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Orally active α-Ketohydroxypyridine Iron Chelators: Studies in Mice

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SUMMARY

Several *N*-substituted 3-hydroxypyrid-2-one and *N*-substitued 2-methyl-3-hydroxypyrid-4-one chelators were screened for ^{59}Fe removal using iron-overloaded mice labeled with ^{59}Fe. The most effectice chelators were found to be the *N*-methyl, *N*-ethyl, and *N*-propyl derivatives of 2-methyl-3-hydroxypyrid-4-one. When the above three chelators were administered intragastrically or intraperitoneally (200 mg/kg) to mice, they caused equivalent ^{59}Fe excretions to intraperitoneal desferrioxamine (200 mg/kg). These results increase the prospects for the use of the α-ketohydroxypyridine chelators in the treatment of iron overload.

Iron is essential for normal growth and development. Under normal conditions iron is regulated by gut absorption and the erythropoietic activity of the bone marrow. It is transported in the serum by transferrin and stored intracellularly as ferritin and hemosiderin. The major abnormalities of iron metabolism are iron overload and iron deficiency anemia. Although the latter is more frequent, the former is more severe because of the associated iron toxicity. Iron overload could arise from increased iron absorption from the gut or regular blood transfusions, e.g., in thalassemia patients. In contrast to the simple venesection treatment of iron overload arising from increased iron absorption, the treatment of transfusional iron overload in thalassemia, an orphan disease (1), requires the use of a chelator. It is estimated that 100,000 children are born every year with some form of thalassemia (2). The treatment of transfusional iron overload using subcutaneous (8–10 hr/day) desferrioxamine, the only clinically available iron chelator, is so expensive that most of the thalassemia patients living in poor countries could not use it. Furthermore, increased noncompliance is observed during adolescence in developed countries because of the difficulties of the daily subcutaneous injections. The use of an oral (3) or a suppository form of desferrioxamine (4) has had a limited success and signs of toxicities were observed when this chelator was used in rheumatoid arthritis patients (5) and at high doses in thalassemia patients (6, 7).

Several other chelators have been tried to treat iron overload but were unsuccessful because of the side effects, e.g., diethylenetriamine pentaacetic acid causes excretion of other metals such as Zn and Mn (8). Promising experimental iron chelators (1) include the pyridoxal isonicotinoylhydrazones (9), 2,4-dihydroxypyridine-*N*-oxide (10), spermidine catecholates (11), deferrithiocins (12), phenolic ethylenediamines (13), and tropolones (14). One of the most promising groups of chelators seems to be the α-ketohydroxypyridine chelators, which are neutral (pK_a 8.5–10) and form neutral 3-chelator to 1-iron complexes at pH 7.4, mobilize iron from transferrin and ferritin *in vitro* and from rabbits and mice in vivo (10, 15–19).

Materials and Methods

Preparation of ^{59}Fe lactoferrin. Human lactoferrin was prepared from human milk as previously described (20) and was found to be 20% iron saturated. ^{59}Fe lactoferrin (90% iron saturated) was prepared by adding, first, ^{59}Fe citrate to the isolated lactoferrin and, then, carrier iron citrate to a final ratio of 1.26 μg of iron to 1 mg of lactoferrin (10).

Iron loading and ^{59}Fe labeling of mice. Male T/O albino mice were loaded with iron by injecting intraperitoneally 2 mg of iron/week for 4 weeks in the form of iron dextran. Two weeks after the last iron dextran injection the mice were labeled with ^{59}Fe by using ^{59}Fe lactoferrin (2 μCi, 1 mg, 0.5 ml/mouse) which was injected via the tail vein. It has been shown previously that this method of labeling causes ^{59}Fe to be deposited initially in the liver as ferritin (21), but 2 weeks later ^{59}Fe is also found in hemoglobin and in other organs.[1] During this period the ^{59}Fe excretions are stabilized (10) and the amount of ^{59}Fe lost from this or following an ^{59}Fe transferrin or ^{59}Fe citrate labeling is very small (3%>) in comparison to the ^{59}Fe label injected (22).

Chelator administration and ^{59}Fe excretion studies. Mice were caged individually in metabolic cages (23) and their urine and feces were collected separately at midday every 24 hr. Immediately after the third day, excretion collection (or 18 days after the ^{59}Fe labeling), the chelators were administered intragastrically or intraperitoneally to mice at a dose of 200 mg/kg. The mice excretions were collected for an additional day after the chelator administration, and then the ^{59}Fe contents of all of the excretions were counted using an LKB 1800 Ultragamma counter. For each mouse, the mean ^{59}Fe excretion of 3 days preceding the administration of the chelator was taken as 100%

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[1] G. J. Kontoghiorghes, unpublished observation.

and, subsequently, all of the excretions were compared against this mean. This method of comparison has been chosen because of the observed mouse variation in the mean ^{59}Fe excretion which was shown to lie between 1500 and 2500 cpm (10).

