Oral Iron Chelators: Present Dilemmas and Future Prospectives

Chaim Hershko, Abraham M Konijn and Gabriela Link

Department of Medicine, Shaare Zedek Medical Center and Department of Human Nutrition and Metabolism, Hebrew University Hadassah Medical School, Jerusalem, Israel P O Box 3235

Correspondence: C Hershko, Department of Medicine, Shaare Zedek Medical Center Jerusalem, Israel P O Box 3235
Fax 972-2-6555610; Phone 972-2-6555111; E-mail hershko@szmc.org.il

CONFERENCIA

HEMATOLOGIA, Vol. 3 N° 2: 81-85
Mayo - Octubre, 1999

Despite the well-documented usefulness of deferoxamine (DF), not all patients are willing to cope with the rigorous requirements of the long-term use of portable pumps. Moreover, the high cost of this treatment is a serious obstacle to its more widespread use. In view of these considerations, there is a great need for the development of alternative, orally effective iron chelating drugs. Within recent years more than one thousand candidate compounds have been screened in animal models. These efforts have led to the identification of several interesting compounds, a few of which may be of possible clinical usefulness. The present discussion will be limited to the most outstanding of these compounds including deferiprone (L1); the polyanionic amines, and; the substituted polyaza compounds.

DEFERIPRONE (L1).

The family of 3-hydroxypyrid-4-one bidentate chelators, designed by Hider and Kontoghiorghes (1,2) binds to iron in a 3:1 ratio with a stability constant of 37, about six orders of magnitude higher than DF. The most important compound of this family is 1,2-dimethyl-3-hydroxypyrid-4-one, (deferiprone or L1). Although clinical reports on the use of deferiprone (L1) in thalassemic and other patients have been published as early as 1987, detailed animal studies on toxicity and on pharmacokinetics have only become available subsequently. At a daily dose of 200 to 300 mg/kg, i.e. 3 to 4 times the dose recommended in man, given for several months, L1 caused bone marrow aplasia in mice, rats, dogs and monkeys, involution of lymphatic tissues and adrenal steatosis. These alterations were associated with high rates of mortality (3).

Pharmacokinetic studies have shown that peak plasma L1 concentrations are achieved within one hour or less of oral administration (4). Glucuronidation is an important mechanism of drug inactivation and is more efficient in man than in rats, a difference that may explain the observed discrepancies in drug efficacy and toxicity between clinical and animal studies (1,5).

The results of long-term iron chelating therapy with L1 in thalassemic patients (6-8) have been summarized in recent years in several reviews (9,10) and the combined experience of the 4 major European and Canadian groups pioneering the clinical use of L1 up to June 1994 has been described in a report of the International Study Group for Oral Iron Chelators (ISGOIC) (11). The study involved 84 patients, 74 with thalassemia major or intermedia, representing a total of 167 patient-years of L1 treatment. Compliance was rated as excellent in 48%, intermediate in 36% and poor in 16% of patients. On an L1 dose of 73 to 81 mg/kg/d, urinary iron excretion was stable, at around 0.5 mg/kg/d with no indication of a diminishing response with time. Serum ferritin showed a very steady decrease with time from an inital mean ±1SD of 4207 ±3118 to 1779 ±1154 after 48 months ($p < 0.001$). 17 patients abandoned L1 therapy. Major complications of L1 requiring permanent discontinuation of treatment included agranulocytosis (3), severe nausea (4), arthritis (2) and peristent liver dysfunction (1). The remaining patients abandoned treatment because of low compliance (3) and conditions unrelated to L1 toxicity.

Lesser complications permitting continued L1 treatment included transient mild neutropenia (4), zinc deficiency (12) transient increase in liver enzymes (37), moderate nausea (3) and arthropathy (16). There was no treatment-associated mortality. Two patients died, both while off treatment: one of hemosiderotic heart disease, and the other of pneumocystis carinii pneumonia with AIDS. This study demonstrates the efficacy of L1 in long-term use for the treatment of transfusional iron overload, but also underlines the complications associated with such treatment.

