Reduction of Tissue Iron Stores and Normalization of Serum Ferritin During Treatment With the Oral Iron Chelator L1 in Thalassemia Intermedia

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In patients with thalassemia intermedia in whom hyperabsorption of iron may result in serious organ dysfunction, an orally effective iron-chelating drug would have major therapeutic advantages, especially for the many patients with thalassemia intermedia in the Third World. We report reduction in tissue iron stores and normalization of serum ferritin concentration after 9-month therapy with the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in a 29-year-old man with thalassemia intermedia and clinically significant iron overload (SF 2,174 µg/L, transferrin saturation 100%; elevated AST and ALT, abnormal cardiac radionuclide angiogram) who was enrolled in the study with L1 75 mg/kg/day after he refused deferoxamine therapy. L1-Induced 24-hour urinary iron excretion during the first 6 months of therapy was (mean ± SD, range) 53 ± 30 (11 to 109) mg (0.77 mg/kg), declining during the last 3 months of L1 to 24 ± 14 (13–40) mg (0.36 mg/kg), as serum ferritin decreased steadily to normal range (present value, 251 µg/L). Dramatic improvement in signal intensity of the liver and mild improvement in that of the heart was shown by comparison of T1-weighted spin echo magnetic resonance imaging with images obtained immediately before L1 administration was observed after 9 months of L1 therapy. Hepatic iron concentration decreased from 14.6 mg/g dry weight of liver before L1 therapy to 1.9 mg/g liver after 9 months of therapy. This constitutes the first report of normalization of serum ferritin concentration in parallel with demonstrated reduction in tissue iron stores as a result of treatment with L1. Use of L1 as a therapeutic option in patients with thalassemia intermedia and iron overload appears warranted.

P R O G R E S S I V E O R G A N dysfunction, often leading to mortality, is a consequence of transfusional or absorptive tissue iron loading in patients with homozygous β-thalassemia.1 Regular chelation therapy with deferoxamine reduces hepatic iron and arrests hepatic fibrosis,2 stabilizes iron-related cardiac disease,3 and prevents gonadal failure when started before age 10 years.4 Administration of standard therapy with deferoxamine is problematic, however; the drug requires prolonged nightly subcutaneous (SC) infusion, frequently associated with local irritation. Compliance with this regimen decreases dramatically during adolescence,5 and many patients with cardiac iron overload still die.5,6 Moreover, the cost of deferoxamine renders it unavailable to thousands of thalassemia patients in the Third World. It has long been acknowledged7 that the added convenience of an orally available chelator would simplify treatment of patients with transfusion-dependent thalassemia, and should also simplify management of patients with sickle cell disease who are maintained on a transfusion program in an effort to prevent or arrest specific disease complications.

Although transfusion-dependent anemias have focused attention on the worldwide need for a safe, orally active iron-chelating agent, disorders of increased iron absorption should also benefit from their development. Individuals with nontransfusion-dependent thalassemia (thalassemia “intermedia”) exhibit excessive dietary iron absorption that can lead to serious iron loading by the second or third decade of life.10 The cost to these patients of neglecting iron loading is the development of life-threatening tissue damage, and infrequent infusions of deferoxamine may suffice to prevent iron accumulation and its complications.11 An orally effective iron-chelating drug would have major therapeutic advantages for these patients, especially for Third World patients with thalassemia intermedia.12

Several orally available, iron-chelating compounds have been developed in recent years; few have been evaluated in human trials. Among those that have, 1,2-dimethyl-3-hydroxypyrid-4-one (CP20 or L1) has been demonstrated to promote clinically significant urinary iron excretion in animal studies13,14 and in human trials in the United Kingdom,15,16 Europe,17 India,18 and Canada.19 In a short-term randomized, cross-over study, L1’s ability to induce urinary iron excretion was demonstrated to be comparable to that of SC deferoxamine and well tolerated20; recent studies have demonstrated its continued long-term efficacy.16,17

We report reduction in tissue iron in the liver and heart, as demonstrated by magnetic resonance imaging (MRI) of both organs, and percutaneous liver biopsy, after 9 months of therapy with L1 in a patient with thalassemia intermedia at high risk of progressive iron-induced morbidity. This constitutes the first report of normalization of serum ferritin concentration and demonstrated reduction of tissue iron stores as a result of treatment with L1.

