Role of Iron Chelation Therapy for Beta-Thalassemia Major: A Review

Naeem Shahid, Fouzia Bibi, Muhammad Usman, Rubab Nasir, Ghulam Mustafa Shah, Hafiz Muhammad Arshad and Abdul Qadir

Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan
Department of Biochemistry and Molecular Biology, University of Gujrat, Pakistan
Department of Management Sciences, COMSATS Institute of Information Technology, Sahiwal, Pakistan
College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan

Received: June 19, 2014
Accepted: August 18, 2014

ABSTRACT

Thalassemia is a hereditary blood disorder which is caused by mutation of either alpha (α) or beta (β) globin gene which results in defective hemoglobin synthesis. Thalassemia can be classified based on genotypic diagnosis into two groups: α-thalassemia and β-thalassemia. They can also be categorized based on the clinical degree of severities into three types: thalassemia major, intermedia, and minor. β-thalassemia major (β-TM) is the most severe type of thalassemia for which no effective approach available that can be used for all the patients. These patients have severe anemia and need regular blood transfusion. Regular transfusion therapy leads to iron overload related complications. Iron chelation therapy is initiated when the patient has received 10-20 transfusions. The three commonly used iron chelators are Deferoxamine (DFO), Deferiprone (DFP) and Deferosirox (DFX). Deferosirox is usually preferred over the two because of its limited toxicities. A new compound having iron chelating activity, 1-(N-acetyl-6-aminohexyl)-3-hydroxypyridin-4-one (CM1) in iron-loaded C57BL6 mice has been studied. However, the effectiveness and toxicity of the CM1 need to be investigated extensively in iron overloaded patients.

KEYWORDS: β-thalassemia major, blood transfusion therapy, iron chelation therapy, deferoxamine, Deferiprone, Deferosirox and 1-(N-acetyl-6-aminohexyl)-3-hydroxypyridin-4-one.

INTRODUCTION

Thalassemia is an inherited blood disorder [1]. The word “thalassemia” is a combination of two Greek words “Thalassa” means sea and “Haema” means blood [2]. Dr. Thomas Cooley gave the first description of thalassemia in 1925. Normally, hemoglobin (Hb) is made up of four α-globin genes and two β-globin genes [3]. Sickle-cell disease (SCD) is caused by a specific mutation of β-globin genes whereas thalassemia is caused by mutation of either α or β-globin gene [4].

The Thalassemic patients can suffer from anemia because of the shortening of red blood cell (RBCs) survival period which is caused by hemolysis and the death of erythroid precursors in bone marrow called ineffective erythropoiesis [5]. Generally thalassemia is prevalent in those populations that are evolved in humid climates which also have malarial endemic. It affects all races and protects people from malaria because it degrades the blood cells easily [4]. Now a day, 1 out of 14 people are thought to be the carriers for different subtypes of thalassemia [6]. Every year almost 400,000 infants are born with serious hemoglobinopathies and the frequency of carriers is about 270 million [7, 2].

CLASSIFICATION

The thalassemia is classified according to the genes of the hemoglobin which is being affected: (i) α-thalassemia and (ii) β-thalassemia.
α-Thalassemia

If a mutation in α-globin gene occurs, the condition is called “α-thalassemia” [8]. Individuals with this disorder have reduced RBCs and their RBCs are usually smaller than normal. Each chromosome 16 carries four genes which control α-globin chain production [9]. There are four types of α-thalassemia based on degree of severity: (i) Silent carrier (ii) α-thalassemia trait (iii) Hemoglobin H and (iv) α-thalassemia major. α-thalassemia is very common in people having same ancestors from Thailand, Vietnam, China, Philippines, Laos, Cambodia and some other Asian countries [2]. It is also common in the people having African ancestry, which include West Indian and African American [10].

β-Thalassemia

If mutation occurs in β-globin gene, then it is called “β-thalassemia” [11]. More than 180 different mutations of the β-globin genes have been reported in β-thalassemic patients [12]. Mutation may occur during transcription, processing of the primary messenger ribonucleic acid transcript, translation, or post-translational stability of the gene product. β-globin synthesis is controlled by two genes on each chromosome 11 [3]. There are three types of β-thalassemia: (i) β-Thalassemia minor (ii) β-Thalassemia intermediate and (iii) β-Thalassemia major.

