Efficacy of Deferiprone in the Treatment of Acute Iron Intoxication in Rats

Frank F. Fassos, MSc; Matitiahu Berkovitch, MD; Nick Daneman; Lee Koren; Ross G. Cameron, MD, PhD; Julia Klein, MSc; Corrado Falcitelli; Patrick St. Louis, PhD; Richard Daneman; Gideon Koren, MD

The Hospital for Sick Children (FF, MB, ND, LK, JK, CF, PSL, RD, GK); The Toronto Hospital (RC); Toronto, Ontario

ABSTRACT

Background: Deferiprone [(1,2-dimethyl-3-hydroxypyrid-4-one) (L1)], is the first orally active iron chelating agent to reach clinical trials in patients with chronic iron overload. Its efficacy in preventing morbidity and mortality in acute iron poisoning has not been tested. Objective: To determine whether deferiprone can reduce the mortality of rats following toxic oral doses of iron. Methods: Rats were administered 612 mg/kg elemental iron by gavage, corresponding to the LD₅₀. A parallel group received the same oral dose of iron followed by deferiprone intraperitoneally at 400 mg/kg (loading dose), followed by additional intraperitoneal injections of 200 mg/kg, 100 mg/kg and 100 mg/kg of deferiprone at one hour intervals. Results: Coadministering deferiprone with the iron decreased mortality from 58% (11/19) to 15% (3/20) (p = 0.013). The administration of deferiprone was associated with urinary excretion of iron (which did not occur with iron alone) and the production of the red deferiprone-iron complex. On histological examination there appeared to be less iron in the liver and gastrointestinal tract. Conclusion: The coadministration of deferiprone can decrease morbidity and mortality caused by acute iron overdose. Deferiprone holds promise for the treatment of iron poisoning but additional study is required.
INTRODUCTION

Recently, there has been both an increase in the number of reported iron intoxications\(^1\) and of iron poisoning deaths.\(^2\) Ipecac-induced emesis, gastric lavage with sodium bicarbonate or sodium phosphate, administration of activated charcoal and whole bowel irrigation are used to prevent absorption of the iron.\(^3\) Yet these techniques do not efficiently eliminate this metal.\(^4,6\)

Deferoxamine (DFO) is currently the most effective agent in eliminating excess iron after its absorption.\(^7\) One molecule of DFO effectively neutralizes all six active coordination sites on one ferric iron atom,\(^8\) thus eliminating potentially harmful free radical reactions. However, DFO is limited to use in industrialized nations due to its cost. For use in cases of iron poisoning, DFO is further limited to use in a hospital environment equipped for IV therapy and supportive emergency care. For a victim several hours away from a medical center, initiating therapy as quickly as possible may be life-saving.

The orally effective iron chelating agent deferiprone [1,2-dimethyl-3-hydroxypyrid-4-one (L1)], has been shown to be efficacious in removing body iron in homozygous \(\beta\)-thalassemia patients with chronic iron-overload.\(^9-10\) There is currently no study that has examined the therapeutic potential of deferiprone in acute iron intoxication. If proven effective, deferiprone could be an alternative treatment to DFO in cases of iron poisoning. Its excellent oral bioavailability would permit administration outside of a hospital setting.

As a first step in evaluating the potential use of deferiprone for acute iron poisoning, we report our findings of its efficacy in a rat model of acute iron intoxication.

MATERIALS & METHODS

Protocol

The protocol was approved by the Hospital for Sick Children Animal Care Committee.

Rats

Male Wistar rats weighing 150 g to 250 g, were purchased from Charles River Canada, Inc. (Quebec, Canada). They were double-housed in plastic shoe box-type cages and were food deprived for approx...
Deferiprone in the Treatment of Iron Intoxication

Table 1

<table>
<thead>
<tr>
<th>Organ</th>
<th>Location</th>
<th>Iron Only</th>
<th>Iron and Deferiprone</th>
</tr>
</thead>
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<tr>
<td>Liver</td>
<td>Kupffer cells</td>
<td>&lt; 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>endothelium (periportal)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>capillary/vein/arteriole endothelium</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>tubules (crystals)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>capillary endothelium</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>myocardium</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>pleural lining</td>
<td>2-3</td>
<td>2</td>
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<tr>
<td></td>
<td>alveolar lining</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Stomach</td>
<td>superficial mucosa/lumen</td>
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<td>4</td>
</tr>
<tr>
<td></td>
<td>capillary, vein endothelium</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
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<td>muscle layers</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>intermyocytic</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>serosa</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
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<tr>
<td></td>
<td>serosa</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Islets</td>
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</tr>
<tr>
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<tr>
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<td>superficial mucosal lumen</td>
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</tr>
<tr>
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<td>crypts</td>
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<td>capillary, vein endothelium</td>
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</tr>
<tr>
<td></td>
<td>intermuscles</td>
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</tbody>
</table>

0 = no iron, 4 = maximal iron score.

imately 14 h prior to iron dosing. The rats were assigned to one of three groups. Group I rats (n = 19) received iron by gavage alone, while those in group II (n = 20) received iron by gavage followed by four intraperitoneal (IP) injections of deferiprone solution. Group III was handled in a similar manner but did not receive either iron or deferiprone. This group served as a control for the various biochemical and histological endpoints.

