Iron-Balance and Dose-Response Studies of the Oral Iron Chelator 1,2-Dimethyl-3-Hydroxyypyrid-4-One (L1) in Iron-Loaded Patients With Sickle Cell Disease

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Several life-threatening complications of the common disorder sickle cell disease require management with red blood cell transfusions and, hence, long-term iron-chelating therapy. The efficacy of the oral iron chelator 1,2-dimethyl-3-hydroxyypyrid-4-one (L1) has not previously been determined in patients with sickle cell disease. We compared the efficacy of L1 to that of standard-dose subcutaneous deferoxamine in four regularly transfused patients with homozygous sickle cell disease, who had evidence of severe iron overload and a history of poor compliance with deferoxamine. Determination of 24-hour urinary iron excretion conducted over 5 days immediately after transfusion showed that the mean daily urinary iron excretion induced by L1 at 75 mg/kg/d (0.48 ± 0.23 mg/kg) was equivalent to that induced by deferoxamine at 50 mg/kg/d (0.39 ± 0.06 mg/kg). In two of three patients studied, a significant (P < .025) increase in mean daily urinary iron excretion was achieved when the dose of L1 was increased to 100 mg/kg/d. Total iron balance studies, which quantitated both urinary and stool iron excretion on L1 and deferoxamine, determined that mean total daily iron excretion induced by deferoxamine (0.88 ± 0.05 mg/kg) was significantly greater (P < .05) than that induced by L1 (0.53 ± 0.17 mg/kg), attributable to the significantly greater stool iron excretion during deferoxamine treatment (0.50 ± 0.16 mg/kg/d) compared with that measured during L1 treatment (0.12 ± 0.08 mg/kg/d, P < .01). Stool iron excretion accounted for a significantly greater percentage of total iron excretion during deferoxamine treatment (59% ± 20%) than during L1 treatment (23% ± 14%, P < .01). These iron balance studies are the first to compare total iron excretion induced by L1 with that achieved by deferoxamine. They demonstrate that the mean total daily iron excretion during L1 treatment (0.53 ± 0.17 mg/kg) is sufficient to maintain net negative iron balance in most regularly transfused patients with sickle cell disease. Because long-term compliance with L1 has been shown previously to be superior to that with deferoxamine in patients with homozygous β-thalassaemia, the use of L1 should increase the long-term effectiveness of iron chelation in patients with sickle cell disease.

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IT IS WELL-RECOGNIZED that the institution of a program of regular red blood cell (RBC) transfusions can markedly reduce disease complications, and improve quality of life, in patients with sickle cell disease. The indications for RBC transfusion programs in the sickling disorders include stroke, intractable recurrent painful crises, and progressive pulmonary disease. Recently, clinically asymptomatic central nervous system infarcts occurring in the first decade of life in a high percentage of patients with sickle cell disease have been reported. In addition, abnormal velocities of blood flow determined by transcranial ultrasonography in children with sickle cell disease have been observed to be predictive of an increased incidence of clinical stroke. Thus, the indications for transfusion in patients with sickle cell disease, to attempt to prevent these and other complications, may be extended in the future.

The cost of an RBC transfusion program is, however, the development of transfusional iron overload, and the requiring of iron-chelating therapy. The currently available iron-chelating drug, deferoxamine, is expensive, is associated with both local and systemic toxicity, and requires daily parenteral administration. These factors must be considered as a significant obstacle to the institution of RBC transfusions in patients with sickle cell disease, most of whom are introduced to this difficult chelation regimen in late childhood and adolescence, when compliance with chronic medical therapy is often erratic. The availability of an effective orally available iron chelator would make regular transfusion a more attractive therapeutic option in the prevention, and management, of the complications of sickle cell disease.

1,2-Dimethyl-3-hydroxyppyrid-4-one (L1) is an orally active bidentate iron chelator capable of inducing negative iron balance and decreasing tissue iron in iron-loaded patients with homozygous β-thalassaemia. Although reported in patients with thalassaemia, its efficacy as an iron chelator in patients with sickle cell disease has not been determined. We report the results of cross-over and dose-response studies of urinary iron excretion and total iron-balance studies comparing urinary and stool iron excretion induced by L1 and by deferoxamine, in patients with sickle cell disease.

SUBJECTS AND METHODS

Patients. Four female patients, 15.4 ± 2.7 years of age (range, 11 to 17 years), were selected for short-term studies of L1 because of previous noncompliance with subcutaneous deferoxamine therapy. All patients had homozygous sickle cell disease; all were managed on a regular program of RBC transfusions. The indications

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The completeness of 24-hour urine collections was assessed by daily conducted over 45 consecutive days per patient, all of whom were reviewed of each patient's technique, as well as by comparison of 24-hour admitted to the Clinical Investigation Unit of the Hospital for Sick was measured during an initial (baseline) phase during which no admission. Preweighed amounts of a standardized hospital diet were monitored by the Medication Event Monitoring System (MEMS) device (Aprex pugamma CS; Wallac, Turku, Finland). All patients had evidence of moderate iron overload (Table 1). This study was approved by the Human Subjects' Review Committee (The Hospital for Sick Children, Toronto, Canada). Written informed consent was obtained from each patient or parent.

