

Iron Chelators

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Progressive iron (Fe) loading is a major, inevitable consequence of regular blood transfusions and of an inappropriate excessive dietary Fe absorption that results in cellular damage and organ dysfunction and ultimately leads to an early death. Excess body Fe affects predominantly the liver, endocrine organs, and myocardium.

Accumulation of intracellular Fe would facilitate hydroxyl radical generation. Lipid peroxidation consequently occurs and causes membrane damage. Fe within the acid milieu of lysosomes causes disruption of the lysosomal membranes and release of hydrolytic enzymes which are potentially cell-damaging substances to the cytosol.

A significant proportion of Fe unbound to transferrin (Tf) is present in sera in which Tf is fully saturated or oversaturated with Fe. This non-transferrin bound Fe (NTBI) is claimed to be the toxic form of Fe as it is expected to be more readily available for parenchymal deposition and can take part in free radical-mediated reactions. This form of Fe is detectable in patients with severe thalassemia and with hereditary hemochromatosis (HH).

Facilitation of Fe excretion is the only viable approach for management of Fe overload. The goal of Fe chelation therapy is to reduce the Fe stores and subsequently to maintain the body Fe at normal or low levels. It is also reasonable to reduce the reactive, labile Fe in both intra- and extra-cellular sites where it causes tissue injury. Constant exposure of chelator to tissue may be sufficient to chelate labile Fe in form of a nontoxic, stable Fe chelate complex, which would benefit patients by reducing Fe toxicity. On the other hand, larger doses of chelators may rapidly reduce Fe stores, and if the Fe chelated form is able to catalyze the free radical reactions or to redistribute to normal metabolic pathways or to the vital parenchymal tissues, it may cause undesirable adverse effects.

The measurement of NTBI may be used to detect a severe degree of Fe loading and would be useful to monitor the reactive Fe during chelation therapy.

Ideal Fe Chelation

Several hundred compounds, both naturally occurring and synthetic, have been developed. Only desferioxamine (DFO), a hydroxamate siderophore produced by *Streptomyces pilosus* that belongs to the hexadentate ligand's group, is an effective and rather safe Fe chelator used worldwide in patients who suffer from iron overload. Properties of ideal Fe chelators required by clinicians include:

1. Specific for Fe or high affinity for Fe³⁺, does not chelate various competing divalent cations or other trace elements.
2. Non toxic, free of both immediate and long-term side effects.

3. Highly effective in promoting net Fe removal to meet negative Fe balance.
4. Fe chelator complex should totally excrete and should not redistribute to more vulnerable tissues.
5. Easy to administer (preferably in oral form) in order to increase compliance.
6. Should be inexpensive, since patients require long-term administration until body Fe returns to normal level

Ideal properties of Fe chelators for investigators include:

1. Chelate Fe from Tf, which is the strongest Fe-binding protein. Removing Fe from Tf is strongly influenced by the presence of mediating substances such as citrate, nitroacetic acid.
2. Easily enter into the cells to chelate Fe from the labile Fe pool and from the ferritin core. The latter may need an appropriate intermediate substance as a reducing agent such as ascorbic acid.
3. Inhibit Fe uptake by ferritin.
4. Should not inhibit Fe uptake by heme.
5. No inhibition of DNA synthesis.
6. Chelated material leaves cells freely and is rapidly excreted.
7. Not utilised for other metabolic needs.
8. Does not promote the growth of microorganisms

Up to now, no ideal Fe chelator has been available for clinical use. However, desferioxamine possesses many of these properties.

Desferioxamine

DFO is a chelating agent with a strong affinity for Fe, which was developed by CIBA Laboratory and the Swiss Federal Institute of Technology in Zurich in 1960. The active substance is desferioxamine B in the form of methane sulpho-nate. DFO is now a reference chelator for comparisons of the efficacy of new chelators.

Pharmacology

The half-life of an intravenous injection of DFO in humans is only 5-10 minutes, and it rapidly disappears from plasma. It is distributed to the intracellular space, is metabolised via an enzyme-mediated mechanism, and is excreted through urine and bile. The peak level of ferrioxamine (FO) appears in the plasma within 1-2 hours after DFO infusion. Continuous infusion either intravenously or subcutaneously is more effective than other routes of administration. Increases in fecal excretion are observed when marrow erythropoiesis is suppressed, high doses of DFO are used, and when Fe store is reduced.

Source of chelatable Fe

Potential sites of chelator action are:

Plasma Fe	• Transferrin bound • Nonspecifically bound
Membrane Fe	
Intracellular Fe	• Reticuloendothelial (RE) • Parenchymal

Fe chelated by DFO is excreted as FO. There is evidence that DFO removes Fe from both hepatic parenchymal cells and RE stores. It has been suggested that Fe from multiple nonspecific sites is also chelated. An intracellular labile Fe pool is believed to exist, and this should be easily chelated.

