Lack of progressive hepatic fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent beta-thalassemia

Ian R. Wanless, George Sweeney, Amar P. Dhillon, Maria Guido, Antonio Piga, Renzo Galanello, M. Rita Gamberini, Elias Schwartz, and Alan R. Cohen

Patients with thalassemia major require lifelong chelation therapy to prevent iron-induced organ damage. The orally active chelator deferiprone has been proposed as an alternative for patients unable or unwilling to use deferoxamine. One report has concluded that deferiprone may worsen hepatic fibrosis in patients with thalassemia, whereas others have found no detrimental effect. A panel of 3 pathologists evaluated 112 coded liver biopsies obtained from 56 patients before and after deferiprone therapy. Fibrosis was scored with the Laennec and Ishak systems. The mean interval between liver biopsies was 3.1 years (range, 1.2-4.9 years). In 11 patients seronegative for hepatitis C, fibrosis scores before and after therapy were 1.12 ± 1.07 and 0.97 ± 0.84 (P = .42) with the use of the Ishak system, and 0.71 ± 0.65 and 0.70 ± 0.53 (P = .91) with the Laennec system. Among 45 patients seropositive for hepatitis C, fibrosis scores before and after therapy were 1.91 ± 1.13 and 2.04 ± 1.30 (P = .43) with the use of the Ishak system and 1.26 ± 0.73 and 1.35 ± 0.90 (P = .41) with the Laennec system. When the data set was limited to biopsies that each contained 6 or more portal tracts (31 patients), analysis still showed no significant change in fibrosis with time. With the use of the Laennec system, the fibrosis score did not increase by more than one level in any patients without hepatitis C; it increased by more than one level in 1 patient with hepatitis C; and it did not decrease by more than one level in any of the 56 patients. This analysis of the largest collection of liver biopsies reported to date in patients receiving deferiprone demonstrates no evidence of deferiprone-induced progression of hepatic fibrosis during long-term therapy. (Blood. 2002; 100:1566-1569)

Introduction

Patients with beta-thalassemia major develop iron overload related to the effects of chronic anemia and repeated transfusions of red blood cells. Iron overload in the liver is associated with the development of fibrosis that may progress to cirrhosis. The development of fibrosis is exacerbated by chronic viral hepatitis, especially hepatitis C, which is present in the majority of patients who received regular blood transfusions prior to the introduction of routine screening for this virus. Chronic iron chelation therapy with deferoxamine (DFO) can prevent or ameliorate organ damage. To adequately control the body iron load, DFO must be given 5 to 7 days per week by a prolonged subcutaneous infusion. Because of poor compliance, adequate therapy may be achieved in only 50% of thalassemia patients.

Deferiprone is an orally active iron chelator and superoxide radical scavenger that is presently the only alternative to DFO. At a dose of 75 mg/kg/d, deferiprone reduces or maintains serum ferritin and hepatic iron concentrations in the majority of patients with thalassemia; although levels may remain unacceptable high in some patients. Adequacy of therapy in poor responders may be improved by higher doses or by alternating therapy with DFO.

Deferiprone has been associated with complications, including neutropenia, agranulocytosis, arthropathy, gastrointestinal symptoms, zinc deficiency, and increased transaminases. These complications are usually minor, but some may require cessation of therapy. One study reported progressive hepatic fibrosis in 5 of 14 patients treated with deferiprone for a median of 2.3 years. Four of the patients with progression were anti–hepatitis C virus–positive (anti-HCV). A review of the same biopsies did not confirm the progression of fibrosis. Although several subsequent long-term studies (12 to 99 months) have found no progression of hepatic fibrosis attributable to deferiprone, the small number of patients and the absence of pretreatment biopsies in some of the studies limit the strength of these findings. In an attempt to resolve the question of deferiprone-related hepatotoxicity, we have examined 112 liver biopsies from 56 patients treated with deferiprone for a median of 3.5 years, looking for evidence of toxicity, particularly the development of hepatic fibrosis.

