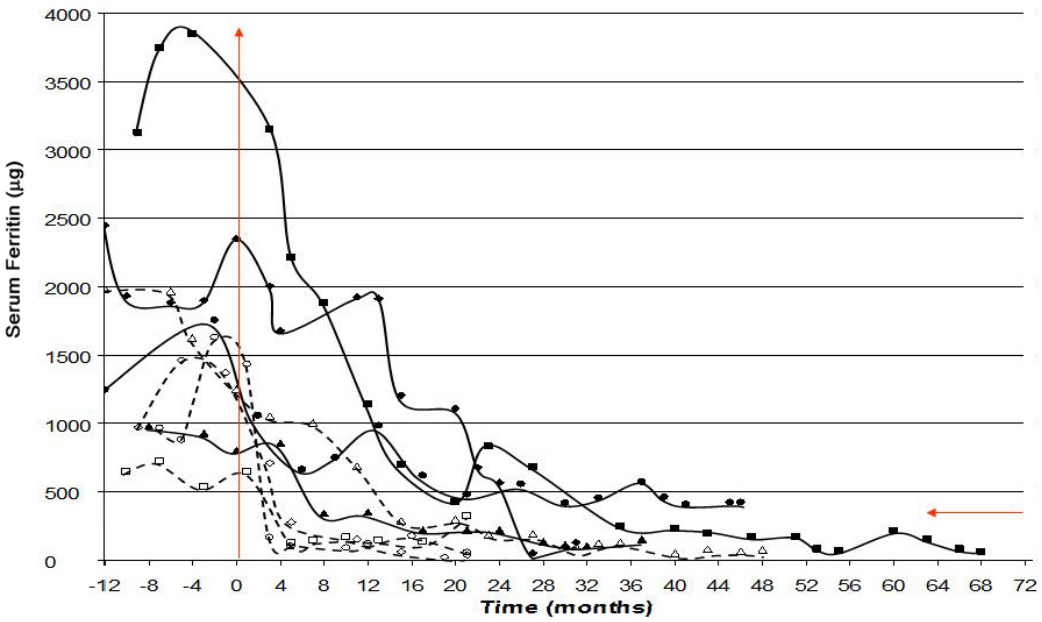

Figure 2. Normalisation of serum ferritin levels using the ICOC deferiprone (75-100 mg/kg/day) and deferoxamine (50-60 mg/kg, at least 3 days/week) combination therapy in thalassaemia major patients. Serum ferritin changes in four male (straight line) and four female (dotted line) patients treated with the ICOC protocol. The combination chelation therapy was initiated at time 0 and lasted 21-68 months. Normal serum ferritin levels are considered to be lower than 350 µg/L. Adapted with permission from reference 13.

medicine for most iron loaded patients worldwide are generally selected at random (20-26).

However, despite the absence of consensus in the selection of chelation therapy protocols there is increasing evidence from a number of clinical studies suggesting that the elimination of excess iron can be safely achieved in almost all categories of patients with different rates of body iron intake from transfusions (13-15,27). Within this context, the use of the ICOC combination protocol of L1 (75-100 mg/kg/day) and DF (40-60 mg/kg at least 3 days per week) and other similar protocols are sufficient for achieving NIS (Figure 2-4) (13-15). Similarly, in most patients the use of L1 monotherapy is sufficient for maintaining NIS (Figure 3-4) (13,27). The introduction of the ICOC and similar protocols leading to the achievement and maintenance of NIS could be considered as the optimum chelation therapy for thalassaemia patients (Table 1) (13,24). Furthermore, based on these initial clinical studies which resulted in the complete removal of excess deposited iron, the current period can be characterised as the golden era for iron chelation therapy in thalassaemia (28).

More diverse experience will be needed in relation to the use of selective personalised protocols for achieving and maintaining NIS in more thalassaemia patient groups, including unusual cases with increased transfusion requirements, as well as cases with other complications such as infections, idiosyncratic drug reactions, hepatocellular carcinoma etc. The continuation of these efforts over longer periods could lead to the major objective namely the establishment of an overall long term strategy for the safe, effective and complete treatment of iron overload in all different categories of patients with thalassaemia and other transfusion related conditions.