Chelators. The 3-hydroxypyrid-4-one derivatives **I–VII** (Table 1) were prepared according to a previously described method (10), similar to a method used for the preparation of other pyridone derivatives (24). Other methods of preparation were also previously reported (25, 26). The 3-hydroxypyrid-2-one derivatives **VIII–XII** (Table 2) were prepared according to a previously described method (10) similar to the method of preparation of 1-methyl-3-hydroxypyrid-2-one (27). Desferrioxamine was obtained from Ciba Geigy, Horsham, U.K.

Results

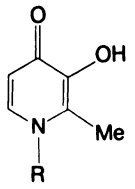
The ^{59}Fe excretion caused by the chelators ranged from 100 to 500% in comparison to the background excretion (100%) and to the controls. Most of the ^{59}Fe (97%) seems to be excreted in the feces, with or without the chelator treatment. In general, the increase in ^{59}Fe excretion varied with the chelator used, the dose, and the route of administration (Fig. 1).

All of the neutral 2-methyl-3-hydroxypyrid-4-one chelators caused substantial increases in ^{59}Fe excretion when administered intraperitoneally or intragastrically. In particular, the *N*-alkyl derivatives (**I, II, III**) were the most effective, causing equivalent ^{59}Fe excretions (250–400%) (Table 1) to intraperitoneal desferrioxamine (350%) (Table 2). In contrast to the *N*-alkyl-substituted chelators, the charged 3-hydroxypyrid-4-one chelators **V** and **VI**, did not cause any appreciable increase in ^{59}Fe excretion and those with hydroxyalkyl side chains **IV** and **VII** have had an intermediate effect (110–175%).

TABLE 1

The effect of *N*-substituted 2-methyl-3-hydroxypyrid-4-ones on daily ^{59}Fe excretions in ^{59}Fe-labeled mice

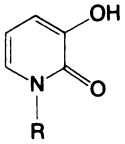
Male T/0 mice were iron loaded using iron dextran (8 mg of iron/mouse, intraperitoneally, over 4 weeks) and, 2 weeks later, ^{59}Fe lactoferrin labeled (2 μCi of ^{59}Fe, 1 mg of lactoferrin/mouse, intravenously). The chelators were administered either intraperitoneally or intragastrically, 18 days after the ^{59}Fe labeling. The mean (± standard error) percentage of ^{59}Fe excretions of the mice treated with the same chelator (200 mg/kg, intraperitoneally or intragastrically) is derived from the mean ^{59}Fe count of three daily excretions in each mouse before the chelator administration, which was taken as 100% and compared to the ^{59}Fe count of the excretion following the chelator administration.

Chelator	Number of mice	Route of administration	Percentage of ^{59}Fe excretion (±SE)
			
R = —Me	8	IP[a]	265 ± 22
(I)	6	IG[b]	291 ± 31
—Et	9	IP	329 ± 37
(II)	9	IG	402 ± 42
—Pr	5	IP	309 ± 29
(III)	5	IG	279 ± 22
—Et (2-OH)	10	IP	174 ± 15
(IV)	10	IG	165 ± 15
+ —$(CH_2)_2$ NH_3	12	IP	115 ± 5
(V)	12	IG	100 ± 4
—CH_2 COO^-	6	IP	96 ± 4
(VI)	5	IG	96 ± 4
—$(CH_2)_5$ OH	9	IP	149 ± 17
(VII)	9	IG	111 ± 7

[a] IP, intraperitoneal.
[b] IG, intragastric.

TABLE 2

The effect of *N*-substituted-3-hydroxypyrid-2-ones on daily ^{59}Fe excretions in ^{59}Fe-labeled mice[a]

Chelator	Number of mice	Route of administration	Percentage of ^{59}Fe excretion (±SE)
			
R = —Me	8	IP	146 ± 22
(VIII)	8	IG	139 ± 10
—Et	11	IP	197 ± 11
(IX)	11	IG	215 ± 20
—Pr	13	IP	169 ± 14
(X)	13	IG	149 ± 16
—C(=O)—Me	8	IP	136 ± 12
	8	IG	137 ± 16
(XI)			
—CH_2—C(=O)—OEt	12	IP	90 ± 3
	12	IG	92 ± 4
(XII)			
Desferrioxamine	15	IP	349 ± 27
	15	IG	104 ± 7
Control	22		100 ± 4

[a] The methods used and the abbreviations are the same as those described in Table 1.

The response of the mice treated with the *N*-substituted 3-hydroxypyrid-2-one chelators is shown on Table 2. In almost all of these chelators there was an increase in ^{59}Fe excretion (chelators **VIII–XI**) following their intragastric or intraperitoneal administration, but this was smaller in comparison to the *N*-alkyl-3-hydroxypyrid-4-ones or desferrioxamine. The ester derivative of 3-hydroxypyrid-2-one (**XII**) was ineffective. No apparent side effects or deaths were observed with all of the chelators during these mouse studies.

