Erratum

Drug evaluation: Deferitrin (GT-56-252; NaHBED) for iron overload disorders


In the drug evaluation entitled Deferitrin (GT-56-252; NaHBED) for iron overload disorders, published in IDrugs (2007) 10(4):270-281, several inaccuracies were present in the text owing to the incorrect assignment of the compound NaHBED as a synonym for deferitrin. The sections of the drug evaluation concerning chemistry and preclinical studies were rewritten when this error was realized, and the corrected evaluation is published here in its entirety.

Drug evaluation: Deferitrin for iron overload disorders

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Deferitrin (GT-56-252) is the first drug in a class of desferrithiocin-derived hexadentate iron chelators. Genzyme Corp is developing this compound as an oral drug for the treatment of severe iron overload in people who require repeated erythrocyte transfusion for management of chronic anemia such as β-thalassemia major. In phase I clinical trials in adults with β-thalassemia, deferitrin promoted iron excretion in a dose-related manner and was well tolerated as both a liquid and capsule in fed and fasted states. There were no serious adverse events or significant laboratory abnormalities. The author concludes that deferitrin may be useful as chelation monotherapy or as part of combination or doublet chelation therapy for the treatment of severe iron overload in patients with β-thalassemia major if its favorable pharmacokinetic profile, efficacy, safety and tolerability are confirmed in more extensive clinical trials. A phase I/II clinical trial that began in September 2003 has reportedly completed recruitment.

Introduction

Iron overload develops in a high proportion of people with severe forms of heritable or acquired chronic anemia, such as severe β-thalassemia or sickle cell disease. Iron overload develops because such people require repeated transfusion of red blood cells (containing a high proportion of iron), because some people with chronic anemia absorb increased quantities of iron from the gastrointestinal (GI) tract, and because physiologic mechanisms to excrete iron are very limited. In people with severe β-thalassemia, for example, both chronic transfusions and increased iron absorption resulting from ineffective erythropoiesis and decreased levels of hepcidin (a polypeptide produced in the liver that is an important regulator of iron homeostasis in humans) [760258] contribute to the development of iron overload.

Cardiac siderosis is the most important sequel of severe iron overload, leading to heart failure and arrhythmias, and the incidence and prevalence of fatal cardiomyopathy is greatest in patients with β-thalassemia major [581803], [760261], [760262], [760263]. Progressive iron overload also injures the liver, endocrine organs, joints and other target cells and tissues. This process eventually causes liver dysfunction, cirrhosis and primary liver cancer, gonadal failure, retarded growth, diminished performance status, decreased quality of life and premature death in many patients [60151], [96727], [581803], [581809], [581811], [581812]. The prevention of death resulting from cardiac siderosis is the most important potential benefit of iron chelation therapy, although maintaining normal hepatic function and preventing hepatic cirrhosis is another objective. Preventing iron-related injury to endocrine organs is critical in children, especially those with β-thalassemia major. Altogether, successful treatment or prevention of severe iron overload increases quality of life and survival in many patients [60151], [96727], [581803], [581809], [581811], [581812].

Treatments for iron overload must be safe, effective and compatible with coexisting medical conditions, lifestyle and dietary preferences. Patients with severe anemia and iron overload cannot achieve iron depletion by the periodic removal of blood (therapeutic phlebotomy) because they cannot tolerate exacerbation of anemia that occurs as an unavoidable consequence. Strategies to bind iron chemically...
in the GI tract and thus diminish its absorption are rational and partially effective [760310], but are overall ineffective in alleviating iron overload. Chelation therapy has been the primary means of reducing iron overload in persons with severe forms of heritable or acquired chronic anemia since the introduction of deferoxamine B (DFO) [760311], a siderophore isolated from Streptomyces pilosus, more than 30 years ago [760322], [760324], [760326], [760332], [760335], [760338]. DFO is typically administered as a subcutaneous or intravenous infusion for 9 to 12 h each day [61392], [86714], [760350]. Lack of compliance (because of the lengthy parenteral administration and painful infusion-site reactions that can occur), cost and physician dissatisfaction are the major impediments to successful DFO treatment [86716], [90050], [109116], [760351], [760356], [760359], [760362], [760366]. These limitations have led to a quest to design more acceptable alternatives to DFO.

Iron forms coordination compounds with three types of ligands: bidentate (3:1 complex of ligand:iron), tridentate (2:1 complex) and hexadentate (1:1 complex). DFO and many other hexadentate ligands are poorly absorbed or relatively inactive when administered orally. In contrast, bidentate and tridentate ligands are generally well absorbed via the GI tract because of their smaller molecular size. Some chelators bind undesired parenchymal cell or storage iron with relative specificity, although others may bind iron in metalloenzymes and thus inhibit important metabolic pathways. The metal-binding specificity of iron chelators is not absolute, and many chelators have avidity for certain non-ferrous metals, including metals necessary for normal physiologic function [113323], [670626], [760375]. The development of iron-chelating compounds that are readily absorbed and remain active after oral administration are of greatest interest for potential clinical applications [113323], [760375]. At present, two oral iron chelation drugs have been licensed for routine use in various countries: deferoxprone (DFP; 1,2 dimethyl-3-hydroxypyrid-4-one), an orally administered bidentate iron chelator [760593], and deferasirox (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl] benzoic acid) an orally administered tridentate iron chelator [588079], [760616]. Both DFP and deferasirox are indicated for the treatment of iron overload in patients with transfusion-dependent β-thalassemia major or other forms of heritable anemia who cannot be treated adequately with DFO [505908], [588079], [760600], [760608], [760616].