MATERIALS AND METHODS

Case Report

In 1989, a 29-year-old Italian male was evaluated for liver disease, presumed to be secondary to iron overload. The son of

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nonconsanguineous parents from Calabria, he had been diagnosed with homozygous β thalassemia and had undergone a splenectomy in 1964 at age 3 years. In 1980, he underwent cholecystectomy after repeated episodes of abdominal pain associated with radiologic demonstration of gallstones; RBC transfusions were administered before both operations. He had never received transfusions since then. In 1980, observation of abnormal levels of AST and ALT and an increased serum ferritin concentration prompted initiation of a program of nightly SC deferoxamine therapy; the patient complied with this erratically and discontinued it after 6 months, although the risk of potential iron-related morbidity had been explained to him. He administered no iron-chelating therapy in the next 10 years.

In 1990, he sought medical attention because of worsening fatigue. Physical examination showed him to be deeply pigmented and icteric, with mild muscle wasting. The liver was enlarged and firm. Height was at the fiftieth percentile for an adult male; external genitalia were fully developed. Laboratory hematologic studies were consistent with previous assessments of steady-state hemoglobin concentrations of 75 to 85 g/L, increased total WBC and platelet counts, and a hemoglobin electrophoresis demonstrating 98% hemoglobin F and 2% hemoglobin A₂, consistent with β₀ thalassemia. Fasting glucose, testosterone, TSH, thyroxine, calcium, phosphate and PTH measurements were within normal limits. Serum AST and ALT levels were increased at 139 and 98 U/L, respectively (normal for both < 40 U/L); prothrombin time, PTT, total protein, and albumin were within normal limits. Transferrin saturation was 95% to 100%; serum ferritin concentration was 2,000 μg/L (normal < 300 μg/L). Analysis of DNA from peripheral blood lymphocytes (PBL) demonstrated homozygosity for the codon 39 C-T mutation responsible for β₀ thalassemia, a normal α-globin gene cluster, and homozygosity for the presence of the C to T substitution at position 158 to the Gv gene.21 Resting electrocardiogram demonstrated atrial bigeminy; radioisotope angiogram of the heart showed a normal resting ejection fraction (EF), but no increase in EF with exercise, and mild abnormalities of diastolic function.

The evidence for and risk of progression of iron-related organ damage was explained in full to the patient, who nevertheless declined to resume SC deferoxamine or to begin a program of continuous intravenous (IV) deferoxamine infusions; 9 months later, he was offered enrollment in the Canadian trial of the oral iron chelator L1. At this time, serum ferritin concentration was increased at 2,174 μg/L; transferrin saturation was increased at 100% (normal < 30%); serum AST and ALT were increased at 126 and 94 U/L, respectively; and serum triglyceride level was increased at 3.74 mmol/L (normal 0.34 to 1.58 mmol/L). Antinuclear antibody and rheumatoid factor were negative. ACTh stimulation testing demonstrated normal baseline ACTh and cortisol, normal cortisol levels 30 and 60 minutes after ACTh stimulation, and two normal urinary free cortisol quantitations. Baseline 24-hour urinary iron excretion was 2.9 mg. After undergoing baseline MRI of the liver and heart and percutaneous liver biopsy, the patient began therapy with L1 at 75 mg/kg body weight per day, administered in three divided doses at 8-hour intervals in the fasting state.