(i) β-Thalassemia minor

Individuals having mutations in one β-globin gene have β-Thalassemia minor. Their RBCs are small. It causes a mild “microcytic anemia” that does not need treatment. It is asymptomatic [13].

(ii) β-Thalassemia intermediate

It is a condition intermediate between major and minor. It is considered a complicated type, needing hematologist attention. Effected individuals can often manage a normal life and usually don’t need lifelong transfusions to survive past 20 years of age [14].

(iii) β-Thalassemia major

Individuals having mutations in both β-globin genes have β-Thalassemia major also known as “Cooley anemia”. Individual with β-thalassemia major are not symptomatic at birth because of the presence of fetal hemoglobin (HbF), but symptoms start to develop by six months of age. This is the most severe type of thalassemia for which no effective approach available that can be used by all the patients therefore needs much attention.

β-thalassemia is most common in the people of Middle Eastern, African, Mediterranean, Southeast Asian, Southwest Asian (Indian, Pakistani etc.) and Chinese descent [15,16]. β-thalassemia minor is more frequent showing geographical differences particularly in Mediterranean area and among the people of Italian, Middle Eastern, Greek, Southeast Asian, Chinese and African descent [17].

TREATMENT STRATEGIES FOR β-THALASSEMAIA MAJOR

β-TM patients are usually treated with frequent blood transfusions however it leads to iron overload for which iron chelation therapy is done. Both of these strategies are reviewed in this paper.

Blood Transfusion Therapy

β-TM patients come to medical attentions at the age of two years and require regular blood transfusion to survive. Transfusion therapy is initiated when patients have severe anemia i.e. Hb< 7 g/dl. However, patients with Hb> 7 g/dl should consider other factors which include facial changes, poor growth, bony expansion and increase splenomegaly. Blood is usually transfused every two to four weeks. The quantity of blood to be given depends on patient’s weight, target increase in Hb level and hematocrit of blood unit. This quantity can be calculated by appropriate graphs and formulae [18]. Although transfusion therapy extends survival, reduce complications of anemia and support growth and development in β-TM patients [19] but frequent blood transfusions can lead to iron deposition in vital organs and if not treated, death occurs [20,21].

Iron Chelation Therapy

As the body has no efficient ways for the removal of iron, the only way to get rid of the excess iron is to use iron binders known as “iron chelators” which excretes iron in the stool or urine. Generally iron chelation therapy is started when the patient has done 10-20 times transfusions or when the serum ferritin level rises greater than 1000ng/mL [18].

Deferoxamine

Deferoxamine (DFO) is a hexidentate iron chelator which was produced by bacterium Streptomyces pilosus, and first used in 1962 [22]. The hexidentate structure shows relative high stability for iron shown by high PM value of 27.7 [23].

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It is rapidly cleared by oral route because of poor absorption; therefore, it is administered either subcutaneous (SC) or intravenous (IV) for 8 to 10 hours via a battery operated portable pump daily or 5 to 7 days a week. SC route is preferred except in cardiac vascular patients for whom high intensive chelation i.e. 24 hours continuous IV route is recommended [24, 25].

Recommended dosage depends on the age and serum ferritin concentration of patients. Average dose for children is 20-40mg/kg/day and for adult are 30-50mg/kg/day [18, 26]. In high risk cases, the dose increases to 50-60mg/kg/day before the use of the combined therapy with DFO and deferiprone [25].

Single molecule of DFO binds single iron atom and forms a complex known as feroxamine that is metabolically inert. Kidney removes plasma iron chelated with DFO. Hepatocytes readily take up DFO that chelate the hepatocellular iron and excretes the feroxamine complex in bile. Within cells, excess iron is removed by DFO induced lysosomal degradation of cytosolic ferritin [27].

Olivieri et al. [28] reported that patients with serum ferritin level \( < 2500 \mu g/L \) has 91% heart disease free survival after 15 years of DFO treatment whereas patients with serum ferritin level \( > 2500 \mu g/L \) has only 20% heart disease free survival after 15 years. Therefore, chelation therapy should be started as soon as serum ferritin level rises \( > 1000 \mu g/L \).

**Toxicity**

1) **Skin Redness**
Patients treated with DFO usually have local reactions with skin reddening and soreness at the infusion site.

2) **Ophthalmic Toxicity**
High dose of DFO results in loss of central vision, night blindness and amaurosis because of retinal and optic nerve disturbance. Under these conditions, the drug is discontinued to restore the vision [29, 30].