The proportion of chelated Fe in urine as compared to the stool varies in different species. In patients with thalassemia with Fe overload, approximately two-thirds of Fe chelated by DFO is excreted in the urine and one-third via the bile in the stool. Studies in animals and humans reveal that RE Fe is the main source of urinary Fe chelated by DFO and that fecal Fe is from hepatic parenchymal cells. The magnitude of removal of Fe from RE may be proportional to the rate of red cell destruction, since a high correlation between degree of hemolysis and amount of urinary Fe is observed. This may also be explained by the reciprocal changes between urinary and stool Fe excretion during chelation when erythropoietic activity is suppressed in patients with thalassemia who are maintained at high hemoglobin levels. Urinary Fe is also derived from hepatic origin in patients with HH.

Recently, the abnormal deposition of free Fe (non-heme, non-Hb, non-ferritin) in red cell ghosts from thalassemic patients with Fe overload was demonstrated. This red cell membrane Fe is chelatable by the orally effective chelator-deferiprone both *in vitro* and *in vivo*. The fall off or disappearance of this form of Fe was demonstrated in non-transfusion-dependent patients with β -thalassemia during long-term, low-dose chelation treatment with deferiprone (DFP).

Route of administration

DFO is not absorbed when given orally. Intramuscular injection is less effective than slow, continuous subcutaneous or intravenous infusion. The rectal suppository route is ineffective. Attempts to develop red cell ghosts containing DFO and the liposomes have been made. These two means of DFO delivery systems have been studied in humans, and increments in Fe chelating efficiency were achieved.

Dose

DFO has to be given by parenteral route either by intravenous or subcutaneous infusion 10-12 hours a day and 5-6 days a week. This regimen is usually practiced as a home chelation therapy. The optimal daily dose is 25-50 mg/kg. In patients with cardiac complications due to severe Fe load, intravenous infusion using a central venous catheter and portable infusor with a reservoir is recommended. Prolonged continuous intravenous DFO infusion has been proven to

be safe and effective in the management of cardiac complications in thalassemia patients.

Effectiveness of long-term DFO chelation

Over 20 years experience in the West using continuous DFO infusion in thalassemia patients who are transfusion-dependent demonstrated that patient survival was significantly improved, body Fe was maintained or decreased, and a better quality of life was achieved.

In well-chelated thalassemia patients who are not transfusion-dependent, DFO chelation is able to maintain Fe stores at near normal amounts, using serum ferritin levels and hepatic Fe concentration as a means of assessment.

Cardiac function is shown to be improved after long-term chelation with DFO. If chelation starts early in childhood, Fe-induced organ dysfunction is preventable. The well-chelated patients have a significantly lesser risk of complications compared to poorly chelated group. Several studies have shown that early and intensive chelation improves growth and pubertal development. If chelation starts after organ damage it reduces the Fe burden and stops progression of tissue damage such as liver fibrosis. However if most endocrine cells are destroyed and fibrosis develops, reversal of organ function is unlikely, so early and intensive chelation is advised.

Factors influencing DFO-induced Fe excretion in humans

DFO-induced Fe excretion is modified by several factors:

1. Dose of DFO: Urinary Fe excretion reaches a plateau level with increasing dose whereas stool Fe excretion shows a linear increase.
2. Degree of Fe load: There is relationship between the amount of excreted Fe, both in urine and stool, and the amount of Fe load assessed by liver Fe concentration, serum ferritin levels, and number of blood transfusion.
3. Activity of erythropoiesis: Very active erythropoiesis found in severe thalassemic patients enhances increased urinary Fe excretion and decreased stool Fe excretion. A reciprocal pattern of excretion is found when the erythropoiesis is suppressed by blood transfusion.
4. Ascorbic acid: Replenishment of ascorbic acid increases DFO-induced urinary Fe excretion but has no effect on fecal Fe excretion. The dose of vitamin C recommended is not higher than 100-200 mg/day. Doses higher than this do not help to further increase Fe excretion. Toxicity is increased, especially to the heart, when high doses of ascorbic acid are used. It has been speculated that a large amount of Fe is mobilised from the non-toxic pool and redistributed to more sensitive tissues.
5. Obstruction of bile flow: In animals, ferrioxamine excretion in urine is increased when biliary occlusion occurs. It is possible that in humans with cirrhotic livers diversion of Fe chelated to a urinary route of excretion occurs.

Adverse Effects of DFO

Generally, side effects of DFO are not serious. Skin irrita-

tion, pruritis, erythema, urticaria, and subcutaneous nodules are observed at the site of infusion. Antihistamines or hydrocortisone added to the infusion is helpful to prevent or reduce reactions. Rotation of infusion sites is advised. Recently, an infusion set with a filter membrane has been developed. This filters a minute precipitate of DFO that causes skin irritation. A more dilute DFO solution is also recommended.