Discussion

In searching for oral iron chelators, several features of the molecular structure should be considered, such as the charge, lipid/water solubility, size, and affinity for iron at physiological pH, which would facilitate oral effectiveness and *in vivo* iron removal (10). The α-ketohydroxy chelating site in tropolones was previously identified to have increased iron selectivity at physiological pH (14). The tropolones, however, were found to be toxic *in vivo*, probably because of their lipophilicity. In an attempt to overcome the lipophilic toxic effects of tropolones, new groups of hydrophilic heteroaromatic chelators containing the α-ketohydroxy-binding site were synthesized using a new method (10) and were identified as effective and strong iron chelators using a new, simple *in vitro* and *in vivo* screening system (10, 15).

In contrast to other chelators, the oral activity observed with some 3-hydroxypyrid-2-ones and -4-ones may be the result of

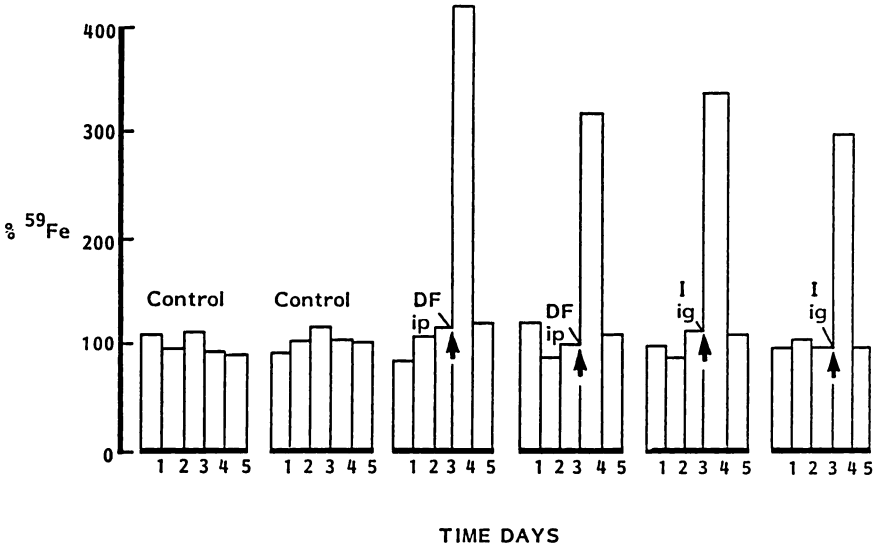


Fig. 1. Mobilization of ^{59}Fe from ^{59}Fe-labeled mice using desferrioxamine and 1,2-dimethyl-3-hydroxypyrid-4-one. Increased percentage of ^{59}Fe excretion is shown on day 4 in the two groups of mice treated with desferrioxamine intraperitoneally (*ip*) and 1,2-dimethyl-3-hydroxypyrid-4-one intragastrically (*ig*) at a dose of 200 mg/kg, in contrast to two mice which did not receive any chelators (controls). For each mouse the mean ^{59}Fe excretion of the first 3 days (days 1–3) was taken as 100% and all daily ^{59}Fe excretions were plotted against this mean. The first daily excretion (day 1) was collected 15 days after the ^{59}Fe labeling.

their increased gut absorption and limited biotransformation which may be related to the neutral charge, small size, and aromatic character of their molecular structure and stability of their iron complexes. Since lipophilic chelators may be toxic, and because some are known to transport iron across membranes and may increase iron absorption (10), it can be suggested that chelators with lipid/water partitions similar to those of the chelators **I, II,** and **III** (K_{par} = 0.2–3.2) would be useful in the treatment of iron overload. Dimeric and trimeric forms of these chelators could also be proved to be effective because of their increased iron-binding capacity. The differences between the 3-hydroxypyrid-2-ones and -4-ones may be related to the greater efficiency of the latter chelators in mobilizing iron from transferrin and ferritin *in vitro* (10, 16–18). The *N*-alkyl substitution in both groups of chelators seems to increase their *in vivo* efficacy in ^{59}Fe removal. In contrast, charged and non-N-alkyl substituent chelators are less effective, probably because of a decrease in their membrane permeability and iron mobilization, and also their rapid excretion and metabolism.

High iron-binding properties were also observed with mimosine (18) and 1-methyl-3-hydroxypyrid-4-one (28), two naturally occurring compounds with a 3-hydroxypyrid-4-one structure. In a preliminary study the daily intraperitoneal or intragastric administration of **I** and **II** (200 mg/kg) to normal and iron-loaded mice for 4 weeks did not cause any apparent ill effects, and the mice continue to live 5 months following that treatment. Since these chelators fulfill many of the criteria of an effective oral iron chelator, such as high selectivity for iron over other metals of biological importance, e.g., Cu, Zn, Mg, and Ca (19), have high iron binding constants, oral activity in three animal species [i.e., mice, rats (22) and rabbits (19)], and, finally, have inexpensive synthesis and no apparent acute toxic effects, the prospects for their use in humans is increased and further efforts for their development into oral drugs is required.

In conclusion, the results of this work clearly indicate the effectiveness of α-ketohydroxypyridines in removing iron *in vivo* and point to the *N*-alkyl-2-methyl-3-hydroxypyrid-4-ones as possible alternative chelators to desferrioxamine which may be used for the treatment of iron overload and other diseases of iron imbalance. Further toxicological studies and other investigations on iron metabolism using these chelators are in progress.

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