Iron Preparation

One hour prior to the experiment, hydrous ferrous sulphate (FeSO₄·7H₂O; Fisher Scientific, NJ) was dissolved in double-distilled Milli-Q water (Millipore, MA) for a final concentration of 0.082 g/mL of elemental iron. At the commencement of the experiment, Groups I and II animals were gavaged with a volume of ferrous sulphate solution corresponding to 612 mg/kg of elemental iron.

**Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one)**

Deferiprone was synthesized at the University of Toronto, department of Chemistry, by Dr. R. McClelland according to previously published methods. In the afternoon prior to the experimental procedure, crystalline deferiprone was dissolved in distilled water for a final concentration of 10 mg/mL. Upon dissolving, deferiprone solution was sterilized via suction-filtration through a GS 0.22 micron filter (Millipore, MA) in a laminar flow hood. The filtrate was stored at room temperature in sterile 100 mL injection vials (Bencard Labs,
Figure 2. Iron staining of the stomach shows complete absence of intracellular iron and substantial reduction in serosal iron in deferiprone-treated animals. A) control, B) iron alone, C) iron plus deferiprone.
Deferiprone in the Treatment of Iron Intoxication

Ontario). Following completion of each experiment, excess volumes of the deferiprone solution were discarded.

Experimental Procedure

Following the administration of the iron solution by gavage, animals were closely observed. Immediately after the iron gavage they were returned to their housing cages and permitted food. Fifteen minutes after the iron gavage, the rats in group II were injected IP with an initial loading dose of 400 mg/kg of deferiprone solution. Thereafter, group II rats received an additional three IP injections of deferiprone solution at one hour intervals in doses of 200, 100 and 100 mg/kg, respectively. Eight hours following the administration of the initial injection of deferiprone to animals in group II, the rats were placed in smaller, clean plastic cages without bedding. Single-time urine specimens were collected from the plastic cage floor using plastic transfer pipettes (Samco Scientific Inc., CA), and were transferred to labelled Eppendorf tubes (Rose Scientific Ltd., Alberta). Animals were then returned to their original cages. Animals that died during the observation period were dissected immediately. Animals that remained alive until the completion of the observation period were euthanized with Ethrane gas (Anaquest, Ontario) in a gas delivery apparatus (Ohio Medical Products, WI). Cardiac blood samples were drawn via syringe and centrifuged at 2500 rpm for ten minutes. Serum was collected by plastic transfer pipettes, frozen temporarily at -20°C and later transferred to a -80°C freezer. Residual bladder urine was collected by needle aspiration, frozen at -20°C and then at -80°C.

Pathology

Cross-sectional slices of the following organs were obtained for pathological examination: heart, liver, lung, stomach, duodenum, and kidney. Tissue samples were stored in pre-labelled vials containing 10% buffered formalin solution (BDH Chemicals Inc., Mississauga). These samples were prepared for hematoxylin/eosin and Prussian Blue iron staining. Photographs of the organ slices were taken using 64 ASA film with a 35mm camera mounted atop a microscope. Slides of iron staining were blindly scored 0 (no iron) to 4 (maximal iron score) by one of the authors, a pathologist.
Figure 3. Iron staining of the duodenum shows complete absence of villous and intracellular iron in deferiprone-treated animals. A) control, B) iron alone, C) iron plus deferiprone.
Deferiprone in the Treatment of Iron Intoxication

Analysis

Serum iron samples were analyzed on a Kodak Ektachem 700 Analyzer (Rochester, NY) at the time of death or at 14 h and urine iron samples on a SpectrAA-10 atomic absorption spectrometer (Varian Techtron Pty Ltd., Australia). This method measures iron bound and unbound to deferiprone. Plasma samples were analyzed for deferiprone content by an HPLC method previously developed by us. Survival between groups was compared by means of a two-tailed Fisher’s exact test. Various laboratory values among groups were compared by the Mann-Whitney U test.

RESULTS

Survival

At the conclusion of the 14 h observation period, only 15% (3/20) of the rats that were given iron and treated with deferiprone had died, in comparison to 59% (11/19) of the rats receiving only iron (p = 0.013). None of the rats that received deferiprone died within the first four hours after the administration of iron, whereas seven of the rats that did not receive deferiprone during this initial period died (p = 0.0064) (Figure 1). In all animals of Group II, there was strong red discoloration of urine, typical of the deferiprone-iron complex. No such color was evident in the urines collected from the rats in Group I or from the rats in the control group, which did not receive any drug.

Serum Iron

The mean serum iron (mean ± SD) was significantly higher for animals receiving 612 mg/kg ferrous iron (7819.53 ± 15383.27 μmol/L) than controls (52.72 ± 25.94 μmol/L) (p = 0.016). The group receiving iron followed by injections of deferiprone, had intermediate concentrations (1803.87 ± 2700.67 μmol/L), not significantly different from that of animals receiving iron alone.