Urinary iron excretion. The urinary iron excretion studies, performed first, compared 24-hour urinary iron excretion induced by deferoxamine at 50 mg/kg/d administered subcutaneously over 12 hours, by L1 at 75 mg/kg/d, and by L1 at 100 mg/kg/d, with both L1 dose levels administered three times daily at 0700, 1500, and 2300 hours. Each phase of the study was conducted over the 5 days immediately after three successive blood transfusions in each patient, ensuring that each chelation regimen was studied at a similar hematocrit. Previously described variations in urinary and faecal iron excretion with changes in hemoglobin level were thereby avoided. On all 5 days of each arm of the study, all urine was passed into acid-washed containers, allowing five 24-hour urinary iron excretions to be measured for each chelation regimen. All deferoxamine infusions were commenced under direct medical supervision in clinic every morning, and the first 8 hours of the subcutaneous infusion were supervised in clinic. Each infusion was completed at home. Each patient was contacted by phone by one investigator, every night during this phase of the study, to document difficulties with completion of an infusion (none were reported). Compliance with L1 was monitored by the Medication Event Monitoring System (MEMS) device (Aprex Corp, Berkeley, CA) as described previously, and by reminder phone calls to the patient at the time of every dose. The completeness of 24-hour urine collections was assessed by daily review of each patient's technique, as well as by comparison of 24-hour absolute creatinine excretion to that predicted by weight and sex.

Total iron-balance studies. These studies were subsequently conducted over 45 consecutive days per patient, all of whom were admitted to the Clinical Investigation Unit of the Hospital for Sick Children for the duration of the study. Each patient was maintained on a low iron diet (10.7 ± 1.8 mg of iron per day) throughout admission. Preweighed amounts of a standardized hospital diet were provided by one dietitian, and accurate recording of food intake by the patients was directly supervised at each meal. Total iron balance was measured during an initial (baseline) phase during which no chelator was administered, during the administration of L1 at 75 mg/kg/d in three divided doses, and subsequently during the administration of deferoxamine at 50 mg/kg/d as a 12-hour subcutaneous infusion. At the commencement of each phase of the study, a dose of 10 μCi of chromium-51-labeled chromic chloride was administered orally in 25 mL apple juice. Stool radioactivity was counted to measure excretion of 51Cr using a gamma counter (LKB 1282 Compugamma CS; Wallac, Turku, Finland). After 95% of the label had been recovered in the stool, all urine and stools were saved in acid-washed containers to permit determination of iron content. Three 24-hour urine iron determinations and one 72-hour stool iron determination were collected during the baseline phase. During administration of each drug, nine 24-hour urine collections and three 72-hour stool collections were analyzed for iron content. Use of stool labeling with 51Cr established that, at the start of each stool collection, all iron consumed before the institution of the iron-restricted diet, and all stool formed before the institution of the chelating drug under study, had passed out of the gastrointestinal tract.

Throughout both urinary iron excretion and total iron-balance studies, the mean peripheral hemoglobin concentration was maintained at greater than 100 g/L in all patients (121 ± 14 g/L), whereas peripheral hemoglobin S levels were maintained at less than 20% (12.2% ± 4.5%) to ensure that endogenous erythropoiesis was suppressed. Laboratory evaluation before commencement of the study, and at weekly intervals, included hemoglobin, platelet and differential white blood cell counts, serum electrolytes, urea and creatinine, total protein, albumin, aspartate and alanine transaminases, calcium, magnesium, phosphorous, copper, and zinc.

L1 was synthesized locally as described previously. Urine iron quantitations were measured by direct atomic absorption spectrometry using a Varian Spectra AA-300 atomic absorption spectrophotometer (Varian Techtron, Mulgrave, Victoria, Australia). Stool was processed for iron analysis by shaking with an equal volume of water in tared plastic collection containers until a homogeneous suspension was obtained. Triplicate 2-mL aliquots were digested by gentle heating in 15 mL of concentrated nitric acid (GR; Merck, Darmstadt, Germany). After volume reduction, the procedure was repeated three times with 15 mL of 1:1 nitric acid and hydrogen peroxide (BDH). The final concentrate was diluted to 25 mL with Milli Q water (Millipore, Bedford, MA). Iron was measured by flame atomic absorption spectroscopy (Varian AA-775). Method blanks were prepared with each batch of samples.

The quantitative measurements of iron excretion before and during chelation treatment were compared using the Student's t-test for paired data. Results of urinary iron excretion during the cross-over study were considered as paired 5-day collections, because there was a consistent trend in urinary iron excretion over the 5 days, with iron excretion increasing between days 1 and 5. Correlation between variables was tested by linear least squares regression analysis. Values are expressed as the mean ± SD (range).

RESULTS

Urinary iron excretion. During administration of L1 at 75 mg/kg/d, 24-hour urinary iron excretion of 0.42 ± 0.20 mg/kg body weight was similar to that during deferoxamine treatment (0.40 ± 0.13 mg/kg; P = NS, Fig 1). Increasing the dose of L1 to 100 mg/kg/d increased urinary iron excretion in two of three patients (Fig 1). In two patients, administration of L1 at 75 mg/kg/d achieved significantly (P < .025) greater iron excretion than did deferoxamine. In a third patient, administration of L1 at 100 mg/kg/d achieved significantly (P < .005) greater urinary iron excretion than deferoxamine. In the fourth patient, who had the most modest degree of iron overload (as determined by the lowest calculated transfusional iron load, transferrin saturation, and serum ferritin concentration) of all the patients, L1-induced urinary iron excretion was significantly (P < .005) less than that achieved by deferoxamine (Fig 1). Serum ferritin concentration in this group of patients correlated significantly with transfusional iron load (P < .005). There was a highly significant correlation between serum ferritin concentration and 24-hour
urinary iron excretion during administration of L1 at 75 mg/kg/d ($r = .96, P < .005$).

**Total iron-balance studies.** Total iron-balance studies demonstrated that the mean daily iron excretion during administration of deferoxamine (0.88 ± 0.05 mg/kg/d) was significantly greater than that during L1 (0.53 ± 0.17 mg/kg/d, $P < .05$). Because no significant difference was demonstrated in the ability of the two chelators to induce urinary iron excretion, the difference in iron excretion was accounted for by a higher mean stool iron excretion during deferoxamine administration (0.50 ± 0.16 mg/kg/d compared with that during L1, 0.12 ± 0.08 mg/kg/d, $P < .005$, Table 2). Stool iron excretion accounted for 59% ± 20% (32% to 77%) of total iron excretion during deferoxamine administration, but only 23% ± 14% (3% to 33%) during L1 administration ($P < .01$, Table 2 and Fig 2).

During the initial urinary iron excretion dose-response studies, compliance with L1 taken at home, as assessed by MEMS device, was 98% ± 2.8% (93% to 100%) drug taken of that prescribed. Compliance with deferoxamine, administered under direct medical supervision, was 100%. No adverse clinical effects, hematologic changes, or biochemical changes were observed during this short-term study.

**DISCUSSION**

There is clear evidence that regular iron-chelation therapy with deferoxamine reduces the morbidity and mortality of transfusional iron overload$^{26-29}$ in regularly transfused patients. However, a well-recognized problem with deferoxamine is the frequently observed decline in compliance with the drug during adolescence, when transfusional iron accumulation is accelerated.$^{30}$ A significant factor contributing to erratic compliance is deferoxamine’s requirement for prolonged nightly parenteral infusions. Certainly, an orally available iron-chelating agent has the potential to make long-term therapy more acceptable to the patient and to improve compliance, and thus long-term effectiveness, of chelation therapy.

The studies reported here demonstrate that L1 induces sufficient mean iron excretion to maintain negative iron balance$^{30}$ in transfusion-dependent patients with homozygous sickle cell disease. Although total iron excretion induced by L1 was less than that achieved by deferoxamine in this short-term exposure, the improved compliance reported with daily use of L1 over 2 years of therapy in patients with thalassemia major$^{14}$ would suggest that this drug should provide long-term chelating effectiveness that is equivalent, or superior, to that of deferoxamine in both cohorts of patients.

This study represents the first controlled total iron excretion study directly comparing deferoxamine and L1, and permits each patient studied to serve as her own control. Such direct comparisons between the two drugs were not possible to date, because of differences in methodology in previously reported studies. Our finding that 32% to 77% of total iron excretion was excreted in the stool during deferoxamine treatment is consistent with previously described values of 37%$^7$ and 32% to 62%.$^{31}$ Similarly, our finding of stool iron excretion induced by L1 of 3% to 33% (of total iron excretion) is consistent with previous reports.$^{11,12}$ The reasons for the wide range in L1-induced stool iron excretion remain to be determined. Possibly, differences in body iron load may affect relative excretion of iron into urine and stool; alternatively, the differences in proportion of stool iron excretion may reflect a difference in the source of chelated iron.

The relative indications for programs of regular RBC transfusion for the treatment, or prevention, of several life-threatening complications of sickle cell disease are broaden-
ing. Given the difficulties associated with administration of deferoxamine, a safe, effective oral iron-chelating agent is desirable for patients requiring long-term therapy. The present study demonstrates that deferoxamine is undoubtedly the more efficacious iron chelator. However, because of the excellent compliance observed in patients with thalassemia administering L1 in long-term studies, in contrast to the erratic compliance observed with desferrioxamine, administration of L1 should result in at least equal long-term effectiveness. This can only be determined by controlled, prospective long-term studies of this promising agent in transfused patients with sickle cell disease.

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