A Comparison of the Iron-Clearing Properties of 1,2-Dimethyl-3-Hydroxypyrid-4-One, 1,2-Diethyl-3-Hydroxypyrid-4-One, and Deferoxamine

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A comparative study of the iron-clearing properties of subcutaneously (SC) administered deferoxamine (DFO) with those of orally administered 1,2-dimethyl-3-hydroxypyrid-4-one (CP20) and 1,2-diethyl-3-hydroxypyrid-4-one (CP94) is presented. The studies were performed in both a non–iron-overloaded, bile duct–cannulated rat model and an iron-loaded *Cebus* monkey model. All three drugs performed well in the rodent, promoting the excretion of iron in both the urine and the bile, with total iron output efficiencies of 2.8%, 1.2%, and 7.1%, respectively. The efficiency of DFO increased slightly in the *Cebus* model, while that of the hydroxypyridones was essentially the same in the monkey, with total iron output efficiencies of 5.5%, 2.1%, and 7.4%, respectively. Iron balance studies showed that both DFO and CP94 were able to maintain the animals in a negative iron balance, while CP20 had little impact.

IRON METABOLISM in primates is characterized by a highly efficient recycling process,[1-4] with no specific mechanism for eliminating this transition metal. Because it cannot be effectively cleared, the introduction of "excess iron"[5-7] into this closed metabolic loop leads to chronic overload and ultimately to peroxidative tissue damage. In a number of iron-overload associated diseases (eg, thalassemia), the only treatment is to maintain patients on chelation therapy. However, the currently accepted protocol, subcutaneous (SC) infusion of deferoxamine (DFO), is not completely satisfactory, as many patients must be maintained on this regimen for most of their natural lives: compliance becomes a major problem. Furthermore, the technology required for DFO infusion is not widely available in some Third World countries, rendering the population areas in the greatest need untreatable. Both of these problems would be overcome with the discovery of an orally effective iron chelator.[8]

While numerous ligands have been shown to be effective at removing iron in cell culture models[9] and have been promoted to the next level of evaluation, the rodent model,[10] most of these compounds have failed at a clinical level. This suggests that the rat model is not necessarily the best indicator of how ligands will perform in humans. In this regard, it has recently been demonstrated that the *Cebus* monkey[11,12] can serve as an excellent intermediate screen for evaluating iron chelators prior to human studies.

In recent years, due in part to the pioneering work of Hider et al[13] and Kontoghiorghes et al,[14] hydroxypyridones have held the attention of the scientific and medical community as potential orally active iron chelators. However, there has been some controversy regarding the efficiency and toxicity of these compounds. Not only is there some contention about the efficiency of the hydroxypyridones as a group, but about the differences in efficiency among the different hydroxypyridones. Evidence is often anecdotal or data are held up in controversy. In an attempt to settle some of these issues, we describe a comparative study of subcutaneously administered DFO with orally administered hydroxpyridones 1,2-dimethyl-3-hydroxypyrid-4-one (CP20) and 1,2-diethyl-3-hydroxypyrid-4-one (CP94).

MATERIALS AND METHODS

Materials. DFO was supplied by Ciba-Geigy, Basel, Switzerland, in the form of the methanesulfonate salt (trade name: Desferal). The ligands CP20 and CP94 were obtained from the same source. Sprague–Dawley rats were purchased from Charles River, Wilmington, MA. *Cebus apella* monkeys were obtained from World Wide Primates, Miami, FL. All reagents and standard iron solutions were obtained from Aldrich Chemical, Milwaukee, WI. Nalgene metabolic cages, rat jackets, and fluid swivels were purchased from Harvard Bioscience, South Natick, MA. Intramedic polyethylene tubing was obtained from Fisher Scientific, Pittsburgh, PA. Atomic absorption measurements were made on a Perkin-Elmer model 5100 PC, Norwalk, CT. Ultrapure salts were obtained from Johnson Matthey Electronics, Royston, England. Imferon, an iron dextran solution, was obtained from Fisons, Bedford, MA. All hematological screens were performed by Allied Clinical Laboratories, Gainesville, FL. Cremophor RH-40 was obtained from BASF, Parsippany, NJ.

Non–iron-overloaded bile duct–cannulated rat. Male Sprague–Dawley rats averaging 400 g were housed in Nalgene plastic metabolic cages during the experimental period and were given free access to water. The animals were anesthetized using sodium pentobarbital (50 mg/kg), administered intraperitoneally (IP). The bile duct was cannulated using 22-gauge polyethylene tubing, approximately 1 cm from the duodenum. The cannula was inserted approximately 2 cm into the duct, and once bile flow was established, the cannula was tied snugly in place. A skin-tunneling needle was inserted from the shoulder area around to the abdominal incision. The cannula was threaded through the needle until it emerged from the shoulder opening.

The cannula was then passed from the rat to the swivel inside a metal torque-transmitting tether, which was attached to a rodent jacket around the animal's chest. The cannula was directed from the rat to a Gilson microfraction collector by a fluid swivel mounted above the metabolic cage. This system allowed the animal to move freely in the cage while continuous bile samples were being collected. Bile samples were collected at 3-hour intervals. Urine samples were taken every 24 hours. Sample collection and handling are as previously described.[15]

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Primate metabolic cages. During the evaluation of various iron chelators, the animals were moved from normal primate cages to specially constructed metabolic cages. These metabolic cages were fabricated from Plexiglass and were approximately 4 ft × 4 ft × 3 ft. Five of the faces had 1-in diameter holes every 2 in. The bottom of the cage consisted of a floor made of 1-in diameter Plexiglass rods spaced every ¾ in apart. Beneath the floor was a plastic screen, which separated the feces from the urine. The very bottom of the cage was appropriately tilted so that the urine flowed out through a spigot, where it was collected in a bottle. Furthermore, the back of the cage was equipped with a second ventilated wall so that the monkeys could be squeezed to the front of the cage for administration of injections when necessary. The animals were housed in these cages for 7 days before exposure to the chelator of interest and throughout the course of the experiment.