The selection of effective and safe iron chelation protocols is very important for the long term survival and the quality of life of thalassaemia and other patients with transfusion dependent refractory anaemias (Table 1). Most importantly, an increase in survival related to a decrease in fatal cardiomyopathies was observed in thalassaemia patients in many countries following the introduction of L1, as a monotherapy or in combination therapies with DF (29-37). Many thalassaemia patients using such effective chelation therapy protocols and in particular L1, have a reduction in iron toxicity related complications such as cardiomyopathies. They have also normal life activities such as jobs, have families and some have exceeded 50 years of age and are reaching life spans similar to those of normal individuals (26, 38,39).

Despite that the ICOC protocols are safely applied in most thalassaemia patients for achieving and maintaining NIS, there are a number of patients who experience toxic side effects with either DF or L1 or both. In these cases, the overall doses of either L1 or DF in the combination may increase to compensate for the overall reduction or withdrawal of the other drug (Table 1) (24). The introduction of DFRA as monothera

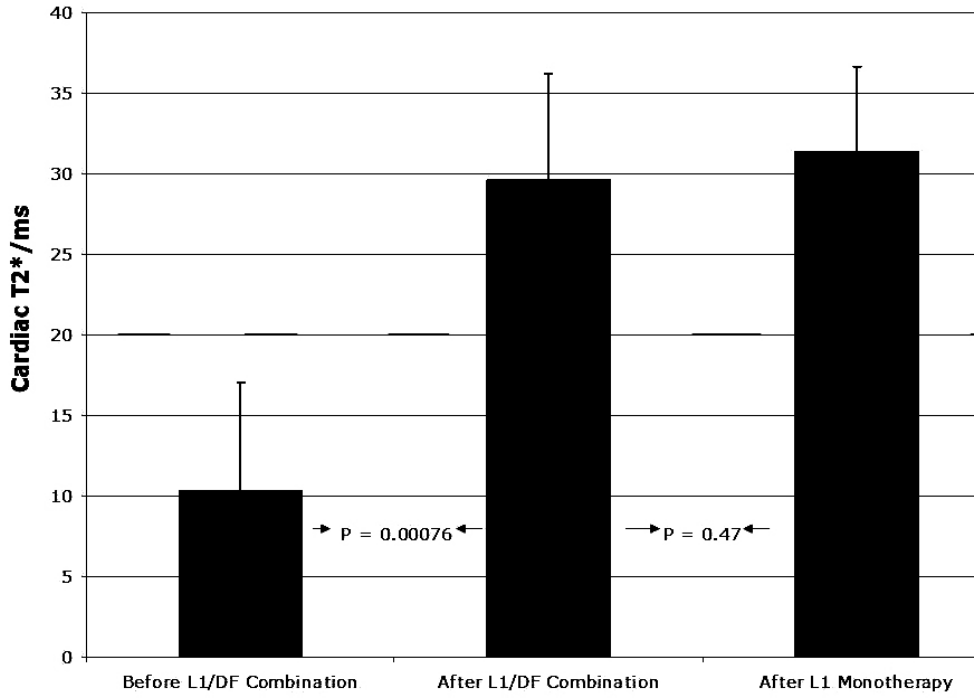

Figure 3. Normalisation of cardiac iron as measured by MRI T2* in the same cohort of thalassaemia patients treated with the ICOC deferiprone/deferoxamine combination therapy for 21-68 months as described in Figure 2 and maintenance of normal iron stores with deferiprone monotherapy (80-100 mg/kg/day) for 2-26 months. The geometric mean (columns), standard deviation and p values are shown. Normal cardiac T2* relaxation time levels are considered to be higher than 20 ms. Adapted with permission from reference 27.