Urine Iron

In control animals (not yet injected with either deferiprone or gavaged with iron), mean urine iron was 10.20 ± 0.78 μmol/L. Most of the animals receiving iron at 612 mg/kg alone had urine irons below 10 μmol/L, with a mean of 21.88 ± 27.37 μmol/L. Conversely, animals receiving iron with IP injections of deferiprone solution had urine iron...
concentrations of 3022.26 ± 968.66 μmol/L at eight hours postinjection (p = 0.0001, compared to rats receiving iron alone) and urine iron concentrations of 1266.00 ± 1596.50 μmol/L (p = 0.013, compared to rats receiving iron alone).

**Deferiprone Concentrations**

The mean deferiprone levels present in the rats that received iron and four IP injections of deferiprone solution were 10.29 ± 14.45 μg/mL 14 h after the iron administration (or at death).

**Tissue Analysis (Table 1)**

When deferiprone was coadministered, iron was not identified in the liver, kidney, and heart tissues. Pancreatic iron staining scores also dropped dramatically with deferiprone treatment.

In the stomach and duodenum, iron was still present in the superficial lining, yet there was less iron in the capillary endothelium, muscles, serosa and an absence of iron in nuclei when deferiprone was coadministered. Iron stain showed complete absence of iron crystals in kidney tubules after deferiprone administration. With iron treatment alone, large iron crystals were deposited in the kidney tubules. There were no signs of tubular or glomerular damage in any group.

Lung tissue of animals treated with iron alone showed iron deposition in the serosa but nowhere else. In the presence of deferiprone there appeared to be greater amounts of iron in the lungs. There was no evidence of alveolar damage in any group.

Histologic stains of the pancreas showed iron deposition in the endothelium and serosa in the absence of deferiprone with almost complete absence when deferiprone was coadministered.

Liver iron staining was localized in the endothelium and in Kupffer cells. There were no noticeable traces of iron deposition in hepatocytes. The administration of deferiprone treatment was associated with the absence of all traces of iron from the endothelium. In the stomach of rats given only iron, there was iron deposition in the serosa as well as within cells (specifically in the cytoplasm and within the nuclei). With concomitant administration of deferiprone, iron staining was reduced in the serosa lining and was virtually nonexistent in the nuclei. While the iron continued to adhere to the stomach surface lining, iron was virtually nonexistent within the gastric cells (Figure 2).

Iron staining of the duodenum was a similar pattern to that of the stomach (Figure 3). Iron was bound to the villous surface and was present in the nuclei, cytoplasm, serosa, and smooth muscle layers. In animals treated with deferiprone, there was virtually no iron within the cells or blood vessels and iron appeared to be completely absent in cell nuclei.

**DISCUSSION**

In cases of iron poisoning, DFO has been used to eliminate the excess of the metal from the body. DFO is not absorbed orally, and in many cases its parenteral administration is confined to hospitals. Hence, victims from isolated areas can lose valuable time during their transport to a medical center. Early initiation of chelation therapy may be instrumental in preventing organ damage and may also prevent mortality.

In the present study, 58% of the male Wistar rats that received 612 mg/kg oral elemental iron solution by gavage died before the 14 h observation period was over, compared to 15% of the rats that received deferiprone. These data indicate that deferiprone reduced and delayed mortality in rats following toxic doses of iron. The decrease in mortality with deferiprone was associated with several biochemical and pathological measures indicating effective chelation of the excess iron. The urine took on a distinctive red color indicating the presence of the deferiprone-iron chelate and an increased urine iron was found in the deferiprone-treated animals. Deferiprone-treated animals had less iron in their tissues (Table 1). We did not, however, distinguish between chelated and free serum iron.

The drug was administered parenterally to ensure that its effects (if any) are due to the systemic chelation of iron rather than from binding to iron in the gastrointestinal tract. However, human studies conducted by us and others clearly show that deferiprone is readily absorbed from the gut.9-11

Serum deferiprone of the group receiving the drug with iron was 10.29 ± 14.45 μg/mL at 14 h after administration. These levels are consistent with those measured in patients treated with deferiprone for chronic iron-overload associated with thalassemia.11
Deferiprone in the Treatment of Iron Intoxication

The increased survival of animals receiving deferiprone with iron was associated with evidence of reduced tissue iron, particularly critical of the stomach, intestine and liver, the organs most affected by iron intoxication (Table 1).

The tendency towards increased lung iron with deferiprone may suggest potential lung toxicity from prolonged deferiprone therapy as has been shown for DFO. Our chronic deferiprone studies of patients with thalassemia have not shown lung toxicity.

Since deferiprone has been used for several years in patients with thalassemia and sickle cell disease, its oral use in acute iron poisoning seems relatively safe. Human studies are needed to document the efficacy of deferiprone in the treatment of acute iron poisoning.

ACKNOWLEDGEMENT

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REFERENCES