Primate iron loading. After intramuscular (IM) anesthesia with ketamine, an intravenous (IV) infusion was started in a leg vein. The iron dextran was added to approximately 90 mL of sterile normal saline and administered to the animals at a dose of 200 to 300 mg/kg. The iron solution was infused over 45 to 60 minutes. Two to three infusions separated by 10 to 14 days were necessary to load the monkeys to a level of 500 mg/kg of iron. This brought the serum transferrin iron saturation to 70% to 80%. The serum half-life of iron dextran in humans is 2.5 to 3 days.[16] We waited 20 half-lives, or 60 days, before using any of the animals in iron-clearing experiments.

Primate low-iron diet. A low-iron liquid diet was prepared by first mixing the following ingredients: casein, 180 g; sucrose, 194 g; dextrin, 194 g; dextrose, 194 g; cellulose fiber, 90 g; vitamin mix, 5 g; methionine, 5 g; flavoring, 2 g; choline chloride, 2 g; and cholesterol, 1 g. A solution of the following liquids was added to portions with mixing: corn oil, 45 g; coconut oil, 45 g; and soy lecithin, 20 g. The following ultrapure grade salts were added to 1,350 mL of distilled deionized water: sodium chloride, 5.68 g; manganese sulfate, 0.04 g; calcium carbonate, 10.16 g; potassium dihydrogen phosphate, 11.93 g; and magnesium sulfate, 3.45 g. Finally, the monkeys were given a fixed amount of the diet according to their weight, 50 mL/kg. The iron level in the diet was measured for every preparation.

Primate iron balance studies. Animals were maintained on the low-iron liquid diet for 7 days before drug administration. The animals were given food according to their body weight. Intake was carefully monitored.

Three days before drug administration (days −2 to day 0), baseline iron intake and output values were measured. These same measurements were made for days +1 through +3. The total amount of iron intake was compared with the total iron output.

Primate hematological screen. Animals were placed in metabolic cages 7 days before the administration of the drug, and started on the low-iron diet. Blood samples were taken at this time for testing. The blood samples were always drawn at the same time of day due to the diurnal variability in some of the measurements, ie, unsaturated iron-binding capacity (UIBC), plasma iron, etc. The assays performed included iron profile (iron, UIBC, total iron-binding capacity [TIBC], percent saturation), complete blood cell count (CBC: white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], platelet count), differential, and chemistry profile (glucose, sodium, potassium, chloride, CO_2, blood urea nitrogen [BUN], creatinine, calcium, phosphorous, total protein, albumin, alkaline phosphatase, aspartate aminotransferase [AST], alanine aminotransferase [ALT], total cholesterol, total bilirubin, globulin, γ-glutamyl transpeptidase). The evaluation of these parameters allowed us to assess the health of the animals going into the experiment and to measure any subtle changes that the test drug may induce in an animal. Any animals showing screen values outside of control values were immediately removed from the experiment. Finally, a postdrug blood sample was taken from each animal on the last day of the experiment.

Primate fecal and urine samples. Fecal and urine samples were collected at 24-hour intervals. The collections began 4 days before the administration of the test drug. Fecal samples were assayed for the presence of occult blood, then weighed and mixed with distilled deionized water and autoclaved for 30 minutes. The mixture was then freeze-dried, and a known portion of the powder was mixed with low-iron nitric acid and refluxed for 24 hours. Once any particulate matter in the digested samples was removed by centrifugation, iron concentrations were determined by flame atomic absorption (AA). Monkey urine samples were acidified and reconstituted to initial volume after sterilization, if necessary.

Drug preparation and administration. DFO was administered SC to the rats in 40% Cremophor RH-40. The hydroxypyridones were also prepared in 40% Cremophor and administered orally. In the rats, DFO was administered at 150 μmol/kg, while the hydroxypyridones were administered at 150 and 450 μmol/kg.

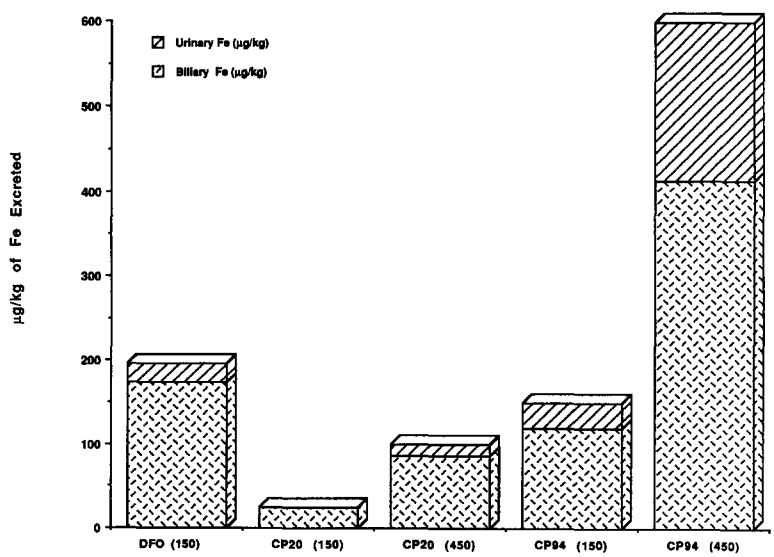

Fig 1. Response of non–iron-overloaded bile duct–cannulated rats to DFO 150 μmol/kg SC, or CP20 and CP94 150 and 450 μmol/kg orally. There is a clear dose-response with both CP20 and CP94.