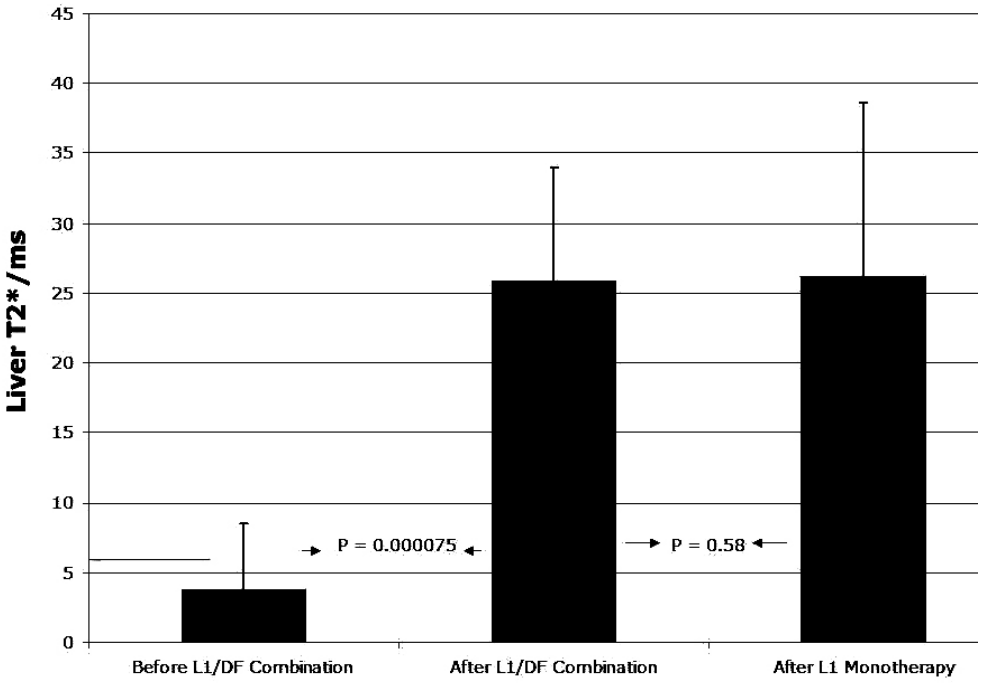

Figure 4. Normalisation of liver iron as measured by MRI T2* in the same cohort of thalassaemia patients treated with the ICOC deferiprone/deferoxamine combination therapy as described in Figure 2 and maintenance of normal iron stores with deferiprone monotherapy (80-100 mg/kg/day) for 2-26 months. The geometric mean (columns), standard deviation and and p values are shown. Normal liver T2* relaxation time levels are considered to be higher than 6.3. ms. Adapted with permission from reference 27.

Table 1. Optimum iron chelation therapy protocols in thalassaemia

Treatment	Name / drugs	Description	Suggested therapy
A] First line treatment	The ICOC deferiprone and deferoxamine combination protocol	Main aim is the complete elimination and prevention of iron overload and toxicity. Secondary aim is the rapid achievement of normal iron stores for the prevention of iron overload cardiomyopathy. Tertiary aim is the prevention of other organ damage caused by iron overload toxicity	Combination of deferiprone and deferoxamine eg the ICOC deferiprone (75-100 mg/kg/day) and deferoxamine (40-60 mg/kg/day, at least 3 days per week) combination protocol for the achievement of normal iron stores.
B] Second line treatment	Deferiprone or deferoxamine monotherapy or their selected combinations.	In these cases the first line treatment of the ICOC combination of deferiprone and deferoxamine could not be implemented due to toxicity and or overall low efficacy by the two drugs. Primary aim is the achievement of normal cardiac iron levels for preventing cardiomyopathy. Effort should also be made to reduce other organ and the total body iron burden.	Combination therapy of deferiprone and deferoxamine at lower tolerated dose protocols than the ICOC combination protocol. Depending on circumstances deferiprone or deferoxamine monotherapy may be used and or alternating therapies by using sequential administration of each of the two drugs.
C] Third line treatment	Deferiprone, deferoxamine and deferasirox monotherapy or selected combinations	Major aim is the maximum iron removal especially from the heart in patients where the first and second line chelation therapy protocols have failed due to toxicity or low efficacy of combinations or monotherapies with deferiprone and deferoxamine.	Alternating sequential chelation of each of deferiprone, deferoxamine and deferasirox. Establishment of the most tolerated chelation monotherapy or deferiprone / deferoxamine or deferiprone / deferasirox combination therapy.
D] Fourth line treatment	Deferasirox monotherapy and deferasirox combination therapies	Main aim is the maximum iron removal, when the first, second and third line treatment with deferiprone and deferoxamine combinations or monotherapies cannot be tolerated due to toxicity.	Deferasirox monotherapy up to the maximum tolerated dose of 40 mg/kg/day. Deferasirox combination therapies with either deferiprone or deferoxamine or both.
E] Chelation therapy for patients with normal iron stores	Mainly deferiprone monotherapy protocols are used for the maintenance of normal iron stores.	Main aim is the safe maintenance of normal iron stores. Deferiprone can be used at 0-100 mg/kg/day in most patients. Deferiprone and deferoxamine combinations may also be used depending on the rate of body iron intake from transfusions.	The deferiprone dose is adjusted according to serum ferritin levels and can be intermittently withdrawn when the serum ferritin levels are about 100 – 300 μg/l. Deferoxamine and deferasirox monotherapies are not recommended for these patients.

