Therapeutic iron chelators and their potential side-effects

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Abstract

A number of iron-chelating agents are currently being considered as orally active alternatives to desferrioxamine (DFO), the therapeutic agent for the treatment of body iron overload that is available at present. These include bidentate hydroxypyridinones (HPO), tridentate desferrithiocin (DFT) analogues and hexadentate aminocarboxylate (HBED) chelators. All chelating agents have the potential to induce toxic effects when iron homoeostasis is affected within the body. This can arise when the absorption, distribution and utilization of iron is affected. Alternatively, chelating agents can induce toxicity by directly interfering with iron-dependent metalloenzymes located within the body. These effects are, however, mainly localized to non-haem enzymes such as ribonucleotide reductase and lipooxygenase. The resultant iron complexes also have the ability to induce toxicity. Depending on the coordination geometry and donor atoms associated with the metal centre, redox cycling of the iron centre with the corresponding generation of free radicals can result.

Redox activity of iron

There is considerable clinical interest in the redox active metals such as iron due to the postulated involvement of hydroxyl radicals in normal physiological responses as well as in a range of disease states [1]. Iron has two important chemical properties which have rendered it a critically important element to virtually all life forms. Iron possesses two oxidation states, iron(II) and iron(III) and the redox potential between these is such that oxidation processes centred on the iron atom can be readily coupled to metabolic reactions. Iron also has a high affinity for oxygen atoms. These two properties are utilized widely by iron-containing proteins, for instance, as electron transfer proteins in the mitochondria, as hydroxylating enzymes and as oxygen transport proteins such as haemoglobin [2]. These important properties also endow iron with the potential of being toxic,
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Scheme 1. Iron catalysed Haber–Weiss reaction. Redox cycling of iron between the ferric [Fe(III)] and ferrous [Fe(II)] states and in the process generating toxic hydroxyl radicals.

this being particularly true should the iron be non-specifically bound to the surface of proteins and membranes. Such weakly coordinated iron can redox cycle between the two oxidation states thereby generating a range of oxygen radicals including the hydroxyl radical \([3]\) (Scheme 1). The hydroxyl radical ('OH) is highly reactive and is capable of interacting with most types of organic molecules, including sugars, lipids, proteins and nucleic acids. Consequently, the production of hydroxyl radicals is undesirable and there are a number of protective measures adopted by cells to protect against its formation, foremost among these is the tight control of iron absorption, transport and storage within multicellular organisms.

\(\beta\)-Thalassaemia major

Several blood diseases require the regular transfusion of red blood cells. One of the most important of these on a worldwide basis is thalassaemia. Thalassaemia is a genetic disorder that is characterized by a defect in the synthesis of haemoglobin, a vital oxygen carrier molecule of the blood \([4]\). It is estimated that there are up to 200 million carriers of the various forms of the disease worldwide.

The only effective treatment of \(\beta\)-thalassaemia major is to increase haemoglobin levels by regular blood transfusions, without which, the majority of patients die within the first year of life. Repeated blood transfusion will lead inevitably to an excess accumulation of iron in the body due to the inability of man to excrete this metal produced from the breakdown of haemoglobin \([5]\). The excess iron found in thalassaemic patients is distributed throughout the body but is found in highest concentrations within the liver and other highly perfused organs. The unregulated accumulation of iron causes tissue damage and failure of organs such as the liver and heart, eventually leading to death. Complications associated with the toxicity of iron following blood transfusion can, to a large extent, be alleviated by the use of specific metal-scavenging agents or chelating agents, to trap and allow excretion of excess and potentially toxic forms of iron from the body.
Chelation therapy

Desferrioxamine (DFO) (Fig. 1; 1) has been available for the treatment of iron overload for over 30 years [6]. DFO is highly selective for iron with a stability constant of $10^{31}$ and only minimal affinity for other metals such as copper, zinc, calcium and magnesium ($10^2$–$10^4$). The major limiting factor of DFO is that it is inactive when administered orally and only causes sufficient iron excretion to keep pace with transfusion regimes when given either subcutane-

Fig. 1. Chemical structure of various iron chelators. 1, Desferrioxamine (DFO); 2, acetohydroxamic acid (ACH); 3, diethylenetriamine penta-acetic acid (DTPA); 4, hydroxybenzyl ethylenediamine (HBED); 5, 3-hydroxy-pyridine-4-one (HPO); 6, desferrithiocin (DFT); 7, ethylene diamine tetra-acetic acid (EDTA); 8, nitriloacetic acid (NTA).
ously or intravenously over 8–12 h, 5–7 days a week. For this reason, many patients find it difficult to comply with the treatment and some even stop taking the drug altogether. Additionally, DFO and the associated equipment required for treatment such as infusion pumps and syringes are prohibitively expensive to patients in developing countries. There is, therefore, no doubt that an affordable orally active chelating agent is needed to treat patients on lifelong transfusion programmes. The development of an oral iron chelator might also allow the extension of the therapeutic use of red-cell transfusions in sickle-cell anaemia.