From the Department of Laboratory Medicine and Pathobiology, Toronto General Hospital and University of Toronto, ON, Canada; McMaster University, Hamilton, ON, Canada; Department of Histopathology, Royal Free and University College Medical School, London, United Kingdom; Dipartimento di Anatomia Patologica e Università degli Studi di Padova, Padua, Italy; Department of Pediatrics, Università degli Studi di Torino, Turin, Italy; Instituto di Clinica e Biologia Dell’Ela-Evolutive, Cagliari, Italy; Divisione Pediatrica, Azienda Ospedaliero di Ferrara, Italy; and the Department of Pediatrics, Jefferson Medical College, and the Department of Pediatrics, Children’s Hospital of Philadelphia, PA.


Supported solely by grants from the Fondazione Italiana Thalassemia and Thalassemia International Federation.

This work was reported, in part, at the 42nd annual meeting of the American Society of Hematology, San Francisco, CA, December 4, 2000.

Reprints: Ian R. Wanless, Department of Laboratory Medicine and Pathobiology, Toronto General Hospital, 200 Elizabeth St, Toronto, ON, Canada; e-mail: ian.wanless@utoronto.ca.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2002 by The American Society of Hematology
**Patients and methods**

From 1994 to 1995, 187 patients with transfusion-dependent beta-thalassemia from 3 centers in Italy and 1 center in the United States enrolled in an open-label uncontrolled trial designed to evaluate the safety and efficacy of deferiprone.13 Entry into this study required either a serum ferritin level greater than 2000 µg/L or liver iron content exceeding 4 mg/g dry weight. From 58 patients at the Italian centers, liver biopsies were obtained fewer than 6 months prior to initiating deferiprone therapy. The reasons for obtaining a biopsy varied. At 2 of the centers, biopsies were obtained to assess liver iron content in those patients who did not have serum ferritin greater than 2000 µL/L or as incidental biopsies at surgery. At the Turin center, all study patients underwent biopsies before entry. No patients from Philadelphia underwent biopsy.

In 1996, 160 of the 162 patients who had completed the first year of study (LA-02) were enrolled in a long-term follow-up study, designated LA-06, with the same dosage schedule. In 1997, the issue of possible hepatotoxicity due to deferiprone was raised.14 At this time, the focus of the study was expanded to include liver morphology. Therefore, the 58 patients with initial biopsies were asked to undergo rebiopsy. After informed consent, biopsies were obtained from 56 of these patients while they were still being treated with deferiprone. The total cohort treated for 4 years with deferiprone at the original dosage and with no exposure to other chelators consisted of 84 patients. Ethical approval of the original and amended protocols was obtained at all study sites.

All patients in this study had received numerous blood transfusions and DFO. Deferoxamine was discontinued before deferiprone therapy was begun. Study patients were given 25 mg/kg oral deferiprone 3 times daily. Compliance was in excess of 95% on the basis of pill counts or a microprocessor-equipped dispenser. HCV status was tested within a few months of entry and at the time of final biopsies by means of the most advanced commercially available kits. Forty-four patients were anti-HCV positive at the time of entry and one more patient was positive at the time of the final biopsy. The remaining 11 patients were seronegative for HCV at entry and end of the study. No patients were treated with interferon or another antiviral therapy. All patients were negative for HIV. Serum alanine aminotransferase (ALT) measurements were available at entry and at the time of the second biopsy. Wedge biopsies obtained during splenectomy were used in 7 instances. All other biopsies were obtained percutaneously.