Recent experience with L1 has been summarized in a report of a major multicenter study empoying the Apotex formulation of L1, involving 187 patients from Cagliari, Torino, Ferrara, Philadelphia and Toronto (the LA-02 study) (12,13), as well as in 3 recent reports by Olivieri (14), Hoffbrand and Wonke (15) and Tondury (16). All patients received a daily L1 dose of 75 mg/kg. The mean duration of treatment was 1.61 years for the study reported by Tricta, and 7.14 for that of Tondury, with the rest ranging from a mean of 3.28 to 4.60 years. Comparing these data with the ISGOIC study terminated in mid-1994 one can see that, with the exception of the Tondury study, there was little or no overlap with the patients participating in the earlier report. Likewise, with the exception of the Tondury study, there was no evidence of a consistent decrease in mean serum ferritins or liver iron concentrations comparing pretreatment values with subsequent measurements. The percentage of patients in whom liver iron concentrations remained above 15 mg/g dry weight (identified in previous studies as the limit above which a significant risk of cardiac complications continues to exist) was 18 (16), 37 (14) and 59 (15) percent. Agranulocytosis developed in 6 patients and transient neutropenia in 19. Although the mechanism of neutropenia in unknown, in some cases it is clearly an immune reaction to L1, as illustrated by the simultaneous development of agranulocytosis, systemic vasculitis, alterations in humoral and cell mediated immune function, and the presence of circulating immune complexes (17).

In three of the above 4 reports the proportion of patients abandoning treatment has been specified. Of a total of 92 patients 36 (39%) discontinued L1 therapy. Six patients died. Of particular concern is the observation that four of the patients died with congestive heart failure due to iron overload, a complication which was shown previously to be prevented by effective deferoxamine therapy. Other important causes of L1 discontinuation were agranulocytosis or neutropenia (6 patients) arthropathy (5 patients) nausea (5 patients), or unsatisfactory response to L1 (8 patients) defined as low compliance (2), rising serum ferritins (4), request to resume DF (1) and change of residence (1).

Thus , by comparison with the ISGOIC study summarizing L1 experience up to June 1994, these recent reports indicate a higher rate of treatment discontinuation (39 vs 20 %), failure to decrese serum ferritin and liver iron concentrations to levels assuring significant cardioprotection in a substantial proportion of cases and, indeed the continued presence of cardiac mortality, a complication of transfusional iron overload which has already been largely eliminated by effective DF treatment. The failure to achieve a steady decrease in storage iron with L1 is explained by the difference in efficacy between the two drugs on a weight per weight basis. As shown by a recent metabolic balance study comparing combined urinary and fecal iron excretion in thalassaemic patients receiving either 60-mg/kg DF or 75 mg/kg p.o. L1, mean iron excretion on L1 was only 65% of that on DF (18). However, in some patients L1 was as effective, or better than L1.

Collectively, these data imply that oral L1 treatment alone will not ensure sufficient protection in all patients and, that close monitoring is required to identify patients in whom additional conventional chelating treatment with DF is indicated. Indeed, the combined use of oral L1 and DF infusions given 2 to 6 times weekly to patients with an unsatisfactoy response to L1 alone, has been advocated in a recent report by Wonke et al (19).

Concerns related to the accelerated development of hepatic fibrosis have been expressed based on observations made on patients on long-term L1 therapy at the Toronto Hospital for Sick Children. Because in thalassemia, viral hepatitis is an important cause of cirrhosis, one shold focus on HcV negative patients to evaluate the potential hepatotoxicity of L1 (14-16). Of the 14 evaluable patients from the Toronto study, 5 developed progression of hepatic fibrosis on L1 treatment. Four of these patients were HcV positive. Only 1 of the 8 HcV-negative Toronto patients developed progression of hepatic fibrosis. By comparison, none of the 17 HcV negative patients reported by Tondury and by Hoffbrand developed this complication. Nevertheless, the potential hepatotoxicity of L1 is still an issue of intense controversy. This important question is likely to determine the future of L1 in clinical medicine and needs urgent clarification by reviewing the hepatic status of all other patients on long-term L1 treatment, such as the large group of patients who participated in the Italian-American multicenter (LA-02) trial (12,13). Until this question is settled, it may be prudent to avoid using L1 in HcV-positive thalassemic patients with active liver disease.

THE POLYANIONIC AMINES (HBED)

The search for improved, orally effective iron chelating compounds has led to the rediscovery of a very powerful polyanionic amine synthesized over 30 years ago by the group of Martell (20): N,N'-bis (2-hydroxybenzoyl) ethylenediamine -N,N'-diacetic acid (HBED). It forms a hexadentate ligand with ferric iron by its secondary, or tertiary nitrogens and hydroxyl and carboxyl groups. The affinity constant for iron(III) of HBED is 39.6, and its affinity for other metals is relatively low. Conversion of the carboxylic groups of HBED to methyl esters results in a marked improvement in its intestinal absorption and a further increase in iron excretion.

HBED and dimethyl-HBED are remarkably nontoxic and their LD_{50} in rats is in excess of 800 mg/kg (21). Our studies in hypertransfused rats have shown that at the dose range of 25 to 50 mg/kg HBED and dimethyl HBED were 12 to 15 times more effective than DF. Likewise, in iron-loaded Cebus monkeys, s.c. HBED was near three times more effective than s.c. DF (22). Iron balance studies performed in a small number of thalassemic patients treated with HBED (23) have shown increased iron excretion following oral treatment both in the urine and stool. Daily total iron excretion with 40 mg/kg/d HBED was 6 to 11 mg, but no further increase in excretion was achieved at a dose of 80 mg/kg/d. These limited amounts of iron excretion represented 28 to 48% of the excretion needed for achieving negative iron balance. Because the prodrug dimethyl-HBED showed improved absorption and bioavailability in animal studies, this compound is now regarded as a promising candidate for clinical evaluation. Likewise, a new prodrug formula of HBED has been developed by Novartis and, in company with two other orally effective compounds developed by the same group, is presently undergoing intensive evaluation (24).

THE SUBSTITUTED POLYAZA COMPOUNDS (IRCO11)

The substituted cyclic polyaza compound IRC011 was synthesized by Israel Resources Corporation Ltd., Haifa Israel (25). IRCO11 has a number of promising features: (a) It is a hexadentate chelator binding Fe(III) at a 1:1 ratio and therefore without the risk of possible toxic intermediate complexes, in contrast with L1 which is a bidentate chelator requiring 3 molecules of the drug for each Fe(III) ion (b) IRC011 is water soluble and its stability constant with Fe(III) is over 1,000 times that of DFO; (c) Its acute LD_{50} in mice exceeds 4.0 mM/kg compared to 0.44 mM/kg for DF and; (d) Unlike DF, polyaza compounds with the structure of IRC011 do not contain any readily hydrolyzable covalent bonds, and are anticipated to resist *in vivo* biotransformation.

The major source of iron mobilized by IRC011 is catabolism of RBC hemoglobin in the RE system (26). Because in thalassemia the contribution of RBC catabolism to the influx of iron to the plasma is incomparably higher than that of all other sources combined, it is reasonable to assume that RBC catabolism is the predominant source of the toxic and readily chelatable non-transferrin bound plasma iron (NTBI) compartment. Thus IRC011 with its superior ability to interact with catabolic RBC iron may not only improve the rate of urinary iron excretion in thalassemia, but possibly also offer better protection of vital tissues from peroxidative damage by preventing the formation or eliminating NTBI. Since IRCO11 is a water-soluble compound, its membrane permeability is limited but it may be regarded as a useful parent compound to a wide spectrum of polyaza analogues. Substitution of its synthetic arms by more lipophilic ligands may result in improved interaction with hepatocellular iron stores, and possibly also better oral activity . Such studies are presently under way.

FUTURE DIRECTIONS

In the search for new and improved chelators, it is useful to remember some of the basic principles determining the safety and efficacy of iron chelators as defined in an important recent review by Hider et al (27):

(a) Hexadentate chelators bind iron at a 1:1 ratio forming a neutral, stable complex which prevents iron from participation in harmful reactions producing hydroxyl radicals. By contrast, bidentate chelators such as L1 require 3 molecules to one ferric iron to form a stable, neutral complex (28). Hence, at suboptimal concentrations of L1, when either tissue iron concentrations are very high or drug concentrations too low, incomplete 1:1 or 2:1 complexes may be formed which may result in enhanced mobilization of potentially toxic iron.

(b) The relative solubility in water and lipids (partition coefficient), determines the ability to cross lipid membranes. High water-solubility improves gastrointestinal absorption. Conversely, increasing lipophilicity results in efficient drug clearance from the portal circulation by the liver. Unfortunately, increasing lipohilicity also improves the penetration of the blood-brain barrier and increases drug toxicity (29).

(c) Size of the molecule: New chelators may be ineffective if their size interferes with their intestinal absorption. To achieve greater than 70% absorption, the molecular weight should be less than 300. Thus, by virture of their lower molecular weights, bidentate and tridentate ligands are predicted to have higher absorption efficiency than hexadentates.

(d) Non-polar prodrugs would be efficiently absorbed and subsequently cleared from the portal system by the liver. Following conversion into the active hydrophilic metabolite, they may chelate liver iron *in situ* to be excreted in the bile or, following release into the systemic circulation would interact with non-transferrin plasma iron and excreted in the urine. Protection against the harmful effects of circulating NTBI is optimal when the chelator is permanently present in the plasma. This effect may be achieved conveniently by employing orally effective prodrugs in slow-release preparations.

The high cost and rigorous requirements of desferoxamine therapy, and the significant toxicity of deferiprone underline the need for the continued development of new and improved orally effective iron chelators. Such development, and the evolution of improved strategies of iron chelating therapy require better understanding of the pathophysiology of iron toxicity and the mechanism of action of iron chelating drugs.

REFERENCES

1. Hider RC, Singh S, Porter JB, Huehns ER. The development of hydroxypyridin-4-ones as orally active iron chelators. In: A. Bank, Ed. Sixth Cooley's Anemia Symposium, **Ann N Y Acad Sci**, 1990, *612*: 327-328.
2. Kontoghiorghes GJ. Design, properties, and effective use of the oral chelator L1 and other a-ketohydroxypyridines in the treatment of transfusional iron overload in thalassemia. In: A. Bank, Ed. Sixth Cooley's Anemia Symposium, **Ann N Y Acad Sci**, 1990, *612*, 339-350.
3. Grady RW, Srinivasan R, Dunn JB, Coursey MP, Lemert RF, Calvano SE, et al. Evidence of toxicity due to 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in normal rats. **Drugs of Today**, Suppl. A. 1992, *28*, 73-80.
4. Al-Refaie FN, Sheppard LN, Nortey P, Wonke B, Hoffbrand AV. Pharmacokinetics of the oral iron chelator deferiprone (L1) in patients with iron overload. **Brit J Haematol**, 1995, *89*, 403-407.
5. Porter JB, Abeysinghe RD, Hoyes KP, Barra C, Huehns ER, Brooks PN, et al. Contrasting interspecies efficacy and toxicology of 1,2-diethyl-3-hydroxypyrid- 4-one, CP94, relates to differing metabolism of the iron chelating site. **Brit J Haematol**, 1993, *85*, 159-168.
6. Olivieri NF, Brittenham JM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, Iron-chelation therapy with oral deferiprone in patients with thalassemia major. **New Engl J Med**, 1995, *332*, 918-922.
7. Tondury P, Kontoghiorghes GJ, Ridolfi-Luthy A, Hirt A, Hoffbrand AV, Lottenbach AM, et al. L1 (1,2-dimethyl-3-hydroxypyrid-4-one) for oral iron chelation in patients with beta-thalassaemia major. **Brit J Haematol**, 1990, *76*, 550-553.
8. Agarwal MB, Gupte SS, Viswanathan C, Ramanathan J, Desai N, Puniyani RR et al. Long-term assessment of efficacy and safety of L1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. **Brit J Haematol**, 1992, *82*, 460-466.
9. Olivieri NF. Long-term therapy with deferiprone. Acta Haematol. **1996**, *95*, 37-48.
10. Al-Refaie FN, Hoffbrand AV. Oral iron-chelating therapy: the L1 experience. **Clin Haematol**, 1994, *7*, 941-964.
11. Al-Refaie FN, Hershko C, Hoffbrand AV, Kosaryan M, Olivieri NF, Tondury P et al. Results of long-term deferiprone (L1) therapy: a report of the International Study Group on Oral Iron Chelators. **Brit J Haematol**, 1995, *91*, 224-229.
12. Tricta F, Spino M. Deferiprone: the long term cumulative experience. (Abstract) 8th International Conference, Oral Chelation in the Treatment of Thalassaemia and Other Disease. **Corfu.**1997, p.44.
13. Cohen A, Galanello R, Piga A, Vullo C, Tricta F. A multi-center safety trial of the oral iron chelator deferiprone. Cooley's Anemia Seventh Symposium. **Ann NY Acad Sci**, 1998, *850*, 223-226.
14. Olivieri NF, Brittenham GM, McLaren CE, Templeton DM, Cameron RG, McClelland RA, Burt AD, Fleming KA. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. **New Engl J Med** 1998, 339, 417-423.
15. Hoffbrand AV, Al-Refaie F, Davis B, Siritanakathul N, Jackson BFA, Cochrane J et al. Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. **Blood** 1998, *91*, 295-300.
16. Tondury P, Zimmermann A, Nielsen P, Hirt A. Liver iron and fibrosis during long-term treatment with deferiprone in Swiss thalassaemic patients. **Brit J Haematol**. 1998, *101*, 413-415.
17. Castriota-Scanderberg A, Sacco M. Agranulocytosis, arthritis and systemic vasculitis in a patient receiving the oral iron chelator L1 (deferiprone). **Brit J Haematol**. 1997, *96*, 254-255.
18. Grady RW, Hilgartner MW, Giardina PJ. Deferiprone: Its efficacy relative to that of desferal. Thirty-Eighth Annual Meeting , American Society of Hematology. Orlando, FL. **Blood** 1996, Suppl. 1. Abstract # 1230.
19. Wonke B, Wright C, Hoffbrand AV. Combined therapy with deferiprone and desferrioxamine. **Brit J Haematol** 1998, *103*, 361-364.
20. L'Eplattenier F, Murase I, Martell AE. New multidentate ligands. VI. Chelating tendencies of N,N'-Di(2-hydroxybenzoyl) ethylenediamine- N,N'-diacetic acid. **J Amer Chem Soc**, 1967, *89*, 837-843.
21. Hershko C, Grady RW, Link G. Phenolic ethylenediamine derivatives: a study of orally effective iron chelators. **J Lab Clin Med**, 1984, *103*, 337-346.
22. Bergeron RJ, Wiegand J, Brittenham GM. HBED: a potential alternative to deferoxamine for iron-cheleating therapy. **Blood** 1988, *91*, 1446-1452.
23. Grady RW, Salbe AD, Hilgartner MW, Giardina PJ. Results from a phase I clinical trial of HBED. **Adv Exper Med Biol**, 1994, *356*, 351-359.
24. Schnebli HP, Acklin P, Buhlmayer P, Crowe A, Faller B, Jin Y, et al. Progress towards new, orally active iron chelators. (Abstract # 216) Thalassaemia: 6th International Conference on Thalassaemia and the Haemoglobinopathies, 1997, St. Paul's Bay, Malta.
25. Winchell HS, Klein JY, Simhon ED, Cyjon RL, Klein O, Zaklad H. Compounds with chelation affinity and selectivity for first transition series elements: Use in medical therapy and diagnosis. Patent Cooperative Treaty (PCT) document 96/10785, 1996.
26. Rivkin G, Link G, Simhon E, Cyjon RL, Klein JY, Hershko C.

IRC11, a new synthetic chelator with selective interaction with catabolic red blood cell iron. Evaluation in hypertransfused rats with hepatocellular and reticuloendothelial radioiron probes and in iron-loaded rat heart cells in culture. **Blood** 1997, *90*, 4180-4187.

27. Hider RC, Choudhury R, Rai BL, Dehkordi LS, Singh S. Design of orally active iron chelators. Acta Haematol, **1996**, *95*, 6-12.
28. Porter JB, Huehns ER, Hider RC. The development of iron chelating drugs. **Clin Haematol**, 1989, *2*, 257-292.
29. Porter JB, Morgan J, Hoyes KP, Burke LC, Huehns ER, Hider RC. Relative oral efficacy and acute toxicity of hydoxypyridin-4-one iron chelators in mice. **Blood**, 1990, *76*, 2389-2396.