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Comparison of Iron Chelator Efficacy in Iron-Overloaded Beagle Dogs and Monkeys (*Cebus apella)*

Raymond J. Bergeron, PhD,[*] Jan Wiegand, William R. Weimar, PhD,[†] Tanaya C. Lindstrom, Tammy L. Fannin, and Katie Ratliff-Thompson

Rodents and dogs are frequently used for preclinical toxicologic assessment of candidate iron chelators. Although the iron-clearing profile of a ligand often is known in rodents, and sometimes in primates, such information in dogs is rarely, if ever, available. Because of this, toxicity studies in dogs could be misleading; chelators that may otherwise be suitable for human clinical studies may be abandoned as being unacceptably toxic, simply because, unknown to the investigator, these drugs remove more iron in this species than would have been expected on the basis of iron clearance results in other species. This is a scenario that we encountered during toxicity trials of (*S*)-β,β-dimethyl-4'-hydroxydesazadesmethyldesferrithiocin in dogs. Thus, we developed an iron-overloaded dog model in which it is possible to evaluate iron-clearing efficiencies of potential therapeutic ligands. Seven deferration agents have been screened in this model, and the results were compared with the iron-clearing efficiency of the same ligands in an iron-loaded *Cebus apella* monkey model. The data suggest that while the iron-clearing efficiencies of most of the drugs were similar between the two species, there can be profound differences. This is consistent with the idea that caution needs to be exercised when carrying out preclinical toxicity evaluations of a chelator in dogs without first measuring the drug's iron-clearing efficiency in this species.

Iron serves as an essential redox component in nearly all life forms. However, because of its poor solubility in the biosphere and, therefore, its poor accessibility, nature has had to develop mechanisms for uptake and processing. For example, microorganisms have developed low molecular weight chelators, siderophores, along with the required ancillary transport and processing systems, to access the metal (29, 49, 53). The microorganisms secrete these ligands into their environment to sequester the ferric iron [Fe(III)]. The microorganism is now virtually bathed in a utilizable form of iron, and the metal complexes are subsequently incorporated and processed. Not surprisingly, the iron management systems in humans are far more complex, utilizing proteins for transport (e.g., transferrin) and storage (e.g., ferritin) (6, 60, 67).

Interestingly, transferrin, ferritin, and other components of iron metabolism are themselves tightly regulated by iron concentration. For example, a number of the iron-regulatory proteins and their transport partners are controlled at the translational level by iron response elements (IREs) in their corresponding mRNAs (33). When the metal is appropriately managed, as by ferritin or transferrin, or is serving as a prosthetic in various biological redox systems (59), it does not present a problem. However, unbound or weakly bound iron, particularly ferrous iron [Fe(II)], reduces hydrogen peroxide to the hydroxyl radical ($HO^{\bullet}$) and hydroxide anion, liberating Fe(III), a process known as the Fenton reaction (39, 47). This radical reacts at a diffusion-controlled rate with other nearby molecules, generating longer-lived radicals, which initiate additional chain processes that cause damage to DNA, cell membranes, and mitochondria, and promote the formation of hypochlorous acid and other carcinogens (2, 39, 40). Furthermore, many physiologic reductants, such as ascorbate, reduce Fe(III) back to Fe(II) for further reduction of peroxide and radical generation. The progress of disorders as diverse as β-thalassemia (57, 71), Parkinson's disease (45, 51), and reperfusion injury (5, 42) have thus been documented to be iron mediated. The solution to these iron-mediated diseases is simply to remove the excess metal.

In primates, iron metabolism is highly efficient (30, 34). Because it cannot be effectively cleared, the introduction of "excess iron" into this closed metabolic loop leads to chronic overload. Unmanaged iron then leads to Fenton-mediated peroxidative tissue damage (50, 56, 68). For example, patients with severe hemolytic anemias such as β-thalassemia require continued blood transfusions, increasing their body iron by 200 to 250 mg/unit. Unless these individuals receive chelation therapy, they frequently die in their third decade from complications associated with global iron overload. Although there is any number of iron-mediated diseases, the most difficult problems are associated with disorders that require long-term transfusion therapy. In this arena, the chelator's toxicity profile, mode of administration, and compliance by the patient become major issues. A highly efficient iron chelator would solve many of these problems.

A ligand's iron-clearing efficiency is the amount of iron excretion above background induced by a ligand, divided by the theo-

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Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610
[†]Present address: Department of Pharmaceutical Sciences, South University School of Pharmacy, 709 Mall Blvd., Savannah, Georgia 31406
[*]Corresponding author.

retical iron clearance; this number is given as a percentage. For example, deferoxamine B (DFO), which is currently used to treat iron overload, forms a tight 1:1 complex with Fe(III). Thus, one might expect that a dosage of 100 mg (150 μmol) of DFO per kg of body weight should promote the excretion of 150 μg-atom of Fe(III) per kg. Unfortunately, the efficiency of DFO in patients is only about 5% (58).

Since its introduction nearly 40 years ago, deferoxamine, a hydroxamate siderophore produced by large-scale fermentation of *Streptomyces pilosus* (25), has nevertheless been the drug of choice for patients with transfusional iron overload. Although the drug's efficacy and long-term tolerability are well documented, it still suffers from several other shortcomings associated with its poor gastrointestinal absorption and short half-life, necessitating prolonged parenteral (e.g., subcutaneous or intravenous infusion), generally for 9 to 12 h/d, 5 or more days weekly (48, 58, 61). Not surprisingly, patient compliance with such a regimen is problematic. An orally effective iron chelator, or a parenterally administered chelator more efficient than DFO, has been a therapeutic target for many years.

Several families of iron chelators have been identified as potential alternatives to DFO, including pyridoxal isonicotinoyl hydrazones (PIHs) (4, 27, 62), hydroxypyridinones (L1/CP20) (44, 63), *N*,*N*'-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*'-diacetic acid (HBED) (37, 38, 43), 3,5-diphenyl-1,2,4-triazoles (ICL670A) (54, 55, 65), and the desferrithiocins (3, 7, 70). Once iron-clearing efficacy has been documented in rodent (19, 41, 46, 52) and/or primate (12, 13, 65, 69) models, the candidate ligand is eligible for toxicity studies. Bear in mind that, because of the life-long treatment required in many chelation therapy situations, the drug-related toxicosis must be minimal. However, by virtue of what iron chelators are designed to do—remove iron—toxicity evaluations must be carefully designed. Any deleterious effects observed in the course of preclinical toxicity assessments may derive from either or both of two sources: toxicity implicit in the structure of the drug and/or toxicity derived from the designed function of the drug.

In the United States, before a new drug can be tested in humans, the Food and Drug Administration requires preclinical toxicity assessments in two species, one a rodent and one a mammalian non-rodent (35, 36). The most commonly used rodent species for these preclinical evaluations have been rats or mice; dogs have been commonly used as a second species in the preclinical evaluation of new drugs (18, 32, 73). Dogs are the species of choice over others (e.g., monkeys) for a number of reasons, including their availability, price, size, and temperament; dogs also are a less senescent species than are non-human primates.

Although frequently there is extensive information on how much iron a given chelator will remove from a rat (8, 11, 19) or a primate such as the *Cebus apella* monkey (12, 17, 20), this information is not often available for dogs. It is clear from those and other studies in rodents and primates that the interspecies differences in iron-clearing efficiency of a chelator can be profound; the situation comparing dogs and primates was unclear.

In 30-day toxicity trials of (*S*)-β,β-dimethyl-4'-hydroxydesazadesmethyldesferrithiocin (chelator {**4**}, Fig. 1) and (*S*)-4'-hydroxydesazadesferrithiocin (chelator {**7**}) in rodents, the animals were given daily doses of 130 and 119 μmol/kg, respectively. These doses were each sufficient to induce excretion of 450 μg of iron/kg of body weight when administered orally to the *C. apella* primates. This number represents the amount of iron needed to be excreted daily to prevent an additional iron burden in patients with transfusion-dependent anemias (27). Under these conditions, neither drug elicited any overt toxic effects in the rodents, and all histopathologic findings were indistinguishable from those of age-matched controls. When a similar study was performed in iron-loaded dogs, chelator {**7**} was well tolerated. However, the dimethyl analogue (chelator {**4**}) was far more toxic than chelator {**7**}. We found this somewhat curious.

