Iron chelating agents for iron overload diseases

Guido Crisponi,1 Valeria Marina Nurchi,1 Maria Antonietta Zoroddu2
1Department of Chemical and Geological Sciences, University of Cagliari, Monserrato (CA); 2Department of Chemistry and Pharmacy, University of Sassari, Italy

Abstract

Although iron is an essential element for life, an excessive amount may become extremely toxic both for its ability to generate reactive oxygen species, and for the lack in humans of regulatory mechanisms for iron excretion. Chelation therapy has been introduced in clinical practice in the seventies of last century to defend thalassemic patients from the effects of iron overload and, in spite of all its limitations, it has dramatically changed both life expectancy and quality of life of patients. It has to be considered that the drugs in clinical use present some disadvantages too, this makes urgent new more suitable chelating agents. The requirements of an iron chelator have been better and better defined over the years and in this paper they will be discussed in detail. As a final point the most interesting ligands studied in the last years will be presented.

Introduction

Although iron is an essential element for life, an excessive amount may become extremely toxic for the human body both for its ability to generate reactive oxygen species (ROS), and for the lack in humans of regulatory mechanisms for iron excretion.1 Human protection from iron induced damages involves the degradation of H2O2 to water and oxygen by peroxidases and catalase, the regulation of total unbound iron by ferritin bound iron which via Fenton reaction easily leads to ROS production, and in scavenging iron stores from organs and tissues in which exert their toxic action. The quantification of iron stores in tissues can be accomplished by iron determination in liver biopsies,6,10 or by magnetic resonance imaging11 or by superconducting quantum interference device magnetic susceptibility.12,13

The first used iron chelator has been desferoxamine (DFO): this chelating agent, in spite of all its limitations, has dramatically changed both life expectancy and quality of life of patients preventing the complications of iron overload.14,15,19 as stated by Bernhardt7 the β-thalassemic patients now in their 50’s … are living proof of the value of this drug. The principal drawbacks of desferoxamine are the lack of oral activity, its high cost and the low compliance. The joined research efforts of clinicians, biochemists and chemists to improve the knowledge of iron metabolism and of the requisites of iron chelators has led to the introduction in clinical use of two new oral chelators deferiprone (DFP) and deferasirox (DFX) (Figure 1) at the beginning of this century. These drugs are extremely useful in the treatment of iron overload, but they too present some disadvantages, which make urgent the need of new chelating agents more suitable from a clinical point of view.2,4 In the Novartis Advices for DFXa a warning is reported against renal toxicity of this drug: Exjade can cause acute renal failure, fatal in some patients and requiring dialysis in others. Postmarketing experience showed that most fatalities occurred in patients with multiple comorbidities and who were in advanced stages of their hematological disorders. According Hider17 DFX, although able to readily enter the cells because of its lipophilic, forms at physiological pH a negatively 3- charged iron complex that cannot easily efflux from cells. This feature of iron complex could explicate the nephrotoxic effects of DFX. The formation of stable polymeric complexes with Zn2+ can further contribute to toxic action of DFX. The notably high pZn of DFX (9.44), larger than those of DFO (6.01) and DFP (6.24), has to be remarked.

In the last seven years a number of papers were published on the comparison of cost effectiveness of chelation therapy in transfusion dependent thalassemia patients.18,20 The common results for the US19 and UK20,21 health-care system perspectives on the cost effectiveness of oral DFX vs infusional DFO suggest that DFX is cost effective compared to standard chelation with DFO, while a different conclusion is reached by Luangasanatip et al.22 in Thailand. The two studies that perform a cost analysis of DFP,22 both suggest that DFP is the most cost-effective cure for the treatment of iron overload in β-thalassemia patients.

The design of new improved iron chelators must take into account specific chemical properties.

The requirements of an iron chelator, better and better defined over the years, can be outlined as:
- Favorable toxicity profile of chelating agent and of its complexes;
- Stability of its complexes, higher than that with endogenous ligands;
- Selectivity toward iron;
- Suitable redox potential of complexes;
- It should not be transformed into inactive metabolites in the body;
- Good intestinal absorption and bioavailability to the target cells;
- Fast kinetic exchange of iron between chelator and endogenous ligands;
- Factors favoring excretion of the formed complexes;
- It must not disturb the metabolic homeostasis in the body fluids.

Further issues govern the suitability of an iron chelator, as its cost and the patient com-
and of the stoichiometry of the formed species. Each complex-formation reaction in a system containing a metal ion M, a ligand L and the proton H is described by the general equation:

\[ pM + qL + rH ⇌ M_{p}L_{q}H_{r} \]  \hspace{1cm} (1)

and the corresponding formation constant, at given temperature and ionic strength, is given by:

\[ \beta = \frac{[M_{p}L_{q}H_{r}]}{[M]^{p}[L]^{q}[H]^{r}} \]  \hspace{1cm} (2)

where charges and coordinated solvent molecules are omitted for simplicity. The terms in square brackets are the molar concentrations of the complex and of the free components; the coefficient \( r \) assumes negative values when the number of protons released from the ligand is higher than the number of protons released in absence of metal ion, or when hydroxylated species are formed.

Actually the real effective binding capacity of a ligand cannot be directly inferred from the stability constant, but it depends on a variety of factors, the principal ones being the competition between metal and proton for the same basic sites on the ligand, and the stoichiometry of the formed complexes. This real efficacy is generally evaluated by parameter \( \beta \), defined as \(-\log[M]\) at \([M]_{\text{tot}} = 1\times10^{-6} M\) and \([L]_{\text{tot}} = 1\times10^{-5} M\) at pH 7.4, where \([M]\) is the concentration of free metal ion and \([M]_{\text{tot}}\) and \([L]_{\text{tot}}\) are the total concentrations of metal and ligand, respectively. Iron chelators are normally compared on the basis of their log K values calculated from data at 25°C and 0.1 M ionic strength. A pFe value >20 is required for efficiently scavenging iron from biological matrices. Some conventional ways of calculating this value have to be remembered, which, if not properly used, can produce misleading results: in the model used for the calculation of pFe the protonation constants of the ligand and the formation constants of all formed complexes have to be taken into account, but the hydroxide formation constants must not be considered, otherwise all the ligands whose pFe is lower than that due to the hydroxide formation, should result in a similar pFe.

**Effect of substituent**

Interesting good linear correlations are found when the protonation constants and the iron complex formation constants of given classes of ligands are considered. In particular, examining the protonation and the stability constants of pyridinones, reported in Table 1,23-26 good linear correlations are found between the first protonation constant and the formation constants log \( K_{11} \), log \( K_{12} \), and log \( K_{13} \), relative to FeL, FeL2 and FeL3 complexes respectively, as well as with the second protonation constant log \( K_{2} \) (Figure 2A).

These correlations are a clear indication that the same properties determine proton and iron binding. If one assumes that the log \( K_{1} \) values of pyridinone ligands can be modulated by proper substituents, the log \( K_{2} \) and log \( K_{11} \), log \( K_{12} \), log \( K_{13} \), also determined by the effect of substituents, can be estimated by the
parameters of the above straight lines. These values allow to evaluate the pFe values of substituted pyridinones as a function of log $K_1$, reported in Figure 2B.

The main feature of Figure 2B is the break point that can be observed at log $K_1 = 7.4$. This implies that, when starting from a pyridinone ligand characterized by a pK1 < 7.4, a substituent that increases the pK1 value till to 7.4, and all the related constants, has the effect of increasing the pFe. On the contrary, when starting from a ligand with pK1 > 7.4, an increase of the pFe can be obtained introducing substituents whose inductive and resonance effects lead to a decrease of pK1 till to 7.4. The effect of pK1 in determining the pFe value has been also remarked by Pyamangkol et al. They described a number of 2- and 6-amido-3-hydroxy-pyridin-4-ones, all characterized by lower pK1 values than that of deferiprone, because of the inductive effect of the amido group. Moreover, the pK1 values of 1-nonsubstituted pyridinones containing the 3-hydroxy group are dramatically lower than those of the corresponding 1-alkyl analogues. This is due to a strong hydrogen bond between the 2-amido function and the 3-oxygen anion, stabilizing the anion. The pFe values of this group of molecules result higher than that of deferiprone as a consequence of the decreased proton competition.

**Suitable redox potential of complexes**

As pointed out in a previous section, the toxic action of a redox-active metal ion as Fe$^{3+}$ depends on the formation of reactive oxygen species that cause remarkable injuries to tissues and organs. To prevent ROS formation in the organism, the quantity of unbound iron is limited by iron binding proteins.

**Good intestinal absorption and good bioavailability**

According Hider the three main factors governing diffusion through biological membranes are molecular size, lipophilicity and net charge. In particular the molecular weight of drugs to be absorbed in the human gut should not exceed 500 Da. Lipophilicity is generally estimated by the water-octanol partition coefficient (P). These factors have been proposed by Lipinski et al. to evaluate membrane permeability by a four parameter analysis. According these authors a good absorption is likely when:

---

**Table 1. Protonation constants (log $K_1$, log $K_2$) and iron complex formation constants (log $K_{11}$, log $K_{12}$ and log $K_{13}$) for some pyridinones.**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Log $K_1$</th>
<th>Log $K_2$</th>
<th>Log $K_{11}$</th>
<th>Log $K_{12}$</th>
<th>Log $K_{13}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Hydroxy-2-pyridinone</td>
<td>5.86</td>
<td>1.2</td>
<td>10.61</td>
<td>9.5</td>
<td>7.1</td>
<td>23</td>
</tr>
<tr>
<td>1-Hydroxy-2-pyridinone</td>
<td>5.78</td>
<td>1.0</td>
<td>10.3</td>
<td>9.0</td>
<td>7.6</td>
<td>24</td>
</tr>
<tr>
<td>3-Hydroxy-4-pyridinone</td>
<td>9.01</td>
<td>3.34</td>
<td>14.2</td>
<td>11.6</td>
<td>9.3</td>
<td>24</td>
</tr>
<tr>
<td>1,2-Dimethyl-3-hydroxy-4-pyridinone</td>
<td>9.82</td>
<td>3.66</td>
<td>15.01</td>
<td>12.3</td>
<td>10.1</td>
<td>25</td>
</tr>
<tr>
<td>1,2-Diethyl-3-hydroxy-4-pyridinone</td>
<td>9.93</td>
<td>3.81</td>
<td>15.21</td>
<td>11.76</td>
<td>9.78</td>
<td>26</td>
</tr>
</tbody>
</table>

---

*This Table is reprinted from Coordination Chemistry Reviews, Vol 252, Crippa G, Renelli M, Iron chelating agents for the treatment of iron overload, Pages No. 1225–1240, Copyright (2008), with permission from Elsevier.*
i) molecular weight <500 Da; ii) log P<5; iii) less than 10 hydrogen bond donors (sum of OH and NH groups) are present in the drug; iv) less than 10 hydrogen bond acceptors (sum of O and N atoms) are present in the drug.

**Fast kinetic exchange of iron between chelator and endogenous ligands**

The behavior of a chelator depends, besides the thermodynamics of complex formation, on kinetic factors, connected to: i) degradation of the chelating agent; ii) complex formation between the chelator and the free metal ion in the plasma; and iii) exchange reaction between the metal bound to endogenous molecules and the chelating agent.

i) Many chelating agents are metabolized in the body to species that lose the chelating properties of the parent molecule. These reactions can be very different, from the gluconidation of hydroxypyridinones, to the acetylation of Trien, or the formation of −S-S-bonds between BAL and SH-containing ligands. The correct choice of drug administration becomes of vital importance when this kind of metabolic transformation is rapid, as for example the subcutaneous infusion of deferoxamine.

ii) The circulating toxic iron ions in plasma are generally bound by different endogenous molecules, ranging from large macromolecules as transferrin to low molecular weight ligands as citrate.

iii) The kinetic of the exchange reaction between the ferric ion bound to endogenous molecules and the chelators depends on a variety of factors, among which the structure, the denticity and the size of the chelator. The knowledge of the different kinetic behaviors of the chelators in use has conducted to improved schemes of therapy. In case of iron overload therapy, in which a more than 30 years experience has been accumulated on deferoxamine action, and at least 20 years for deferiprone, the knowledge of the differences in organ distribution and bioavailability, in target biomolecules and in kinetic and thermodynamic properties, has led to a combination therapy in which the two chelators exert a synergistic action.39,40

**Recent research achievements on iron chelating agents**

In this section a surely not exhaustive survey of recent progresses in the study of new iron chelators will be presented. A number of new ligands appeared in literature in the last 10 years, reporting thorough studies of complex formation equilibria and, in some cases, biological evaluations. Particular attention has been paid to 3-hydroxy-4-pyridinone (3,4-HP) ligand family. The 3,4-HPs are mono-anionic N-heterocyclic molecules that bind FeIII with the two oxygen atoms (bindentate {0,0} chelators) with high affinity. This family of ligands can be easily extra-functionalized with the aim of improving their properties, above all the bioavailability and the chelating ability. The group of Hider37,41 has proposed a variety of bidentate or polydentate 3,4-HP chelators. In particular they synthesized and studied the chemical properties of different 2- and 6-amido-3-hydroxyypyridin-4-ones; it is interesting to remark that all these ligands exhibit lower pKs values than deferiprone due to the inductive effect of the amido group. These lower pKs lead to a decreased proton competition, so to better chelating properties in comparison with the parent deferiprone. Some ligands of the HP family were also studied for the treatment of toxicity of other hard metal ions: aluminium, by Santos et al.43 and plutonium by Fukuda.44 A number of tetradeinate 3,4-HP chelators have been studied by the group of Santos45-46 in one case two 3,4-HP chelating moieties were connected by an iminodiaceatic acid (IDA) scaffold, with a 1,4-disubstituted arylpiperazine on the nitrogen atom in the linker. The same authors also presented:

- Two hexadentate ligands, based on the KEMP acid scaffold to which three 3-hydroxy-4-pyridinone chelating moieties are attached via two differently sized spacers, the tris-hydroxyypyridinonate based compounds KEMPPr(3,4-HP)3 and MPBu(3,4-HP)3.47 These ligands are very strong chelating agents not only towards iron but also for group III metal ions, with potential clinical applications in metal chelation therapy.