**Methods**

This study was approved by The Hospital for Sick Children’s Human Subject Review Committee and the Health Protection Branch, Health and Welfare Canada (File No. 9427-11117-41C, HPB, Ottawa, Canada). Written informed consent was obtained from the patient. L1 was synthesized according to previously published methods by the direct reaction in aqueous solution of methylamine and maltol, as described previously.20,24

**Hepatic iron quantitation.** Percutaneous liver biopsy specimens were divided with a wooden splint and weighed immediately to determine fresh weight. One portion was then overdried (105°C, 8 hours), reweighed and wet-ashed with hot concentrated HNO₃ (Suprapur; Merck, Darmstadt) in a screw-cap Teflon vessel, for analysis of total iron by Zeeman-corrected electrothermal atomic absorption (Varian SpectrAA-300; Techtron Pty, Ltd, Malgrave, Australia). The other portion was homogenized with a plastic pestle in a microcentrifuge tube in 0.50 mL cold 0.25 mol/L sucrose/3 mmol/L imidazole HCl, pH 7.2. Total iron recovered in the homogenate, as compared with that determined in the dried sample, was 98% to 98%. After sonication (Branson Sonifier W-350; power setting 3, 15 pulses at 20% duty cycle), a 100-mL aliquot was applied to a carboxymethylcellulose column and eluted in stepwise fashion according to the method of Selden and Peters.22 Fractions eluting in the order of transferrin, ferritin, hemoproteins, and hemosiderin were analyzed for iron by atomic absorption.

**Histologic examination of the liver.** Cores of liver tissue were fixed in 10% buffered formalin, embedded in paraffin, and stained with Prussian blue for iron. Tissue was fixed in universal fixative (2% glutaraldehyde plus 4% formaldehyde) and processed using standard methods for transmission electron microscopy using a Phillips 400 electron microscope.

**Magnetic resonance imaging.** The patient underwent magnetic resonance imaging (MRI) in a 1.5T superconducting magnet using a spin-echo technique.26 The patient was positioned supine with simultaneous cardiac and respiratory gating. Initial coronal and transverse images were taken to determine the Euler angles to image the heart, liver, and peripheral muscle simultaneously along the cardiac short axis. T₂-weighted spin echo was performed using cardiac gated sequence consisting of R-R interval/2 TR (time of pulse repetition)/TE (time to echo)/number of acquisitions] spanning from the apex to the base of the heart at 1-cm center-to-center separations and 7-mm slice thickness.

A midheart slice position was then selected as the reference for the T₂-weighted spin echo acquisition. This was a gated multiecho sequence consisting of [x R-R/20-40-60-80/2 (TR/TE)/number of acquisitions] spanning the apex to the base of the heart and the shoulder muscles.

**Image analysis.** The signal intensities from the cardiac septum, posterior wall, liver, and peripheral muscle were calculated from identically positioned slices of the T₂-weighted spin echo and gradient echo images. A 0.25-cm² of the region of interest was applied over these organs, and an average signal intensity was obtained. The ratio of signal intensities between the heart and peripheral muscle and the liver and peripheral muscle were used subsequently for comparison. Image-derived T₂ values were obtained by fitting the signal intensities from each echo of the multiecho sequence to an exponential function:

\[ SI = S₀ \cdot e^{-\frac{TE}{T₂}} \]

where SI is signal intensity, S₀ is nondecayed signal intensity, and TE is time to echo. The signal intensities were first corrected for variation in image field size and receiver attenuation when applicable.27

**RESULTS**

**Urinary iron excretion.** Urinary iron excretion during the first 6 months of L1 therapy varied between 11.3 and 109 mg iron excreted in 24 hours, with a mean excretion of 53.3 mg, equal to a mean urinary iron of 0.77 mg/kg body weight per day. During the next 6 months of L1 administration, the patient’s urinary iron excretion varied between 4.6 and 39.6 mg in a 24-hour period, with a mean excretion of 18.3 mg.
mg/day, or 0.27 mg/kg/day. The decrease in urinary iron excretion is shown in Fig 1A.