Table 1. Effect of Iron-Chelating Drugs on 24-Hour Cumulative Iron Excretion in Urine and Bile of Non–Iron-Overloaded, Bile Duct–Cannulated Rats

	Iron Excretion									
	DFO (150 μmol/kg SC) (100 mg/kg) n = 6		CP20 (150 μmol/kg orally) (21 mg/kg) n = 5		CP20 (450 μmol/kg orally) (63 mg/kg) n = 5		CP94 (150 μmol/kg orally) (25 mg/kg) n = 6		CP94 (450 μmol/kg orally) (75 mg/kg) n = 5	
Measurement	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Theoretical total	8.37	100	2.79	100	8.37	100	2.79	100	8.37	100
Experimental total	0.232 ± 0.042	2.8	0.022 ± 0.004	0.85	0.100 ± 0.025	1.2	0.148 ± 0.030	5.3	0.597 ± 0.092	7.1
In urine	0.059 ± 0.023	25.4	−0.002 ± 0.00	0	0.014 ± 0.004	14.3	0.030 ± 0.012	20.1	0.185 ± 0.040	31.0
In bile	0.173 ± 0.035	74.6	0.025 ± 0.005	100	0.085 ± 0.024	85.7	0.118 ± 0.027	79.9	0.411 ± 0.082	69.0

DFO was administered to the monkeys subcutaneously in sterile water for injection at 150 μmol/kg. The other chelators were administered to the monkeys orally in low-iron gelatin capsules with the aid of a pilling gun at 300 or 450 μmol/kg. Before chelator administration, the monkeys were anesthetized with ketamine 7 to 10 mg/kg IM and given 2 to 3 mg of Reglan IV (A.H. Robins Co, Richmond, VA) to prevent vomiting.

Both the rats and the monkeys were fasted for 24 hours before dosing.

Efficiency calculations. The efficiency of each ligand was calculated on the basis of a 1:1 DFO-iron complex, or a 3:1 ligand-iron complex for CP20 and CP94. In the monkeys, the numbers were generated by averaging the iron output for 4 days before the administration of the drug, subtracting these numbers from the 2-day iron clearance after the administration of the drug, and then dividing by the theoretical output. The efficiencies in the rodent model were calculated by subtracting the iron excretion of control animals from the iron excretion of the treated animals. This