- Two new tris(3-4-HP) hexadentate chelators, NTa(BuHP)3 and NTP(PrHP)3, in which the three HP units were connected by nitrolotriacetic acid (NTA) and nitrolotripropionic acid (NTP). Their iron complex formation ability has been studied in solution and their scavenging properties from overloaded animals assayed in vivo. A very strong chelating affinity for Fe must be remarked [pFe = 27.9 NTA(BuHP)3; pFe = 29.4 for NTP(PrHP)3].48 Further families of ligands have been considered as chelators for trivalent metal ions. Biaso et al.49 presented two tripodal molecules, O-TRENSOX, formed by three 8-hydroxy-3-sulphonate-quinoline anchored on tris(2-aminoethyl)amine (TREN), as well as analogous triscatechol derivatives TRENCAMS. Impressive pAl values 20.0 and 26.2 for O-TRENSOX and TRENCAMS respectively were reported. Numerous studies on the design, synthesis and complex formation equilibria of variously substituted hydroxypyridin-4-oxo-substituted hydroxypyridin-carboxylates have been presented by the group of Di Marco: these ligands are characterized by two coordinating groups (–OH and –COOH) in various positions (2,3; 3,2; 3,4; 4,3) and differen-

tently methylated.53-55 A study of our group on bishophosphate ligands showed their high efficiency as iron chelating agents, reaching pFe values higher than that of deferiprone.56 To further improve bishophosphate chelating properties the conjugation with other strong coordinating groups was proposed. Ding et al.7 synthesized catechol-bishophosphate conjugates and Bailey et al.58 mixed bishophosphonate-hydroxypyridinonate compounds. With both kinds of ligands the too short linker prevented simultaneous tetradentate coordination.

Fox and Taylor65 experimented an interesting iron chelator, formed by two kojic acid units linked by a methylene group, for the in vitro mobilization of ferritin-bound iron, and proved its high efficacy. This ligand forms stable iron dinuclear complexes, characterized by the extremely high pFe value 20.5.62 On the basis of this interesting chemical result we designed and synthesized a variety of chelators based on two KA units joined by different linkers.65-67 In particular those based on a −CH2-NR-CH2 linker form Fe2L3 complexes in which the three ligands completely satisfy the coordination sphere of two iron ions. The relatively low molecular weight (340-450 Da) characterizes them as possible oral chelators, and the many chances of modulating their binding ability working on proper substituents in the KA units and in the linker offer good perspectives.

Many tetradeinate ligands have been investigated to date as possible FeIII chelators for oral use. In order to completely saturate the six coordination positions on ferric ion, the denticity of these ligands requires formation of polynuclear species, which are invariably found in these systems. In particular, the most common polynuclear complex is the dimer Fe2L2, with a charge depending on ligand structure. An especially high affinity for the ferric ion has been found by Santos et al.59 concerning the new bis(3-hydroxy-4-pyridinone) derivative of iminodiacetic acid, imino-bis(acetyl(1-(3-amino-propyl)-3-hydroxy-2-methyl-4-pyridi- none)), IDAPA (3,4-HP)3. The calculated pFe value is 25.8, of the same order of magnitude as that of DFO. However, in the case of IDAPA (3,4-HP)3, up to eight complex species form in the explored pH range (0.4-9), and their relative amount at a defined pH (e.g. 7.4) depends on the total concentration of both the metal ion and the ligand.
Conclusions

In the last thirty years the chemical research has been deeply involved in the synthesis of a large variety of iron chelators according to the structural requisites for their introduction in clinical practice. Despite the significant improvements made in the cure of iron overload with the introduction of deferiprone and deferasirox, and of combined chelation therapy, the clinical results have not been completely satisfactory for the various drawbacks presented by these chelators. The failure to find the ideal iron chelator can be ascribed to inherent difficulties deriving from the biological and clinical restraints.

We think that the large research efforts on these topics have in any way created a large progress in this field, above all with a deeper knowledge of iron metabolism, drug targets, drug absorption mechanisms, relationships between structure and physical-chemical properties, and basic requirements for the different clinical purposes.

We hope that a continuous dialogue among chemists and clinicians, and an ample support to the research in this field, will lead to the common target of prolonging survival and improving the quality of life of iron-loaded patients.

References

36. Hider RC, Liu ZD. Emerging understanding of the advantage of small molecules such as hydroxypyridinones in the treat-