**Serum ferritin concentration.** Serum ferritin concentration decreased steadily during 6 months of L1 therapy, from a prechelation level of 2,174 to 251 µg/L after 6 months of L1 therapy. The decreases in urinary iron excretion and serum ferritin concentration are shown in Fig 1B.

**MRI of Cardiac and Hepatic Iron**

**Image interpretation.** On the MRI before the start of L1 therapy (Fig 2A), the initial T1-weighted spin echo image in the coronal view identified an enlarged liver, with a vertical span of 18 cm in the right midsagittal line. The most notable feature was complete absence of signal from the liver, compatible with significant field inhomogeneity created by iron deposition. This corresponds to heavy iron deposition in the liver. Similarly, on the cardiac short axis view (Fig 3A) before initiation of chelation therapy, the liver signal was severely depressed. The myocardial signal also was depressed in both views, corresponding to iron deposition in the cardiac septum and posterior wall of the heart.

The MR image after 9 months of L1 therapy demonstrated a distinct improvement in signal intensity from the liver region by T1-weighted spin echo imaging, observed in both the coronal (Fig 2B) and cardiac short axis (Fig 3B) views. Cardiac muscle signal intensity also improved. All these are indications that the iron content in the organs imaged was reduced as compared with that of the previous study.

**Quantitative MR image-derived parameters.** The signal intensity ratio between the liver and the peripheral muscle was less than 0.01 (normal > 1.0) before therapy with L1, owing to the very low-to-absent signal intensity in the liver. After L1 therapy, the same signal ratio improved to 0.72. Before therapy, image-derived T2 relaxation time for the liver was also very short at 9.2 ms (normal 32 to 36 ms). After completion of 9 months of L1 therapy, the T2 relaxation values increased to 17.4 ms. This near doubling of relaxation time can be attributed to a reduction of liver iron content, owing to a fixed relationship between iron concentration and 1/T2.

The myocardial/peripheral muscle ratio was 0.90 before L1 therapy (normal > 1.3). This improved to nearly normal values of 0.96 after 9 months of L1 therapy. Myocardial T2 values before therapy was 33 ms, improving to 35 ms after 9 months of therapy. The less severe iron deposition in the myocardium before chelation therapy rendered the improvement in the signal from this organ less dramatic than that observed in the liver.

**Hepatic Iron Stain and Histology**

Histologic examination of the liver biopsy specimen taken before the start of treatment with L1 (Figs 4 and 5) showed more than 90% of hepatocytes strongly positive for cytoplasmic iron by Prussian blue staining, with iron also abundant in portal macrophages. The liver architecture was altered with moderate portal fibrosis. Light microscopic examination of the liver biopsy specimen taken after 9 months of L1 therapy showed approximately 30% of hepatocytes staining positively for iron, with a marked reduction in the amount of iron within each hepatocyte (Figs 4 and 5B).

**Hepatic Iron Concentration of Liver Biopsy Specimens**

Hepatic iron concentration after 9 months of L1 therapy was 1.9 mg/g dry weight of liver tissue, substantially reduced from the value of 14.6 mg/g dry weight of liver immediately before initiation of chelation therapy. The pattern of iron loading was unchanged, however. Before therapy, 83% of hepatic iron was associated with hemosiderin, 16% was ferritin, and a very small portion was observed in other fractions. After 9 months of chelation, 85% of hepatic iron was still associated with hemosiderin and most of the remainder was associated with ferritin.

**Cardiac Function**

Radionuclide angiography before L1 therapy showed an abnormal resting electrocardiogram with atrial bigeminy,
reverting to normal sinus rhythm during exercise. The left ventricular EF (LVEF) was 61% at rest, with no increase at peak exercise. Mild right ventricular dilatation, and mild abnormalities in diastolic function (peak filling rate, time to peak filling, and atrial contribution) were observed.

After 9 months of L1 therapy, the resting LVEF remained normal (57%), with a normal increase (to 63%) now observed at peak exercise. The abnormalities of right ventricular dilatation and abnormalities in diastolic function did not change during L1 therapy, except for a slight improvement in atrial contribution (14% after L1 therapy, as compared with 17% before therapy).