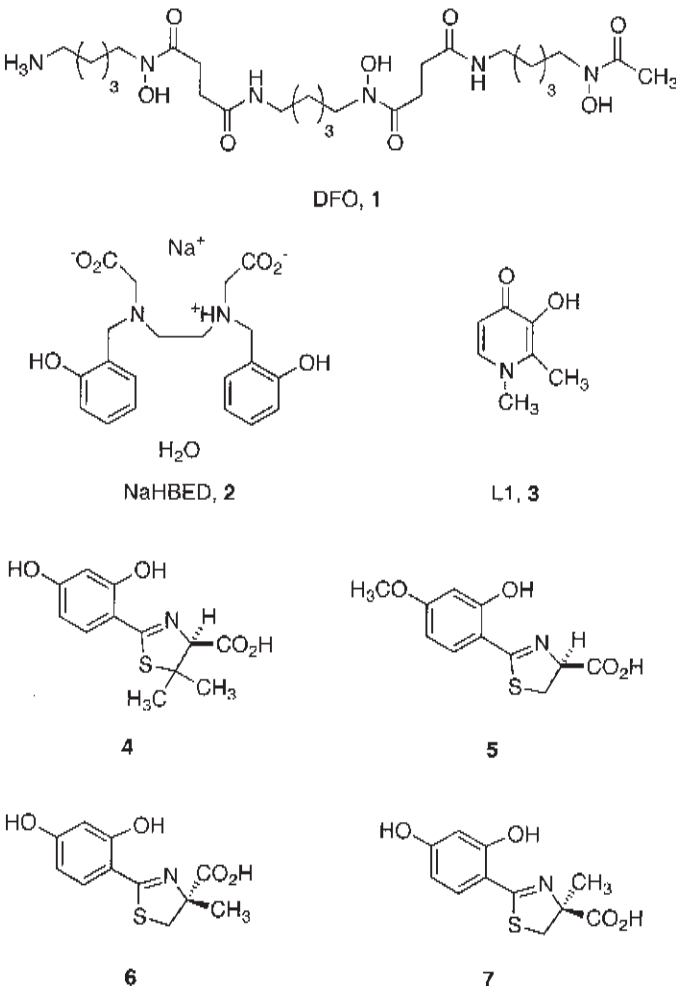

Figure 1. Structures of the iron chelators chosen for evaluation: deferoxamine B (DFO, {**1**}), *N*,*N*'-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*'-diacetic acid, monosodium salt (NaHBED, {**2**}) 1,2-dimethyl-3-hydroxypyridin-4-one (L1, {**3**}), (*S*)-β,β-dimethyl-4'-hydroxydesazadesmethyldesferrithiocin {**4**}, (*S*)-4'-methoxydesazadesmethyldesferrithiocin {**5**}, (*R*)-4'-hydroxydesazadesferrithiocin {**6**}, and (*S*)-4'-hydroxydesazadesferrithiocin {**7**}.

Intensely dark bile was found at euthanasia of dogs administered ligand {**4**}. The color of the bile was suggestive of high concentrations of the ferric complex of chelator {**4**}. This finding is something that we had not seen during the course of investigating the toxicity of numerous other chelators, and raised the possibility that the ligand may have been causing excretion of more iron than would have been predicted on the basis of iron clearance studies performed in the monkeys (20). Since excess iron removal may have contributed to the observed toxicosis, we were compelled to examine the iron clearance of this ligand in canines

more closely than simple biliary iron estimates could provide. Furthermore, general understanding of how iron chelators perform in dogs is lacking. This meant developing an iron-overloaded canine model, in which it was possible to accurately measure chelator-induced biliary and renal iron clearances. The dog model is organized in manner similar to that of our *C. apella* monkey model (10-12, 17, 18) and allows determination of iron-clearing efficiencies in dogs, so that a more informed decision can be made when designing drug dosing regimens for preclinical toxicity evaluations of candidate iron chelators in this species. This is an important issue, as chelators that may otherwise be suitable for formal clinical studies in patients may be dismissed as being unacceptably toxic, simply because they remove too much iron from dogs. The animal husbandry and iron-loading, low-iron diet, and the iron-clearing efficiencies of several iron chelators in monkeys and dogs are described. In addition, the pharmacokinetic parameters of desferrithiocin analogues {**4**} and {**7**} are compared in dogs and primates.

Materials and Methods

Animals and materials. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee, University of Florida. *Cebus apella* monkeys (conventional) were obtained from World Wide Primates (Miami, Fla.). The standard housing for the monkeys was a divided 3.5 × 10 × 5-ft condo cage, in which one monkey is housed in each of two 3.5 × 5 × 5-ft compartments. The base diet for the monkeys was the Harlan Teklad Primate Diet, 25% protein (Harlan Sprague-Dawley, Indianapolis, Ind.), provided as 10 biscuits each morning and evening. Male beagle dogs (conventional) were purchased from Harlan Sprague-Dawley. The base diet for the dogs, the Harlan Teklad Global Diet, 25% protein (Harlan Sprague-Dawley), was provided ad libitum. Vari-Kennels and exercise pens used in the dog studies were acquired from Omaha Vaccine Company (Omaha, Nebr.). Poly-coated floor grates used in the dog cages were purchased from Ryan's Pet Supplies (Phoenix, Ariz.). Ultrapure salts were obtained from Johnson Matthey Electronics (Royston, United Kingdom). All hematologic and biochemical studies (12) were performed at Antech Diagnostics (Tampa, Fla.). Atomic absorption spectroscopic measurement of food mix and fecal and urine samples were made using a Perkin-Elmer model 5100 PC (Norwalk, Conn.). Histologic evaluation of necropsy tissues was performed at Florida Vet Path (Bushnell, Fla.).

Iron chelators. The drugs compared in the two species are shown in Fig. 1. Deferoxamine B (DFO, {**1**}) as the methanesulfonate salt, Desferal (Novartis Pharma, Basel, Switzerland), was obtained from a hospital pharmacy (Gainesville, Fla.). *N,N*'-Bis(2-hydroxybenzyl)ethylenediamine-*N,N*'-diacetic acid, monosodium salt (NaHBED, {**2**}) was converted to this form at SRI International (Menlo Park, Calif.) from the monohydrochloride dihydrate (Strem Chemical Co., Newburyport, Mass.). 1,2-Dimethyl-3-hydroxypyridin-4-one (L1, {**3**}) was a gift from H. Peter, Novartis Pharma (Basel, Switzerland). The desferrithiocin analogues [(*S*)-β,β-dimethyl-4'-hydroxydesazadesmethyldesferrithiocin {**4**}, (*S*)-4'-methoxydesazadesmethyldesferrithiocin {**5**}, (*R*)-4'-hydroxydesazadesferrithiocin {**6**}, and (*S*)-4'-hydroxydesazadesferrithiocin {**7**}] were synthesized in our laboratories as described (20, 21).

Iron loading of *C. apella* monkeys. The monkeys were iron overloaded by use of intravenous iron dextran, as specified in an earlier publication, to provide about 500 mg of iron/kg of body weight (69); serum transferrin iron saturation increased to between 70 and 80%. At least 20 half-lives (60 days [72]) elapsed before any of the animals were used in experiments evaluating iron-chelating agents (body weight at time of experiments, 3.8 to 4.8 kg).

Primate fecal and urine samples. Fecal and urine samples were collected at 24-h intervals and were processed as described (10, 13, 16). Briefly, the collections began four days prior to administration of the test drug and continued for an additional five days after the drug was given. Iron concentration was determined by using flame atomic absorption spectroscopy as given in detail in other publications (13, 24).