apy or combination therapy with either L1 or DF could also be considered in these cases for increasing the overall iron chelation efficacy. Preliminary studies suggest that DFRA, especially in combination therapies may be effective in reducing the excess iron stores in non heavily iron loaded thalassaemia patients (22, 23, 41, 42). However, other studies suggest that DFRA may not be so effective, especially in iron removal from the heart and may also be more toxic than L1 and DF (42-46).

None of the studies so far using DFRA have shown that NIS can be achieved and maintained in thalassaemia patients. In this context, it remains to be proven whether chelation therapy using DFRA may in the long term lead to an increase in the overall survival of thalassaemia patients, as is the case with the cohort of thalassaemia patients treated with the L1/DF combination (29-33). In particular, it remains to be seen whether the unique properties of L1 such as the rapid removal of excess toxic iron from the heart, the elevation of LVEF, improvement of endothelial function and the clinical antioxidant properties, would also be mirrored in patients treated with DFRA and DF and lead to an increase in the overall survival of thalassaemia patients (29-33, 47-50).

4. MONITORING OF IRON OVERLOAD AND CHELATION THERAPY IN THALASSAEMIA PATIENTS

Thalassaemia patients are subjected to a large number of clinical and biochemical tests mainly in relation to red blood cell transfusions, iron overload and toxicity, as well as chelating drug efficacy and toxicity (26,39). There are different levels of body iron overload and particular organ iron distribution and toxicity in thalassaemia and other iron loaded patients (6,7,51-53). In iron overload diseases the damage to organs is usually associated with the level of excess storage iron, as well as the susceptibility of the particular organ to iron toxicity (6,7, 15, 51-54).

Body iron levels in thalassaemia and other regularly transfused patients are routinely monitored using serum ferritin estimations usually every three months. Similarly, excess organ iron deposition mainly in the heart and liver is estimated annually us-

ing MRI relaxation times T2 or T2* (Figure 2-4) (26, 34,35, 39).

In this context, estimations of serum ferritin greater than the normal level (>350 μg/L) suggest excess body iron deposits. Similarly, MRI relaxation times T2* smaller than the normal cardiac (20 ms>) and liver (6.3. ms>) values suggest different levels of excess iron deposition in these organs respectively (16-19). In general, the higher the deviation from the normal levels the higher the levels of excess deposited iron in the body and individual organs. Usually, high serum ferritin levels correspond to high liver iron deposits but not necessarily to heart, spleen or pancreas excess iron deposition (18,19,51-54). An increase in liver, cardiac and pancreatic iron deposition, was observed using MRI T2* in a cohort of paediatric thalassaemia and other regularly transfused patients under 10 years of age (55). Within this context, chelation therapy should aim for the reduction and prevention of iron overload at young ages and also as soon as possible following about 20 units of red blood cell transfusions so that damage to organs can be minimised, reversed and most importantly prevented (26,39).

Different iron overload assessment criteria are used for the clinical and biochemical monitoring of thalassaemia patients receiving intensive chelation therapy or for those who have already achieved NIS levels. Safety measures for these patient categories are also followed for the selection of chelation protocols in order to avoid the possibility of chelating drug overdose and toxicity, as well as iron deficiency complications (26,39,46). Similar safety measures and procedures apply to other non iron loaded categories of patients with NIS receiving long term chelation therapy usually using L1 eg patients with Friedreich Ataxia, Parkinson's disease, renal dialysis etc. (46,56-59).