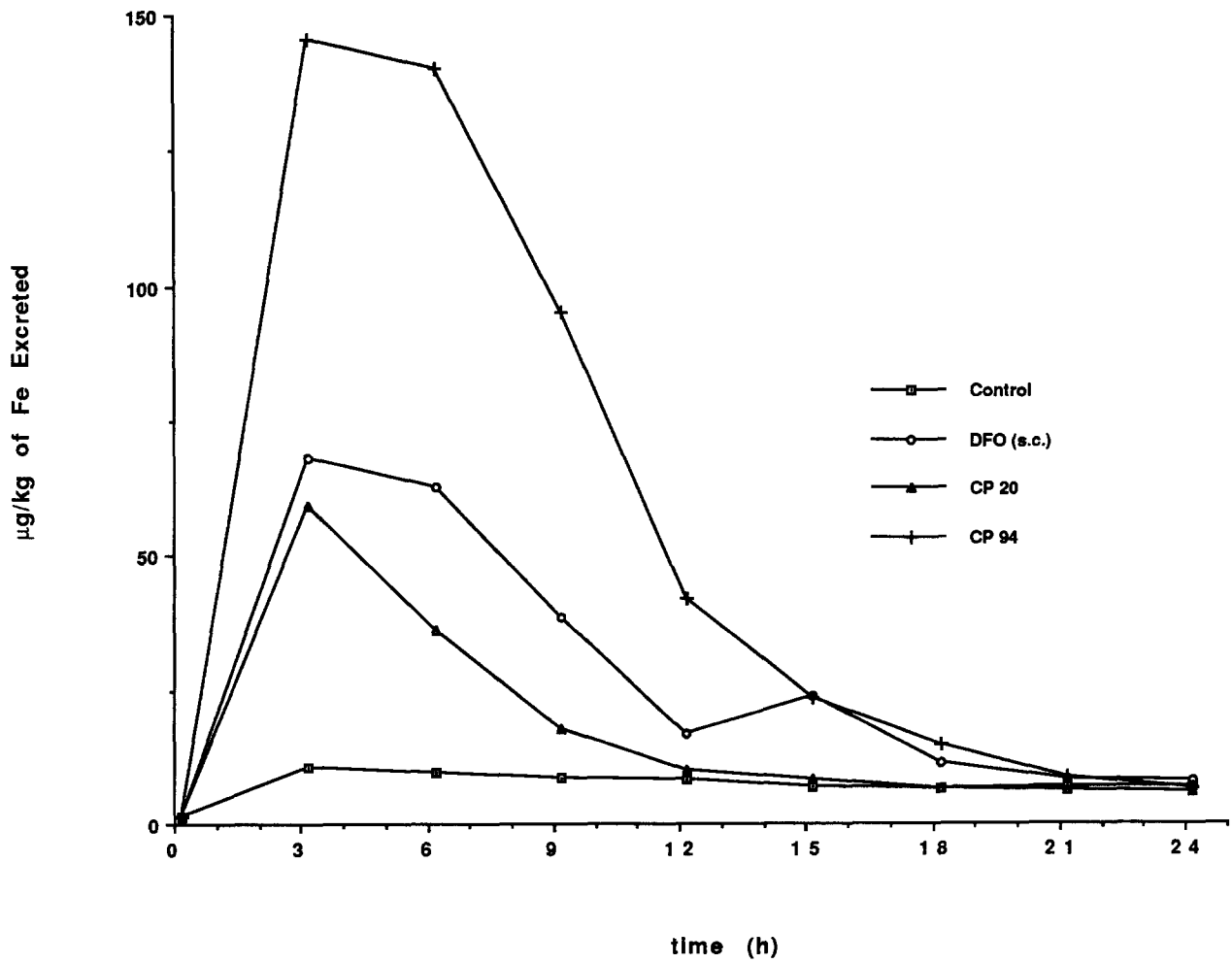

Fig 2. Ferrokinetics of chelator-induced biliary iron clearance in rodents given DFO 150 μmol/kg SC, or CP20 and CP94 450 μmol/kg orally.

number was then divided by the theoretical output to obtain the efficiency.

RESULTS

Chelator-induced iron clearance in rodents. The efficiency of the hydroxypyridones CP20 and CP94 was compared with SC administered DFO as a positive control. The efficiencies of the drugs were calculated based on the assumption that DFO forms a 1:1 complex with iron and that both CP20 and CP94 form a 3:1 complex with the metal. These calculations showed the efficiency of DFO to be 2.8% ± 0.7% (range, 2.1% to 4.1%) when the drug was administered SC. Approximately 75% of the ligand-promoted iron excretion was found in the bile and 25% in the urine (Fig 1 and Table 1). The drug-promoted iron excretion in bile (Fig 2) was complete in 21 hours, while urinary excretion was complete in 24 hours. When comparing these results with the data obtained from the orally administered hydroxypyridones, CP94 proved to be excellent, surpassing SC administered DFO in total iron output. The comparison was made on an equivalent iron-binding capacity basis: 3 mol of CP94 to 1 mol of iron. The pyridone's efficiency calculated on this basis was approximately 2.5 times that of DFO ($P < .002$), with an efficiency of 7.1% ± 2.5% (range, 5.2% to 10.3%). Like DFO, the iron clearance from urine was complete in 24 hours, while the biliary clearance was complete in 21 hours (Fig 2). Again, most of the iron was in bile (69%), with 31% in urine. The CP20 ligand was 0.43 times as efficient as DFO ($P < .005$), with an efficiency of 1.2% ± 0.6% (range, 0.2% to 1.9%), and only 0.17 times as efficient as the diethyl