Collection of monkey samples for pharmacokinetic analysis. After food had been withheld overnight, the monkeys were sedated as set forth elsewhere (23). The animals were intubated, and anesthesia was maintained via inhalation of Isoflurane gas (1.5 to 2%) for the first 4 h of the experiment. A lubricated 5-F, 16-in. urethral catheter was inserted into the bladder to allow collection of urine samples. Compound {**4**} was administered orally (n = 4) as a suspension in water at a dosage of 130 μmol/kg (the dogs were given 65 μmol of the drug/kg twice daily). At postdrug administration hour (PAH) 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8, blood samples (1.8 ml) (22) were taken from a leg vein. The monkeys were allowed to recover after the 4-h time point and were re-sedated briefly with Telazol (Fort Dodge Animal Health, Fort Dodge, Iowa) at the 6- and 8-h time points. Urine was collected via catheter followed by rinsing of the bladder with saline prior to drug administration and at 2-h intervals for 4 h thereafter.

In a separate experiment, eight primates were anesthetized as described previously and were given analogue {**7**} orally at a dosage of 119 μmol/kg (23; the dogs were given the drug at a dosage of 59.5 μmol/kg twice daily). The drug was given as its sodium salt in aqueous solution (n = 4) or as a suspension in 40% Cremophor RH-40/water (n = 4). Blood samples (1.8 ml) were taken at PAH 0, 0.5, 1, 2, 3, 4, 6, and 8. The monkeys were allowed to recover after the 4-h time point and were re-sedated as described previously. Prior to drug administration and at 1- to 2-h intervals for 4 h thereafter, urine was collected as described previously.

Estimated biliary iron clearance in dogs. Bile samples obtained from the gallbladder of dogs during the course of a routine necropsy were processed for iron content, as described for the bile duct-cannulated rats (11, 13, 19). Estimated biliary iron excretion was calculated by multiplying the iron concentration of the bile by bile excretion of 12 ml/kg/d (1).

Iron overloading procedure in dogs. The dogs were iron overloaded as described (18). Iron infusions were repeated every 10 to 14 days until a final iron burden of 300 mg (toxicity trials) or 500 mg (iron clearance experiments) of iron/kg had been achieved. The iron was allowed to equilibrate for at least 30 days before the animals were exposed to an iron chelator (28).

Housing of dogs. Traditional canine metabolic cages were unsuitable for our purposes due to their metal content and poor urine/feces separation. Therefore, the dogs used in the iron clearance experiments (body weight: 11.8 to 13.2 kg at the start of the series of experiments; 12.8 to 14.2 kg at the end) were housed in plastic Vari-Kennels that were sufficiently large for the animals to move around, 32 × 22.5 × 23 in (L × W × H, respectively), but not so large that they were likely to defecate in the cage. On day

–3, the dogs were moved from their kennel runs and were individually housed in the Vari-Kennels. To allow collection of any urine voided in the cages between time points, a poly-coated floor grate mounted on a polyvinylchloride framework was used as an elevated floor in the Vari-Kennels, so that if the dogs urinated while they were in the cages, the urine could still be collected.

Fecal and urine sample collection and preparation. The dogs were kept in the crates at all times except during sample collection (7 a.m., noon, 4 p.m., and 11 p.m.) and supervised play/exercise periods. Every 6 to 8 h, urine samples via midstream sample catch or catheterization (if necessary) were collected into a plastic container. In addition, any urine in the cages was collected by use of a plastic turkey baster; hair was removed using a plastic hair catcher. Once the urine had been collected, the dogs were placed in a 4 × 4-ft exercise pen that had been placed on a linoleum floor, where the dogs would generally defecate quickly. Fecal samples were collected from the linoleum floor using latex gloves.

The urine and fecal samples collected throughout the day were combined (i.e., each dog had one pooled urine and one pooled feces sample per day). Fecal samples were examined for occult blood by using a guaiac test, distilled water was added, and the samples were autoclaved. Fecal preparation was the same as that for the iron-loaded primates (10, 13). Urine sample preparation was the same as for the bile duct-cannulated rats (12, 19). Urine and fecal samples were collected for two days before drug administration and for an additional three days after the drug was given.

Low-iron diet in dogs. The low-iron diet consisted of boiled chicken, boiled brown rice, corn oil, fiber, gelatin, garlic powder, and a low-metal mineral mix (NaCl [25.56 g], $MnSO_4$ [0.2 g], $CaCO_3$ [50.8 g], KH_2PO_4 [71.58 g], and $MgSO_4$ [10.36 g]). The diet was made by boiling two whole chickens (approx. 1.8 kg [4 lb] each) in distilled water. Once the chickens were thoroughly cooked, they were removed from the pan and allowed to cool, then the skin, meat, and fat were separated from the bones. Four boxes of brown rice (Kraft 10-Minute Rice, 420 g/box) and approximately 20 g (four tablespoons) of garlic powder were added to the water that was used to cook the chickens. The rice was cooked according to package instructions.

Each batch of food consisted of: cooked chicken (125 g), cooked rice (500 g), distilled water (275 ml), fiber (5 g), and corn oil (25 ml) that were blended in a food processor until smooth. The batches of food were combined, then the low-metal mineral mix in 500 ml of distilled water and eight 7-g packets of unflavored gelatin dissolved in an additional 500 ml of distilled water were added. The food was thoroughly mixed by hand; the resulting paste-like substance was weighed into 1-liter capacity plastic containers. The food mix, once refrigerated, had a consistency similar to that of canned dog food and contained < 30 ppm iron. The food was kept refrigerated and was warmed to room temperature before feeding. The dogs were offered 900 g of food/d.

To prevent upsetting the dog's digestive system, the animals' regular diet was switched to the low-iron food gradually. The low-iron diet was started nine days before drug administration. The first three days (–9 to –7), the dogs were fed a 50/50 mixture of the low-iron diet and their normal dog chow. The following three days (–6 to –4), the animals were fed only the low-iron diet. On day –3, the dogs were moved from their kennel runs into individual housing (Vari-Kennels). Feeding of the low-iron dog food continued until day +3, when their regular diet and housing status was returned. Iron-free water was provided ad libitum via water bottles affixed to the outside of the cages.

Toxicity trials in dogs. After a two-week acclimation period, young adult male beagles were iron overloaded as described previously. The drug was administered orally in gelatin capsules to the dogs (body weight, 10.7 to 12.1 kg at the start of drug administration) at a daily dosage of 130 μmol/kg (65 μmol/kg twice daily) ({**4**}, n = 5) or 119 μmol/kg (59.5 μmol/kg twice daily) ({**7**}, n = 3) after food had been withheld overnight (i.e., at 9 a.m.). The animals were fed at noon, and the second daily dose was given at 5 p.m. These doses would clear 450 μg of Fe/kg/d in the *C. apella* primate model. During the 30-day trials, the animals were closely monitored (overall condition daily; weight twice weekly; urinalysis and blood biochemical analysis (12), and complete blood count once weekly) for any signs of adverse effects; these parameters also were assessed before drug dosing commenced and immediately before euthanasia between 8 and 24 h after the final dose. In addition, at euthanasia, bile samples were collected from the gallbladder, and extensive tissues (18) were submitted for histologic analysis.

Collection of canine samples for pharmacokinetic analysis. Pharmacokinetic analysis of compounds {**4**} and {**7**} in the dogs was performed on the first {**4**} or second {**7**} day of the toxicity trial. Food was withheld from dogs overnight, and the first daily dose of the compound was administered as described previously. At PAH 0, 0.5, 1, 2, 3, 4, and 8, blood samples (1.8 ml) were taken from a hind limb vein. Urine samples were obtained from the dogs via a urethral catheter at PAH 0, 2, and 4. The second daily dose of the drug was not given until after the 8-h time point samples had been collected.

Drug preparation and administration. (i) Monkeys. The DFO and NaHBED were put into solution with sterile water for injection and saline, respectively, and were administered subcutaneously to the primates at a dosage of 150 μmol/kg. The remaining drugs were administered orally: in capsules, compounds {**3**} and {**7**}; as the monosodium salt (prepared by the addition of the free acid to one equivalent of sodium hydroxide) in an aqueous solution, ligand {**6**}; as a suspension in distilled water, analogue {**4**}; or solubilized in 40% Cremophor RH-40/water (vol/vol), compound {**5**}. The desferrithiocin analogues {**4-7**} were dosed at 150 μmol/kg; L1 {**3**} was given at a dosage of 300 μmol/kg.