The most frequent toxic effects of long-term DFO therapy are growth failure, bone abnormalities, and high-frequency sensorineural hearing loss. These toxic effects are correlated with intensive chelation on a high dose of DFO and are more frequent in younger patients with low serum ferritin levels. A significant improvement in patients with mild defects has been reported after the reduction of the DFO dose. Other complications resulting from very high doses of DFO are acute visual loss, night blindness, color vision abnormalities as a consequence of pigmentary degeneration, and optic nerve disorders. In most cases recovery of vision is observed after DFO treatment is suspended.

Renal and pulmonary toxicity have also been described in patients treated with very high doses of DFO. Opportunistic *Yersinia* infection is a serious complication. This organism can utilize ferrioxamine in the bowel and become highly pathogenic in well-chelated patients.

These adverse effects can be reduced or prevented by avoiding the use of high doses of DFO and by not starting chelation too early in life. When the Fe load is minimal, the body Fe status should be regularly monitored, and the dose of DFO should be reduced when the serum ferritin level is less than 1000 ng/ml. As recommended by Porter et al, one can use the ratio between the mean DFO daily dose (mg/kg) and serum ferritin level for adjusting the dose of DFO. A ratio of 0.025 is regarded as the toxicity threshold.

Systemic reaction is not common. Hypertension, bradycardia, rigors, headache, and photophobia were described in intramuscular administration. Anaphylactic reactions and acute renal failure have been recorded.

Oral Iron Chelation: Deferiprone

The high cost of the drug, the mode of administration, and the problems of poor patient compliance have limited the use of DFO, especially in the regions where the thalassemia is highly prevalent. Several hundred compounds have been developed as alternative chelators. Among those compounds DFP (1,2 dimethyl-3-hydroxypyrid-4-one, L₁, CP20) is the only oral chelator that has undergone extensive trials in humans. This hydroxypyridone compound, bidentate chelator, was designed by Hider et al in 1984.

The pilot clinical trial was conducted in London, and now clinical studies have been carried out in several centers worldwide. Administration of DFP both in animals and humans has shown the promotion of Fe excretion. In humans, DFP-induced Fe excretes mainly in urine.

In long-term DFP chelation in thalassemia patients who received regular transfusions, prevention of further increases of serum ferritin levels and liver Fe concentration was

achieved with a dose of 75-100 mg/kg/day. A lower dose of DFP at 50 mg/kg/day administered to adult patients with nontransfusion-dependent b-thalassemia was found to be effective as their serum ferritin levels fell to normal or near normal levels within 1-2 years of treatment. The decrease in hepatic Fe concentration and disappearance of red cell membrane free Fe during DFP chelation were recorded. No serious side effects, less frequent or no requirement for blood transfusions, elevation of hemoglobin concentrations of 1-3 g/dl, increased body weight, and a sense of well being were observed during the course of treatment. This regimen would be of benefit to patients with thalassemia intermedia.

DFP is well tolerated up to 150 mg/kg/day without acute toxicity. There have been several reports of side-effects including neutropenia, agranulocytosis, arthralgia, zinc deficiency, and gastrointestinal disturbances. Progressive hepatic fibrosis has been documented only at one center, and this complication is still controversial. DFP has a narrow safety margin; thus, careful precautions are recommended. Frequent and regular monitoring of blood counts during treatment is necessary.

New Development of Fe Chelator

Several compounds have been developed as candidates for promising and effective chelators. These include ICL 670 A [CGP 72670, 4-13-5 Bis (2-hydroxyphenyl)-1,2,4-triazol-1-yl], a novel orally active Fe chelator, and CL 749 B, a depot form of DFO. They were developed by Novartis. These new chelators are currently being investigated.

A combination of DFO subcutaneous infusion and oral DFP was introduced by Grady et al. A synergistic effect on Fe excretion was observed.

It is hoped that in the near future, more effective oral chelators will replace the parenteral forms.

References

1. Development of Iron Chelators for Clinical Use. Arthur E Martell, W. French Anderson, David G. Badman. Elsevier/ North Holland. 1981.
2. Disorders of Iron Metabolism. Alan Jacobs. Clinics in Haematology. Vol 11, No2, 1982. W.B. Saunders Company Ltd.
3. The Development of Iron Chelators for Clinical Use. Raymond J. Bergeron, Gary M. Brittenham. CRC Press. 1994
4. Clinical Disorders of Iron Metabolism. Chaim Hershko. Bailliere's Clinical Haematology: International Practice and Research. Vol 7, No4, 1994 Bailliere Tindall
5. Iron: From Current Biochemistry to New Chelator Development Strategies. National Institutes of Health, Bethesda, Maryland September 21-22, 1998.
6. 9th International Conference on Oral Chelation In the Treatment of Thalassemia and Other Diseases. Universitaet Hamburg. March 25-28, 1999.
7. Long-term chelation with low dose of deferiprone. Pensri Potrakul et al. Iron in Medicine and Biology: Annual Meeting of the European Iron Club and BIOMED Workshop on Iron Chelators. Zeist, The Netherlands, July 9-11, 1998.