CRITICAL REVIEW OF STANDARD AND NEW METHODS OF ASSESSING COMPLIANCE WITH CHELATION THERAPY IN THALASSEMIC PATIENTS

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Pharmacology, University of Toronto

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ABSTRACT

Patients with transfusional dependent β-thalassemia require iron chelation therapy in order to eliminate the excess iron. Desferrioxamine is the only approved iron chelator and, although very effective, its use is reduced due to cumbersome method of administration. Deferiprone (L1), a new iron chelator, has been shown to be efficacious orally and with acceptable adverse effects.

The aims of this study were to evaluate standard (diary, outcome measures, pill count, drug levels) and new electronic (CADD-1 infusion pump, MEMS devices) methods of assessing compliance in thalassemic patients and to compare compliance with DFO and L1 in the context of a randomized controlled trial.

Compliance in the DFO-treated patients was significantly lower than in the L1-treated patients, presumably because of the easier administration of L1. No correlation was found between compliance assessed by pill count, electronic monitors and outcome measures. In the L1-treated arm, MEMS devices provided a more accurate measure of compliance than pill counts.

In β-thalassemia compliance monitoring using several methods is crucial as non-compliance is common and is associated with decreased survival.
# Table of Contents

1. Introduction  
   - I - Beta-thalassemia  
   - II - Iron metabolism  
     - A. Normal iron metabolism  
     - B. Iron containing proteins  
     - C. Intestinal absorption of iron  
     - D. Iron distribution and kinetics  
   - III - Iron overload  
   - IV - Iron chelation therapy  
   - V - Compliance  
     1. Overview  
     2. Factors that influence compliance  
     3. Methods of compliance measurement  

2. Hypothesis  
3. Objectives  
4. Methods  
   - Study design  
   - Patient population  
   - Methods  
   - Statistical analysis  

5. Results  
6. Discussion  
7. Conclusions  
8. Future plans  
9. Figures  
10. Tables  
11. Appendices  
12. References
ABBREVIATIONS

DFO  Desferrioxamine
Fe/gdwlt Iron/gram dry weight liver tissue
HIC  Hepatic Iron Concentration
MEMS Medication Event Monitoring System
SF   Serum Ferritin
SQUID Superconducting Quantum Interference Device
UIE  Urinary Iron Excretion
INTRODUCTION

1. Beta-Thalassemia

Beta-thalassemia is one of the commonest genetic disorders and consists of a reduction in the synthesis of the β globin chain of hemoglobin(1). Although the disease is spread worldwide, it is more prevalent among certain populations, such as Italian, Greek, Indian and Chinese (1,2). The incidence of thalassemia in its risk groups is 10-20/1000 births. Hemoglobin A (adult hemoglobin, HbA) is a tetrameric molecule which contains 2 α chains and 2 β chains. Hemoglobin F (fetal hemoglobin, HbF) consists of 2 α chains and 2 γ chains and is the predominant Hb during prenatal life and shortly after birth. However, immediately after birth, HbF starts to decrease gradually to be replaced by adult hemoglobin. β-thalassemia major becomes symptomatic from 3 to 12 months of age, as HbA fails to replace the declining levels of HbF (2). As the production of β chains is deficient, the bone marrow tries to overcome the defect by synthesizing an excess of α chains, which associate and form insoluble aggregates within newly formed red cells (2). This is injurious to the immature red cell and ultimately results in cell lysis. As the bone marrow fails to correct the resulting peripheral anemia, extramedullary organs such as the liver(3,4), spleen and lymph nodes become involved in erythropoiesis, leading to enlargement of the organs.
At least 91 mutations and several deletional mutations have been identified within or around the β-globin chain gene located on chromosome 11, all affecting the expression of the β-globin chain gene (2). Different levels of expression are associated with different clinical pictures of the disease.

β-thalassemia major is defined as β-thalassemia which requires regular transfusions to sustain life. In 1925, Thomas Cooley, a Detroit pediatrician, described similarities in the appearance and clinical course of the disease in four children of Greek and Italian immigrants (5). The advanced clinical picture which he described, consisting of severe anemia, hepatosplenomegaly, growth retardation and bone deformities, is no longer seen at present in North America due to regular transfusion programs (2). The aim of regular transfusions is to maintain the hemoglobin at a level that prevents hypoxia and ineffective erythropoiesis (6). Although transfusions prevent the consequences of chronic hypoxia, they help these patients to have a normal appearance and to prolong survival, they have created a new problem: iron overload.

II. Iron Metabolism

A. Normal iron metabolism

Iron is a ubiquitous element, essential for many cellular functions. The amount of iron in the body has to be constant. This is accomplished by a balance between
absorption and excretion\(^{(7,8)}\). An alteration of this equilibrium produces either iron deficiency or iron overload. Iron overload is of particular biologic significance, as unbound iron produces oxidative ionic damage to cells (see below).

**B. Iron-containing proteins**

Iron binds to proteins either by incorporation into a protoporphyrin IX ring or by interaction with other protein ligands. These complexes are designated as heme and hematin respectively. Heme-containing proteins constitute one group of iron-containing proteins that bind oxygen \((O_2)\). Hemoglobin is an example of a heme protein (Fig. 1) that transports \(O_2\), whereas myoglobin is a heme-containing protein involved in the storage of \(O_2\)\(^{(7)}\).

Other proteins, such as ferritin and transferrin, which do not contain heme, are involved in iron transport. Transferrin is a \(\beta1\)-glycoprotein, synthesized in the liver with a molecular weight of 78 kDa and has two iron binding sites. Several other metals can bind to transferrin, but its highest affinity is for ferric iron. It is estimated that the association constant of ferric iron to transferrin is approximately \(10^{-22}\)M, although there is interspecies variability. In the usual physiological state, approximately one ninth of all transferrin molecules are saturated with iron at both sites, four ninths of molecules carry iron at either site and four ninths are free of iron\(^{(7)}\).
Ferritin is a crystalline, water-soluble protein, which, although present in serum, is the major protein involved in iron storage(9). It contains an outer polypeptide shell and a ferric core. The ratio of polypeptide to iron is continually changing in order to accommodate excess iron. When the storage capacity of ferritin is exceeded, iron is deposited near ferritin spheres. These amorphous deposits are called hemosiderin. Hemosiderin is mainly found in the liver, although it can also be found in other organs(10).

Another important subgroup of nonheme-containing proteins is represented by ferredoxins, which are iron-containing proteins involved in various enzymatic processes(7).

C. Intestinal absorption of iron

Iron absorption is the most important mechanism involved in maintaining the iron balance under normal conditions. It is well known that the low pH of the stomach favours the reduction of ferric iron to the ferrous state(11). In the small intestine (mainly duodenum) ferrous iron enters the mucosal cell and this uptake is dependent only on the amount of iron presented to the cell(7). The transfer from the mucosal cell to the capillary bed is a process modulated by the body requirements. The amount of iron absorbed usually represents only 5-10% of the total ingested amount. In the presence of iron deficiency the amount absorbed
increases, whereas iron overload decreases the amount absorbed. The exact mechanisms by which iron is transferred into the bloodstream are still poorly understood(7).

Studies in untreated β-thalassemic patients have shown that there is a paradoxical increase in the absorption of iron(12,13). The mechanism is unknown, but two hypotheses have been proposed. The first states that the expanded bone marrow needs more iron than the reticuloendothelial system is able to salvage from senescent red cells, which makes the marrow relatively deficient in iron(14). The second hypothesis is based on the observation that hypoxia, even in the absence of increased erythroid activity, is able to increase iron absorption(11). Both hypotheses were supported by the fact that transfused β-thalassemic patients have normal iron absorption(2).

D. Iron distribution and kinetics.

The normal distribution of iron in a 70 kg adult is schematically shown in Fig. 2. The total iron burden is approximately 4 g(4,14), and is maintained constant as previously discussed. The total red blood cell mass contains approximately 2.5 g of iron incorporated into hemoglobin, with a turnover rate of 25 mg/day. The biggest iron reservoir is the liver, which contains approximately 1 g of iron as ferritin deposits in parenchymal cells(4,14). The liver is also the main organ injured
in acute and chronic iron overload(3,4).