Desferrithiocin (DFT) is a novel tridentate siderophore that was first isolated from cultures of Streptomyces antibioticus DSM 1865, and is structurally unrelated to DFO [795920]. DFT forms a stable 2:1 complex with Fe(III) [795929], [795937]. DFT administered orally possessed good iron-clearing efficiency in both the bile duct-cannulated rat model [795945] and the iron-overloaded Cebus apella primate model [124502], [144376]. Although DFT is severely nephrotoxic [124502], [144376], the oral chelating activity of the compound justified SAR and in vivo iron chelation studies to identify an orally active and safe DFT analog. A large body of literature indicates that DFT derivatives are chelators with adequate oral bioavailability, and have potential use in the treatment of iron overload resulting from transfusion. One tridentate DFT analog, deferitrin, is an orally active iron chelator that is under clinical development by Genzyme Corp for the treatment of iron overload. This compound appears in the literature under several names, including: (S)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazole-carboxylic acid [796114]; (S)-4'-(HO)-DADFT [796114]; 4'-hydroxydesazadesferrithiocin [760411]; and GT-56-252 [472082]. Deferitrin has been evaluated in phase I clinical trials [573932], [669865], and began phase II trials in September 2003 [739436], although no results have been published. The US FDA has awarded Orphan Drug status to deferitrin for the treatment of iron overload [739335].

**Synthesis and SAR**

A range of SAR studies of DFT analogs has been reported. In early studies, the DFT sodium salt, desmethyl DFT, desazadesmethyl DFT sodium salt, desazadesmethyl DFT pivaloyloxyethyl ester and desazadesmethyl-5,5-dimethyl DFT all demonstrated iron-clearing efficiency in the iron-overloaded C apella primate model and non-iron-overloaded, bile-duct-cannulated rat model. However, when administered to rodents for 10 days, DFT sodium salt demonstrated severe renal toxicity, and desazadesmethyl DFT sodium salt and desazadesmethyl DFT pivaloyloxyethyl ester all resulted in severe GI toxicity. Desmethyl DFT did not demonstrate significant toxic side effects [124502].

The enantiomers (R)- and (S)-desmethyl DFT (DMDFT) were demonstrated to be effective iron chelators with minimal toxicity in rodents, but only (S)-DMDFT induced iron clearance in C apella primates; the (R)-enantiomer was also substantially more toxic than the (S)-enantiomer in the primates. Pharmacokinetic data suggested enantioselectivity as a factor in renal clearance of the DFTs and their iron complexes, with the clearance of (S)-DMDFT being 3.5-fold greater than that of (R)-DMDFT, and the clearance of Fe(III) [(S)-DMDFT] being 6.8-fold greater than that of Fe(III) [(R)-DMDFT] [760411], [760414].

In later studies, removal of the pyridine nitrogen and demethylation of DMDFT led to (S)-4,5-dihydro-2-(2-hydroxyphenyl)-4-thiazolecarboxylic acid [(S)-DADMDF]. Although (S)-DADMDF was an effective iron chelator (12.4% efficiency in C apella at a dose of 300 μM/kg), the compound was extremely toxic to the GI tract [314054]. In an attempt to improve iron clearance and reduce toxicity profiles, structural modifications to (S)-desazadesmethyl DFT were undertaken, including the introduction of hydroxy, carboxy or methoxy groups to the aromatic ring; an alteration of the thiazoline ring; increasing the distance between the ligand donor atoms; and benzo-fused aromatic rings. Ligands with > 2% efficacy in a bile-duct-cannulated rat model were subsequently tested in iron-overloaded primates. Lead compounds that exhibited > 3% efficacy in primates were then screened for toxicity in rodents. SAR data from the resulting series of compounds led to the selection of 2-(2,4-dihydroxyphenyl)-Δ-thiazoline-4(5)-carboxylic acid [(S)-4'-((HO)-DADMDFT] as a promising candidate for clinical evaluation, exhibiting an iron excretion efficiency of 2.4% in monkeys and no GI toxicity [314054].
Altering the lipophilicity (ie, partition properties, log Papp) and/or the redox potential of the aromatic ring was demonstrated to alter the toxicity profile, organ distribution properties and potential metabolic disposition of the compounds. Hydroxylation, as in the analogs (S)-4′-(HO)-DADFT (deferitrin) or (S)-4′-(HO)-DADMDFT, was ultimately determined to be compatible with iron clearance in the C apella primate model, and also profoundly reduced toxicity in rats [314054], [520478], [760411]. Whereas DFT caused lethal nephrotoxicity in rats, an abstraction of the pyridine nitrogen from DFT to yield (S)-desazadesferrithiocin resulted in a compound with primarily GI toxicity. When an electron-donating hydroxyl group was added to position 4 of the aromatic ring of (S)-desazadesferrithiocin to yield a 4′-hydroxylated analog (deferitrin), no serious GI toxicity was observed, and an iron chelation efficiency of 17.7% was achieved in monkeys (dosed orally with 150 μM/kg of deferitrin) [760411].

Systematic SAR studies identified the structural elements required for the oral iron-clearing activity of DFT. The distance between the ligating centers cannot be altered without loss of efficacy, and the thiazoline ring must remain intact. Benzo-fused compounds, which were designed to improve the tissue residence time and possibly iron-clearing efficiency of the ligands, were ineffective chelators in vivo. The maintenance of an (S)-configured C(4) carbon is optimal. The introduction of a hydroxy in the 4′-position of the aromatic ring of at least three different desaza-desferrithiocin analogs resulted in moderate-to-small distances between the ligating centers cannot be altered without loss of efficacy, and the thiazoline ring must remain intact. Benzo-fused compounds, which were designed to improve the tissue residence time and possibly iron-clearing efficiency of the ligands, were ineffective chelators in vivo. The maintenance of an (S)-configured C(4) carbon is optimal. The introduction of a hydroxy in the 4′-position of the aromatic ring of at least three different desaza-desferrithiocin analogs resulted in moderate-to-small changes in iron clearing efficacy, but significant reductions in the toxicity of the compounds were observed [796129].