3) **Auditory Toxicity**
Hearing loss may occur in young patients with lower serum ferritin level [31]. Patients with mild hearing defect improve hearing after the withdrawal of drug but patients with severe hearing defect persist this defect even after the withdrawal of the drug [32].

4) **Impaired Growth**
Although treatment with DFO decreases iron overload complications, it itself interferes with the growth. Cartilage dysplasia occurred patients who initiated DFO treatment at very early age with dose higher than 40mg/kg/day due to which stunted growth and ricket like bone changes results [33, 34, 35].

5) **Infections**
There is high risk of *Yersinia* infections in iron overload patients and the risk increases further with the DFO treatment as *Yersinia* uses iron from feroxamine complex to facilitate its growth [36]. These infections can be lethal if not recognized treated on time.

6) **Patients treated intensively with high dose of 10-20mg/kg/hour develops acute pulmonary toxicity and renal failure.**

Different chelator associated toxicity tests must be carried depending on the adverse effects of the particular agent to be used. Therefore, yearly audiometric and ophthalmologic tests are recommended for patients receiving DFO regularly. The use of DFO results in consistent decrease in morbidity and mortality [37]. Due to adverse effects and inconvenient mode of administration, the use of DFO is limited [38].

**Depot Formulation**

DFO depot (ICL 749B) is a novel salt derived from the modification of DFO that is then suspended in a lipid carrier. The reason behind this formulation is to administer a smaller dose of DFO that is released for a prolonged period. Depot formulation increases chelating efficiency and reduces the proportion of “wasted” DFO. The clinical use of ICL 749B started in January, 1997 [39]. Results of phase I single dose, which involved 30 patients showed clinically relevant urinary iron excretion (UIE) with all the doses evaluated. The pharmacokinetics (PK) of UIE and tolerability of depot formulation was compared with the standard DFO formulation [40]. No drug related severe adverse effects were observed in these studies. Tolerability was better with the standard DFO formulation than with the depot DFO formulation and expected drug accumulation did not attain in the depot formulation. Therefore, clinical use of ICL 749B was stopped.

**Long Acting Formulation**

An alternate formulation “long acting DFO” was prepared to resolve the problems of compliance with prolonged daily infusion of DFO. When DFO is chemically linked to a hydroxyethyl starch (HES) polymer, a new
compound DFO-HES is formed. The affinity and specificity for iron of this compound is same as the standard DFO but the vascular retention is 10 to 30 time longer [41]. The clinical use of this high molecular weight chelator in β-TM patients did not show adequate efficacy of this formulation [42]. Both the depot DFO and HES-DFO could not show significant improvement in the iron overload treatment.

**Deferoxamine**

Deferiprone (DFP) is a bidentate oral iron chelator that can easily penetrate through membranes and shuttle iron from tissues to blood because of its small size [43]. Three molecules of DFP are needed to bind single iron atom, each molecule provides two co-ordination sites “bidentate chelation” [44]. Recommended dose of DFP is 75mg/kg/day which are divided into 3 sub-doses given 1 hour before meal. Comparative studies have shown that 75mg/kg/day of DFP are as effective as 40mg/kg/day of DFO [45]. Individuals being treated with DFP have better myocardial magnetic resonance imaging (MRI) pattern and less incidences of developing cardiac disease than those being treated with DFO [46, 47].

MRI is the most recent technique used in the assessment of iron overload to measure cardiac iron overload and to predict risk of iron induced damage. It was first used, more than a decade ago for cardiac iron assessment in iron overloaded patients [48]. MRI also serves as a non-invasive alternative to liver biopsy and is the most accurate technique because it measures iron in the whole organ. R2 and T2* parameters have been validated for liver iron concentration while T2* for cardiac iron concentration. If MRI shows T2* > 20 then it should be done on the done on the annual basis and if T2* < 20 it should be done frequently according to the severity of cardiac iron overload. In general lower the T2* higher the risk of cardiac dysfunction [49, 50].

**Toxicity**

1) Agranulocytosis (0.5-2%) and neutropenia (4%) can result any time after one month treatment [51, 52]. Therefore, weekly monitoring of neutrophil count is recommended and once agranulocytosis has occurred, the drug must be discontinued.