**Conduct of the biopsy review**

In accordance with standard practice, an independent International Safety Monitoring Committee has monitored the multicenter study of deferiprone. In early 2000, this committee commissioned one of us (G.S.) to coordinate a review of liver biopsies from the LA-06 study to search for evidence of chronic liver disease. From 1994 to 1995, 187 patients with transfusion-dependent beta-thalassemia from 3 centers in Italy and 1 center in the United States enrolled in an open-label uncontrolled trial designed to evaluate the safety and efficacy of deferiprone.13 Entry into this study required either a serum ferritin level greater than 2000 µg/L or liver iron content exceeding 4 mg/g dry weight. From 58 patients at the Italian centers, liver biopsies were obtained fewer than 6 months prior to initiating deferiprone therapy. The reasons for obtaining a biopsy varied. At 2 of the centers, biopsies were obtained to assess liver iron content in those patients who did not have serum ferritin greater than 2000 µL/L or as incidental biopsies at surgery. At the Turin center, all study patients underwent biopsies before entry. No patients from Philadelphia underwent biopsy.

In 1996, 160 of the 162 patients who had completed the first year of study (LA-02) were enrolled in a long-term follow-up study, designated LA-06, with the same dosage schedule. In 1997, the issue of possible hepatotoxicity due to deferiprone was raised.14 At this time, the focus of the study was expanded to include liver morphology. Therefore, the 58 patients with initial biopsies were asked to undergo rebiopsy. After informed consent, biopsies were obtained from 56 of these patients while they were still being treated with deferiprone. The total cohort treated for 4 years with deferiprone at the original dosage and with no exposure to other chelators consisted of 84 patients. Ethical approval of the original and amended protocols was obtained at all study sites.

All patients in this study had received numerous blood transfusions and DFO. Deferoxamine was discontinued before deferiprone therapy was begun. Study patients were given 25 mg/kg oral deferiprone 3 times daily. Compliance was in excess of 95% on the basis of pill counts or a microprocessor-equipped dispenser. HCV status was tested within a few months of entry and at the time of final biopsies by means of the most advanced commercially available kits. Forty-four patients were anti-HCV positive at the time of entry and one more patient was positive at the time of the final biopsy. The remaining 11 patients were seronegative for HCV at entry and end of the study. No patients were treated with interferon or another antiviral therapy. All patients were negative for HIV. Serum alanine aminotransferase (ALT) measurements were available at entry and at the time of the second biopsy. Wedge biopsies obtained during splenectomy were used in 7 instances. All other biopsies were obtained percutaneously.

**Histologic grading systems**

Chronic injury in the liver is assessed as a combination of fibrosis and architectural deformation, commonly referred to simply as fibrosis. For fibrosis, 2 grading systems were used to facilitate comparison with other published work. For the Ishak system,22 fibrosis was graded 0 to 6. For the Laennec system,23 a minor modification of the METAVIR system,24 fibrosis was scored in 7 grades, with 0 indicating no definite fibrosis; 1, minimal fibrosis (no septa or rare thin septum; may have portal expansion or mild sinusoidal fibrosis); 2, mild fibrosis (occasional thin septa); 3, moderate fibrosis (moderate thin septa; up to incomplete cirrhosis); 4A, mild cirrhosis, definite or probable; 4B, moderate cirrhosis (at least 2 broad septa); 4C, severe cirrhosis (at least one very broad septum or many minute nodules). Necroinflammatory activity was assessed by means of Ishak’s hepatic activity index.22

**Results**

Clinical features of the patients are summarized in Table 1. The patients were treated with deferiprone for a mean of 3.1 years (median 3.5 years), with 32 of these patients receiving deferiprone for 3 or more years.

The hepatic fibrosis scores are shown in Table 2. In the entry biopsies, fibrosis was mild or absent in the majority of patients and was more severe in HCV**-** patients. The final biopsies showed no significant differences from the entry biopsies, whether HCV**-** or HCV**+.** Because the fibrosis scores are ordered categories rather than continuous variables, analysis was also performed by means of the nonparametric Wilcoxon rank sum test. This test also showed no significant change in scores with time. Because the pathologists varied slightly in their choice of thresholds for the scores, the data for each pathologist were analyzed separately to ensure that averaging the data did not affect the results. However, the data from each of the 3 pathologists showed the same conclusion of no significant change with time (data not shown).