A method for the synthesis of deferitrin was reported in 1999 [760411]. The preparation employed the amino acid (S)-α-methylcysteine, prepared from a hydrolysis of DFT in 6 N HCl at 100°C for 80 min. The resulting hydrolysis product mixture of (S)-α-methylcysteine and 3-hydroxypicolinic acid in degassed methanol was treated with 2,4-dihydroxybenzonitrile, followed by degassed phosphate buffer (pH 6) and NaHCO3. The mixture was stirred under nitrogen at 71°C for 3.5 days to produce deferitrin in 57% yield [760411].

Preclinical development

DFT analogs, including deferitrin, were tested at a dose of 384 μmol/kg/day in a bile-duct-cannulated rat model [760411]: this model was chosen because it provides information on both chelator-induced total iron output and the kinetics of biliary and urinary iron excretion. (S)-desazadesferrithiocin and deferitrin (the corresponding 4′-hydroxylated analog) were observed to behave differently in the model. (S)-desazadesferrithiocin was moderately effective, with an efficiency of 2.7 ± 0.5% according to bile and urine analyses at 24 h after drug administration. Under these conditions, no chelator-induced iron excretion was observed with deferitrin; the efficiency of the molecule was < 0.5%. However, in later studies, the stools of non-iron-overloaded rodents were observed to be red in color after 2 days of treatment with deferitrin at the same dose. The results of guaiac tests were negative, suggesting the presence of excreted iron, but not blood in the stool. Additional experiments with deferitrin and (S)-desazadesferrithiocin (both administered orally at 75, 150 or 300 μmol/kg) were therefore conducted in an iron-overloaded C apella primate model. The iron-clearing efficiencies and modes of iron excretion of the compounds were not observed to differ significantly. The efficiency of (S)-desazadesferrithiocin at a dose of 300 μmol/kg was 13.1 ± 4.0%; the efficiency of deferitrin at a dose of 150 μmol/kg was 13.4 ± 5.8%. In both instances, 86% of the iron was excreted in the stool and 14% was excreted in the urine. At a dose of 75 μmol/kg, the efficiencies of the respective compounds in inducing iron excretion were also similar: 21.5 ± 12% (76% stool, 24% urine) and 17.7 ± 3.9% (79% stool, 21% urine). However, the differences in the toxicity profiles of these compounds was significant, with deferitrin displaying substantially less toxicity. Furthermore, the efficiency of these analogs is not substantially less than the efficiency of the natural product DFT, which exhibited 16.1 ± 8.5% efficiency and 78% fecal and 22% urinary iron excretion at a dose of 150 μmol/kg [760411].

The cellular uptake and iron chelation properties of deferitrin were evaluated alongside the enantiomer (-)-(R)-4′-hydroxydesazadesferrithiocin [796129]. Both isomers were observed to bind iron with a tridentate configuration. Deferitrin was also a more efficient deferration agent than the (-)-(R)-enantiomer in iron-overloaded C apella primates. Studies in L1210 murine leukemia cells evaluated the iron chelation efficacies of the two enantiomers. Differences in iron transport across the cell membrane were observed; the IC50 value of deferitrin was at least 5-fold lower than the IC50 value of the (+)-(R)- compound [796129].

Toxicity

Studies evaluated the toxicology of deferitrin and related analogs in rats administered doses of 384 μmol/kg/day for 10 days [760411]. DFT elicited severe nephrotoxicity; all five animals tested died within 10 days of drug administration. For the (S)-desazadesferrithiocin analog, the primary toxicity was instead in the GI tract, although all animals died by day 5 following either subcutaneous or oral dosing. With deferitrin, by contrast, GI toxicity was not observed. No deaths occurred when the compound was administered at a dose of 384 μmol/kg/day orally for 10 days. A gross inspection of organs at 1 day after the final dose revealed no drug-related abnormalities. However, a histopathological analysis of tissues indicated that the compound was associated with some nephrotoxicity, although to a degree that was not comparable to the severe toxicity observed with DFT. Histologically, the kidneys of rats receiving deferitrin exhibited some vacuolar degeneration in the proximal tubular epithelium that was considered to be reversible; however, one animal in the study displayed possible early necrosis. Notably, because fecal iron clearance values were similar for the three compounds, the differences in toxicity could be readily attributed to the mode of iron clearance [760411].

Further toxicology studies tested the 28-day administration of deferitrin (doses unstated) in both iron-overloaded and
iron-unloaded rats and dogs [472082]. In iron-loaded animals, the general profile of toxicity was not altered and the effects on the target organs were similar, but the severity of effects was reduced. The repeat-dose no effect levels in both species were 30 mg/kg/day. The target organs for adverse effects were reportedly identified in the study, although no details were given [472082]. A brief report of 28-day toxicology studies of orally and intravenously administered deferitrin in rats, dogs and cynomolgus monkeys indicated that the toxicokinetics displayed proportionality between dose and systemic exposure. Repeated administration of the compound did not substantially alter systemic exposure [669866].

Other studies have been reported in brief, without details of dosage. Repeat-dose toxicology studies in non-iron-overloaded and iron-loaded rats and dogs show that the kidneys are the target organs of the drug. In dogs, sporadic, non-dose-related thrombocytopenia has been observed in association with deferitrin administration [669861]. No evidence of in vivo genotoxicity, teratogenicity or reproductive toxicity was observed in primates [669861].

**Metabolism and pharmacokinetics**

In single-dose pharmacokinetic studies of 14C-labeled deferitrin (dosage unstated) in the rat, dog and monkey, the compound was found to be orally available in all three species; half-lives of deferitrin were 3 and 8 h in the rat and dog, respectively. The tissue distribution of deferitrin in the rat was similar after oral and intravenous administration. The highest concentration of deferitrin was detected in the adrenal glands and in the intestines and intermediate concentrations were detected in the kidneys and liver [472082], [669866].

The pharmacokinetic parameters of deferitrin in humans were first assessed in a phase I clinical trial in 18 adults with beta-thalassemia who required chronic transfusion and chelation therapy [669865]. Patients received two doses of deferitrin on consecutive days, as a 3-mg/kg solution (in either a fed or fasted state), a 4.5-mg/kg dose with food (as a solution or a capsule) or an ~ 6-mg/kg dose as capsules (with food or to fasted individuals). Deferitrin was very well absorbed; AUC values were similar when the drug was administered with food or to fasted subjects, suggesting that bioavailability was not impaired by food. Furthermore, systemic exposure was similar for capsules or liquid, although absorption was delayed and Cmax was considerably lower for the capsule [669865].

Pharmacokinetics were assessed in a similar phase I, open-label, parallel-design clinical trial of deferitrin in 26 adults with beta-thalassemia [573932]. Deferitrin was administered at five dose levels from 3 to 15 mg/kg, on 2 consecutive days, to either fed or fasted patients. The drug was given as a liquid at 3.0 and 4.5 mg/kg or as capsules equivalent to 4.5, 8.0, 11.0 and 15.0 mg/kg. AUC values for the fed and fasted states and for the liquid and capsule formulations were similar, again suggesting that the bioavailability of deferitrin is not impaired by food. The AUC increased dose proportionally from 4.5 to 8 mg/kg, but did not increase further with the 11- and 15-mg/kg doses. Between 13 and 48% of the drug was detected in serum as the 2:1 deferitrin:iron complex. The elimination half-life was similar for all doses (approximately 3 to 4 h). Measurement of deferitrin levels in the urine showed that ~ 75% was intact deferitrin at all doses. Approximately 2% of deferitrin in the urine was complexed to iron. Renal excretion of deferitrin uncomplexed with iron was similar to that seen in preclinical studies, which predict iron excretion to be via the fecal route [573932].

**Clinical development**

**Phase I**

Two similar phase I clinical trials assessed the safety and pharmacokinetics parameters of deferitrin in 18 [669865] and 26 [573932] adults with beta-thalassemia who required chronic transfusion and chelation therapy, the details of which are discussed in the 'Metabolism and pharmacokinetics' section.

**Phase II**

A phase I/II, nonrandomized, open-label, active-control, crossover clinical trial is ongoing to compare the safety and iron excretion properties of deferitrin with those of DFO in adults with beta-thalassemia [739436]. This trial reportedly began in September 2003, and had completed patient enrolment, which was intended to be 25 adults with beta-thalassemia and serum ferritin levels > 500 ng/ml. Safety, tolerability and iron excretion in the urine and stool and pharmacokinetic were to be assessed [739436]. At time of publication of this review, no results of this trial had been reported.

**Side effects and contraindications**

In the phase I clinical trial in 18 adult beta-thalassemia patients administered two doses of 3 to 6 mg/kg deferitrin, the drug was well tolerated both as a liquid and capsule and in both fed and fasted states; no serious adverse events, notable laboratory abnormalities or changes in ECGs detected [669865].

The similar phase I clinical trial in 26 adults with beta-thalassemia administered two doses of deferitrin (3 to 15 mg/kg) under equivalent conditions [573932]. Deferitrin was well tolerated in assays of serum chemistry and hematology, coagulation parameters, urinalysis and urine beta2-microglobulin, and in ECGs and physical examinations. An episode of hypoglycemia that occurred in one diabetic patient with beta-thalassemia (3 days after the second 4.5-mg/kg dose of deferitrin) was reasonably attributed to diabetes mellitus and its treatment, rather than to deferitrin [573932].

**Patent summary**

The University of Florida credits Raymond J Bergeron with leading the design, synthesis and evaluation of organ-specific iron chelators, and his name is featured as an inventor on all the relevant documents relating to deferitrin. The applications and patents appear to cover the US, Europe, Australia and Japan. There are no apparent reports of patent life extensions or opposition procedures.
WO-00012493, filed in August 1999 and assigned to University of Florida, claims deferitrin (claim 1) and appears to be the ‘product’ case. Granted US equivalents include US-06083966, US-06521652, US-06525080, US-06559315 and US-07126004 – issued between 2000 and 2006 – and one granted European equivalent, EP-01109795. The technology invented by Dr Bergeron was licensed exclusively to SunPharm Corp (which was subsequently acquired by GelTex Pharmaceuticals Inc and later by Genzyme) for development and marketing. SunPharm in turn entered into a marketing agreement on iron chelators with Schein Pharmaceutical Inc (now Watson Pharmaceuticals Inc). The assignation of WO-00012493 reflects these agreements.

Two ‘use’ cases have also been published for deferitrin by the University of Florida: WO-00016763, filed in September 1999 (and jointly assigned to Columbia University) claims iron-chelating mono- and bis-heterocyclic derivatives for treating Plasmodium infection, and WO-2004017959, filed in August 2003, claims aryl-substituted heterocyclic compounds as antioxidants for the prevention and treatment of inflammatory disorders, neoplasia and ischemia. In June 2005, the latter application was deemed withdrawn by the EPO. Formulations of deferitrin and DFO analogs are disclosed in two applications by the University of Florida – WO-00016765, filed in September 1999, and WO-2005023310, filed in September 2004, which claims a composition comprising a polyamine-metal chelator conjugate.

WO-03097582, filed in May 2003, is a process patent assigned to Genzyme. This application appears to be a key patent family comprising up to 12 corresponding US patents (issued between 2004 and 2006), two European patents (EP-01506162 and EP-01529037) and 15 US patents pending grant. Additionally, EP-01302467, assigned to DSM Chemie Linz GmbH, also appears to be a process case for deferitrin. However, this application was deemed to be withdrawn in November 2006.

**Current opinion**

The development of effective oral iron chelators that cause minimal adverse effects could lead to greater patient and physician acceptance and, therefore, to potentially superior results to those presently obtainable with parenteral DFO. One such agent, deferitrin, has potential advantages over DFO for use in the treatment of severe iron overload as a result of chronic blood transfusion. Deferitrin has been demonstrated to induce iron loss after oral administration in a relatively small number of patients with β-thalassemia, transfusion dependency and iron overload. The bioavailability of deferitrin appears to be good whether administered with food or after fasting, in solution or in capsule form. Furthermore, the quantities of iron excreted and the efficiency of iron excretion induced by deferitrin appear to be dose-dependent within a dose range that seems to be well tolerated. These observations suggest that oral deferitrin could be formulated in several ways that would accommodate the various needs of pediatric and adult patients without stringent regard to dietary habits or lifestyle. Deferitrin may also decrease the availability of unbound iron that could participate in the Fenton reaction and thus could reduce iron-induced oxidative injury, although the overall practical advantages of this property in maintaining or improving target organ function or in prolonging survival have not been demonstrated in vivo. Thus, oral deferitrin may induce efficient iron chelation, although whether the agent can induce a negative iron balance in humans (and the dosage required to do so) has not been demonstrated. Thus far, many of the scientific or medical publications pertinent to deferitrin therapy in humans have appeared in abstract form, and peer-reviewed publications are therefore awaited.

Approximately three-quarters of the iron excreted in β-thalassemia patients treated with deferitrin occurred via the feces, and the present evidence indicates that deferitrin itself is not nephrotoxic. It is therefore plausible that deferitrin could be safer and more effective than presently licensed agents in patients with impaired renal function, although this is unproven. Because deferitrin is a synthetic product, its use is not likely to be associated with the types of uncommon systemic or common localized allergic reactions characteristic of parenteral administration of DFO [59494], [760567], [760573], although it is possible that idiosyncratic reactions to deferitrin will occur in some patients.

A comparison of some clinically pertinent properties of deferitrin with those of the presently licensed agents DFP or deferasirox is informative. Peak plasma concentrations of deferitrin (especially when administered as a liquid) are achieved rapidly after dosing (as with DFP [760581]), whereas deferasirox reaches peak plasma concentrations more slowly, but has a somewhat longer elimination half-life that could enhance clinical benefit [512010]. DFP traverses cell membranes readily and removes iron from a variety of cell types, and from iron-binding proteins [76152], [760593], [760599], [760600]. Deferasirox also enters a variety of cells and chelates intracellular iron readily [743217], [760613]. The entry of deferitrin into target tissues for iron overload in humans has not been reported, although the compound readily enters cells in vitro. However, movement of chelators into target cells is essential for iron mobilization. For example, DFO mobilizes iron deposited in parenchymal cells and macrophages, although iron mobilization from the heart occurs less rapidly than that from the liver and other sites [768115]. DFP removes iron from non-cardiac parenchyma, macrophages, transferrin, ferritin and hemosiderin [76152], [760600]. Deferasirox enters a variety of cells, especially cardiac myocytes, and chelates intracellular iron readily [743217], [760613]. The feces is the predominant route of iron excretion in subjects who have taken either deferitrin or deferasirox [588079], whereas DFP promotes iron excretion predominantly via the urine [76152], [760600]. With all the three drugs, net iron excretion is approximately dose-proportional. The oral bioavailability of deferitrin does not appear to depend on a fed or fasted state, unlike the bioavailability of deferasirox, which increases significantly when the drug is administered with food, placing some limitations on ad libitum dosing of the drug [512010].

The adverse effects profiles of DFP and deferasirox are well characterized. Adverse effects of DFP include...
significantly greater cardiac protection than DFO in patients for cardiac disease and death. DFP therapy provides treatment that offers the greatest reduction in incidence rates β malignancy. joint disease; (v) trace-metal deficiencies; (vi) increased rate in pediatric patients); (iv) occurrence or progression of pulmonary injury; (iii) endocrine function (including growth anemia or other blood dyscrasias; (ii) cardiac, hepatic, renal or deferitrin. These include (but are not limited to): (i) aplastic events that could be potentially caused or facilitated by years of treatment are needed to identify uncommon adverse effects with deferitrin, 10 to 15% of patients treated have a skin rash and mild, non-progressive elevations in serum creatinine levels occur in ∼ 35% of patients [760615]. Thus far, patients treated with deferitrin have not reported skin rash and evidence of renal toxicity of deferitrin is lacking. The risk of renal failure (including some fatal cases), reported in postmarketing reports of patients treated with deferasirox [767043], might be increased by pre-existing renal disease or the simultaneous administration of other medications that impair renal function. Auditory and ocular abnormalities, growth abnormalities in pediatric patients and neutropenia or agranulocytosis have occurred in association with deferasirox treatment [760616]. Altogether, short-term clinical trials suggest a low propensity for adverse effects with deferitrin, although the prevalence of adverse events associated with prolonged administration of deferitrin is unreported. Reports of deferitrin administration to animals [472082], [760415] and oral deferitrin administration to humans have not shown evidence of severe GI or renal toxicity that occurred in animal studies with some other DFT analogs [314054], [760411], [760414]. Long-term clinical trials based on many patient years of treatment are needed to identify uncommon adverse events that could be potentially caused or facilitated by deferitrin. These include (but are not limited to): (i) aplastic anemia or other blood dyscrasias; (ii) cardiac, hepatic, renal or pulmonary injury; (iii) endocrine function (including growth rate in pediatric patients); (iv) occurrence or progression of joint disease; (v) trace-metal deficiencies; (vi) increased susceptibility to infection; and, (vii) development of malignancy.

A crucial issue in the management of patients with β-thalassemia major is the employment of chelation treatment that offers the greatest reduction in incidence rates for cardiac disease and death. DFP therapy provides significantly greater cardiac protection than DFO in patients with β-thalassemia major who have not developed symptoms or other abnormalities attributable to cardiac siderosis [760263]. At present, however, there are no reports describing the effect of either deferitrin or deferasirox on cardiac function or iron levels in patients with β-thalassemia major. No published data document deferitrin-induced reductions in iron deposits in certain critical target parenchyma in humans, for example, cardiac myocytes, anterior pituitary cells or pancreatic islet cells. Additional observations on cardiac and endocrine function in patients before and after treatment are therefore needed. Further studies to document the effect of deferitrin treatment on iron stored in macrophages in liver and bone marrow would also be informative. Multivariate analyses of large patient datasets are needed to identify patient characteristics that are associated with greater benefit, greater susceptibility to adverse events or to unacceptableity of deferitrin therapy. Taken together, additional clinical observations are needed to substantiate the mechanisms of action, tissue specificity, safety and effectiveness of long-term deferitrin therapy, and to define its practical clinical utility in children and in adults with severe forms of heritable anemia who require management with chronic transfusion.

A range of clinical trials should be undertaken to characterize the efficacy of deferitrin further. In severe β-thalassemia, long-term, single-arm or comparative clinical trials are needed to estimate overall survival or iron overload event-related intervals in deferitrin-treated patients. Future clinical trials should be considered to evaluate the potential of deferitrin for the alleviation of iron overload in patients with conditions such as sickle cell disease, rare forms of heritable anemia, myelodysplasia and hemochromatosis. There is good evidence from short-term clinical trials that patients with rare forms of anemia and iron overload can be treated effectively with DFO, DFP and oral chelation drugs [760610], [760617], [760619], although results of long-term follow-up of large groups of patients thus treated have not been reported [760620]. In patients with myelodysplastic syndrome (MDS), there was a significant inverse correlation of transfusion and survival [760624]. Some investigators have recommended that chelation therapy in patients with MDS be initiated after 20 to 30 units of transfusions, or when serum ferritin exceeds 2500 μg/l [760626]. Data from a phase II clinical trial of patients with MDS indicate that deferasirox therapy was effective and well tolerated [760630], and retrospective analysis of a MDS case series suggests that DFO chelation therapy may have improved overall survival [760632]. The results of long-term clinical trials of large cohorts of patients with MDS and iron overload have not been reported, and thus the value of iron chelation therapy in reducing morbidity or mortality significantly is unknown. Therefore, chelation therapy should be used only in patients who have both a high risk of developing iron overload and good prognoses.

Deferitrin may also be useful for management of other conditions. There is fair evidence to support DFO chelation treatment of patients with hemochromatosis and severe iron overload who are unable to undergo phlebotomy, or as an adjunct for managing early age-of-onset hemochromatosis, especially in patients with cardiomyopathy [760620], [760635]. In short-term clinical trials of a few adults with hemochromatosis, DFO treatment was as effective as phlebotomy of 500 ml weekly in removing iron from the liver [760635]. However, there are presently no reports of clinical trials of oral iron chelation drugs in people with hemochromatosis and iron overload. In addition, evidence presently available is insufficient to support the routine use of chelation therapy of any type in patients with either HFE hemochromatosis or other types of adult age-of-onset hemochromatosis, African iron overload, or African American iron overload. Likewise, it seems prudent to treat iron overload in patients with normal erythropoiesis post-transplantation or post-chemotherapy with phlebotomy, not chelation.
Future phase III clinical trials should compare the efficacy of deferitrin with that of deferiprone or deferasirox. In comparison with chelation monotherapy, combinations of chelation drugs may enhance iron excretion, achieve net iron balance and possibly decrease cardiac deaths. The combined use of a chelation agent that readily penetrates cells with a second chelator that promotes more efficient excretion of iron may achieve overall results that are superior to those of chelation monotherapy. However, combined chelation therapy may increase the overall incidence of adverse treatment-related events, because it will expose patients to the potential to develop adverse events associated with each of the drugs employed in the treatment regimen. Nonetheless, it seems reasonable that clinical trials of doublet therapy that include deferitrin should be considered if longer-term clinical trials of deferitrin confirm that it is an effective and well-tolerated monotherapy.

A number of compounds with potential utility as iron chelators are at a less advanced stage of development than deferitrin. Three compounds of the 3-hydroxyypyridin-4-ones class (to which DFP belongs) that originated in the laboratory of RC Hider and colleagues at King’s College London have undergone some preclinical studies in animal models. CP-94 (diethyl-3-hydroxyppyridine-4-one) was as much as 8-fold more effective in promoting iron excretion than DFO in hypertransfused rats, and was equally effective when administered orally or parenterally [22787]. CP-94 also appears to be an effective chelator when administered to normal rats [386956] and iron-loaded marmosets [431916]. CP-365, the major metabolite of CP-94, is excreted in both bile and urine and is itself an active chelator with clinical potential owing to its high affinity for Fe(III) [WO-09854138], [768150]. The chelator CP-502 (1,6-dimethyl-3-hydroxy-4-(1f)-pyridinone-2-carboxy-(N-methyl)-amide hydrochloride) enhanced iron chelation activity in a [59Fe]ferritin-labeled rat model, wherein it was more effective than DFP [768150]. Efficacy was seen in bile-duct-cannulated rats [768150] and in normal rats after intravenous and oral dosing [768154], leading to clinical trials by Ciba-Geigy AG (as CGP-46700). Although no development was reported since 1999, CP-502 remains a promising compound for further preclinical development by licensee Apotex Inc [349087].

HES-DFO is derivative of DFO created by coupling DFO with hydroxyethyl starch (HES). In phase I clinical trials, intravenous infusions of HES-DFO (40SD02; Biomedical Frontiers Inc) achieved maximum plasma levels of chelator more than 10-fold higher than those attainable with DFO. Drug residence time in plasma was markedly prolonged, and the infusions increased urinary iron excretion [768163]. Although these results are promising, there are no reported observations of this treatment in humans with iron overload.

Distinct from chelation, strategies to block iron absorption or to promote iron excretion have centered on the mammalian divalent metal transporter-1 (DMT-1; divalent cation transporter-1, DCT-1; natural macrophage resistance-associated protein 2, NRAMP2). DMT-1 isoforms transport iron and other metals across the apical (luminal) aspect of enterocytes to the cytoplasm and from endosomes; DMT-1 participates in or modulates iron uptake by and transport from liver, macrophages, brain and kidney [768167]. The selenium-containing compound ebselen is a potent inhibitor of DMT-1 uptake in HEK293T cells overexpressing DMT-1 [768169], and Xenon Pharmaceuticals Inc is developing small-molecule inhibitors of DMT-1 as a treatment for iron overload disorders [768171]. However, the L-type calcium channel blocker nifedipine, long used for management of hypertension and other cardiovascular disorders, mobilizes hepatic iron in mice with primary and secondary iron overload and enhances urinary iron excretion [768170]. Modulation of DMT-1 function by L-type calcium channel blockers emerges as a new pharmacological therapy for the treatment of iron overload disorders. Among these candidate drugs, nifedipine is appealing, because it has been used with relative safety in humans for many years, and may have the capability of decreasing iron absorption at the level of the intestine and promoting its mobilization from storage sites, thus augmenting iron excretion.

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**Licensing**

**Genzyme Corp**
In November 1996, SunPharm Corp licensed iron-chelator technology from the University of Florida [225898]. In November 1999, GelTex Pharmaceuticals Inc acquired SunPharm in a deal which included the iron chelator technology [739252]. Later, in December 2000, stockholders of GelTex approved the merger agreement with Genzyme [393616].

**Watson Pharmaceuticals Inc**
In October 1999, Schein Pharmaceutical Inc (now Watson Pharmaceuticals) and SunPharm Corp entered into an exclusive licensing agreement. Under the terms of the agreement, Schein was to obtain the rights to develop and market a proprietary iron chelation compound in the US, Europe and certain Far Eastern territories. SunPharm was entitled to receive payments upon reaching certain clinical, regulatory and manufacturing milestones in addition to royalties on sales [342465]. However, in June 2004, Watson refocused its R&D on urology and nephrology and discontinued its programs outside these areas [546588].
### Development status

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### Literature classifications
#### Chemistry

**Synthesis of deferitrin**
A published route of synthesis involved the amino acid (S)-α-methylcysteine, prepared from the hydrolysis of DFT in 6 N HCl at 100°C for 80 min. The resulting hydrolysis product mixture of (S)-α-methylcysteine and 3-hydroxypicolinic acid in degassed methanol was treated with 2,4-dihydroxybenzonitrile, followed by degassed phosphate buffer (pH 6) and NaHCO₃. The mixture was stirred under nitrogen at 71°C for 3.5 days to produce deferitrin in 57% yield.

**SAR**
The distance between the ligating centers and the thiazoline ring were critical to the oral iron-clearing activity of DFT analogs. Benzo-fused compounds were ineffective chelators *in vivo*. The maintenance of an (S)-configured C(4) carbon was optimal. The introduction of a hydroxy group in the 4'-position of the aromatic ring reduced toxicity in several analogs.

**SAR of toxicity**
Whereas DFT caused lethal nephrotoxicity in rats, an abstraction of the pyridine nitrogen of DFT to yield (S)-desazadesferrithiocin resulted in a compound with primarily GI toxicity. The addition of a hydroxyl group at position 4 of the aromatic ring produced deferitrin, which caused no serious GI toxicity.

#### Biology

**In vivo Iron excretion**
Bile duct-cannulated rats administered 384 μmol/kg/day of deferitrin or (S)-desazadesferrithiocin (po or sc) for up to 10 days. Desazadesferrithiocin, but not deferitrin, promoted iron excretion after one day (2.7 and < 0.5% efficiency, respectively). However, deferitrin was apparently effective after 2 days.

**In vivo Iron excretion**
Cebus apella primates administered single oral doses of 75, 150 or 300 μmol/kg of deferitrin or (S)-desazadesferrithiocin. The modes of iron excretion and iron-clearing efficiencies of the compounds were similar. Efficiency was 13.1% for 150 μmol/kg of deferitrin and 13.4% for 300 μmol/kg of (S)-desazadesferrithiocin. In both instances, 86% of the iron was excreted in the stool and 14% was excreted in the urine.

**In vivo Toxicity**
Bile duct-cannulated rats administered 384 μmol/kg/day of deferitrin, (S)-desazadesferrithiocin or DFT orally for up to 10 days. DFT caused lethal nephrotoxicity in five out of five rats within 10 days. (S)-desazadesferrithiocin caused primarily GI toxicity; all animals died by day 5. Deferitrin caused slight GI toxicity and no deaths.

**In vivo Toxicity**
Iron-loaded and iron-unloaded rats and dogs administered deferitrin (doses unstated) for 28 days. In iron-overloaded animals, the general profiles of toxicity and target organs were similar to iron-unloaded animals, but the severity of effects was reduced. The repeat-dose no effect levels in both species were 30 mg/kg/day.

#### Metabolism

**In vivo Pharmacokinetics**
Single unstated dose of ¹⁴C-labeled deferitrin administered to rats, dogs and monkeys. Deferitrin was orally available in all three species; half-lives were 3 and 8 h in the rat and dog, respectively. Deferitrin was detected in high concentrations in the adrenal glands and intestines; intermediate concentrations were found in the kidneys and liver.

**In vivo Pharmacokinetics**
Phase I, open-label, parallel-design clinical trial in 26 adults with β-thalassemia administered deferitrin at five dose levels from 3 to 15 mg/kg, on 2 consecutive days, to either fed or fasted humans. Bioavailability was not impaired by food. AUC increased dose proportionally from 4.5 to 8 mg/kg, but did not at higher doses. Between 13 and 48% of the drug was detected in serum as the 2:1 deferitrin:iron complex. The t¹/₂ was ~3 to 4 h.
Associated patent
Title Thiazoline acid derivatives.
Assignees University of Florida Research Foundation
Publication US-98144103 31-AUG-98
Inventors Bergeron RJ Jr.

References
59404 Rapid desensitisation for desferrioxamine anaphylactic reaction. Miller KB, Rosenwasser LJ, Bessette JA, Beer DJ, Rocklin RE. J ALLERGY CLIN IMMUNOL 1989 83 582-584
76152 1,2-Dimethyl-3-hydroxypropyrid-4-one, an orally active chelator for treatment of iron overload. Kontoghiorghes GJ, Aldouri MA, Sheppard L, Hoffbrand AV. J LAB CLIN MED 1987 109 4 1294-1295
86714 Desferrioxamine-induced iron excretion in humans. Pippard MJ. JAMA 1989 282 2 323-343
86716 The toxic effects of desferrioxamine. Porter JB, Huehns ER. JAMA 1989 282 2 459-474
113323 Development of iron-chelating agents for clinical use. Benthin GM. BLOOD 1992 80 3 569-574
225898 SunPharm expands technology agreement with the University of Florida. SunPharm Inc PRESS RELEASE 1996 November 18
342465 Schein Pharmaceutical licenses iron chelator from SunPharm. SunPharm Corp PRESS RELEASE 1999 October 06
349087 Drug development pipeline: CP94. King's College London COMPANY COMMUNICATION 1999 November 30
388956 Biliary and urinary metabolic profiles of 1,2-diethyl-3-hydroxypropyrid-4-one (CP94) in the rat. Lu SL, Goewiawatana I, Liu ZD, Mallet AI, Hider RC. DRUG METAB DISPOS 2000 28 8 873-879
393615 GelTex Pharmaceuticals announces stockholders approval of merger agreement with Genzyme Corporation. GelTex Pharmaceuticals Inc PRESS RELEASE 2000 December 13
546588 Watson refocuses on urology, nephrology and generics. Watson Pharmaceuticals Inc PRESS RELEASE 2004 28 June
573932 The safety and pharmacokinetics of deferitrin, a novel orally available iron chelator. Donovan JM, Yardumian A, Gunawardena KA, Plone MA, Wonke B. BLOOD 2004 104 11 Abs 504
581803 Clinical consequences of acquired transfusional iron overload in adults. Schafer AI, Cheron RG, Dluhy R, Cooper B, Gleason RE, Soeldner JS, Bunn HF. N ENGL J MED 1981 304 6 319-324
581811 Clinical manifestations and therapy of transfusional haemosiderosis. Gabutti V, Borgia-Pignatti C. BAILLIERES CLIN HAEMATOOL 1994 7 4 919-940
581812 Pulmonary iron overload in thalassemia major presenting as small airway disease. Ooi GC, Khong PL, Lam WK, Trendell-Smith NJ, Tsang KW. BAILLIERES CLIN HAEMATOOL 2002 108 1 43-46
588079 Deferasirox. Barton JC. CURR OPIN INVESTIG DRUGS 2005 6 3 327-335
669865 The safety and pharmacokinetics of GT56-252, a novel orally available iron chelator. Donovan JM, Palmer PA, Plone MA, Wonke B. BLOOD 2004 104 1 Suppl 1 29
669866 Pharmacokinetics of GT56-252, novel orally available iron chelator in rat, dog and cynomolgous monkey. Marquis JK, Audie Dagher R, Bree M, Appelqvist T, Guillamat PO, Forster P. TOXICOL SCI 2003 72 Suppl 1 47
670626 The design of orally active iron chelators. Hider RC, Zhou T. ANNALS NEW YORK ACAD SCI 2005 1054 141-154
739335 Orphan products: FDA. Genzyme Corp INTERNET SITE 2004 April 14

The efficacy and tolerability of ICL670, a once-daily oral iron chelator, in patients with myelodysplastic syndromes (MDS) and iron overload. Gattermann N, Cazzola M, Greenberg P, Maertens J, Soulieres J, Rose C LEUK RES 2005 29 1 S76

Improved survival in patients with myelodysplastic syndrome (MDS) receiving iron chelation therapy. Leitch HA, Goodman TA, Wong KK, Vickars LM, Galbraith PF, Legar CS BLOOD 2006 108 11


Exjade (deferasirox) prescribing information. Novartis Pharma Corp 2006


First human studies with a high-molecular-weight iron chelator. Dragsten PR, Hallaway PE, Hanson GJ, Berger AE, Bernard B, Hedlund BE J LAB CLIN MED 2000 135 1 57-65


Small-molecule screening identifies the selanazal drug ebselen as a potent inhibitor of DMT1-mediated iron uptake. WelI HA, Buckett PD, Wessling-Resnick M CHEM BIOL 2006 13 9 965-972


Drug Discovery Programs – DMT1. Xenon Pharmaceuticals Inc COMPANY WORLD WIDE WEB SITE 2007 February 23


Coordination chemistry of microbial iron transport. 42. Structural and spectroscopic characterization of diastereometric Cr(III) and Co(III) complexes of desferrithiocin. Hahn FE, McMurry TJ, Hugi A, Raymond KN J AM CHEM SOC 1990 112 1854-1860

Metal complex formation of a new siderophore desferrothiocin and of three related ligands. Anderegg G, Raber M J CHEM SOC SER CHEM COMMUN 1990 1194-1196