III. Iron Overload

In the early 1960s, treatment of β-thalassemic patients with regular blood transfusions represented a significant advance towards the cure of a fatal disease. However, soon afterwards the consequences of chronic blood transfusion became evident: the patients developed iron overload. Each unit of blood brings approximately 175 mg of iron into the body(6). Most β-thalassemic patients require 20 to 30 units of blood per year, which translates into 4-5 g of iron per year. At this rate, the amount of iron accumulated exceeds 70 g by the beginning of the second decade of life, an almost lethal dose(6).

Because there is no physiologic route for excretion of excess iron, when the transferrin-binding capacity is exceeded, iron starts to accumulate in organs such as the liver, pancreas, thyroid, parathyroid, the zona glomerulosa of the adrenal gland, the renal medulla, the heart, bone marrow and spleen(15). The clinical consequences of iron deposition are numerous (Table 1)(2). Liver enlargement has been shown to appear after 10 years of age in transfused patients(16). Histologically, intralobular fibrosis and cirrhosis is seen(16-18). Moreover, iron overload increases the risk of developing hepatocellular carcinoma(19,20).

Endocrine disturbances, such as impaired growth hormone production(21),
hypothyroidism(2), and decreased production of mineralocorticoid hormones(22) are usual findings in iron-overloaded β-thalassemic patients. Diabetes mellitus is also a common endocrine complication(2) and is due to both pancreatic hypofunction(23) and/or peripheral insulin resistance(24).

However, the most important consequence of iron overload is iron deposition in the heart(25). Accumulation of iron in myocytes leads to degeneration and intracellular calcification(17). It has been shown that involvement of subepicardial, subendocardial and intraventricular septum is present in animals after 8 to 12 weeks of iron overload. The clinical consequences of these pathologic findings are progressive cardiac failure and rhythm disturbances(26). It is estimated that most β-thalassemic patients die of cardiac complications due to iron overload(27,28).

The exact mechanisms by which iron causes toxicity have received a great deal of attention. It has been shown that free iron, unbound to either ferritin or transferrin, is toxic to cells because it forms free radicals(8). Free radicals induce lipid peroxidation(29). Peroxidation of mitochondrial membranes and hepatocyte microsomes has been documented in vivo in iron-overloaded rats and in the spleen of thalassemic patients(30). Moreover, it seems that even amorphous deposits of hemosiderin can destroy lysosomal membranes(31), which results in the release of hydrolytic enzymes with subsequent cellular damage. Other evidence supporting the
role of free radicals in cellular damage is the finding of decreased levels of vitamin E, an antioxidant, in the serum and red blood cells of β-thalassemic patients(32,33). Both the level of vitamin E and the total serum antioxidant activity bear a strong inverse relationship to the degree of iron overload(32,33). It has also been shown that superoxide production in the neutrophils from thalassemic patients is at least five-fold higher than in normals(34).

Overall, there is strong evidence to suggest that survival in β-thalassemic patients is closely correlated with the total body iron burden of the body(2,27,28).

IV. Iron chelation therapy

Chronic transfusion therapy has transformed thalassemia from an anemic syndrome into a severe disorder of iron overload(35). In the early 1960s it became apparent that thalassemic patients receiving regular transfusions should receive an iron chelator as part of their management, in order to excrete excess iron(6).

A. Desferrioxamine (DFO)

Desferrioxamine (Desferal, Ciba-Geigy Ltd., Basel, Switzerland) was the first chelator shown(36) to induce a negative iron balance in thalassemic patients and is still the only approved agent for this indication. DFO mesylate is a trihydroxamic acid (Fig.3) produced by Streptomyces Pilosus(36); each hydroxamic acid
terminates with a free amino acid group which enables DFO to form salts with organic and inorganic acids. DFO is a hexadentate chelator which means that one molecule of DFO is required for binding of one molecule of ferric iron(36). The stability constant for ferric iron is very high (10^{31})(37) and it is much higher than that for ferrous iron or other metals(36,37). DFO is wrapped around an iron nucleus, encasing it in an envelope of organic material. The DFO-iron complex, called ferrioxamine, is extremely stable and resistant to enzymatic degradation(37). It can readily be excreted in the urine and stool(38).

DFO is able to penetrate cells. The source of iron is represented by the chelatable intermediate iron pool(38,39) from which iron can be either released to the plasma to combine with transferrin or diverted into ferritin stores. DFO has a very low bioavailability, being active only after parenteral administration(36,38,40). The half life is only 5-10 minutes(2). It is mostly metabolized in the liver(2). The metabolites have not been well-characterized. In the urine, the major compound is metabolite C, in which the original amino group of DFO has been replaced by a carboxyl group. Plasma plays also an important role in enzymatic breakdown of DFO. The main mechanisms of excretion consist of glomerular filtration and tubular secretion(38).

Ferrioxamine, the DFO-iron complex, is excreted unchanged in the urine
through glomerular filtration although it is also partially reabsorbed in the tubule. Ferrioxamine is also excreted in bile and subsequently in feces. Biliary excretion of iron constitutes up to 42% of urinary excretion\(^{(41)}\). The amount of iron excreted is dependent upon several factors such as: regimen of DFO administration, magnitude of iron stores, vitamin C status and others\(^{(42)}\). It has been shown that prolonged subcutaneous infusion of DFO over 10-12 hours nightly is the method of administration which results in the greatest excretion of iron\(^{(38,43)}\). However, the amount of iron excreted is directly correlated with the size of iron stores\(^{(44)}\).

Vitamin C, in a dose of 100 mg daily, has been shown to enhance urinary iron excretion by expanding the intermediate chelatable pool and thus making iron more available for chelation\(^{(38,45,46)}\). Moreover, iron overload is frequently associated with ascorbate deficiency due to increased catabolism of ascorbate and its oxidative conversion to oxalate\(^{(47)}\).

Long-term trials of DFO have shown that it can decrease hepatic iron stores\(^{(48)}\), ameliorate cardiac\(^{(49,50)}\), pancreatic\(^{(51)}\) and other organ dysfunction\(^{(6,52)}\), improve growth\(^{(53)}\) and sexual maturation\(^{(54)}\) and increase survival in patients with thalassemia major\(^{(55)}\). A recent study has shown that a sustained reduction of iron as measured a serum ferritin lower than 2500 ng/ml 80% of the time it is measured is associated with a 91% disease-free survival after
15 years. This was in contrast to the 20% disease-free survival after 15 years observed in the group where serum ferritin measurements were above 2500 ng/ml 67% of the time (28). It is also clear that early use of DFO in an amount proportional to the transfusional iron load reduces the iron burden and prevents the development of organ dysfunction(27).

Although very effective(56), the use of DFO is limited by its serious toxicity(57), its cumbersome method of administration(58) and its high cost(40). Prolonged use of high doses of DFO has been associated with ocular toxicity, auditory toxicity (sometimes irreversible)(59-61), and growth retardation due to bone changes(62). Toxicity is inversely correlated with the magnitude of the iron stores(60,63). Therapy with DFO is painful and difficult to administer(40). Due to these factors, and also to a lack of an immediately evident benefit, noncompliance with therapy is very common. In addition, the cost of DFO and of the infusion pump exceeds $20,000/year(40). Most of the worldwide thalassemic population cannot afford this cost. To overcome these problems, efforts have been made to find an iron chelator that is effective orally and is less expensive than DFO.

B. Deferiprone (L1)

An ideal iron chelator has to be effective orally, specific for ferric iron, non-toxic and affordable(64). From among many substances that have been studied over
the past twenty years, a potential useful iron chelator, Deferiprone (L1-Apotex Inc., Toronto, Canada) is the only one which has reached the clinical trial phase.

L1 (1,2-dimethyl-3-hydroxypyrid-4-one) is a bidentate chelator which binds in a 3:1 ratio to iron (Fig.4) at physiological pH(65). L1 has a high specificity for ferric iron, with a stability constant of $10^{37}(65,66)$. L1 has a high bioavailability, and is almost completely absorbed from the stomach within 5-10 minutes(67). Various values for pharmacokinetic parameters have been reported in the literature due to different dosing schedule and formulations of the drug(67-69). In our experience the mean serum half-life has been 3 hours(68). The major metabolic pathway is through glucuronidation in the liver(65). L1-glucuronide has a longer half-life than with the parent compound and is excreted in the urine with more than 80% of the drug being eliminated in the first six hours(67-69). No active metabolites have been found to date, although some of the toxicity of L1 has been attributed to a yet unknown reactive metabolite(Hoffbrand, A.V., personal communication).

L1 is able to chelate iron from plasma and from parenchymal organs(67,70). Various studies ongoing worldwide have shown that L1 is at least as effective as DFO in achieving a negative iron balance(70-75). One proof of efficacy is the amount of iron excreted in the urine. Five studies have demonstrated similar urinary
iron excretion when L1 was used compared to DFO(76-78). It appears that sustained moderate plasma levels of L1 throughout the day are associated with more efficient chelation(79). Doses of 100 mg/kg/day of deferiprone induced an iron excretion greater than 0.5 mg/kg/day, which is sufficient to maintain a negative iron balance(80). Serum ferritin has been also shown to decrease significantly during treatment with L1(70,71). However, direct proof of the efficacy of L1 requires evidence of a decrease in hepatic iron concentration. Olivieri et al demonstrated that the hepatic iron concentration decreased and was maintained at a level below that associated with complications in a group of patients studied for a maximum period of five years(71).

Although effective, L1 is not without side-effects. Animal studies have shown potential severe toxicity such as anemia, neutropenia and thymic aplasia at doses of 300-400 mg/kg(78,81,82). Among the more than 400 patients worldwide who have been treated with L1, one major complication has been observed: agranulocytosis. To date fourteen cases have occurred but only a few have been published(72,83). The mechanism is unknown, although L1 has been shown not to be directly myelotoxic in cell culture(84,85). All patients had an uneventful recovery after L1 was discontinued. Other L1-related adverse effects consisted of arthropathy (71-73,80) and zinc deficiency(86), particularly in diabetic patients.
The data available support the use of L1 as an alternative chelating agent in patients unable or unwilling to take DFO. The present randomized study will definitively compare the efficacy of L1 and DFO, and provide information regarding their relative toxicities and the compliance with the two regimens.

V. Compliance

1. Overview

Compliance is defined as adherence to a prescribed therapeutic regimen because of a perceived self-benefit and a positive outcome (87). Noncompliance with therapy is one of the biggest threats to successful treatment and one of the most common problems encountered in clinical practice. Overall noncompliance with medical treatments is estimated to be close to 40% (88), justifying the fact that it is now considered "America's other drug problem" (89, 90). Noncompliance accounts for approximately 50% of variance in an individual drug response, and, together with pharmacokinetic differences, constitutes the main factors creating variability in an individual's drug response (91). From an economic point of view, failure to comply with treatment is the cause for an increased number of clinic visits, hospital admissions, emergency room visits and use of other health care services (92-94).

Detection of noncompliance is a prerequisite for adequate treatment. It is also
crucial to assess compliance during clinical trials since negative results can be erroneously attributed to lack of efficacy of the treatment, instead of failure to take the medication(95). It has become clear in the last years that no data regarding the efficacy of a new drug can be interpreted without monitoring compliance.

2. Factors that influence compliance with therapy (Table 2)

Disease

Motivation of the patient to comply with a therapy is influenced by several factors such as chronicity and severity of the disease and the presence or absence of complications. For example, in a patient with a chronic disease with few or no symptoms, adherence to a certain regimen is very poor. The attitude towards disease, the acceptance of the sick role are also reflected in compliance with therapy. Mental disorders or severe disabling illnesses interfere also with the ability of the patient to comply with the therapy.

Therapeutic regimen

The behaviour of taking or not taking the medications is clearly determined by the difficulty and duration of the treatment- the longer and the more frequently a drug has to be taken, the less likely is it that the patient will comply. Multiple drug therapy, and complex treatments that interfere with daily life are also reasons for noncompliance. Disabling or intolerable adverse effects are additional limiting
factors in compliance with therapy. The cost of therapy will be a major concern for a patient with limited resources.

**Interaction between the patient and the health care professional**

A health care professional who is caring, concerned and supportive will likely increase the patient's compliance. Good communication and counselling increase the patient's understanding of the therapy and result in a positive attitude towards it.

**Socio-economic factors**

The age extremes, lack of material resources, interference of the treatment with work/school schedule and lack of family support are key factors determining compliance with drugs. Old people living alone, with limited finances and requiring multiple drugs are more likely not to comply with therapy.

**3. Methods of compliance measurement**

There are many ways to evaluate compliance with therapy, and this reflects the fact that no "gold standard" method exists. Indirect information regarding compliance with therapy is gathered through history taking, counting pills and using a patient's diaries. However, it is well known that the information reported by patients, either orally or in writing, is unreliable due to either inability to remember or false reporting in order to please or to avoid the disapproval of the
physician(96). An estimate of compliance based on counting pills can also be misleading. Pill dumping, meaning that the patient discards unused tablets, is a common phenomenon in a setting in which the patient is aware that compliance is being monitored(97).

Another proposed and useful tool for assessing compliance is a successful outcome such as the measurement of a decrease in blood pressure(98). Being a short-term outcome, measuring the blood pressure gives information regarding the immediate previous compliance. The so-called "toothbrush effect" or "lab-coat" effect, meaning adherence to the prescribed therapy for one or two days prior to the clinic visit, is a limitation of this method(99). It is well recognized that compliance decreases between clinic visits(100).

More direct information about adherence to the prescribed treatment is obtained through monitoring of biological markers (e.g. measuring the blood glucose in a diabetic patient) and of drug levels. Measurement of the blood concentration of the drug is feasible for certain drugs. However, individual differences in drug metabolism, variable times of sampling relative to ingestion of the drug, and the failure of patients to follow the exact instructions prior to sampling are a few limitations to the wide use of measuring blood concentrations as an indicator of compliance(101).
It is clear that an accurate, sensitive and nonsensitizing method to measure compliance was needed. In the late 1980s, a U.S. company marketed an electronic device called medication event monitoring systems (MEMS, APREX Corporation, Fremont, CA.) (Fig. 5). The device consists of a cap provided with a microprocessor which records the time and date of each bottle opening. Although the method does not prove actual ingestion of the drug, there is general agreement that it gives a reliable estimate of compliance(102,103). It is unlikely that the patient remembers to open the bottle at a certain time and to close it without taking the drug. The great majority of patients do not purposefully deceive the physician. They simply fail to remember to take the drug. MEMS devices constitute a major advance in monitoring compliance with tablets or capsules. CADD-1 devices (SIMS Smiths Industries Medical System, Canada) (Fig. 6) are new electronic infusion pumps that dispense a constant amount of drug over a defined period of time. The biggest advantage of this pump is that the amount to be dispensed can be programmed, and the patient cannot interfere with it. At the end of the study period the pump shows the amount that was actually used and on this basis the compliance can be calculated.
HYPOTHESES

1. Due to the cumbersome parenteral administration of DFO, the compliance in the DFO group will be lower than in the L1 group, because L1 is administered orally.

2. Standard methods of assessing compliance (pill count, diaries) are inferior to electronic devices (CADD-1 pump, MEMS).

OBJECTIVES

1. To assess the compliance with two therapy regimens (one standard, administered subcutaneously, the other experimental, administered orally) using standard and new electronic methods.

2. To compare the compliance between the two therapy arms.

3. To assess the compliance with each therapy using drug levels and the outcome of the disease of the patient.
METHODS

Study Design

Patients with diagnosed transfusion-dependent β-thalassemia were approached regarding possible participation in a randomized prospective trial of a new investigative iron chelator (L1) and desferoxamine (DFO). After informed consent (Appendix I), the patients were stratified into high (>7 mg Fe/g dry weight liver tissue) and low iron-overloaded (<7 mg Fe/g dwlt) according to their hepatic iron concentration as assessed either by liver biopsy and/or a Superconducting Quantum Interference Device (SQUID). After stratification, the patients were assigned, by a research pharmacist who did not know the patients, to receive either L1 (75 mg/kg/day in 3 divided doses) or DFO (50 mg/kg/night, 4-7 nights/week). Enrolled patients will be followed for a study period of two years. This trial is meant to compare the relative efficacy, safety and compliance of L1 with the standard iron chelator DFO.

Patient Population

Number

The number of patients to be enrolled in this trial will be 66, which will give this study the power to detect at least 20% difference in effectiveness of the two
iron chelators. To date 55 patients have been enrolled, 29 in the L1 group and 26 in the DFO group. Eight patients have been withdrawn from the study due to adverse events (2), family reasons (1), psychiatric disorder (1), chronic neutropenia prior to starting on L1 (2), bone marrow transplantation (1) and non-compliance with the study protocol (1). 25 patients on L1 and 26 patients on DFO have been used in the present analysis.

**Inclusion Criteria**

Patients who are 10 years of age or older, diagnosed with homozygous β-thalassemia as confirmed by hemoglobin electrophoresis and/or DNA analysis, and willing to participate in the study, were eligible for enrollment.

**Exclusion Criteria**

Patients who refused to participate in the screening, who had been previously treated with L1, who had had prior serious adverse reactions to DFO, who failed to attend >20% of the visits in the first three months of the study, who were receiving other investigational drugs, who had a past history of malignancy, who had a medical, psychological or psychiatric risk and for whom therapy with an investigational drug would be unwise or who were pregnant or breast feeding or were not using a reliable birth control method were excluded from the study.

**Methods**
A patient's compliance was assessed using both traditional methods and new electronic monitoring devices (Table 3).

**Indirect methods**

**Diary**

For both L1 and DFO groups the patients were instructed to fill out a monthly booklet with information regarding the number of doses taken per day for the L1 group, the amount of medication infused for the DFO group, other concomitant medications, and their comments about chelation therapy (Appendix II).

**Pill Count**

At every visit, ranging between 3 and 5 weeks according to transfusional requirements, the patients received a supply sufficient for the interval to the next appointment plus extra tablets for a week. They were asked to return the remaining tablets at the time of the next clinic visit. We assessed their compliance as the percentage of pills prescribed which were taken:

\[
\text{Compliance (\%) = \frac{\text{Number of pills taken}}{\text{Number of pills prescribed}} \times 100}
\]

\[
= \left( \frac{\# \text{ of tabs prescribed} - \# \text{ of tabs returned}}{\# \text{ of tabs prescribed}} \right) \times 100
\]

**MEMS devices**

Medication Event Monitoring System (MEMS, Aprex Corporation, Fremont,
California) devices are standard pill containers with microprocessors in the capable to record the timing and frequency of bottle openings. The major limitation of this device consists of the fact that an opening of the bottle is recorded as an event whether or not the patient actually took the drug. Patients were told how a MEMS device functions and were instructed to dispense the drug from the MEMS containers at eight-hour intervals. It was emphasized that they are not allowed to dispense more than one dose with one bottle opening and that failure to do so should be reported to the physician. Patients were allowed to supplement only one missed dose at the time of the subsequent dose ( recorded as a delay, but not as noncompliance). At every monthly visit, the patients had a MEMS reading, data from the monitor being transferred to a computer screen which showed a calendar plot with information regarding the number of bottle openings for each day and a dose-time list with the exact time when the bottle was opened (Appendix III). Compliance was assessed as the ratio of the number of openings to the number of doses prescribed.

**CADD1-PUMP devices**

Desferrioxamine has to be infused overnight with a pump which dispenses a constant amount of drug over a 12 hour interval. The main and unique advantage of the CADD1 pump ( SIMS Smiths Industries Medical System, Toronto, Canada)
is that it allows assessment of the exact amount of drug infused over a monthly period. The pump can be set up electronically to dispense a determined amount every time the pump is started. The pump is set by the research nurse every time the patient comes to the clinic and the patient cannot interfere with the programming. The patients were aware of the fact that their compliance was monitored. The compliance with DFO was assessed monthly as the ratio of the amount infused to the amount prescribed.

Direct Methods

L1 concentration

Blood was drawn for measurement of L1 plasma trough levels every three months during the duration of the study. The patients were instructed to take the last dose at 10 p.m. the day prior to sampling and not to take the morning dose. In order to ensure that the patients followed the instructions, the MEMS recordings for the day of sampling and the preceding day were checked. The levels that did not represent an actual trough level (MEMS recordings showed a dose taken in the morning prior sampling) were not included in the analysis. Two mL of heparinized blood were obtained each time. The plasma was immediately separated and kept at -20°C until analyzed within four weeks. The analysis was performed using a simple, fast and sensitive high-performance liquid chromatography (HPLC) method.
developed in our laboratory. The mean recovery of L1 was 81±6.5 % and the detection limit was 0.5 ug/ml L1 when 0.25 ml of serum was analyzed (104).

**Outcome measures**

The primary end-point of chelation therapy is a reduction in the body iron burden. The standard test used worldwide for assessment of iron overload is serum ferritin. We used the reduction in serum ferritin levels as an indicator of compliance with therapy. However, it is well known that serum ferritin is an acute phase reactant and infections or liver disease can falsely elevate it (105). In order to overcome this limitation we used the hepatic iron concentration as assessed either by liver biopsy or Superconducting Quantum Interference Device (SQUID) susceptometer (105, 106) as an indicator of compliance with the therapy. SQUID is a new device that noninvasively measures the amount of iron in the liver based on the magnitude of the magnetic field generated by the iron. However, in order to achieve a negative iron balance years of good compliance with the therapy are needed. Since this analysis was done only after approximately 12 months of therapy, we also measured the urinary iron excretion as an immediate indicator of compliance.

**Statistical Analysis**

Data are presented as means ± S.D. The mean compliances for the two
therapeutic regimens were compared using the unpaired Student's t-test. The mean compliances in the L1 group as determined using the pill count method and the MEMS device were compared using the paired Student's t-test. Correlation between values was studied by least square regression analysis. For all statistical analysis a p=0.05 was considered significant.

RESULTS

Among a total number of 51 patients (25 in the L1 arm and 26 in the DFO arm), results of 5 patients in the DFO group were not analyzed due to lack of availability of compliance data (the patients failed to bring the CADD pump for reading at every monthly visit). Data on the other 46 patients were analyzed for a study period of 11 ± 4.2 months (2-15) for the L1 group and 11.63 ± 3.26 (2-15) for the DFO group. For part of the analysis, (correlation between compliance and successful outcome) 5 patients in the L1 arm and 2 patients in the DFO arm were excluded due to the fact that the duration of treatment was less than six months.

The characteristics of the patients in the two treatment groups were similar and are presented in Table 4.

Comparison of various methods for assessing compliance

In the DFO-treated group, three methods of compliance monitoring were
used: monthly CADD-1 pump reading, monthly diary and successful outcome measures such as urinary iron excretion, a decrease in serum ferritin and in hepatic iron concentration as measured by liver biopsy and/or SQUID device susceptometer. The compliance reported by the patients in the monthly diary is not yet available. The mean compliance with DFO as assessed by CADD-1 pump reading was 66.17 ± 11.45% (range 40.65-84.47). No correlation was found between compliance and urinary iron excretion (r=0.06, p value=0.83) during the study period (Fig.7). It is well known that urinary iron excretion is influenced by the total body iron burden. To control for this confounding factor, the DFO arm was divided into two subgroups, one with hepatic concentrations in excess of 7 mg Fe/g dry weight liver tissue (dwlt), and the other with hepatic iron concentrations below 7 mg/g dwlt. Due to, possibly, the small number of patients in each group, this stratification failed to increase the correlation coefficient (r=0.35 for the group with <7 mgFe/gdwlt (Fig.8) and r=0.08 for the group with >7 mg Fe/g dwlt (Fig.9)).

The correlation between the mean compliance with DFO and the mean percent reduction in the hepatic iron concentration was also poor (r=0.24, p=0.42) (Fig.10). The mean compliance with DFO in the patients who had a decrease in hepatic iron concentration was 66.72%, which was not significantly different from
the patients who registered no change or an increase in hepatic iron concentration. The mean compliance in this latter group was 64.24 (\( p = 0.71 \), using a t test for unpaired samples).

There was also no correlation between compliance and the mean serum ferritin (\( r=0.34, \ p=0.16 \)) (Fig.11).

In the L1-treated arm, apart from diaries and successful outcome, electronic monitoring by MEMS devices, pill count and L1 trough concentrations were used to measure compliance. The mean compliance assessed by pill count was 91.42 ± 5.93 (76.26-99.00), significantly higher than the value obtained by MEMS devices, which was 82.93±14.71 (40.7-98.16) (two-tailed \( p \) value=0.017, using the t test for paired samples). The mean compliances as measured by MEMS reading and pill count are compared to the other estimates of compliance in Table 5. No correlation was found between compliance assessed by electronic monitoring and standard pill counting with L1 trough levels, reduction in hepatic iron concentration or urinary iron excretion (Fig.12-21). There was a trend towards a correlation between L1 trough levels and compliance in the months preceding sampling, as assessed by pill count (\( r=0.38, \ p=0.06 \))(Fig.12). Compliance measured by MEMS reading was 91.28% in the patients whose hepatic iron concentration decreased during the study period, significantly higher than the 83.09% compliance in the patients whose
hepatic iron concentration remained unchanged or increased (p value=0.04, using the t-test for unpaired samples). However, the compliance as monitored by pill count did not differ between the two groups (93.33% vs 89.71%, p value = 0.11). The mean serum concentrations failed to correlate with compliance assessed by pill count(r=0.11,p=0.64- Fig.22) and by MEMS reading (r=0.16,p=0.48- Fig.23).

Comparison of compliance with the two therapy regimens

One of the end-points of this study was to compare the compliance in the two treatment groups. CADD-1 pump reading was used as an objective measurement of compliance in the DFO group. MEMS readings were used to monitor the compliance with L1. The compliance in the DFO group was 66.17±11.45% which was significantly lower than the 72.93±14.71% compliance in the L1 group (two-tailed p value=0.0003, using the t-test for unpaired samples).
DISCUSSION

Overview

β-thalassemia is a single gene disorder with a high prevalence worldwide(2). The major defect consists of a decreased or absent production of the β-chain of hemoglobin which results in anemia(1). Patients who are homozygous for the defect require regular blood transfusions as frequently as 3 to 4 weeks in order to maintain their hemoglobin close to 100 g/L(6). Due to the fact that the human body is equipped with mechanisms of iron preservation but not of iron excretion(2), the excess transfusional iron builds up gradually in different organs leading to functional disruption and eventually organ failure(2). The only way to overcome the accumulation of potentially toxic amounts of iron is to accompany transfusional therapy with iron chelation(6).

Desferrioxamine is the only iron chelator presently available. Although very effective in reducing the body iron burden(27,28,48,55) its use is limited due to serious adverse events such as auditory and retinal toxicity(59,60) and growth retardation(62), as well as its high cost(40), which makes it unavailable to most of the worldwide thalassemic population, and its cumbersome administration(58). DFO is effective only when administered parenterally. Most North American patients are treated with subcutaneous DFO using infusion pumps which dispense an equal
amount of drug over a 10-12 hour period. This modality of treatment, although effective, is very painful and locally irritating(58). Thus, it is not surprising that compliance with such a difficult treatment decreases over time. During childhood parents bear the responsibility for infusing the medication. When the patient reaches puberty, the strive for autonomy brings him or her to the decision of not continuing this treatment(107,108). It is estimated that compliance decreases by half after twenty years of age(109). Death following non-compliance with chelation therapy for ten years is a common event in thalassemia(27,28).

There are several factors that influence compliance such as: severity of the disease, difficulty of the treatment, socioeconomic factors, age, knowledge about disease and drug, and patient-physician relationship(89,110). Each factor plays a different role in an individual case.

In this population, the lack of compliance is primarily influenced by the difficulty of the treatment and the absence of an immediate visible benefit.

The physician treating a thalassemic patient has had to estimate the compliance with DFO based only on the patient's report. Once clinical deterioration has occurred, taking steps to improve compliance is no longer beneficial. There is no report in the literature regarding the use of electronic pumps in monitoring the compliance with DFO treatment.
It was clear that a new orally active iron chelator was urgently needed. L1 (1,2,-dimethyl-3-hydroxypyrid-4-one) is the only chelator that has reached the clinical trial phase. Due to its oral effectiveness(70,71), low cost and relative low toxicity(71,80,86,111), L1 will probably represent a major therapeutic advance for thalassemic patients. Previous reports have shown that compliance with L1 is high(103,112), likely because of its easier administration. However, no study has been done trying to monitor compliance adequately in DFO-treated patients and compare the compliance to that in L1-treated patients.

Assessment of Compliance

**DFO-treated patients**

The compliance in the DFO arm of the study (n=19) was assessed using CADD-1 pump monitoring, diary and positive outcome events, such as urinary iron excretion, change in serum ferritin and decrease in hepatic iron concentration, measured by either SQUID or liver biopsy. The compliance as assessed by CADD-1 pump reading was low (66.17±11.45%). This study was the first one in which adherence to the DFO treatment was objectively measured. Due to the fact that the patients were aware that their compliance was monitored, their compliance was likely higher than that of patients who are not monitored. Is this compliance adequate to induce a negative iron balance and to prolong disease-free survival in
thalassemic patients? The answer is difficult, since no long-term compliance monitoring has ever been attempted in thalassemic patients.

Urinary iron excretion (UIE) as an indicator of short-term compliance did not correlate with the values provided by the CADD-I pump reading. This was not surprising since it is known that UIE varies greatly, and is influenced by many factors, such as the total body iron burden (39, 44), the available chelatable iron pool (38, 42) and the vitamin C status (45, 46). It has been documented that the higher the body burden of iron, the higher the urinary excretion. The available chelatable pool of iron is also a significant factor influencing UIE since only circulating free iron can bind to DFO. The pool is constantly changing. It has been shown that vitamin C increases the intermediate chelatable iron pool by blocking iron deposition into reservoirs such as liver (38). Most thalassemic patients are vitamin C-deficient (46), so replacement with vitamin C is warranted as an adjuvant to chelation therapy. The vitamin C status of our study population is not yet available, although all patients in the DFO group received vitamin C supplements. The chelatable iron pool is also influenced by the hemoglobin level. When the hemoglobin is low, iron is used for hematopoiesis, with the result that less iron is available for chelation. The patients in the study were requested to collect urine for one or two days prior to a clinic visit, at which time their hemoglobin was at a
nadir, just prior to receiving a blood transfusion. This means that urinary iron excretion was usually measured when their hemoglobin was low and less iron was available for chelation.

The change in serum ferritin and hepatic iron concentration also failed to correlate with compliance. Both serum ferritin and HIC are indicators of body iron stores and are predictors of long-term compliance with chelation. It is well-documented that in order to achieve a negative iron balance several years of chelation therapy are required(6). Thus, one would not expect to see any correlation between the change in body iron stores and compliance after a few months of chelation therapy. Even when patients were stratified into those with an increase in HIC and those with a decrease in HIC during the study period, no difference in compliance was found between the two groups, which is likely related to small groups of patients in each group.

**L1-treated patients**

New (MEMS devices) and standard methods (pill count, diary, L1 trough concentrations, positive outcome measurements) were used to assess the compliance with this therapy.

The compliance assessed by pill count was higher than that electronically measured by MEMS device. There are two possible explanations: either the pill
count overestimated or the MEMS device underestimated the compliance. Several studies have shown that pill count is an unreliable indicator of compliance, a major limiting factor being pill-dumping. In this group of motivated patients, pill dumping, although possible, is unlikely. Another possible explanation for the erroneously high pill count are errors in pharmacy drug dispensing. MEMS devices are electronic tools available for measuring compliance. The major limitations is the assumption that the patient always takes the drug when the bottle is opened. Another inconvenience is the fact that the patient needs to carry the device and take the pill only from that particular bottle. Taking more than one dose at a time from the bottle can be responsible for an underestimation of the real compliance. However, the fact that the compliances measured by pill count were all clustered around 80% as opposed to the wider, more realistic variability in compliances measured with MEMS devices, suggests that the latter method is more accurate.

Similar to the DFO group, no correlation was found between compliance as assessed by pill count and MEMS devices and mean serum ferritin and changes in HIC, even when patients were divided in two subgroups with high and low HIC. However, when compliance by MEMS devices was compared between patients who had an increase and those who had a decrease in their HIC a statistically significant difference was found, suggesting that at least 85% of daily L1 dose has to be taken
to assure reduction in iron stores. If this difference in compliance, between patients with an increase and a decrease in HIC, as measured by MEMS device but not by pill count, will persist to the end of the study, it will further support the superiority of the MEMS device over pill count in assessing compliance.

Using L1 trough levels as an immediate indicator of compliance also failed to correlate with the values obtained by pill count and/or MEMS devices. The major problems consisted of individual variability in drug handling. A valid L1 trough level provides information regarding the compliance for the one day prior to sampling. Failure to follow instruction prior to sampling, such as not taking the morning dose, is also associated with an erroneously elevated trough level.

**Comparison of compliance with the two therapy regimens**

Compliance assessed by MEMS devices was significantly higher than compliance with DFO as measured by CADD-1 pump. It is conceivable that compliance in DFO-treated patients outside of the clinical trial is even lower than that obtained in this study. More clinic visits, more time spent by the research team (physician, nurse) with every patient and awareness of the fact that compliance is monitored are grounds for the assumption stated above. It is estimated that a compliance of 85% with L1 is sufficient to induce a negative iron balance(71). In our study group, the compliance was close to this value, which enables us to
estimate that at least this group the patients complied not only better than in the DFO group. but that they also had an adequate compliance to induce a decrease in HIC. In a recent report assessing a group of 20 patients on L1 followed for up to five years the compliance was $83\pm2\%$, which strongly suggests that even in long-term studies the compliance with L1 is adequate to maintain a non-toxic level of iron(71). It was previously stated that MEMS readings might underestimate compliance. There is also possible that CADD-I pump overestimates compliance (e.g. infusing the drug in a pillow). Based on these, the difference in the adherence to the two regimens is even more impressive.
CONCLUSIONS

Assessment of compliance in thalassemic patients is critical. The lack of an immediate benefit from a life-long therapy which can be very difficult and painful to administer are the factors accounting for non-compliance in this group of patients.

Outside a clinical trial, the evaluation of compliance can help predict which patients will suffer organ damage secondary to iron overload because of non-compliance with chelation therapy. For DFO-treated patients which account for the majority of thalassemic patients, it is mandatory to use an infusion pump. The CADD-1 pump is the only pump available that can monitor compliance. However, its high cost is prohibitive for most patients.

L1, available now only for experimental use, constitutes an alternative treatment to DFO. As it is active orally and cheaper, it represents a significant advance for thalassemic patients. This study and previous ones have shown that the overall compliance with L1 is only approximately 80%. Thus, it is important to monitor compliance even in this group of patients.

No gold standard methods exist for assessing compliance with tablets or capsules. Our results suggest that MEMS devices can give a more realistic
estimation of compliance. However, some patients will be unable to afford the $150/year/device cost of MEMS devices. For these patients, it is still important to estimate compliance by pill count, keeping in mind the limitations of this method.

During a clinical trial especially, it is critical to use more than one method in order to measure compliance as accurately as possible, so that valid conclusions about the efficacy of the treatment can be drawn.

The results of this study are likely applicable to other chronic disorders. The reasons for noncompliance such as lack of immediate benefit, prolonged treatment which interferes with daily life, severe side-effects are common features in many other chronic diseases. Monitoring the compliance is essential in such conditions since the final outcome is likely influenced by the adherence to the therapeutic regimen.
FUTURE PLANS

The study aimed at following patients for a minimum period of two years. It is conceivable that the final results will be different from those presented here, although, in terms of managing the patients, the short-term results are probably more important. In addition to the final analysis, we are interested in evaluating the factors that play a role in compliance in this particular patient population. In order to accomplish this, a questionnaire (Appendix IV) will be filled out by each patient in the study. Information such as socio-economic status of the patient, age of diagnosis, complications due to disease or therapy, type of chelator agent used, dose and duration of treatment, knowledge about disease and drug, doctor-patient interaction, all will likely provide a better insight into the issue of noncompliance with a prescribed therapy.
Fig. 1. Pathway for heme biosynthesis

CE - β-carboxyethyl (propionic); CM - carboxymethyl (acetic); M - methyl;
V - vinyl
Fig. 2. Total body iron in an adult male of 70 kg is about 4 g and this is maintained by a balance between absorption and body losses. Although the body absorbs only 1 to 2 μg/day to maintain equilibrium, the internal requirement for iron is greater (20-25μg). A red blood cell has a life-span of 120 days so that 0.8% are destroyed each day and must be replaced. A man with 5 liter blood volume has 2.5 g of iron incorporated into hemoglobin with a 5 mg turnover of iron for hemoglobin degradation and another 5 mg for other purposes. (adapted after Conrad & Umbreit- Am J Hematol 42:67, 1993)
Fig. 3.  a) Chemical structure of desferrioxamine

b) Hexadentate ligand 1:1; Desferrioxamine is able hexacoordinate all 6 sites of iron. Once bound to desferrioxamine Fe is difficult to dissociate, even in dilute solution (10^-5)

(adapted from Porter, et al., Bailliere's Clin Haematol, 2;2:257,1989)
Fig. 4. a) 1,2-dimethyl-3-hydroxypyrid-4-one (L1)

b) Bidentate chelator; Iron shares only two molecules of oxygen with L1; Three molecules of L1 are required to occupy all iron sites.

Fig. 5. Medication Event Monitoring System (MEMS)
Fig.6. CADD-1 infusion pump
Fig. 7.
Correlation between UIE and compliance by CADD-1 pump

$r=0.06, p=0.83$
Correlation between UIE and compliance by CADD-1 pump
(patients with HIC <7mg Fe/gdwlt)

$r=0.35$, $p=0.39$
Fig. 9.
Correlation between UIE and compliance by CADD-1 pump

(patients with HIC >7mg Fe/gwlt)

$r=0.08, p=0.85$
Fig. 10.
Correlation between % reduction in HIC and compliance
by CADD-1 pump

\[ r = 0.24, \ p = 0.43 \]
Fig. 11.

Correlation between serum ferritin and compliance by CADD-1 pump

$r=0.34$, $p=0.16$
Fig. 12.
Correlation between compliance by pill count and L1 trough level

$r=0.38, p=0.06$

Compliance with L1 by pill count in the month of sampling (%)
Fig. 13.

Correlation between compliance by MEMS device and L1 trough level

$r = 0.16, p = 0.43$

Compliance with L1 by MEMS device in the month of sampling (%)
Fig. 14.

Correlation between % reduction in HIC and compliance by pill count

$r=0.26, p=0.27$
Fig. 15.
Correlation between % reduction in HIC and compliance by MEMS
Fig. 16.

Correlation between UIE and compliance with L1 by pill count

$r=0.03$, $p=0.91$
Fig. 17.

Correlation between UIE and compliance with L1 by MEMS device

$r=0.18, p=0.47$
Fig. 18.

Correlation between UIE and compliance by pill count
(patients with HIC <7mg/gdwlt)

$r=0.33, p=0.46$
Fig. 19.
Correlation between UIE and compliance by pill count

(patients with HIC >7mg Fe/gdwit)

$r=0.20, \ p=0.56$
Fig. 20.

Correlation between compliance by MEMS and UIE
(patients with HIC < 7mg/gdwlt)

\[ r = 0.005, \ p = 0.99 \]
Fig. 21.
Correlation between UIE and compliance by MEMS devices (patients with HIC >7mg Fe/g dwLt)

$r=0.29, p=0.39$
Correlation between compliance with L1 by pill count and serum ferritin

$\rho = 0.11, p = 0.64$
Fig. 23.

Correlation between compliance by MEMS device and serum ferritin

\[ r = 0.16, \ p = 0.48 \]
TABLE 1. CONSEQUENCES OF IRON OVERLOAD

<table>
<thead>
<tr>
<th>ORGAN INVOLVED</th>
<th>PATHOLOGIC FEATURES</th>
<th>CLINICAL PICTURE</th>
</tr>
</thead>
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<td>HEART</td>
<td>- hemosiderin deposits</td>
<td>- arrhythmias</td>
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<td></td>
<td>- fibrosis</td>
<td>- cardiac enlargement</td>
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<td></td>
<td></td>
<td>- cardiac failure</td>
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<tr>
<td>LIVER</td>
<td>- hemosiderin deposits</td>
<td>- cirrhosis</td>
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<tr>
<td></td>
<td>- intralobular fibrosis</td>
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<tr>
<td>ENDOCRINE</td>
<td>- iron deposits</td>
<td>- growth retardation</td>
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<td>- hypothyroidism</td>
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<td></td>
<td></td>
<td>- hypoparathyroidism</td>
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<td></td>
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<td>- adrenal insufficiency</td>
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<td>- diabetes mellitus</td>
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<td>FACTORS THAT INFLUENCE THE COMPLIANCE WITH THERAPY</td>
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<tr>
<td>1.</td>
<td>DISEASE</td>
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<td>- severity</td>
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<td>- mental disorders</td>
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<td>THERAPY</td>
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<td>- duration</td>
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<td>- side-effects</td>
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<td>- cost</td>
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<td>- multiple drug therapy</td>
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<td>- interference with the life-style</td>
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<td>KNOWLEDGE ABOUT DISEASE &amp; THERAPY</td>
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<td>- good communication</td>
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<td>- counselling</td>
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<td></td>
<td>- patient involved in decision</td>
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<td>- doctor is supportive and caring</td>
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<td>4.</td>
<td>INTERACTION BETWEEN DOCTOR AND PATIENT</td>
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<tr>
<td></td>
<td>- age</td>
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<td></td>
<td>- no family support</td>
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<tr>
<td></td>
<td>- employment status</td>
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TABLE 3. METHODS OF ASSESSING COMPLIANCE

<table>
<thead>
<tr>
<th>DIRECT METHODS</th>
<th>L1</th>
<th>DFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>- L1 trough levels</td>
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<td></td>
</tr>
<tr>
<td>- Positive outcome</td>
<td>- Positive outcome</td>
<td></td>
</tr>
<tr>
<td>(HIC, SF, UIE)</td>
<td>(HIC, SF, UIE)</td>
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<table>
<thead>
<tr>
<th>INDIRECT METHODS</th>
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<tbody>
<tr>
<td>- Diary</td>
<td>- Diary</td>
</tr>
<tr>
<td>- Pill count</td>
<td>- N/A</td>
</tr>
<tr>
<td>- MEMS device</td>
<td>- CADD-1 pump</td>
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</tbody>
</table>

HIC- Hepatic iron concentration, SF- Serum ferritin,
UIE- Urinary iron excretion
TABLE 4. CHARACTERISTICS OF PATIENTS IN EACH TREATMENT GROUP

<table>
<thead>
<tr>
<th></th>
<th>DFO</th>
<th>L1</th>
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<tr>
<td>NUMBER</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>DURATION OF TREATMENT</td>
<td>11.63±3.26</td>
<td>11±4.2</td>
</tr>
<tr>
<td>FEMALE/MALE RATIO</td>
<td>11:15</td>
<td>11:14</td>
</tr>
<tr>
<td>MEAN SERUM</td>
<td>2089±1048</td>
<td>2194±1251</td>
</tr>
<tr>
<td>FERRITIN (ug/L)</td>
<td>(882-3944)</td>
<td>(447-6452)</td>
</tr>
<tr>
<td>HEPATIC IRON</td>
<td>7.43±3.59</td>
<td>9.56±4.77</td>
</tr>
<tr>
<td>CONCENTRATION (mg Fe/gdwlt)</td>
<td>(2.4-15.7)</td>
<td>(2.7-21.1)</td>
</tr>
</tbody>
</table>

Values represent means± SD (range)
TABLE 5. COMPARISON OF METHODS FOR ASSESSING COMPLIANCE IN THE L1-TREATED GROUP

<table>
<thead>
<tr>
<th>METHOD</th>
<th>MEMS READING</th>
<th>PILLCOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 TROUGH</td>
<td>$r=0.16$, $p=0.43$</td>
<td>$r=0.38$, $p=0.06$</td>
</tr>
<tr>
<td>% REDUCTION IN HIC</td>
<td>$r=0.1$, $p=0.67$</td>
<td>$r=0.26$, $p=0.27$</td>
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<tr>
<td>MEAN SERUM FERRITIN</td>
<td>$r=0.16$, $p=0.48$</td>
<td>$r=0.11$, $p=0.64$</td>
</tr>
<tr>
<td>MEAN UIE</td>
<td>$r=0.18$, $p=0.47$</td>
<td>$r=0.02$, $p=0.91$</td>
</tr>
<tr>
<td>UIE in pts with &lt;7 mg Fe/gdwt</td>
<td>$r=0.01$, $p=0.99$</td>
<td>$r=0.33$, $p=0.46$</td>
</tr>
<tr>
<td>UIE in pts with &gt;7 mg Fe/gdwt</td>
<td>$r=0.29$, $p=0.39$</td>
<td>$r=0.2$, $p=0.56$</td>
</tr>
<tr>
<td>Pts with decreased HIC</td>
<td>91.29</td>
<td>93.33</td>
</tr>
<tr>
<td>Pts with increased HIC</td>
<td>83.09</td>
<td>89.71</td>
</tr>
</tbody>
</table>

UIE - Urinary iron excretion, HIC - Hepatic iron concentration
Appendix I.  

**CLINICAL INFORMATION FORM**

<table>
<thead>
<tr>
<th>Research Project:</th>
<th>Randomized Trial of L₁ and Deferoxamine in Thalassemia Major</th>
</tr>
</thead>
</table>
| Investigator(s):  | Dr. Nancy Olivieri (416) 813-6823  
|                   | Dr. Gidcon Koren (416) 813-5781                                   |
| Sponsoring Company: | Rh Pharmaceuticals, Winnipeg, Manitoba |
| Patient Identification: | Name: ____________________________  
|                     | HSC#: ____________________________  
|                     | D.O.B.: ____________________________ |

Thalassemia major is a severe anemia, requiring life-long transfusions which are associated with harmful iron loading in the tissues of the body. The purpose of this study is to determine whether a new oral chelator, 1,2-dimethyl-3-hydroxy-4-pyridine, commonly known as L₁, can chelate or remove iron from the tissues of the body as well as can standard therapy with nightly subcutaneous deferoxamine (DFO). DFO has been shown to remove iron from the tissues of the body and to prevent serious iron-related disease if it is used regularly. However, DFO can be irritating, inconvenient to use, and may have associated toxicities with intensive use and can be very difficult to comply with. Therefore, we are trying to develop a new oral chelator L₁.