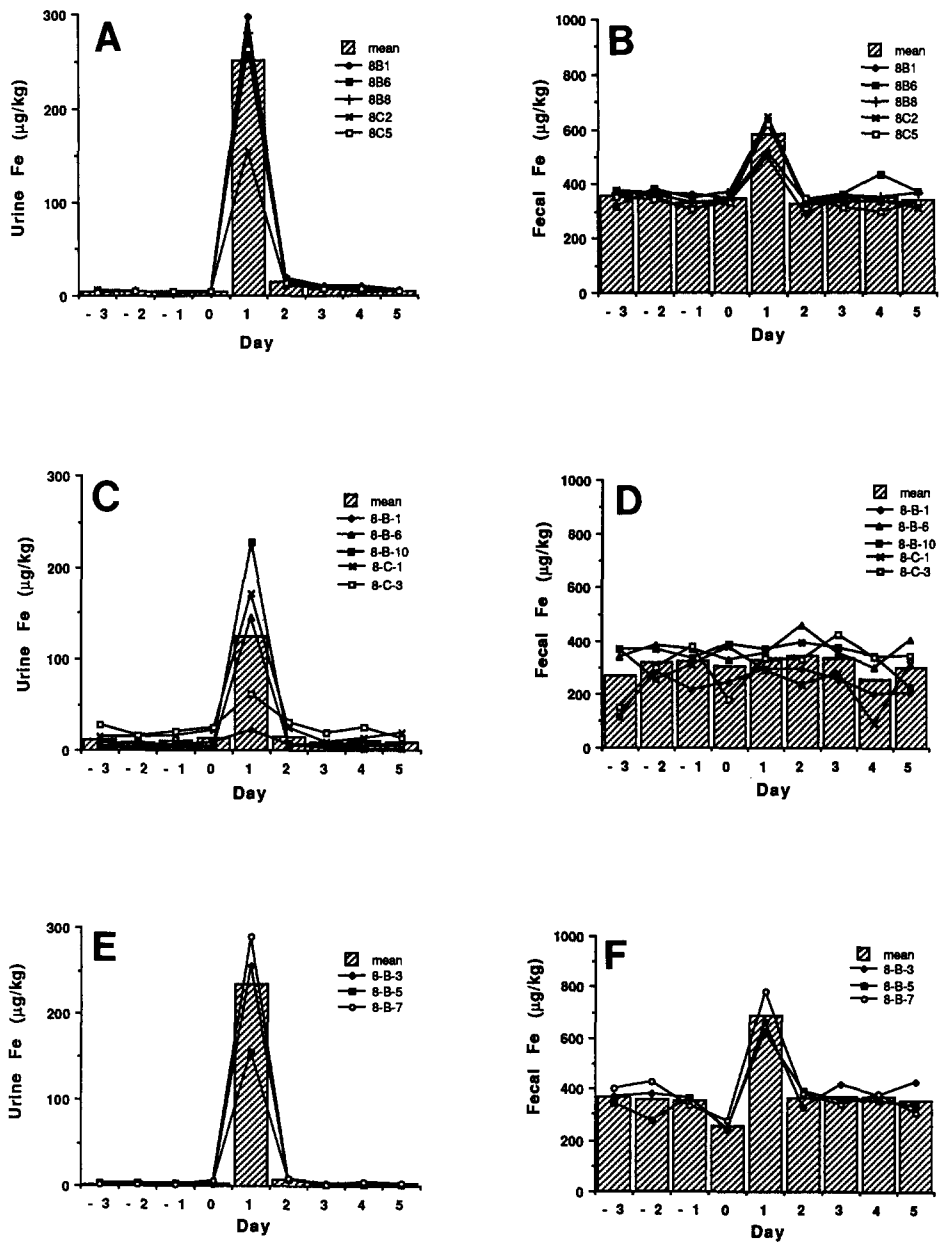

Fig 3. Chelator-induced iron excretion reported in micrograms of iron excreted per kilogram of body weight in urine (A, C, E) and feces (B, D, F) of iron-overloaded *Cebus* monkeys. Drugs tested were DFO 150 µmol/kg SC (A and B), CP20 450 µmol/kg orally (C and D), and CP94 450 µmol/kg orally (E and F).

analogue ($P < .001$). The CP20-induced iron clearance was also complete in 24 hours in urine and in 12 hours in bile (Fig 2), with 86% of the total excretion in the bile and 14% in the urine (Fig 1 and Table 1). Both of the hydroxypyridones demonstrated a dose-dependent response (Fig 1 and Table 1). We noted hypersalivation in 40% of the rats treated with CP20 and in 60% of those treated with CP94 after the administration of a dose of 450 μmol/kg.

Chelator-induced iron clearance in primates. The *Cebus* monkeys responded differently to the ligands than did the rats. The variability in ligand-induced iron clearance was higher in the monkeys than in the rats. However, with each animal serving as its own control, effective chelators were easily identified (Fig 3 and 4, and Tables 2 and 3). Although the mode of excretion was different (Fig 5 and Table 2), with 55% of the iron in urine and 45% in feces, the efficiency of DFO increased from 2.8% ± 0.7% in the rats to 5.5% ± 0.9% (range, 4.4% to 6.6%) in the primates. The efficiency of both of the hydroxypyridones, when administered at 450 μmol/kg, was essentially the same, increasing from 1.2% ± 0.6% in the rodent to 2.1% ± 1.1% (range, 0.4% to 3.0%) in the case of CP20, and from 7.1% ± 2.5% in the rats to 7.4% ± 1.2% (range, 6.6% to 8.8%) with CP94. In the primates, CP94 was 1.3 times as efficient as DFO ($P < .04$), while CP20 was only 0.4 times as efficient ($P < .001$). When comparing the efficiencies of the hydroxypyridones, CP94 was found to be approximately 3.5 times as efficient as CP20 ($P < .001$).

There was a clear dose-response with CP94, but not with CP20. The efficiency of CP94 when administered at 300 μmol/kg was 7.3% ± 1.3% (range, 5.3% to 8.5%), with