2) Painful swelling of the joints, especially the knees also observed in some patients. The swelling is due to the formation of partially redox reactive complexes of deferipone and iron i.e 1:1 or 1:2. These compounds induce inflammatory changes, possibly mediated by free radicals [53]

3) Neurologic abnormalities occur with the use of high dose of DFP [54].

4) Others include diarrhea, hepatic fibrosis, fluctuation in liver enzymes level and gastrointestinal disturbances [55].

Patients who receive DFP should assess their complete blood counts (CBCs) weekly and serum amino transferases monthly.

**“Shuttle Hypothesis” Combination of DFO and DFP**

This concept was first hypothesized in Herschkoo in 2000 [56]. DFP although weaker chelator, has ability of entering cardiac cells more easily than DFO. DFP then transfers chelated cardiac iron to the DFO, a strong chelator that results in significant decrease in cardiac iron overload. DFO and DFP are used in combination when use of either drug alone cannot achieve targeted level of iron excretion without increase in toxicity [57, 58]. The usual dose of DFO used is 50 mg/kg/day via SC infusions for 2 days a week and that of DFP is 50 mg/kg/day orally divided in 3 doses for rest of the 5 days of the week.

**Deferasirox**

Deferasirox (DFX) is tridentating orally active iron chelator developed by computer modeling [59]. It is highly specific for iron and does not chelate other metals like zinc or copper [60]. Liquid iron concentration (LIC) measured every 3 weeks showed maximum removal with 20mg/kg/day dose of DFX than 10mg/kg/day dose of DFO [61]. A phase III study was done in 2004 which also proved the effectiveness of DFX in 586 β-MT patients who were not properly treated with DFO.

Two molecules of DFX bind a single iron atom. Like DFO, DFX makes complex with plasma iron but unlike DFO, these complexes are removed via hepatobiliary route. Hepatocytes efficiently take up DFX that chelate hepatocellular iron and then excrete in the bile [62, 63]. Within the cells, DFX chelates cytosolic iron through liposomal degradation of ferritin by proteasome [64].

DFX tablet is taken along with water or orange juice at least 30 minutes before meal. Recommended dose of DFX is 20mg/kg/day. Patients who receive more than 4 units of packed cells monthly require 30mg/kg/day of DFX and those who receive less than 2 units of packed cells monthly need 10mg/kg/day. The elimination time of drug is 11 to 16 hours that makes it feasible to administer one time a day.

DFX is well absorbed from the GI tract and is slowly cleared [65, 66]. DFX is 5 times more effective than DFO and 10 times more effective than DFP [67].
Toxicity

1) Renal Toxicity
DFX causes renal tubular epithelial cell damage in animal models [68]. A non-progressive increase in serum creatinine observed in >33% of patients receiving DFX[69, 70]. If there is an increase in serum ferritin, the drug must be discontinued.

2) Increase in Serum Transaminases
Increase in serum transaminases has observed in few patients but it does not necessarily require drug withdrawal.

3) Others
Skin rashes and mild to moderate gastrointestinal disturbances may also occur.

Monthly serum creatinine, serum aminotransferases, bilirubin levels and CBCs must be checked in patients receiving DFX.

Combination of DFX and DFO
Combined theory of DFO and DFX has been effective in patients failing to single drug therapy [71]. DFX works as an intracellular chelator whereas DFO works as an extracellular chelator [67]. However, combination of DFX with DFO is yet approved.

Newer chelators in clinical trials
A new compound having iron chelating activity 1-(N-acetyl-6-aminohexyl)-3-hydroxypyridin-4-one (CM1) in iron-loaded C57BL6 mice has been studied [72]. Mouse was given a ferrocene-supplemented diet (Fe-diet) that caused iron overload in liver and spleen tissues. CM1 treatment for 3 months reduced the levels of the membrane non-heme iron, non-transferrin bound iron (NTBI), labile plasma iron (LPI) and the plasma malondialdehyde (MDA)[73]. Thus, CM1 can be considered as an alternative chelator for the removal of excess iron from blood and other vital organs however toxicity of the CM1 is need to be studied in more detail. The challenge for the future is to ensure that people who are born with a severe form of thalassemia will continue to thrive, while effective prevention efficiently decreases the number of severely affected patients worldwide.

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ACKNOWLEDGEMENT
We would like to acknowledge the assistance provided by Dr. Amber Afroz, Assistant Professor, Department of Biochemistry and Molecular Biology, University of Gujrat, Pakistan, during the planning and writing of this review article.
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