Chelating drug toxicity in transfused patients with normal or near normal iron store parameters is widely reported. In this context only L1 appears to be safe for thalassaemia or other categories of patients with NIS. In contrast the use of DF or DFRA is not generally recommended in the drug labels of the manufacturers for patients with NIS and especially with serum ferritin levels less than 500 μg/L due to possible drug toxicity complications associated with the reduced iron stores (46, 60,61). Ocular and auditory toxicities have been previously reported in DF treated thalassaemia patients with low iron stores. In addition, fatal cases of mucormycosis have been reported in haemodialysis patients treated with DF for aluminium removal (62-64). Fatal renal, hepatic, bone marrow and gastric haemorrhagic cases have been reported in different categories of patients with NIS, treated with DFRA (46,60,61). Renal toxicity monitoring using mainly serum and urine creatinine is widely used for patients treated with DFRA for preventing associated nephrotoxicity since earlier reports in animal and human studies appear to suggest that the kidney is one of the major toxicity target organ for DFRA treated patients (46,60,61).

In relation to excess chelation in non heavily iron loaded patients, cardiac toxicity due to iron deficiency has been reported in a thalassaemia patient using intensive DF and L1 combination therapy (65). Similarly, neurotoxicity in thalassaemia patients has been reported for using L1 overdose (250 mg/kg/day) for more than a year (65,66). The mandatory monitoring using weekly or fortnightly blood counts for preventing L1 induced agranulocytosis or the monitoring of other toxic side effects such as neutropenia, joint/ musculoskeletal pains, zinc deficiency etc, are also important for the safety of patients with NIS treated with L1 (25,26,35,36,46). The regular monitoring of chelating drug toxic side effects as well as of body iron levels eg by monthly serum ferritin estimations is crucial for the short and long term safety for categories of non transfused or regularly transfused patients with NIS (39).

5. FACTORS AFFECTING THE RATE OF BODY IRON INTAKE AND REMOVAL IN THALASSAEMIA PATIENTS

Many genetic, dietary, immunological and other factors appear to affect the rate of body iron intake, deposition and toxicity, as well as the removal of excess body iron in thalassaemia and other regularly transfused patients (53). In general, the major determinant factor in body iron intake in regularly transfused patients is the rate of red blood cell transfusions, whereas for body iron removal it is the efficacy of the iron chelation therapy (Table 2) (67).

There are many differences in the rate of transfusions among patients with thalassaemia and other refractory anaemias. In general, in these patients 1-4 units of packed red blood cells are transfused every 1-4 week periods. Each unit transfused contains 200-250 ml of packed red blood cells. The transfusion of 1ml of packed red blood cells is approximately equivalent to 1mg body iron intake. The toxic side effects of iron overload in regularly transfused patients begin from childhood and are usually detected when 50-100 of packed red blood cells have been transfused (55,67).

In some cases of thalassaemia patients with splenomegaly, the rate of transfusions and consequently the amount of body iron intake could be as much as two to three times higher than other patients with normal spleen size (68-70). Similarly, the rate of body iron intake is much lower in comparison to genetic variant cases of thalassaemia such as thalassaemia intermedia patients (71-74). Most

Table 2. Major factors affecting the iron load and removal in Thalassaemia patients

Factors	Examples
Factors affecting body iron load and distribution	• Rate of RBC transfusions. Rate of iron absorption. Rate of iron excretion. • Transferrin saturation. Nontransferrin-bound iron. Number and type of transferrin receptors in different cell types and organs. • The size and function of liver and spleen as the major iron storage organs. Organ size, vascularity and iron storage capacity in each organ. Splenectomy. • Rate of hemolysis of RBC. Red blood cell antibodies. Haptoglobin levels and function. • Organ specificity for iron uptake and storage: liver, spleen > bone, brain. • Rate of iron deposition and removal in different organs. Organ function. Antioxidant capacity. Ageing.
Factors affecting total body and individual organ iron removal	• Iron chelation therapy using deferoxamine, deferiprone, deferasirox and their combinations. The deferiprone / deferoxamine, ICOC combination could reduce the iron load in the liver and heart and also serum ferritin to normal levels. • Differential removal from the iron pools and organs. Deferiprone and iv deferoxamine are more effective in iron removal from the heart than deferasirox. All three chelators are effective in iron removal from the liver. Iron removal from the brain was shown using only deferiprone. • Non-uniform organ distribution of stored iron during chelation therapy and identification of intense iron foci. The principle “last in first out” mobilization of iron deposits by chelators usually apply. • Dose of chelator or chelator combinations and route of administration. Route of excretion of chelator and iron complexes. Combination therapy kinetics. • Iron removal from transferrin by deferiprone but not deferoxamine or deferasirox. Exchange of the deferiprone iron complex with apo-transferrin. No exchange of deferoxamine and deferasirox iron complexes with apo-transferrin. • Absorption, distribution, metabolism, excretion and toxicity of chelator, iron complexes and metabolites (ADMET). • Effects of dietary factors, dietary iron, metals other than iron, drugs and nutrients with chelating properties. • Drug interactions. Effects of diuretics and coagulants. The effect of other drugs on iron metabolic and chelation pathways. • Exercise. Sweating.
Genetic factors	• Metallomics, proteogenomics, nutrigenomics, pharmacogenomics, metabolomics
Hormonal function	• Erythropoietin levels. Erythropoietic activity of the bone marrow. Hepcidin levels. • Male/female hormonal activity and secondary events, e.g. iron loss during menstruation and child bearing
Disease factors affecting organ iron load and redistribution	• Anaemia • Hypoxia • Inflammation • Malignancy • Infection
Genetic and acquired conditions affecting organ iron load	• Thalassaemia intermedia. • Idiopathic haemochromatosis. • Atransferrinaemia. • Anaemia of chronic disease. • Parkinson’s and Alzheimer’s diseases with brain iron accumulation. • Acute iron poisoning.