Safety of L1. The patient was reviewed in the clinic weekly. He tolerated L1 well, and reported no joint pain or
Fig 3. MRI of cardiac iron before L1 therapy (A). Initial T₁-weighted spin echo MRI in the cardiac short axis view demonstrated a depressed myocardial signal. (B) After 9-month L1 therapy, T₁-weighted spin echo MRI in the cardiac short axis view demonstrated improvement, indicating that the iron content was reduced as compared with that in the previous study.

No change in hematologic or biochemical parameters was observed during 9 months of therapy. ACTH testing repeated 4 months after baseline showed no change in the values observed before L1 administration. Antinuclear antibody and rheumatoid factor have remained negative.

DISCUSSION

Thousands of Third World patients with major thalassemia syndromes, including those who develop iron-related toxicity in association with gastrointestinal hyperabsorption of iron, exhibit serious iron-related morbidity and mortality. For these patients, an orally active iron-
chelating agent would provide life-saving chelation therapy. For patients of the developed world, freedom from nightly, often painful infusions would represent a welcome normalization of lifestyle. An orally available iron-chelating agent has thus been sought actively for more than a decade.

The bidentate hydroxypyridones show considerable potential as orally available iron chelators. Administration of 1,2-dimethyl-3-hydroxypyrid-4-one or L1 resulted in urinary iron excretion comparable to that obtained with SC deferoxamine in thalassemic patients. Although conflicting observations of acute toxicity have been reported with use of L1 in animals,\textsuperscript{31,32} administration of the drug has been associated with infrequent but serious complications in human trials, including agranulocytosis and thrombocytopenia which reversed after withdrawal of L1,\textsuperscript{33} and in long-term clinical trials hematologic parameters will have to be monitored carefully to determine the risk of agranulocytosis and whether the drug is acceptably safe for clinical use.

We report normalization of serum ferritin concentration and reduction in tissue iron stores in a patient with thalassemia intermedia maintained on L1 for 9 months. Like this patient, individuals with thalassemia intermedia

\textbf{Fig 4.} Hepatic iron stain and histology at low and high power (A) before L1 therapy. Histology of the liver showed more than 90\% of hepatocytes strongly positive for cytoplasmic iron by Prussian blue staining. Iron was also abundant in groups of portal macrophages; liver architecture was altered with moderate portal fibrosis. (B) After 9 months of L1 therapy, light and electron microscopic examination of the liver showed approximately 30\% of hepatocytes staining positively for iron, with a marked reduction in the amount of iron in each hepatocyte.
who maintain a high peripheral hemoglobin concentration without regular RBC transfusions may show variable degrees of excessive food iron absorption which may lead to serious iron loading by middle life. These patients represent a major public health problem in populations in which thalassemia is common. Although the degree of iron overload is less in such patients than in patients with transfusion-dependent thalassemia, our patient had evidence of iron-related organ toxicity with early hepatic cirrhosis and mild cardiac diastolic dysfunction with lack of systolic increase with exercise. L1 therapy has reduced his tissue iron load substantially, as demonstrated by reduction in transferrin saturation and serum ferritin concentration, improvement in liver function, and increase in LVEF with exercise after 9-month L1 therapy, improvement in MRI of cardiac and hepatic iron, and reduction in hepatic iron concentration. Continued monitoring of urinary iron excretion and trends in serum ferritin concentration in response to a reduced daily dose of L1 will be necessary in this patient to prevent further iron-related organ damage.

Our report is the first to present evidence in humans for L1-induced reduction of iron in the liver and heart, the organs involved in the most serious iron-related morbidity and mortality. Although the safety and efficacy of long-term use of L1 has been the subject of much debate, governmental approval for clinical trials of L1 is now forthcoming in several countries, including the United States, and should be supported by the encouraging result observed in our patient.

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