**Orally active iron-chelating agents**

The design of an orally active, non-toxic chelator has been a goal of many medicinal chemists over the past 20 years. Unfortunately, the above goal has not yet been achieved since a wide range of requirements need to be met before such an agent becomes available.

**Requirements for selective iron chelation**

**Iron selectivity**

To achieve effective and safe chelation *in vivo*, a compound with a high iron binding constant and a high degree of specificity in relation to other metals is required. Iron exists in two oxidation states, iron(III) or ferric iron, and iron(II) or ferrous iron. Under aerobic conditions, iron(III) is the more stable and chelators with high affinity for this form of iron are therefore of much greater use. Iron(III) forms the most stable bonds with oxygen-containing chelating agents; it is for this reason that the majority of siderophores utilize the dioxo ligands catechol and hydroxamate.

**Bidentate versus hexadentate ligands**

The stability of the metal complex is also influenced by the number of covalently linked arms on the chelator (Fig. 2). Due to entropy considerations, hexadentate

![Bidentate ligand 3:1 and Hexadentate ligand 1:1](image)

*Fig. 2. Bidentate and hexadentate iron complexes.* Iron is most stable when bound by six oxygen atoms arranged octahedrally around the metal ion. A bidentate ligand occupies two of the above positions requiring three molecules to totally encompass the iron atom. In contrast, all six coordination positions are occupied by a single hexadentate molecule.
ligands possess a much higher kinetic stability than that of the corresponding bidentate ligands. Thus the affinity constant of iron for the bidentate acetohydroxamic acid (Fig. 1; 2) and hexadentate desferrioxamine (DFO) (Fig. 1; 1) are $10^{28}$ and $10^{33}$ respectively. Hexadentate ligands also retain appreciable iron-binding power at low concentrations. This is in contrast to bidentate ligands where a third-order concentration dependence applies, as three ligands are required to occupy all co-ordination positions around the iron atom.

As a general rule, oligodentate ligands are kinetically more inert than their bidentate analogues. Thus a potential disadvantage of bidentate ligands which, due to their co-ordination of iron in a stepwise fashion (eqn. 1), can lead partially dissociated (2:1 and 1:1) complexes to form at low concentrations. In principal, these incomplete iron complexes can generate hydroxyl radicals.

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\text{Fe}^{3+} \rightleftharpoons [\text{Fe}^{3+}-\text{L}]^{2+} \rightleftharpoons [\text{Fe}^{3+}-\text{L}_2]^{+} \rightleftharpoons [\text{Fe}^{3+}-\text{L}_3]\]  

(1)

Ideally a kinetically inert molecule should be used for chelation therapy, where, once chelated, the iron remains tightly bound to the ligand ensuring minimal redistribution of iron. However, in practice, there is a fine balance as kinetically inert ligands are less able to chelate oligomeric and polymeric forms of iron (for instance haemosiderin and certain forms of non-transferrin-bound iron). Thus hexadentate ligands may be less effective than bidentate compounds when solubilizing aggregate forms of iron. The disadvantage of increased kinetic lability faced by bidentates relative to hexadentates can be minimized by the use of ligands with high affinities for iron(III).

**Oral absorption**

A key property for an oral iron chelator is its ability to cross biological membranes enabling it to be absorbed from the intestinal tract. Most drugs enter cells by simple diffusion through the hydrophobic region of the cell membrane and consequently uncharged drugs permeate more rapidly than charged molecules. Thus neutral chelators are more likely to cross epithelial cells of the intestine and be orally active than charged molecules. Similarly such chelators should also be able to penetrate into the cytoplasm of cells. Clearly for oral activity to be achieved, the chelator must also be designed to resist the acidity of the stomach and enzymic cleavage.

Oral bioavailability and penetration of biological membranes, in addition to being affected by parameters such as octanol/water partition coefficients and ionic state, are also influenced by molecular size. Generally molecules with molecular masses > 400 Da only poorly penetrate biological membranes by simple diffusion. A major distinguishing feature between bidentate and hexadentate ligands is molecular size; bidentate ligands typically fall in the molecular-mass range 100–250 Da, whereas the larger hexadentate ligands fall in the range 400–1000 Da. Most siderophores, including DFO, have molecular masses between 600 and 900 Da. Diethylenetriamine penta-acetic acid (DTPA) (Fig. 1; 3) (393 Da) and hydroxybenzylethylene diamine (HBED) (Fig. 1; 4) (364 Da) are probably close to the minimum size possible for effective hexadentate iron(III) ligands. Thus by virtue of their lower molecular masses, bidentate molecules are likely to have a much higher bioavailability than hexadentate ligands. This is certainly the
experience with 3-hydroxypyridin-4-ones such as CP20 (Fig. 1; 5a) and CP94 (5b), which have been studied in man and are efficiently absorbed [7].

**Metabolism and pharmacokinetic properties of chelating agents**

The metabolism and pharmacokinetics of chelating agents are likely to play a critical role in determining both the efficacy and toxicity of therapeutic agents. It is important to ensure that the chelating agent is not metabolically degraded to metabolites which lack the ability to bind iron. This will inevitably require the use of higher drug levels, increasing the risk of inducing toxic side-effects.

Further therapeutic benefit can be gained by ensuring that the chelating agent is delivered to target sites at an appropriate concentration, rate and duration. Ideally for maximal chelation, a drug must be present within the body at both a reasonable concentration and duration to ensure interception of iron from either extracellular or intracellular iron pools. Compounds with short-plasma half lives are thus likely to be less effective due to the limited pool of chelatable iron present within the body at any one time.

**Design of orally active chelating agents**

The obvious method of choice is to model novel structures on natural hydroxamate and catechol siderophores [8]. Coordination of iron for both hydroxamates and catechols occurs with oxygen donor atoms which give rise to chelators which possess extremely high affinities for iron(III). Catechol-based ligands are unfortunately prone to oxidation in the intestine as well as generally being poorly absorbed. A further disadvantage of this ligand is that its iron complex has a net negative charge preventing it from efficiently crossing biological membranes. Hydroxamate ligands in contrast form neutral iron complexes but are metabolically labile and only poorly absorbed by the oral route.

A number of iron-chelating agents are currently being considered as orally active alternatives to DFO, the presently available therapeutic agent for the treatment of body iron overload resulting from frequent blood transfusion. These compounds include bidentate hydroxypyridinones (HPOs) (Fig. 1; 5), tridentate desferrithiocin (DFT) analogues (Fig. 1; 6) and hexadentate aminocarboxylate chelators, for instance HBED (Fig. 1; 4) where 3, 2 and 1 of the above ligand(s) are coordinated with ferric iron respectively. The above ligands can also be classified by the donor atoms associated with the metal centre. High-spin trivalent ferric ion, due to its high charge density, forms most stable interactions with weakly polarizable atoms such as oxygen. Ferrous ion, in contrast, has a preference for nitrogen donor atoms. DFT and HBED, unlike HPO and DFO, bind iron via a mixture of oxygen and nitrogen donor atoms and thus are less specific for iron(III).

**Potential toxicity of iron chelators**

Chelating agents have the potential to induce toxic effects when they affect iron homoeostasis within the body. This can arise when the absorption, distribution and utilization of iron is affected. This safety margin may be increased in the presence of iron overload but as not all cells will be equally iron overloaded, some
will be more susceptible to iron deprivation than others. Alternatively, chelating agents can induce side effects by directly interfering with iron-dependent metalloenzymes located within the body. The resultant iron complexes also have the ability to induce toxicity. Under certain circumstances, redox cycling of the iron centre with the corresponding generation of free radicals can result. The affinity constant of the ligand for iron is also important, since the lack of stability of complexes can lead to redistribution of iron from relative non-toxic iron stores to potentially toxic sites in the body, eventually leading to tissue damage.

**Lack of iron selectivity**

Unfortunately many ligands which possess a high affinity for iron(III) also have high affinities for other metals. Aminocarboxylate ligands such as DTPA, used in patients who develop toxic side-effects with DFO due to its relative lack of selectivity for iron(III), lead to zinc depletion. In an attempt to enhance the selectivity of this class of chelator for iron(III), Martell and co-workers have synthesized several hexadentate analogues which contain both carboxyl and phenolic ligands [9]. A particularly useful compound from this series is HBED which is significantly more effective than DFO when given intramuscularly to iron-overloaded rats. However, HBED is not efficiently absorbed via the oral route and by virtue of its two carboxyl functions, retains a relatively high affinity for zinc.

Another class of chelating agent which has been extensively evaluated over the past decade is DFT, a siderophore isolated from *Streptomyces antibioticus*. This compound is a tridentate molecule which forms a 2:1 complex with iron(II1) [10]. DFT, however, retains an appreciable affinity for other metals, particularly copper and shows toxic effects in hepatocytes at high doses [11]. Long-term studies in non-iron-overloaded rodents (20 mg/kg per day) and dogs (30 mg/kg per day) reveal progressive signs of toxicity (reduced body weight gain and food consumption, nephropathy and neurotoxicity) even at relatively low doses. Because of the above toxicity problems, this compound is no longer considered to possess clinical potential as a chelating agent. However, a number of synthetic analogues, where the methyl group on the thiazoline ring has been replaced by a hydrogen atom and which lack some of the toxic effects of the parent drug, are presently being investigated [12]. Unfortunately, the metal selectivity of these new analogues is unlikely to differ significantly from that of the parent compound.

HPOs, which combine favourable aspects of both hydroxamate and catechol groups, give rise to compounds which possess high selectivity for iron(III). Both the free ligand and iron complex remain neutral over the physiological pH range of 5–9 and are hence able to readily cross biological membranes. A range of analogues of these compounds can also be made by varying substituents at positions 1- (R₁) and 2- (R₂) of the molecule. The considerable early promise displayed by HPOs in animal studies has been reflected in preliminary clinical studies conducted both in the U.K. and elsewhere [7,13,14].

**Interaction with iron-containing enzymes**

Even with a selective chelator for iron(III), toxicity may occur because the compound is capable of interacting with iron-containing enzymes. Iron-containing
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Metalloenzymes can be divided into haem and non-haem types. In general, chelating agents are not effective inhibitors of haem-containing enzymes for two reasons: (i) the avid interaction between the porphyrin nucleus and iron and (ii) that bidentate interaction between the ligand and porphyrin-bound iron is not possible [15]. In contrast many non-haem iron-containing enzymes are extremely susceptible to inhibition by chelators. Iron centres dominated by oxygen and imidazole ligands are particularly susceptible. Such iron centres are found in ribonucleotide reductase, and lipoxygenase family of enzymes and tyrosine hydroxylase. Clearly iron(III) chelators should be designed to possess a low affinity for the active sites of these and other such enzymes.

Selective distribution of chelating agent and its iron complex

A systematic study of Levin [16] clearly indicated that not only is the $K_{\text{part.}}$ value of critical importance for the ability of a compound to penetrate the blood–brain barrier (BBB), but also the molecular mass. Thus although there is an excellent linear relationship between partition coefficient and permeability for molecules with molecular mass $<300$ Da, there is no such relationship with larger molecules (molecular mass $>500$ Da). Thus, most hexadentate ligands are predicted to penetrate the blood–brain barrier inefficiently, at best. In contrast bidentate ligands will penetrate relatively easily — an undesirable feature for most therapies. However, penetration of low-molecular-mass molecules is strongly dependent on $K_{\text{part.}}$ values and molecules with values $<0.05$ penetrate poorly [17].

Thus the same properties which maximize oral absorption will also endow the molecule with the ability to efficiently penetrate the blood–brain barrier. For this reason, it is important to ensure that chelating agents are directed to target tissue such as the heart and liver while minimizing exposure to critical organs/cells. Once intracellular iron has been chelated by the ligand, the resulting iron complex must also be able to cross cell membranes to remove iron from the cell. Thus both the free ligand and the iron complex should be water soluble and yet possess no charged groups. Consequently the iron complex should also be uncharged. Of the high-affinity iron chelators, only hydroxamate and HPO ligands possess this property.

Minimal redistribution of iron

In principle, the redistribution of iron can occur if both the ligand and its iron complex freely cross biological membranes. The affinity constant of a ligand for iron is also important, since the lack of stability of iron complexes can lead to redistribution from iron stores to potentially toxic sites in the body, eventually leading to tissue damage. Ideally, metal chelate complexes should be excreted rapidly in the faeces or urine with no redistribution of iron from relatively non-toxic sites such as the liver to more harmful ones such as the heart. Complexes formed intracellularly should not accumulate within cells, but should be able to freely permeate from cells. In the case of liver cells, this should result in significant excretion of iron in the bile. Clearly the biliary iron-chelator complex should not then be reabsorbed from the gut.
Toxicity of iron complexes

Depending on the coordination geometry and donor atoms associated with the iron centre, redox cycling of the metal with the corresponding generation of free radicals can result. Iron should be co-ordinated by the chelator in such a manner as to prevent direct access to oxidants and reductants (e.g. hydrogen peroxide and superoxide). If this is achieved, then hydroxyl radical production will be reduced to a minimum. Unfortunately this is not always so readily achieved. The relative ability of different ligands to generate free radicals is highly variable and will depend on a number of parameters [18]. The availability of the free coordination site on the metal is required for catalysis; thus the ability or otherwise of ligands to completely occupy the entire metal cation surface will strongly influence the efficiency of hydroxyl radical generation. The absolute affinity constant \( K_1 \) or \( \beta_3 \) of the ligand for iron can also influence hydroxyl radical generation. A high affinity usually implies reduced access for oxidants/reductants.