(ii) Dogs. The DFO and NaHBED were put into solution with sterile water and were given subcutaneously to the dogs at a dosage of 150 μmol/kg. The desferrithiocin analogues {**4-7**} were loaded into gelatin capsules and were given orally at a dosage of 150 μmol/kg. The L1 was weighed into gelatin capsules and was administered orally at a dosage of 300 μmol/kg.

Calculation of iron chelator efficiency. The theoretical iron outputs of the chelators were generated on the basis of a 1:1 ligand:iron complex for DFO and NaHBED, a 3:1 ligand:iron complex for L1, and a 2:1 ligand:iron complex for the desferrithiocin analogues {**4-7**}. Efficiencies in the monkeys were calculated by averaging the iron output for four days before administration of the drug, subtracting these numbers from the two-day iron clearance after administration of the drug, then dividing by the theoretical output; the result is expressed as a percentage (10).

The iron-clearing efficiency in the dogs was generated in a similar manner (i.e., by averaging the iron output for two days before administration of the drug, subtracting these numbers from the two-day iron clearance after administration of the drug, then dividing by the theoretical output); the result is expressed

as a percentage.

Plasma and urine analytical methods. Plasma and urine pharmacokinetic samples were prepared for high-performance liquid chromatographic (HPLC) analysis, as described elsewhere (23). In brief, the samples were extracted with methanol, centrifuged, and filtered through a 0.2-μm filter. Analytical separation was performed using a C_{18} reversed-phase HPLC system, with UV detection at 310 nm, as described previously (22). The mobile phases and chromatographic conditions for chelator {**4**} and analogue {**7**} were as presented in another publication (23). The concentrations were calculated from the peak area fitted to calibration curves by using nonweighted, least squares linear regression with Rainin Dynamax HPLC Method Manager software (Rainin Instrument Co., Woburn, Mass.). The method had a detection limit of 0.5 μ*M*, and was reproducible and linear over a range of 1 to 1,000 μ*M*.

Pharmacokinetic analyses. The model-independent pharmacokinetic parameters, including the area under the time-concentration curve (AUC) from time zero to the time of the last measured plasma concentration (8 h), the total area under the first-moment curve (AUMC), mean residence time (MRT), maximal concentration (C_{max}), and time of peak concentration, were estimated from plasma concentration-time data as reported earlier (15, 22, 23).

Statistical analysis. Data are presented as mean ± SEM. For comparison of the means for the two species, a one-tailed, two-sample *t* test (without the assumption of equality of variances) was performed. A significance level of $P \leq 0.05$ was used.

Results

Toxicity of compounds {4} and {7}. Iron-overloaded dogs were given either compound {**4**} (130 μmol/kg/d) or compound {**7**} (119 μmol/kg/d) in what were intended to be 30-day toxicity trials; these doses caused the excretion of 450 μg/kg of iron in the primates. The dogs treated with ligand {**7**} tolerated the drug well throughout the 30-day dosing period, no hematologic or serum biochemical abnormalities were observed, and all histopathologic results were indistinguishable from those of iron-loaded, untreated dogs. In contrast, although the dogs given analogue {**4**} appeared to be unaffected during the first 3 weeks of drug dosing, their appetite decreased shortly thereafter, and they began vomiting. Owing to the rapidly deteriorating condition, three of the five treated dogs were euthanized before the planned 30-day course could be completed. The serum biochemical results and blood counts were unremarkable, except for increases in blood urea nitrogen, creatinine, and phosphorus concentrations in two of the five treated dogs. All of the treated animals had renal proximal tubule damage; otherwise, the histopathologic results were undistinguished.

Estimated biliary iron clearance in dogs. Interest in a canine iron clearance model began when, at euthanasia at the close of the aforementioned toxicity trial, intensely dark bile was found up to PAH 24 in the gallbladder of the dogs treated with chelator {**4**}. Previous necropsy of other dogs exposed to a variety of ligands did not uncover anything of this nature. This raised the possibility that the ligand may have been causing excretion of more iron than would have been predicted on the basis of iron clearance studies performed in the *C. appella* monkeys (20). Estimated biliary iron excretion (Fig. 2) implied that ligand {**4**} was causing excretion of 3.6 mg of Fe/kg in a dog euthanized at PAH 8 (last dose), and excretion of > 2 mg of Fe/kg in animals euthanized at PAH 24, when given at a dose which, in primates, caused excretion of only 450 μg of iron/kg. This is considerably higher than the estimated biliary iron content of untreated iron-loaded control dogs and iron-loaded dogs treated with NaHBED (18) or desferrithiocin analogue {**7**} (Fig. 2). The biliary iron concentration of the latter chelators had largely returned to that in the control dogs by the time the animals were euthanized between PAH 18 and 48 (final dose).

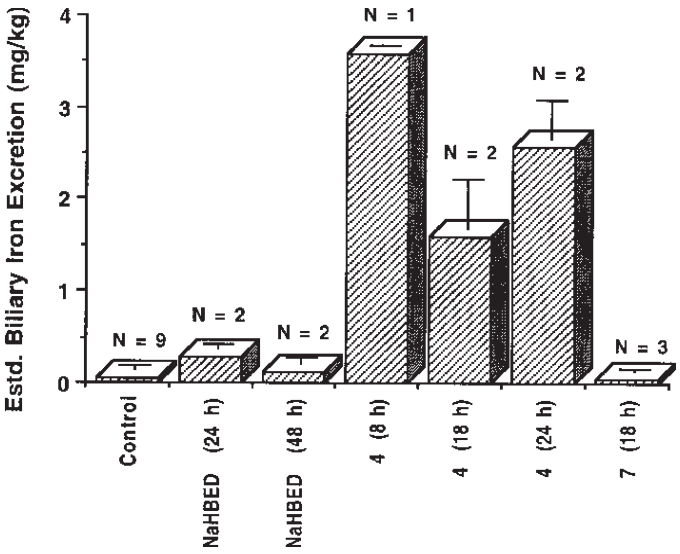

Figure 2. Estimated biliary iron excretion induced by NaHBED, {**4**}, and {**7**} in iron-loaded dogs euthanized at 8-48 PAH. Biliary iron excretion (mg/kg, *y*-axis) was calculated by multiplying the iron concentration of the bile by bile excretion of 12 ml/kg/d. Untreated iron-overloaded dogs served as controls; the details of the NaHBED toxicity evaluation are described elsewhere (18).

Pharmacokinetics. In the course of the canine toxicity trials (day 1 {**4**} or day 2 ({**7**}), pharmacokinetic parameters also were evaluated. Comparison of the plasma concentration-time curves in the dogs and monkeys for compounds {**4**} and {**7**} indicated that the plasma concentration of the drugs reached a higher value in the dogs than it did in the monkeys (Table 1). The AUC and C_{max} for ligands {**4**} and {**7**} in the dog were greater than eight times the same parameters in the monkey, even though the monkeys received twice the dose as that given to the dogs. The C_{max} of chelator {**4**} in the dogs was 55.1 ± 15.1 μ*M*, whereas in the primates, it was 4.6 ± 2.3 μ*M*. The C_{max} of agent {**7**} was 27.8 ± 7.3 μ*M* and 3.4 ± 1.4 μ*M* in the dogs and primates, respectively.

Urinary excretion of the analogues in the two species also was different. Renal excretion of both drugs in the primates was low, whereas in the dogs, the renal excretion of both ligands was higher (Table 1). In fact, during sample collection, a brownish color indicative of the iron complex was detected in the urine of the chelator-treated dogs between PAH 2 and 4.

Efficiency of ligands in dogs. The unexpected biliary iron clearances and toxicosis associated with desferrithiocin analogue {**4**} in dogs, coupled with the absence of data on how chelators in general perform in canines, prompted us to look more closely at this animal. Accordingly, the model used in *C. apella* primates was modified for use in dogs. We evaluated the efficiencies of seven ligands (Fig. 1) in this model and compared these results with those derived from the primates.

Table 1. Pharmacokinetic parameters compared in *Cebus apella* primates versus beagle dogs

Cmpd	Species	n	Dosage, μmol/kg	Pharmacokinetic parameter[a] AUC, h-μmol/L	AUMC, h²-μmol/L	C_{max}, μmol/L	TPC, h	MRT, h	UE, % of total dose
{**4**}	Monkey	4	130[b]	14.7 ± 4.4	79.8 ± 29.8	4.6 ± 2.3	5.5 ± 1.0	5.3 ± 0.6	0.024 ± 0.031
	Dog	5	65[c]	241 ± 72	1,409 ± 1,097	55.1 ± 15.1	1.8 ± 1.3	5.0 ± 2.0	11.4 ± 4.0
{**7**}	Monkey[d]	8	119	10.8 ± 2.8	58.4 ± 14.3	3.4 ± 1.4	6.5 ± 0.9	5.4 ± 0.4	0.72 ± 0.34
	Dog	3	59.5[c]	83.1 ± 15.9	233.2 ± 78.8	27.8 ± 7.3	2.3 ± 1.2	2.8 ± 0.7	42.9 ± 17.9

[a]Mean ± SD is reported for each parameter calculated from time zero to the time of the last measured plasma concentration (8 h).
[b]In the monkeys, compound {**4**} was administered as a suspension in water.
[c]In the dogs, each compound was administered in capsules.
[d]Previously published (23); compound {**7**} was administered as either the monosodium salt in water (n = 4) or as a suspension in 40% Cremophor (n = 4).
Cmpd = Compound; TPC = time of peak concentration; MRT = mean residence time; UE = urinary excretion.

Table 2. Dosing regimens, routes, and iron clearance compared in *C. apella* primates versus beagle dogs

Compound	Species	Vehicle	Iron clearance, μg/kg[a]	Feces/urine ratio, %	Efficiency, % mean[a]	Range	*P* value
DFO	Monkey[b]	Water	435 ± 115	64/36	5.1 ± 1.3	3.3–6.6	< 0.05
	Dog	Water	1,251 ± 575	68/32	14.7 ± 6.7	7.9–23.4	
NaHBED	Monkey[b]	Saline	1,139 ± 383	68/32	13.6 ± 4.5	7.7–19.9	= 0.05
	Dog	Water	1,559 ± 296	60/40	18.5 ± 3.5	15.3–23.6	
L1	Monkey[b]	Capsule	177 ± 77	30/70	3.1 ± 1.4	1.1–4.3	NS
	Dog	Capsule	334 ± 483	95/5	6.0 ± 8.6	< 0–16.6	
{**4**}	Monkey	Water	622 ± 221	77/23	14.8 ± 5.3	7.9–20.1	< 0.02
	Dog[c]	Capsule	996 ± 294	95/5	23.7 ± 7.0	14.4–36.6	
{**5**}	Monkey[b]	40% Cremophor	679 ± 134	82/18	16.2 ± 3.2	12.2–19.6	NS
	Dog	Capsule	844 ± 273	92/8	20.1 ± 6.5	12.4–27.0	
{**6**}	Monkey[b]	Sodium salt/H_2O	162 ± 61	89/11	3.9 ± 1.4	2.2–5.1	NS
	Dog	Capsule	36 ± 147	65/35	0.9 ± 3.5	< 0–6.0	
{**7**}	Monkey	Capsule	607 ± 304	85/15	14.5 ± 7.2	5.4–21.7	NS
	Dog	Capsule	357 ± 215	95/5	9.3 ± 5.5	4.7–17.0	

All test chelators (structures shown in Fig. 1) were administered as a single dose subcutaneously (DFO, NaHBED) or orally (L1, desferrithiocin analogues {**4-7**}) at a dosage of 150 μmol/kg, except for L1, which was administered at a dosage of 300 μmol/kg.
[a]Reported as mean ± SEM. In the dogs, n = 4, except for DFO, where n = 5. In the monkeys, n = 4 except for DFO and NaHBED, where n = 5 and n = 6, respectively.
[b]Monkey data published previously: DFO (16), NaHBED (17), L1 (CP20) (12), {**5**} (20), and {**6**} (23).
[c]Cumulative over two experiments using the same four animals each time.
DFO = deferoxamine; NaHBED = *N,N*'-bis(2-hydroxybenzyl)ethylenediamine-*N,N*'-diacetic acid, monosodium salt; NS = not significant.

(i) Deferoxamine B (DFO). Whereas the efficiency of DFO in iron-overloaded monkeys at a dosage of 150 μmol/kg, given subcutaneously (Table 2) was 5.1 ± 1.3% (16), the efficiency in the dogs at this dosage is higher, 14.7 ± 6.7%. Although iron clearance in the dogs was nearly three times that seen in the monkeys ($P < 0.05$), the mode of iron excretion was similar. Approximately 60% of the iron was excreted in the feces of each species; the remainder was excreted in the urine.

(ii) *N,N*'-Bis(2-hydroxybenzyl)ethylenediamine-*N,N*'-diacetic acid, monosodium salt (NaHBED). At a dosage of 150 μmol/kg, given subcutaneously, the iron-clearing efficiency of NaHBED in the primates (17) was 13.5 ± 4.5% (Table 2). It was slightly higher in the dogs (18.5 ± 3.5%, $P = 0.05$). Approximately 30 to 40% of the iron was excreted in the urine of each species, and the remainder was excreted in the feces.

(iii) 1,2-Dimethyl-3-hydroxypyridin-4-one (L1). In the monkeys (12), L1 administered orally at a dosage of 300 μmol/kg had an efficiency of 3.1 ± 1.4% (Table 2). At this same dosage, iron clearance in the dogs was within the error of that seen with the primates, efficiency of 6.0 ± 8.6% ($P > 0.05$). The large variability in dogs was due to moderate iron-clearing efficiency in one dog, mild response in two dogs, and the total lack of efficacy of the drug in one dog. The mode of excretion was different between the two species. In the dogs, the majority of the iron was found in the feces, 95%, but in the primates, most of the iron was located in the urine, 70%.

(iv)(*S*)-β,β-Dimethyl-4'-hydroxydesazadesmethyldesferrithiocin {4}. The efficiency of chelator {**4**} given to the primates at a dosage of 150 μmol/kg per os (p.o.) was 14.8 ± 5.3% (Table 2). The same dose of the drug administered to the iron-loaded dogs was 24.7 ± 8.0%. This experiment was repeated approximately six months later to ensure that iron excretion was stable in the test animals. In that study, the iron clearance results obtained were similar to those obtained the first time the drug was tested, 22.7 ± 6.9% ($P > 0.05$). The combined data for the two experiments (Table 2) resulted in an iron-clearing efficiency of 23.7 ± 7.0% ($P < 0.02$ versus monkeys). Finally, the mode of excretion between the two species also was different. The monkeys excreted 23% of the iron in the urine and 77% of the iron in the feces. The dogs also excreted the majority of iron in the feces, but the overall proportion was higher: 97% and 94% for the first and second time (respectively) the drug was evaluated. Only 3 to 6% of the iron was excreted in the urine.

(v) (*S*)-4'-Methoxydesazadesmethyldesferrithiocin {5}. In the primates (20), chelator {**5**} was given at a dosage of 150 μmol/kg, p.o.; the iron-clearing efficiency was 16.2 ± 3.2%. The efficiency of the drug administered to the dogs at the same dose was similar (Table 2), 20.1 ± 6.5% ($P > 0.05$). In addition, the mode of excretion also was similar; the majority of the iron was excreted in the feces, 82% for the primates and 92% for the dogs.

(vi) (*R*)-4'-Hydroxydesazadesferrithiocin {6}. The iron-clearing efficiency of analogue {**6**} was 3.9 ± 1.4% when administered to the primates at a dosage of 150 μmol/kg, p.o. (24). The efficiency in the dogs was lower (Table 2), but within error of that of the monkeys, 0.9 ± 3.5% ($P > 0.05$). The majority of the iron was excreted in the feces of both species, 89% for the monkeys and 65% for the dogs.

(vii) (*S*)-4'-Hydroxydesazadesferrithiocin {7}. Although the (*R*)-enantiomer {**6**} did not induce excretion of appreciable amounts of iron from monkeys or dogs, the (*S*)-enantiomer {**7**} was more effective when administered at the same dosage to either species (Table 2). The iron-clearing efficacy of analogue {**7**} in the monkeys was 14.5 ± 7.2%. The efficiency of the drug in the iron-loaded dogs at the same dosage was similar (9.3 ± 5.5%, $P > 0.05$). The mode of excretion between the two species also was similar. In each instance, the largest proportion of the iron was excreted in the feces (85% for the monkeys and 95% for the dogs). The pronounced difference in the iron-clearing behavior between the (*R*)- and (*S*)-enantiomers of this drug in the primates has been reported elsewhere (23).

Discussion

The dog has been commonly used as a second, non-rodent species in the preclinical evaluation of new drugs. Dogs are frequently chosen over other species (e.g., monkeys) for a number of reasons, including availability, price, size, and temperament; dogs are also a less senescent species than are non-human primates. Although dogs have proven themselves of considerable value in the testing of new drugs, they do not always respond to the drugs the same way that primates do (26, 64, 66). For example, the anesthetic propofol is significantly glucuronidated in microsomes prepared from human and marmoset liver, but is not directly glucuronidated by dog liver microsomes (66). In addition, the glucuronidation of several uridine diphosphate glucuronosyltransferase substrates in kidney microsomes indicated that, although microsomes from humans and marmosets glucuronidated a range of compounds, of the compounds tested in dog microsomes, only bilirubin was glucuronidated (66).

This was underscored in our laboratories during the preclinical evaluation of N^1,N^{11}-diethylnorspermine (DENSPM), a polyamine antineoplastic analogue now undergoing clinical trials in patients. Pharmacokinetics studies in dogs (14) indicated that, after intravenous administration of a bolus, approximately 50% was excreted unchanged in the urine during the first 4 h after the drug was given. In addition, the plasma half-life ($t_{1/2}$) was 1.21 h. The dosing schedules for humans were initially based on the results obtained from the dog model. However, when the drug was administered to patients, the plasma half-life was much shorter than that observed in dogs (31), and < 1% of the drug was excreted in the urine. A series of experiments was then performed in the *C. apella* monkey model (9) and, similar to that in humans, the plasma half-life was rapid, 0.07 to 0.14 h versus 1.21 h in the dogs. Furthermore, again similar to that in the human subjects, only a tiny fraction of the dose (0.005 to 0.022%) was detected in the urine. In summary, the plasma pharmacokinetics and the urinary excretion profiles of DENSPM in the monkey are virtually identical to those observed in humans, and are clearly different from those in dogs.

These interspecies differences also carry over into the handling of excess iron. Many species eliminate excess iron readily enough that hemochromatosis does not develop (28). However, this is not the case in humans; in several diseases, severe, lethal iron overload can develop. Although phlebotomy can be used to manage primary hemochromatosis, transfusional iron overload, as seen in thalassemia and sickle cell anemia, requires chelation therapy.

In the ongoing search for therapeutic iron chelators, there are unique issues regarding toxicity evaluations. Among these is the toxicity implicit in what iron chelators are designed for, deferration. Removal of too much iron, either systemically or focally, can be deleterious. Clearly, any chelator that removes iron effectively from an animal, if administered long enough, must, by definition, be toxic. Thus, in the design of toxicity trials, the first cautionary note is that normal and iron-overloaded animals should be used in toxicity studies, regardless of species. Further, the doses of chelators administered must be set according to some understanding of how effectively the ligand removes iron from a given species. Often, the iron-clearing efficiency of a particular test agent in rodents and primates is known. Unfortunately, toxicity trials are most often carried out in rats and dogs, not in rats and primates. Owing to the profound difference in the toxicity between two desferrithiocin analogues in dogs, a dichotomy that was not observed in rodents, we chose to develop an iron clearance model in beagle dogs. It soon became obvious that little was known about how iron chelators perform in dogs.

Over several weeks, dogs were first loaded with iron dextran to a value of 500 mg/kg. A palatable, reproducible, low-iron diet, specialized husbandry conditions, and appropriate urine and fecal sample collection schemes minimized iron contamination during iron clearance experiments. The iron-clearing efficiencies of seven ligands, including the reference chelator, DFO; a synthetic hexacoordinate compound, NaHBED; a bidentate oral chelator that is in clinical use in several countries, L1; and four analogues of desferrithiocin, one of which {**7**} is currently undergoing clinical trials, were evaluated in this model. The results obtained in the beagles were compared with historical primate values.

There were no appreciable differences in overall iron-clearing efficiency between primates and beagles for L1 or desferrithiocin analogues {**5-7**} (Table 2). Interestingly, however, there were significant differences between the two species for DFO, NaHBED, and desferrithiocin analogue {**4**}. The assessment of ligand {**4**} in iron-loaded beagles confirmed that the compound was twice as active in dogs as it was in monkeys ($P < 0.02$). The other notable exceptions were NaHBED, which was slightly more active in dogs than primates ($P = 0.05$), and DFO, which was nearly three times more active in dogs than primates ($P < 0.05$). Interestingly, the fraction of chelator-induced iron excreted in the feces of dogs treated with DFO or NaHBED (60 to 68%) was lower than that found in the dogs treated with desferrithiocin analogues {**4**}, {**5**}, or {**7**} (92 to 95%); this difference between the types of compounds was more profound than that seen in the monkeys.

In the dogs, ligand {**4**} was nearly 2.5 times more efficient than ligand {**7**}. There were several noteworthy results in the pharmacokinetic evaluations of chelators {**4**} and {**7**} (Table 1), in which the dogs were given half the dose as that received by the primates. The AUC values for compounds {**4**} and {**7**} were 16 and 7.7 times higher, respectively, in the dogs than in the primates. In similar manner, the C_{max} values were 12 and 8.2 times greater in the dogs for compounds {**4**} and {**7**}, respectively. Most interestingly, the fraction of total drug excreted in the urine in the dogs was 60 times higher than that in the primate urine for compound {**7**} and was 475 times greater than that in the primate urine for compound {**4**}. All of this suggests that dogs absorb these compounds more effectively than do primates, resulting in higher exposure to certain desferrithiocin analogues in dog plasma than might otherwise be anticipated from results of primate experiments.

Thus, there are several profound differences in the way the

dog handles ligand {**4**} (and possibly other iron chelators) versus the way primates handle them, not the least of which are increased iron-clearing efficiency and prolonged plasma exposure to the ligand. Whereas such disparity may not be an issue in a single dosing scenario, it can be problematic under a multiple dosing regimen. Exact reasons for these interspecies differences remain to be explored. This observation is also consistent with the idea that, if dogs are to be used in the evaluation of a chelator's toxicity profile, the compound's iron-clearing efficiency in this species for the dosages of the drugs selected needs to be taken into account. Increased iron clearance in dogs would only exacerbate iron-mediated toxicosis in a prolonged trial; the importance of this measurement would certainly be exaggerated in animals with normal iron stores. In conclusion, because of the nature of the function of iron chelators and the potential for markedly different iron-clearing efficiencies between dogs and primates (human and non-human), the iron-clearing profile of the ligand under consideration should be evaluated in dogs prior to toxicity studies in this species.

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References

1. **Altman, P. L.** 1961. Blood and other body fluids. Federation of American Societies for Experimental Biology, Washington, D.C.
2. **Babbs, C. F.** 1992. Oxygen radicals in ulcerative colitis. Free Radic. Biol. Med. **13:**169-181.
3. **Baker, E., A. Wong, H. Peter, and A. Jacobs.** 1992. Desferrithiocin is an effective iron chelator in vivo and in vitro but ferrithiocin is toxic. Br. J. Haematol. **81(3):**424-431.
4. **Becker, E. and D. Richardson.** 1999. Development of novel aroylhydrazone ligands for iron chelation therapy: 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogs. J. Lab. Clin. Med. **134(5):**510-521.
5. **Bel, A., E. Martinod, and P. Menasche.** 1996. Cardioprotective effect of desferrioxamine. Acta Haematol. **95(1):**63-65.
6. **Bergeron, R. J.** 1986. Iron: a controlling nutrient in proliferative processes. Trends Biochem. Sci. **11:**133-136.
7. **Bergeron, R. J., G. Huang, W. R. Weimar, R. E. Smith, J. Wiegand, and J. S. McManis.** 2003. Desferrithiocin analogue-based hexacoordinate iron(III) chelators. J. Med. Chem. **46(1):**16-24.
8. **Bergeron, R. J., Z.-R. Liu, J. S. McManis, and J. Wiegand.** 1992. Structural alterations in desferrioxamine compatible with iron clearance in animals. J. Med. Chem. **35:**4739-4744.
9. **Bergeron, R. J., R. L. Merriman, S. G. Olson, J. Wiegand, J. Bender, R. R. Streiff, and W. R. Weimar.** 2000. Metabolism and pharmacokinetics of N^1,N^{11}-diethylnorspermine in a *Cebus apella* primate model. Cancer Res. **60(16):**4433-4439.
10. **Bergeron, R. J., R. R. Streiff, E. A. Creary, R. D. Daniels, Jr., W. King, G. Luchetta, J. Wiegand, T. Moerker, and H. H. Peter.** 1993. A comparative study of the iron-clearing properties of desferrithiocin analogues with desferrioxamine B in a *Cebus* monkey model. Blood **81(8):**2166-2173.
11. **Bergeron, R. J., R. R. Streiff, W. King, R. D. Daniels, Jr., and J. Wiegand.** 1993. A comparison of the iron-clearing properties of parabactin and desferrioxamine. Blood **82(8):**2552-2557.
12. **Bergeron, R. J., R. R. Streiff, J. Wiegand, G. Luchetta, E. A. Creary, and H. H. Peter.** 1992. A comparison of the iron-clearing properties of 1,2-dimethyl-3-hydroxypyrid-4-one, 1,2-diethyl-3-hydroxypyrid-4-one, and deferoxamine. Blood **79(7):**1882-1890.
13. **Bergeron, R. J., R. R. Streiff, J. Wiegand, J. R. T. Vinson, G. Luchetta, K. M. Evans, H. Peter, and H. B. Jenny.** 1990. A comparative evaluation of iron clearance models. Ann. N. Y. Acad. Sci. **612:**378-393.
14. **Bergeron, R. J., W. R. Weimar, G. Luchetta, R. R. Streiff, J. Wiegand, J. Perrin, K. M. Schreier, C. Porter, G. W. Yao, and H. Dimova.** 1995. Metabolism and pharmacokinetics of N^1,N^{11}-diethylnorspermine. Drug Metab. Dispos. **23(10):**1117-1125.
15. **Bergeron, R. J., W. R. Weimar, and J. Wiegand.** 1999. Pharmacokinetics of orally administered desferrithiocin analogs in *Cebus apella* primates. Drug Metab. Dispos. **27(12):**1496-1498.
16. **Bergeron, R. J., J. Wiegand, and G. M. Brittenham.** 1998. HBED: a potential alternative to deferoxamine for iron-chelating therapy. Blood **91(4):**1446-1452.
17. **Bergeron, R. J., J. Wiegand, and G. M. Brittenham.** 1999. HBED: the continuing development of a potential alternative to deferoxamine for iron-chelating therapy. Blood **93(1):**370-375.
18. **Bergeron, R. J., J. Wiegand, and G. M. Brittenham.** 2002. HBED ligand: preclinical studies of a potential alternative to deferoxamine for treatment of chronic iron overload and acute iron poisoning. Blood **99(8):**3019-3026.
19. **Bergeron, R. J., J. Wiegand, J. B. Dionis, M. Egli-Karmakka, J. Frei, A. Huxley-Tencer, and H. H. Peter.** 1991. Evaluation of desferrithiocin and its synthetic analogues as orally effective iron chelators. J. Med. Chem. **34:**2072-2078.
20. **Bergeron, R. J., J. Wiegand, J. S. McManis, J. Bussenius, R. E. Smith, and W. R. Weimar.** 2003. Methoxylation of desazadesferrithiocin analogues: enhanced iron clearing efficiency. J. Med. Chem. **46(8):**1470-1477.
21. **Bergeron, R. J., J. Wiegand, J. S. McManis, B. H. McCosar, W. R. Weimar, G. M. Brittenham, and R. E. Smith.** 1999. Effects of C-4 stereochemistry and C-4' hydroxylation on the iron clearing efficiency and toxicity of desferrithiocin analogues. J. Med. Chem. **42(14):**2432-2440.
22. **Bergeron, R. J., J. Wiegand, K. Ratliff-Thompson, and W. R. Weimar.** 1998. The origin of the differences in (*R*)- and (*S*)-desmethyldesferrithiocin: iron-clearing properties. Ann. N. Y. Acad. Sci. **850:**202-216.
23. **Bergeron, R. J., J. Wiegand, W. R. Weimar, J. S. McManis, R. E. Smith, and K. A. Abboud.** 2003. Iron chelation promoted by desazadesferrithiocin analogues: an enantioselective barrier. Chirality **15(7):**593-599.
24. **Bergeron, R. J., J. Wiegand, M. Wollenweber, J. S. McManis, S. E. Algee, and K. Ratliff-Thompson.** 1996. Synthesis and biological evaluation of naphthyldesferrithiocin iron chelators. J. Med. Chem. **39(8):**1575-1581.
25. **Bickel, H., G. E. Hall, W. Keller-Schierlein, V. Prelog, E. Vischer, and A. Wettstein.** 1960. Metabolic products of actinomycetes. XXVII. Constitutional formula of ferrioxamine B. Helv. Chim. Acta **43(8):**2129-2138.
26. **Brazzell, R. K., Y. H. Park, C. B. Wooldridge, B. McCue, R. Barker, R. Couch, and B. York.** 1990. Interspecies comparison of the pharmacokinetics of aldose reductase inhibitors. Drug Metab. Dispos. **18(4):**435-440.
27. **Brittenham, G. M.** 1990. Pyridoxal isonicotinoyl hydrazone (PIH): effective iron chelation after oral administration. Ann. N. Y. Acad. Sci. **612:**315-326.
28. **Brown, E. B., D. Rubenia, D. E. Smith, C. Reynafarje, and C. Moore.** 1957. Studies in iron transportation and metabolism X. Long-term iron overload in dogs. J. Lab. Clin. Med. **50**(6)**:**862-893.
29. **Byers, B. R. and J. E. Arceneaux.** 1998. Microbial iron transport: iron acquisition by pathogenic microorganisms. Met. Ions Biol. Syst. **35:**37-66.
30. **Conrad, M. E., J. N. Umbreit, and E. G. Moore.** 1999. Iron absorption and transport. Am. J. Med. Sci. **318(4):**213-229.
31. **Creaven, P. J., R. Perez, L. Pendyala, N. J. Meropol, G. Loewen, E. Levine, E. Berghorn, and D. Raghavan.** 1997. Unusual central nervous system toxicity in a phase 1 study of N^1N^{11}-diethylnorspermine in patients with advanced malignancy. Invest. New Drugs **15(3):**227-234.
32. **Ecobichon, D. J. (ed.).** 1997. The basis of toxicity testing. CRC, Boca Raton, Fla.

33. **Eisenstein, R. S. and K. L. Ross.** 2003. Novel roles for iron regulatory proteins in the adaptive response to iron deficiency. J. Nutr. **133(5 Suppl 1):**1510S-1516S.
34. **Finch, C. A. and H. A. Huebers.** 1986. Iron metabolism. Clin. Physiol. Biochem. **4(1):**5-10.
35. **Gad, S. C. (ed.).** 1995. Safety assessment for pharmaceuticals. Van Nostrand Reinhold, New York.
36. **Gad, S. C.** 2002. Drug safety evaluation. Wiley, New York.
37. **Grady, R. W. and C. Hershko.** 1990. An evaluation of the potential of HBED as an orally effective iron-chelating drug. Semin. Hematol. **27(2):**105-111.
38. **Grady, R. W., A. D. Salbe, M. W. Hilgartner, and P. J. Giardina.** 1994. Results from a phase I clinical trial of HBED. Adv. Exp. Med. Biol. **356:**351-359.
39. **Halliwell, B.** 1994. Free radicals and antioxidants: a personal view. Nutr. Rev. **52(8 Pt 1):**253-265.
40. **Hazen, S. L., A. d'Avignon, M. M. Anderson, F. F. Hsu, and J. W. Heinecke.** 1998. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to oxidize α-amino acids to a family of reactive aldehydes. Mechanistic studies identifying labile intermediates along the reaction pathway. J. Biol. Chem. **273(9):**4997-5005.
41. **Hershko, C., R. W. Grady, and A. Cerami.** 1978. Mechanism of iron chelation in the hypertransfused rat: definition of two alternative pathways of iron mobilization. J. Lab. Clin. Med. **92(2):**144-151.
42. **Hershko, C.** 1994. Control of disease by selective iron depletion: a novel therapeutic strategy utilizing iron chelators. Bailliere's Clin. Haematol. **7(4):**965-1000.
43. **Hershko, C., A. M. Konijn, and G. Link.** 1998. Iron chelators for thalassaemia. Br. J. Haematol. **101(3):**399-406.
44. **Hoffbrand, A. V., F. Al-Refaie, B. Davis, N. Siritanakatkul, B. F. A. Jackson, J. Cochrane, E. Prescott, and B. Wonke.** 1998. Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. Blood **91(1):**295-300.
45. **Jellinger, K. A.** 1999. The role of iron in neurodegeneration. Prospects for pharmacotherapy of Parkinson's disease. Drugs Aging **14(2):**115-140.
46. **Kontoghiorghes, G. J.** 1986. Dose response studies using desferrioxamine and orally active chelators in a mouse model. Scand. J. Haematol. **37(1):**63-70.
47. **Koppenol, W.** 2000. Kinetics and mechanism of the Fenton reaction: implications for iron toxicity, p. 3-10. *In* D. G. Badman, R. J. Bergeron, and G. M. Brittenham (ed.), Iron chelators: new development strategies. Saratoga, Ponte Vedra Beach, Fla.
48. **Lee, P., N. Mohammed, L. Marshall, R. D. Abeysinghe, R. C. Hider, J. B. Porter, and S. Singh.** 1993. Intravenous infusion pharmacokinetics of desferrioxamine in thalassaemic patients. Drug Metab. Dispos. **21(4):**640-644.
49. **Leong, S. A. and G. Winkelmann.** 1998. Molecular biology of iron transport in fungi. Met. Ions Biol. Syst. **35:**147-186.
50. **Lieu, P. T., M. Heiskala, P. A. Peterson, and Y. Yang.** 2001. The roles of iron in health and disease. Mol. Aspects Med. **22:**1-87.
51. **Loeffler, D. A., J. R. Connor, P. L. Juneau, B. S. Snyder, L. Kanaley, A. J. DeMaggio, H. Nguyen, C. M. Brickman, and P. A. LeWitt.** 1995. Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions. J. Neurochem. **65(2):**710-716.
52. **Longueville, A. and R. R. Crichton.** 1986. An animal model of iron overload and its application to study hepatic ferritin iron mobilization by chelators. Biochem. Pharmacol. **35(21):**3669-3678.
53. **Neilands, J. B.** 1995. Siderophores: structure and function of microbial iron transport compounds. J. Biol. Chem. **270(45):**26723-26726.
54. **Nick, H. P., P. Acklin, B. Faller, Y. Jin, R. Lattmann, M.-C. Rouan, T. Sergejew, H. Thomas, H. Wiegand, and H. P. Schnebli.** 2000. A new, potent, orally active iron chelator, p. 311-331. *In* D. G. Badman, R. J. Bergeron, and G. M. Brittenham (ed.), Iron chelators: new development strategies. Saratoga, Ponte Vedra Beach, Fla.
55. **Nisbet-Brown, E., N. F. Olivieri, P. J. Giardina, R. W. Grady, E. J. Neufeld, R. Sechaud, A. J. Krebs-Brown, J. R. Anderson, D. Alberti, K. C. Sizer, and D. G. Nathan.** 2003. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet **361(9369):**1597-1602.
56. **O'Connell, M. J., R. J. Ward, H. Baum, and T. J. Peters.** 1985. The role of iron in ferritin- and haemosiderin-mediated lipid peroxidation in liposomes. Biochem. J. **229:**135-139.
57. **Olivieri, N. F. and G. M. Brittenham.** 1997. Iron-chelating therapy and the treatment of thalassemia. Blood **89(3):**739-761.
58. **Pippard, M. J.** 1989. Desferrioxamine-induced iron excretion in humans. Bailliere's Clin. Haematol. **2(2):**323-343.
59. **Ponka, P.** 1994. Physiology and pathophysiology of iron metabolism: implications for iron chelation therapy in iron overload, p. 1-29. *In* R. J. Bergeron and G. M. Brittenham (ed.), The development of iron chelators for clinical use. CRC, Boca Raton, Fla.
60. **Ponka, P., C. Beaumont, and D. R. Richardson.** 1998. Function and regulation of transferrin and ferritin. Semin. Hematol. **35(1):**35-54.
61. **Porter, J. B.** 2001. Deferoxamine pharmacokinetics. Semin. Hematol. **38(1, Suppl. 1):**63-68.
62. **Richardson, D. R. and P. Ponka.** 1998. Pyridoxal isonicotinoyl hydrazone and its analogs: potential orally effective iron-chelating agents for the treatment of iron overload disease. J. Lab. Clin. Med. **131(4):**306-315.
63. **Richardson, D. R.** 2001. The controversial role of deferiprone in the treatment of thalassemia. J. Lab. Clin. Med. **137(5):**324-329.
64. **Scherling, D., W. Karl, G. Ahr, H. J. Ahr, and E. Wehinger.** 1988. Pharmacokinetics of nisoldipine. III. Biotransformation of nisoldipine in rat, dog, monkey, and man. Arzneimittelforschung **38(8):**1105-1110.
65. **Sergejew, T., P. Forgiarini, and H.-P. Schnebli.** 2000. Chelator-induced iron excretion in iron-overloaded marmosets. Br. J. Haematol. **110:**985-992.
66. **Soars, M. G., R. J. Riley, and B. Burchell.** 2001. Evaluation of the marmoset as a model species for drug glucuronidation. Xenobiotica **31(12):**849-860.
67. **Theil, E. C. and B. H. Huynh.** 1997. Ferritin mineralization: ferroxidation and beyond. J. Inorg. Biochem. **67(1-4):**30.
68. **Thomas, C. E., L. A. Morehouse, and S. D. Aust.** 1985. Ferritin and superoxide-dependent lipid peroxidation. J. Biol. Chem. **260:**3275-3280.
69. **Wolfe, L. C., R. J. Nicolosi, M. M. Renaud, J. Finger, M. Hegsted, H. Peter, and D. G. Nathan.** 1989. A non-human primate model for the study of oral iron chelators. Br. J. Haematol. **72(3):**456-461.
70. **Wolfe, L. C.** 1990. Desferrithiocin. Semin. Hematol. **27(2):**117-120.
71. **Wonke, B.** 2001. Clinical management of β-thalassemia major. Semin. Hematol. **38(4):**350-359.
72. **Wood, J. K., P. F. Milner, and U. N. Pathak.** 1968. The metabolism of iron-dextran given as a total-dose infusion to iron deficient Jamaican subjects. Br. J. Haematol. **14(2):**119-129.
73. **Wu-Pong, S. and Y. Rojanasakul (ed.).** 1999. Biopharmaceutical drug design and development. Humana Press, Totowa, N.J.