thalassaemia intermedia patient categories have variable levels of haemoglobin production and are not severely anaemic in comparison to thalassaemia major patients. In general, thalassaemia intermedia patients have slower rate of iron intake because of less frequent transfusions or no transfusions and also an increase in gastrointestinal iron absorption (71-74).

Many other associated factors and parameters affecting each patient appear to influence the rate of body iron intake as well as the removal of excess body iron in thalassaemia (Table 2) (27,53,75). Such factors include splenectomy, red blood cell antibodies and haemolysis, rate of dietary iron absorption, individual variations on chelating drug absorption, distribution, metabolism, elimination and toxicity (ADMET), chelating drug efficacy, compliance with treatment, infections, pregnancy etc (27,53,75-77). For example, regular red blood cell transfusions continue normally during pregnancy in thalassaemia patients but the use of iron chelation therapy by any of the three chelating drugs is not recommended because of the possibility of embryotoxicity.

Individualised, tailor made effective and safe chelation therapy protocols need to be designed for treating the various categories of transfusional iron loaded patients with different rates of body iron intake (Table 1,2) (24,76,77). In this context, the major therapeutic target of iron chelation therapy is the achievement of negative iron balance, in which the rate of iron removal from the body is higher than the rate of iron intake from transfusions and gastrointestinal iron absorption. This procedure needs the continuous adjustment of the iron chelation protocols until reaching safely the ultimate aim of chelation therapy, namely the stage of achievement and maintenance of NIS (Table 1,2) (24,26).

Thalassaemia patients with NIS are generally devoid of the iron overload toxicity damage which is

mostly observed in heavily iron loaded patients such as cardiomyopathy, liver fibrosis and cirrhosis, endocrine complications etc. Furthermore, the iron overload toxicity complications are reversible in most patients achieving and maintaining NIS (15,78). Chelating drug doses are usually reduced for the maintenance of NIS and the quality of life in this category of patients is highly improved. A general increase in the survival rate of thalassaemia patients is currently observed in many countries, mainly as a result of improved and more effective iron chelation therapy protocols (26,29).

6. COMPLICATIONS IN THE INTRODUCTION OF EFFECTIVE IRON CHELATION PROTOCOLS

The introduction of effective and safe iron chelation therapy protocols and especially L1 which has cardioprotective effects in iron loaded patients, has lead to an overall increase in the survival and the life span of thalassaemia patients (26,29-33). This trend is progressively changing thalassaemia from a fatal to a chronic disease (26,39). However, the complexity of the iron overload pathology and other associated complications in thalassaemia requires continuous vigilance including the monitoring of toxic side effects and changes in therapeutic protocols including iron chelation therapy (78). Despite the various pathological complications the vast majority of thalassaemia patients can adhere to the ICOC combination therapy of L1/DF and other similarly effective and safe chelation protocols (Table 1,2) (13,14, 20,21).

There are several conditions that may interfere with the application of chelation protocols in thalassaemia patients which include the cost of the therapy, marketing procedures, patients' tolerance and compliance, adverse reactions and many others (25,26,53). The selection of optimal chelation therapy protocols for personalised medicine in thalassaemia may also be influenced by many other environmental, metabolic, immunological, pharmacological, nutritional and genetic factors (Table 1,2) (26,53, 76,77).