Another important property of complexing agents which can influence free radical-generating ability of an iron complex is redox cycling. If the ligating atoms of a complex are mixed, such that some favour the oxidized form of the metal and some the reduced form, then a complex of intermediate redox potential is produced and as such can readily oscillate between oxidized and reduced forms. If the reduced form is readily autoxidized by oxygen then superoxide will be produced. Thus ligands such as DFO, which strongly bind Fe(III) via oxygen donor atoms to form an octahedral iron complex, only poorly generate hydroxyl radicals, in contrast to DFT which binds the metal via a mixture of oxygen and nitrogen donor atoms (Fig. 3).

Iron(III)-EDTA is present in solution as \([\text{Fe(III)EDTA(H}_2\text{O)}]^-\), EDTA being too small to completely encompass the iron mass [19]. This leads to the generation of a seventh coordination site which is occupied by a water molecule but can be easily displaced by oxidants/reductants, facilitating redox cycling of iron leading to the generation of free radicals. Other aminocarboxylate ligands such as DTPA [20], which bind iron in a 1:1 ratio, and NTA (Fig. 1; 8) [21], which binds in a 2:1 manner, like EDTA form complexes with seven coordination positions around the metal centre. However, unlike EDTA, all ligating atoms are derived from the chelating agents accounting for their reduced hydroxyl radical-generating ability compared with the former (Fig. 3). Although DTPA has a higher affinity for iron compared with NTA, it is significantly more efficient in generating hydroxyl radicals. A possible explanation for this is that the redox potential of the iron atom bound by DTPA differs from that of NTA since three of the seven coordination positions around the metal ion are occupied by nitrogen donor atoms. HBED, in contrast to the other aminocarboxylate ligands, only poorly generates hydroxyl radicals (Fig. 3). Molecular modelling of the HBED iron complex suggests that this ligand, largely due to the presence of two phenolate moieties, is capable of forming a six-coordinate complex. This, coupled with its extremely high affinity for iron \( K_1 = 10^{46} \), dramatically reduces its hydroxyl radical-generating ability (Fig. 3).

A complex with high kinetic stability is also essential to minimize free radical generation. Thus bidentate ligands such as HPOs and, to a lesser extent, tridentate
**Fig. 3.** The hydroxyl radical-generating ability of a range of iron complexes with respect to Fe-EDTA. Xanthine oxidase was used as a source of superoxide and hydrogen peroxide. Incubation conditions: 1 mM hypoxanthine, 0.02 unit/ml xanthine oxidase, 1 mM N,N'-((5-nitro-1,3-phenylene)bisglutaramide (NPG), 0.5 mM iron complexed with various ligands; incubation period, 37°C for 30 min. Hydroxylated products of NPG were separated and quantified by reversed-phase HPLC.

Ligands such as DFT, provide optimal coordination number and stereochemistry, but, due to appreciable dissociation rate of the ligand from the complex, there are short intervals of time (typically 1 ms) when the complex will not be fully coordinated. In such a state, oxidants/reductants such as H$_2$O$_2$ and O$_2^-$ can gain access to the metal centre and thereby generate free radicals. This is nicely illustrated by the dramatic decrease in hydroxyl radical generation of bidentate L1 in the presence of excess ligand (5:1) (Fig. 3). Here dissociation of one of the ligands from the metal centre, unlike the case for 3-fold excess of ligand (3:1), is immediately followed by replacement by an identical ligand and prevents oxidants/reductants from gaining access to the metal centre.

**Conclusions**

The introduction of a safe and effective chelating agent for use in the clinic almost inevitably requires compounds to fulfil most if not all the design criteria outlined in this chapter. Unfortunately this goal has eluded medicinal chemists and clinicians alike for over 20 years. Considerable progress has, however, been made over the last decade and the prospects for the emergence of a suitable iron chelator over the next few years with an acceptable therapeutic index to replace DFO is a realistic objective.
References