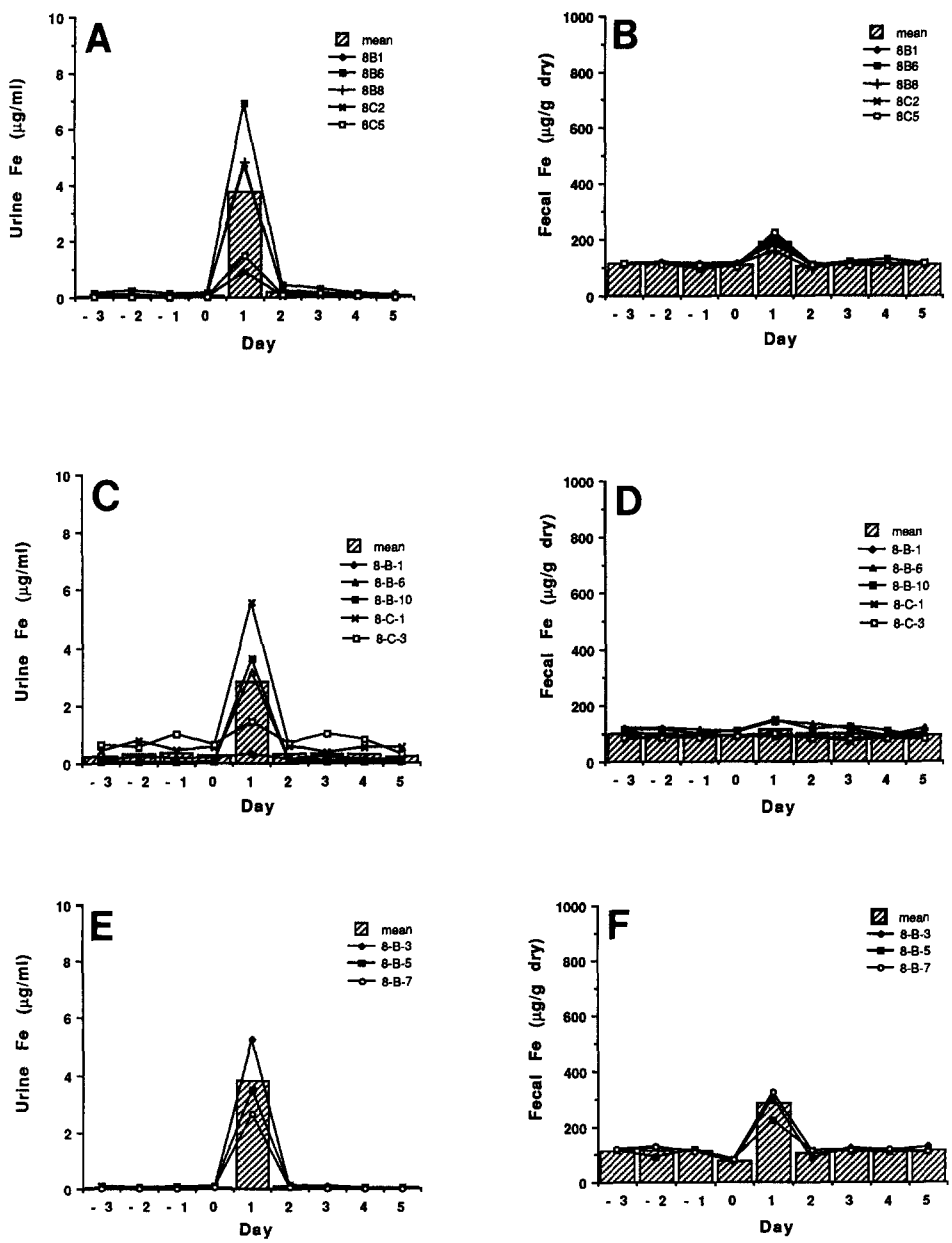

Fig 4. Chelator-induced iron excretion reported in concentration units of μg/mL or μg/g dry weight in urine (A, C, E) and feces (B, D, F) of iron-overloaded *Cebus* monkeys. Drugs tested were DFO 150 μmol/kg SC (A and B), CP20 450 μmol/kg orally (C and D), and CP94 450 μmol/kg orally (E and F).

Table 2. Drug-Induced Iron Excretion in Urine and Feces of Iron-Overloaded *Cebus* Monkeys

	Iron Excretion									
	DFO (150 μmol/kg SC) (100 mg/kg) n = 5		CP20 (300 μmol/kg orally) (42 mg/kg) n = 5		CP20 (450 μmol/kg orally) (63 mg/kg) n = 5		CP94 (300 μmol/kg orally) (50 mg/kg) n = 5		CP94 (450 μmol/kg orally) (75 mg/kg) n = 3	
Measurement	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Theoretical total	8.51	100	5.70	100	8.56	100	5.59	100	8.37	100
Experimental total	0.47 ± 0.07	5.5	0.18 ± 0.08	3.1	0.18 ± 0.09	2.1	0.41 ± 0.07	7.3	0.62 ± 0.10	7.4
In urine	0.26 ± 0.06	55.5	0.12 ± 0.06	69.8	0.12 ± 0.09	64.5	0.14 ± 0.08	35.0	0.24 ± 0.07	38.2
In feces	0.21 ± 0.10	44.5	0.05 ± 0.04	30.2	0.07 ± 0.12	35.5	0.26 ± 0.05	65.0	0.38 ± 0.07	61.8

35% of the induced iron being excreted in urine and 65% in feces, while at 450 μmol/kg the efficiency was 7.4% ± 1.2% (range, 6.6% to 8.8%), with 38% of the iron in urine and 62% in bile (Fig 5). It is notable that although the efficiency of CP94 was approximately 7% when administered at either 300 or 450 μmol/kg, the total amount of iron cleared increased proportionally with the dose. At a dose of 300 μmol/kg, the amount of total iron cleared was 0.41 ± 0.07 mg/kg, while at 450 μmol/kg it was 0.62 ± 0.10 mg/kg ($P < .02$), a difference of approximately 35%.

There was not a clear dose-response with CP20: the efficiency of the ligand when administered at 300 μmol/kg was 3.1% ± 1.4% (range, 1.1% to 4.2%), while at 450 μmol/kg the efficiency was 2.1% ± 1.1% (range, 0.4% to 3.0%). The total amount of iron cleared did not increase significantly with the dosage. At a dose of 300 μmol/kg, the amount of total iron cleared was 0.18 ± 0.08 mg/kg, while at 450 μmol/kg it was 0.18 ± 0.09 mg/kg ($P > .8$). The mode of excretion for each dosage was similar, with the majority of the iron being excreted in urine: 70% at 300 μmol/kg and 65% at 450 μmol/kg.

Although 30% to 35% of the induced iron associated with CP20 at either dose was found in the feces, it is important to note that the observed induced fecal iron excretion for CP20 was statistically insignificant when compared with baseline levels, increasing from a baseline value of 325 ± 49 μg/kg to 377 ± 58 μg/kg ($P > .05$) at 300 μmol/kg, and from 302 ± 84 μg/kg to 328 ± 35 μg/kg at 450 μmol/kg ($P > .5$). However, the urinary iron excretion values were found to be significant. Urinary iron excretion increased from a baseline of 3 ± 2 to 126 ± 64 μg/kg ($P < .001$) when the ligand was administered at 300 μmol/kg, and from 11 ± 8 to 125 ± 84 μg/kg when the ligand was administered at 450 μmol/kg ($P < .001$).

During chelator treatment with DFO and CP94 at either dose, iron excretion was found to increase significantly over the baseline values in both the urine and the feces. With DFO, urinary iron excretion increased from a baseline of 4 ± 1 to 252 ± 56 μg/kg ($P < .001$), while fecal iron excretion increased from a baseline of 350 ± 22 to 583 ± 69 μg/kg ($P < .001$). In the case of CP94 administered at 300 μmol/kg, urinary iron excretion increased from a baseline of 4 ± 1 to 143 ± 75 μg/kg ($P < .001$), while fecal iron excretion increased from a baseline of 356 ± 54 to 602 ± 99 μg/kg ($P < .001$). Urinary iron excretion associated with CP94 administered at 450 μmol/kg increased from a baseline of 3 ± 1 to 234 ± 69 μg/kg ($P < .001$). Fecal iron excretion at this dose increased from a baseline of 335 ± 62 to 689 ± 82 μg/kg ($P < .001$).

Primate iron balance studies. It is critical to point out that in each experiment the level of iron in the food was measured with atomic absorption spectroscopy. An example of the iron status of a monkey in an iron balance study is shown in Table 4. It should be clear from these data that these animals absorb rather substantial amounts of iron under normal circumstances. We have seen this to be the case with all of our monkeys. The results summarized in Table 5 indicate that both DFO and CP94 can hold the monkeys in negative iron balance, whereas CP20 is ineffective. Here we compare the amount of iron absorbed by the untreated animals over a 3-day period with the amount absorbed by treated animals over a 3-day period. Animals in a negative iron balance are putting out more iron than they are taking in.

Primate hematological screens. For all untreated animals, CBCs and kidney and liver profiles, except ferritin levels, were within the accepted normal range of the human values. Monkey ferritin could not be measured using the commercially available human ferritin antibody assay. Under the conditions of the experiments, there were no significant changes in the CBC or kidney and liver profiles.

Drug toxicity. The only unusual effect we observed in the rats was hypersalivation associated with the hydroxypyridones. Of the three drugs evaluated in the primates, only

Table 3. Comparison of Chelator Efficiencies in Rats and Monkeys

Rats			Monkeys		
Compound	Efficiency (%)	Range (%)	Compound	Efficiency (%)	Range (%)
DFO (150 μmol/kg) SC	2.8 ± 0.7	2.1-4.1	DFO (150 μmol/kg) SC	5.5 ± 0.9	4.4-6.6
CP20 (150 μmol/kg) orally	0.9 ± 0.5	0.3-1.7	CP20 (300 μmol/kg) orally	3.1 ± 1.4	1.1-4.2
CP20 (450 μmol/kg) orally	1.2 ± 0.6	0.2-1.9	CP20 (450 μmol/kg) orally	2.1 ± 1.1	0.4-3.0
CP94 (150 μmol/kg) orally	5.3 ± 2.1	2.1-8.8	CP94 (300 μmol/kg) orally	7.3 ± 1.3	5.3-8.5
CP94 (450 μmol/kg) orally	7.1 ± 2.5	5.2-10.3	CP94 (450 μmol/kg) orally	7.4 ± 1.2	6.6-8.8

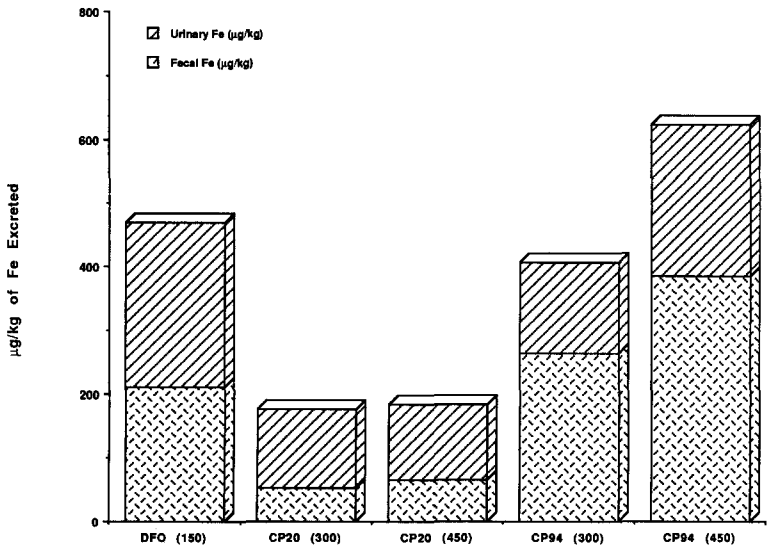

Fig 5. Response of iron-overloaded *Cebus* monkeys to DFO, CP20, and CP94. There is a clear dose-response with CP94, but not with CP20.

CP94 was associated with any toxicity. When four monkeys were given this drug at 450 µmol/kg, one of the animals died after approximately 19 hours. The dead animal tested positive for blood in the urine and feces. Of the 15 compounds that we have evaluated in the *Cebus* system, we had never before experienced an animal death. Postmortem examination showed moderate to severe hemosiderosis of the bone marrow, liver, and spleen, but was unable to unequivocally identify a cause of death.

DISCUSSION

One of the principle difficulties in evaluating the efficiency of iron chelators in primates is associated with the noise level in the measurements. While the concentration of iron in the urine of untreated animals is very low, the fecal iron concentrations are always high and one is confronted with the problem of having to assign meaning to values which represent small differences between large numbers. This is why careful consideration must be given to both the test diet and to data representation.

With the monkeys, each batch of prepared food was evaluated for iron concentration using atomic absorption spectroscopy; we did not depend on calculations derived from tables to generate iron concentrations in the food. Without such measurements, one can never be certain about the validity of stool iron measurements. Our initial studies with commercial "low-iron" monkey food[12] demonstrated how far off such assumptions can be. However, even with a low-iron diet, iron clearance data must be interpreted carefully.

Table 4. Example of a Monkey in an Iron Balance Study Before and After Administration of DFO 150 µmol/kg (SC)

	Iron Balance (Day −2 to Day 0)		Iron Balance (Day +1 to Day +3)	
	Absolute (µg)	(µg/kg)	Absolute (µg)	(µg/kg)
Intake	4,962	1,306	5,052	1,329
Urine output	41	11	1,239	326
Fecal output	4,099	1,079	4,531	1,192
Net (in−out)	822	216	−718	−189

The animal is absorbing iron in the predrug iron balance and is in negative iron balance after drug administration.

Iron clearance numbers can be misleading when reported only as excretion (concentration × fecal or urinary output). These values are dependent on stool weight and urine volume. In this study, iron output is reported as micrograms of iron excreted per kilogram of animal weight, as well as in concentration units. Both parameters taken together allow one to evaluate the true effectiveness of the chelators. Although the variability in chelator-induced iron output was higher in the monkeys than in rats (Table 2), it was still easy to identify effective ligands (Figs 3 and 4, and Tables 2 and 3). Each monkey served as its own control, and fecal and urinary iron backgrounds were low.

The most effective of the chelators in the rodent model was oral CP94, followed by SC DFO, with oral CP20 the least effective (Fig 1). In this test system, the diethyl hydroxypyridone was far more effective than the dimethyl analogue. Furthermore, there was a notable difference in the disposition of the cleared iron between the two ligands: CP94 generated far more iron in bile. Both of the hydroxypyridones behaved qualitatively like DFO in terms of iron clearance rates, as iron excretion in urine was complete in 24 hours, while biliary clearance was complete in 12 to 21 hours (Fig 2). Both CP20 and CP94 demonstrated dose-dependent iron clearance (Fig 1 and Table 1). Although there were some quantitative differences, the rodent model was able to forecast the qualitative behavior of the ligands in the monkeys.

The behavior of DFO in the primates mimicked that seen in human subjects. At a dose of 150 µmol/kg, the ligand's efficiency was 5.5%, approximately twice that seen in the rats ($P < .001$). The induced iron excretion was nearly equally distributed in urine and feces (Fig 5). Induced iron clearance was complete in 24 hours. However, CP94 per-

Table 5. Net Iron Balance in *Cebus* Monkeys

Drug	Dosage (μmol/kg)	Route	Predrug (μg/kg)	Postdrug (μg/kg)	Significance of *t* Test
DFO	150	SC	217 ± 123	−244 ± 142	$P < .001$
CP20	300	Oral	342 ± 159	175 ± 153	$P > .1$
CP20	450	Oral	176 ± 16	110 ± 39	$P > .1$
CP94	300	Oral	306 ± 126	−194 ± 164	$P < .001$
CP94	450	Oral	425 ± 73	−273 ± 133	$P < .001$

The amount of iron absorbed by the untreated animals over a 3-day period is compared with the amount of iron absorbed by the treated animals over a 3-day period. Net iron balance = dietary iron intake − (urinary iron + fecal iron). Animals in a negative iron balance are excreting more iron than they are absorbing.

formed better than the hydroxamate when compared on an iron-binding equivalence basis, 450 μmol/kg of CP94 versus 150 μmol/kg of DFO: 7.4% versus 5.5% ($P < .04$). The efficiency of CP94 was essentially the same in rodents and primates, 7.1% and 7.4%, respectively. However, at a dose of 450 μmol/kg, the efficiency of CP20 did not increase significantly on going from rats to primates. While the CP20-induced iron excretion in the urine was significantly above baseline ($P < .001$), stool iron was not significantly higher than background ($P > .5$). At the dosages evaluated in the primates, CP20 was singularly unimpressive.

Both DFO and CP94 were effective at putting the primates in negative iron balance, whereas CP20 had little impact on this (Table 5). On the average, monkeys treated with 150 μmol/kg of DFO excreted 0.24 mg/kg more iron than they absorbed ($P < .001$), while animals treated with 450 μmol/kg of CP94 excreted 0.27 mg/kg ($P < .001$). It is interesting that iron clearance values and iron balance considerations lead to somewhat different profiles for the chelators. For example, looking at total DFO- (150 μmol/kg) or CP94- (450 μmol/kg) induced iron output, DFO promoted the excretion of 0.47 mg/kg of iron above baseline, while CP94 promoted excretion of 0.62 mg/kg above baseline ($P < .05$). However, although CP20 did clear some iron in the urine, it was not enough to offset the amount of iron absorbed.

As with the rodent model, all chelator-induced iron clearance was complete within 24 hours of drug administration. Finally, it is difficult to definitively connect the monkey's death with CP94. However, a CBC and chemistry profile were within normal limits for this animal before administration of the drug. Furthermore, the animal had never demonstrated any untoward effects from anesthesia and had been given CP20 at the same dose, 450 μmol/kg, 1 year previously with no difficulties.

Results from previous investigations have confirmed that the non–iron-overloaded, bile duct–cannulated rat model can serve as a preliminary screen for iron chelators and will detect every chelator with potential in vivo activity. However, strict correlation between the *Cebus* monkey and rodent models cannot be expected, because the two species of experimental animals differ significantly with respect to their iron metabolism. In our previous investigation, the rat model was predictive for DFO and desferrithiocin behavior in the primates, but not for pyridoxal isonicotinyl hydrazone analogues. In the current study, the rat data were predictive of the outcome in the primates.

The *Cebus* monkey data suggest that, under the experimental conditions used, CP94 is far superior to CP20. Both CP94 and DFO were easily able to hold the monkeys in negative iron balance, while CP20 failed to do so. However, some concern exists regarding the toxicity of CP94. Finally, the data further support the safety and effectiveness of DFO when administered SC.

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