Idiosyncratic reactions to chelating and other drugs and also other treatments related to the underlying disease may interfere with the selection of effective chelating drug protocols. Withdrawal of chelating drugs is recommended for patients with idiosyncratic reactions such as L1 and DFRA induced agranulocytosis, DFRA induced renal damage and DF induced auditory and ocular toxicity. In these cases the selection of chelating drug monotherapy or combination therapy protocols is limited to the most efficient and least toxic selections (Table 1) (24).

Infections instead of cardiomyopathy are now being considered as the main cause of death in thalassaemia following the reversal of congestive cardiac failure episodes, mainly as a result of the introduction of L1 (32,79-81). In this context several parameters may change the chelation therapy protocols in response to potential toxicity such as the possibility of the chelating drug to be used as a siderophore for microbial growth or in exacerbating the neutropenia in immunocompromised patients etc. (82,83). Similar therapeutic approaches and chelating drug protocols need also to be designed for other complications in thalassaemia patients such as hepatitis, HIV-AIDS, hepatocellular carcinoma etc, where chelation therapy may have implications on the treatment of these other diseases (32,79).

Toxicity related complications in the selection of chelation therapy protocols could also be envisaged as a result of drug interactions leading to exacerbation of a particular toxic side effect such as hepatotoxicity, kidney toxicity, neutropenia etc (84). Such drug interactions are feasible because of the many other drugs used by the thalassaemia patients as a result of the pathological complications of the underlying disease (84).

The concomitant administration of aluminium containing antacids is not recommended for DFRA since the formation of lipophilic metal complexes can increase the gastrointestinal absorption of aluminium and other toxic metals (60,61,71). The formation of toxic redox cycling lipophilic iron and other metal complexes, is the basis of the mechanism of the cytotoxic anticancer activity of lipophilic chelators such as omadine, 8-hydroxyquinoline and tropolone and investigational new anticancer drugs such as triapine, VLX600, Dpc and Dp44mT (85-88). In contrast, the formation of hydrophilic metal complexes by hydrophilic chelators such as L1 and DF appear to decrease the gastrointestinal absorption of iron and other metals and also the cytotoxic activity in normal and cancer cells (71,85, 89).

Patient variations in the chelating drug response are observed similar to variations in ADMET for all other drugs (76,77,90). Such effects could influence the overall efficacy and toxicity of the chelating drugs and also their metabolites. For example, the rate of glucuronidation of L1 and DFRA to their respective glucuronide metabolite conjugates, which have no iron binding properties could affect their efficacy for iron binding and removal in thalassaemia patients (84,90, 91).

Changes in iron chelation protocols are also envisaged in response to changes in the rate of body iron intake from transfusions and overall body iron load. For example, higher range of doses of chelating drugs should be applied for achieving negative iron balance in thalassaemia patients with increased trans-

fusion requirements due to splenomegaly or increased levels of red blood cell antibodies, whereas lower overall doses should generally be applied to patients with NIS who have no such complications (13, 27).

The optimal chelation therapy protocols selected for thalassaemia patients including those with increased body iron intake, as well as those not adhering to the ICOC and similar protocols should be based on similar criteria ie for the provision of chelation therapy with maximum efficacy and minimum toxicity (Table 1) (24).

7. CONCLUSION AND FUTURE PROSPECTS

Thalassaemia patients with NIS are devoid of any toxic side effects of iron overload such as cardiomyopathy, liver damage, endocrine and other complications etc (13,14,72). Increases in survival and better quality of life are observed in this category of thalassaemia patients in comparison to other categories of patients with heavy iron load (26). In most cases, many of the toxic side effects of iron overload including cardiomyopathy appear to be reversible following the use of effective chelation therapy protocols involving especially L1 (13-15,32, 36,37,48,5,81,92).

The achievement and maintenance of NIS using individualised tailor made ICOC chelation protocols of the L1/DF combination and L1 monotherapy is a major breakthrough in the treatment of transfusional iron overload and toxicity in thalassaemia (13,14,25,28). These and similar protocols which can achieve and maintain NIS should be used as a first line chelation protocols for the treatment of all thalassaemia patients (Table 1) (13,14, 28,29-33,42).

In general, the rate of reduction of the body iron load to the stage of NIS depends mainly on the initial body iron load, the rate of red blood cell transfusions and the tolerance of the chelation therapy. Despite many complications and variations in the body iron intake and load, the ICOC and similar protocols appear to be generally effective and safe for accomplishing the stage of NIS in most thalassaemia patients (28). The individualised tailored made chelation protocols, the continuous and close monitoring of the body iron stores, as well as the regular biochemical and clinical monitoring of the underlying disease in thalassaemia patients with NIS could assure their long term safety and survival (24).

Different rates in the normalisation of the iron stores have been detected in thalassaemia patients with different iron load. The iron clearance process may take 0.5 - 3.5 years to be achieved in most cases (13,14,27,78). Thereafter, it appears that the excess iron stored in the various organs and the body in general could be easily controlled in most thalassaemia patients with NIS using overall lower chelating drug doses in comparison to moderately or heavily iron loaded patients (13,24,78). This methodology of monitoring and controlling this stage of NIS could be used for the prevention of iron load and effectively reduce the iron overload related morbidity in thalassaemia and other regularly transfused patients (26,38).

The lowering of the overall doses in thalassaemia patients with NIS could also result in substantial reduction in the overall cost of the chelation therapy in comparison to other more heavily iron loaded groups of thalassaemia patients.

The search for optimal chelation protocols for the minority of thalassaemia patients who may not tolerate DF and L1 as well as their combinations still continues. Clinical studies suggest that the use of DFRA as monotherapy or in combination with L1 or DF seems to stabilise the iron load in some of these patients (40,41). However, there is no evidence that such therapies can be effective for accomplishing and maintaining NIS in thalassaemia patients. Furthermore, the long term safety and efficacy of such protocols are still under investigation. In particular, there are serious concerns on the ability of such protocols for the rapid elimination of excess cardiac iron and the long term survival of these patients (42-44). Similarly, there are also concerns regarding the toxic side effects in patients treated with DFRA (47).

The safety and efficacy concerns regarding the use of the approved chelating drugs (DF, L1, DFRA) are also raised for the development and introduction of investigational new chelating drugs in thalassaemia and other transfusional iron overloading conditions. It will be interesting to identify the therapeutic index of such new investigational chelating drugs as well as their combination effects with the clinically available chelating drugs (67,93,94). The introduction of new investigational chelating drugs could benefit some categories of regularly transfused patients especially patients who have adverse reactions and cannot tolerate L1, DF, DFRA and their combinations (67,93,94).

The possibility of development of naturally occurring chelators especially the ones which can become readily available from food products such as mimosine (Figure 1), which can be used as monotherapy or in combination therapies may also increase the prospects of benefiting many groups of thalassaemia and other iron loaded patients (95,96). In this case not only patients who cannot tolerate L1, DF and DFRA but also the vast majority of thalassaemia patients in developing countries can benefit because under the present conditions they cannot afford the high prices of the synthetic chelating drugs (25,67, 97-99).

Many other chelation strategies can be developed for achieving negative iron balance in thalassaemia and related groups of patients such as specific chelator administration for decreasing iron absorption, other routes of chelating drug administration such as DF suppositories, intravenous L1, chelator incorporation in the transfused blood etc (71,75,100). Informas tion on the safety of these strategies could be obtained from the administration of these chelators in thalassaemia patients and patients with other diseases such as HIV-AIDS and Parkinson's disease (101-104). Similarly, many other strategies can also be developed to ease the rate of body iron intake such as dietary control of food iron absorption, reduction of red blood cell antibodies, improved transfusion methods, increased production of Hb F, introduction of antioxidants, improved spleen function, etc (105-110).

8. ACKNOWLEDGEMENTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. The authors would like to thank Dr Christina N. Kontoghiorghe for reading and making comments on the manuscript. The study was supported from internal funds of the Postgraduate Research Institute of Science, Technology, Environment and Medicine, a non-profit, charitable organization. This paper is dedicated to Evdoxia Mantilari Kontoghiorghe who passed away in February 2017 and Anders Rosen who passed away in July 2017.

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Key Words: Thalassaemia, Transfusion, Iron overload, Normalisation of iron stores, Maintenance of iron stores, Deferoxamine, Deferiprone, Deferasirox, Combination therapy, Review.

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