If you agree to join this study, you will be asked to undergo a liver biopsy. This is a procedure in which a small sample of liver is taken through the skin in the right side of the abdomen, after skin freezing. This procedure takes approximately one minute. It will require you to come to the Toronto General Hospital, have your blood checked for its ability to clot since bleeding may complicate liver biopsies, and have local freezing inserted in the skin after which a sample will be taken. You will be required to remain sitting or lying for a period of four hours before you are discharged home. From the biopsy specimen, your doctors will determine how much iron is in the liver as a result of your previous transfusions. You will then be divided into a “high” or “low” iron group, based upon whether there is a great deal (“high” iron) or a very little (“low” iron) amount of iron within the liver. Both “high iron” and “low iron” patients will be then assigned to receive therapy with oral L₁ or with subcutaneous DFO. This will be decided by chance, like a lottery, by an individual who is not your doctor and who does not know you personally. Your doctor will have no say as to which treatment you are assigned. Once you are randomized to therapy, you will remain on this treatment, either L₁ or DFO, for a period of at least one and a half years. At the start of the study you will have a magnetic resonance scan of your liver, heart and pituitary gland, and testing of the function of your liver, heart and pituitary gland will be carried out with exercise and blood tests. Your iron levels will also be taken at this time. These tests will be repeated at one and a half years and if necessary, at three years’ time. However, if either L₁ or DFO is shown to be better than the other drug in decreasing iron in the body by one and a half years, we will not be required to continue the study after this, and either L₁ or DFO will become the only therapy for iron overload.

As long as you remain in the study, you will need to have a blood test drawn every week. Each visit will take about 1/2 hour. Each blood sample will need about two teaspoons of blood. You will continue to be transfused and on a 3-5 week basis, and will continue to see your doctor in clinic.
We will ask you to keep a record every day as to whether you have used L1 or DFO. Once a month, at the time of our transfusion, we will go over your records. Twice a year, we will ask you to fill out a form of how much you can do and how well you feel on either treatment.

The risks of DFO are damage to the eyes and ears, interference with growth, kidney and lung failure which usually occurs only with high dose DFO, and tissue irritation. The potential risk of L1 is a decline in the white cell count to less than normal levels, which can be dangerous because this makes an individual more susceptible to infection. This side effect of L1 has been observed in only 2 out of more than 200 patients treated with L1 over the last 2 years. Both patients' white count have returned to normal once L1 was stopped. This is why we will perform white cell counts every week while you are on L1. Joint aches and pains have also been described with the use of L1.

The potential benefits to this study are that, by comparison with a group of patients treated with DFO over the three years, L1 may be shown to be able to remove iron as well as DFO does, and will become the new therapy for iron overload. Without this trial, we will not be able to tell whether L1 can replace DFO.

If you join the study, it is your own choice. If you refuse to take part in it or leave it at any time, this will not affect your present or future care in the Thalassemia Program.

In the case that there are direct harmful effects suffered by you when you take either L1 or DFO, then you will be switched to the other form of therapy.
CONSENT FORM

Research Project: A Randomized Trial of L1 and Deferoxamine in Thalassemia Major

Investigator(s): Dr. Nancy Olivieri (416) 813-6823
                 Dr. Gideon Koren (416) 813-5781

Sponsoring Company: Rh Pharmaceuticals, Winnipeg, Manitoba

I acknowledge that the research procedures described on the attached form and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study. I also understand the benefits of joining the study. The possible risks and discomforts have been explained to me. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential, and that no information will be released or printed that would disclose personal identity without my permission.

I understand that I am free to withdraw from the study at any time. I further understand that if I do not participate in the study, or if there is withdrawal from it at any time, the quality of medical care for me in the Thalassemia Program at The Hospital for Sick Children will not be affected.

I hereby consent to participate.


The Person who may be contacted about the research is:

Dr. N. Olivieri

Who may be reached at telephone #: 813-6823


Signature of Patient, and Age

Witness

Date
Appendix II.

MEMS® Medication Management System
May 10, 1996

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<td>Site</td>
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**Drug**: L1  
**Duration of Action**: 8 hours  
**Regimen**: Take 2 tablet(s) of L1 every 8 hour(s).

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C = Clinical event
**MEMS® Medication Management System**  
May 10, 1995

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<th>Id #</th>
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**Drug**  
L1

**Duration of Action**  
8 hours

**Regimen**  
Take 2 tablet(s) of L1 every 8 hour(s).

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### Analysis Period:

Apr 12, 1995 06:43 to May 10, 1995 12:31

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- **0** - Doses taken within an hour of the time prescribed
- **□** - Doses taken more than one hour earlier or later than the time prescribed
Appendix III. DIARY

DATE: □ □ • □ □ • □ □ (YY.MM.DD)  Patient Initials: □ □ □  
Patient I.D.: L1 □ □

1. Compliance

(1) Patients receiving L₁

I took my medication today at:
_____ a.m. _____ p.m. _____ p.m.

Did you miss one dose of medication? □ NO □ YES
Did you miss more than one dose? □ NO □ YES

If "Yes" is the answer to either of the above questions, explain why.
_________________________________________________________
_________________________________________________________
_________________________________________________________

(2) Patients receiving Desferal

Please record the time the infusion started and finished.
Starting time: __________       Finishing Time __________

Did you miss a dose of Desferal? □ NO □ YES
Was all the medication infused? □ NO □ YES
If no, how much was infused?

Reason that the infusion was not complete:
_________________________________________________________
_________________________________________________________
_________________________________________________________

2. Starting and Stopping Medications

Did you start any new medication today? □ NO □ YES
Did you stop any medication today? □ NO □ YES

If "Yes" is the answer to either of the above questions, please explain stating the name and amount of the medication, and the reason for starting or stopping.
_________________________________________________________
_________________________________________________________
_________________________________________________________
3. **Response to Study Medication**

Did you notice any changes in your health or side effects today? Are you feeling different from previous days? If side effects were noted, please comment if they were mild, moderate, or severe.

☐ YES  ☐ NO

If “Yes”, explain.

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

4. **Other Comments**

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
Appendix IV.

QUESTIONNAIRE

PERSONAL DATA
NAME: ________________________ AGE(years): ____ DOB: ___/___/___
SEX: F M; ETHNICITY _________

SIBLINGS
How many brothers and sisters do you have? _____
Do you have any other brother or sister diagnosed with thalassemia?
YES NO If yes how many? _____

PARENTS
Are you living with your parents? YES NO
Are your parents separated or divorced? YES NO
What are their occupations? Mother_________ Father_________

SOCIO-ECONOMIC STATUS
Are you a student? YES NO
If yes please circle one of the following: school (grade ___ );
college; university.
Are you working? YES NO
If yes please specify. Part-time Full-time.
Are you married? YES NO
Do you have children? YES NO
If yes how many? _____

DISEASE
When has your disease been diagnosed? ___/___/___ How often are you
receiving a blood transfusion? Every ____ weeks.
Have you ever had any complication (such as diabetes mellitus, delayed
puberty, secondary amenorrhea, liver disease, heart disease, etc) due to disease?  
YES NO

If yes please specify ____________________________________

______________________________________________________

Please describe in your own words what thalassemia is. ________

______________________________________________________

______________________________________________________

THERAPY

What type of chelation therapy are you receiving at the present time?

LI DFO

When have you started on this therapy?__/__/__ Please explain in your own words how this therapy works.___________________________

______________________________________________________

______________________________________________________

______________________________________________________

Have you ever been told by your doctor how this drug works? YES NO

Have you ever had any complication due to the therapy? YES NO

If yes please specify________________________________________

______________________________________________________

Is it important for you to take chelation therapy? YES NO

If yes, why do you think is important.__________________________

______________________________________________________

______________________________________________________

What will happen to you if you do not take your medication?____

______________________________________________________

______________________________________________________

Have you ever been told what the consequences of noncompliance with the therapy are? YES NO
Is there anyone in your family who helps you to take your medication?  

If yes, please specify. ____________________  YES  NO  

How are you being helped?  YES  NO  
- reminding  
- mixing the medication  
- putting the needle in  
- giving the tablets  
- others ____________________  

DOCTOR-PATIENT COMMUNICATION  
How do you grade, on a 1 to 5 scale*, the relationship between you and your doctor?  1 2 3 4 5  

What your doctor can do for you in order to help you to cope with the disease and/or with therapy? ____________________  

* 1- very poor  
2- satisfactory  
3- good  
4- very good  
5- excellent
REFERENCES


1,2-dimethyl-3-hydroxypyrid-4-one, in normal and iron loaded rats. *J Clin Pathol* 40:404.