<table>
<thead>
<tr>
<th>Table 1. Summary of clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All patients</strong></td>
</tr>
<tr>
<td>Patients, no.</td>
</tr>
<tr>
<td>Female-male ratio, no.</td>
</tr>
<tr>
<td>Age at final biopsy, y, mean (range)</td>
</tr>
<tr>
<td>Duration between biopsies, y, mean (range)</td>
</tr>
<tr>
<td>Duration of deferiprone therapy before final biopsy, y, mean (range)</td>
</tr>
</tbody>
</table>

*One patient became HCV**+** after the first biopsy and for purposes of analysis is considered HCV**-**.
†The biopsies were obtained prior to onset of therapy. Data are presented as mean ± SD.
Table 3. Interval changes in hepatic fibrosis scores using various criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>HCV&lt;sup&gt;-&lt;/sup&gt; (n = 11)</th>
<th>HCV&lt;sup&gt;+&lt;/sup&gt; (n = 45)</th>
<th>P&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Better</td>
<td>Worse</td>
<td>Better</td>
</tr>
<tr>
<td>Laennec (1 or more grade change)</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Laennec (greater than 1 grade change)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ishak (1 or more grade change)</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Ishak (greater than 1 grade change)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ishak (1 or more grade change, weighted)†</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

*Proportion with worse fibrosis HCV<sup>-</sup> versus HCV<sup>+</sup> (Fisher exact test, 2 sided).
†Interval changes of 1-2, 3-4, and 5-6 ignored, as employed in Olivieri et al.<sup>10</sup>
Earlier studies that evaluated sequential liver biopsies in patients with thalassemia major treated with subcutaneous DFO reported progression of fibrosis in approximately 30% of the patients. However, information was available at that time regarding the HCV status of the patients. Other studies in patients treated with deferasirox have not observed drug-induced progression of liver fibrosis associated with the use of this agent and suggested that the increase in fibrosis observed in some patients was most likely related to hepatitis C infection and/or to the hepatic iron load.2,14-19 Our finding of no progression of fibrosis while on deferoxamine is in agreement with these smaller studies. The differing conclusion of Olivieri et al10 may be related to the small number of patients in their study and to the difficulties of grading fibrosis in the small biopsies.14,28,29 The treatment period in our study was longer than that in the Olivieri study, making it likely that our study would be more sensitive for the discovery of drug-induced fibrosis if it had occurred.

An inevitable problem in a histological study is the variable size and technical quality of the biopsies. This problem was addressed by requiring the 3 pathologists to document problems with specimen quality. In the overall analysis, all biopsies contained at least 2 portal tracts so that no patients were excluded. The results were unchanged when a more conservative limit of at least 6 portal tracts was adopted or when otherwise deficient biopsies were excluded.

Aminotransferase elevation is known to occur in some patients treated with deferoxamine. A small percentage of patients in the original 1-year trial (LA-02) were withdrawn because of ALT elevation. The course of liver function and histologic structure after continued deferoxamine therapy in the face of severe elevations of ALT has not been studied to our knowledge.

Acknowledgments

The International Safety Monitoring Committee that commissioned this study is an independent body of scientists including Drs Elias Schwartz (chair, US), Samuel Charache (US), Chaim Hershko (Israel), Stuart MacLeod (Canada), and Giuseppe Masera (Italy). This committee was convened by Apotex Inc in accordance with section 5.5.2 of the International Conference on Harmonization Good Clinical Practice guidelines. The members of the Safety Monitoring Committee and the authors have no financial interest in the development of deferoxamine.

References

Lack of progressive hepatic fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent beta-thalassemia

Ian R. Wanless, George Sweeney, Amar P. Dhillon, Maria Guido, Antonio Piga, Renzo Galanello, M. Rita Gamberini, Elias Schwartz and Alan R. Cohen

Updated information and services can be found at:
http://www.bloodjournal.org/content/100/5/1566.full.html

Articles on similar topics can be found in the following Blood collections
  Clinical Trials and Observations (4542 articles)
  Red Cells (1159 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml