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. 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.

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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.\textsuperscript{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 μg/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.\textsuperscript{10} 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 other 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 fibrosis. Three pathologists were recruited to perform a blinded assessment of the biopsies.

Two biopsies from each of 56 patients were received at McMaster University, where each biopsy was assigned a random code and all other identifying marks were covered. Each pathologist received a different set of random numbers from the statistician that established the sequence of biopsy review. The study coordinator held the randomized code until the pathologists had completed their assessments blinded to patient identity, biopsy accession date, and clinical information. Hematoxylin-and-eosin–stained slides were available for all biopsies. A connective tissue stain (Masson trichrome or van Gieson) was available for 103 of the 112 biopsies. While the stains available varied with the institutional source, the same stain was used for the biopsies of each patient in most instances. The mean number of portal tracts was 10.6 ± 7.2 (range, 2-39). Thirty-one biopsies from 25 patients had fewer than 6 portal tracts. After removal of these 31 biopsies and associated paired biopsies, the mean number of portal tracts was 11.2 ± 6.0 (range, 6-35); the mean biopsy length was 8.6 mm; and the mean number of fragments was 2.0.

The 3 pathologists met to discuss details of the grading systems before commencing independent scoring for fibrosis, necroinflammation, and tissue adequacy. To avoid introduction of bias, the biopsies were not reviewed for the purpose of reaching a consensus among the pathologists. For each of the biopsies, the individual pathologists’ scores on each parameter were averaged. Mean fibrosis scores from each pathologist are also reported separately, and interval changes were expressed according to various criteria. Improvement or worsening of fibrosis was considered present if at least 2 pathologists agreed on the direction of the change.

The SAS system (SAS Institute, Cary, NC) was used for statistical analysis. One-sample t tests\textsuperscript{16} on the difference of the averages was used to test the null hypothesis that there was no change in the parameters from the entry to the final biopsy. A Wilcoxon\textsuperscript{21} matched-pairs rank sum test was also used. The effect that hepatitis C status, sex, and clinical center had on the differences was determined by means of a one-way analysis of variance.\textsuperscript{20} Individual Kruskal-Wallis\textsuperscript{21} tests were also used. Two-tailed tests were used throughout. Parametric and nonparametric tests gave effectively identical results in all comparisons, and only the former are shown.

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,\textsuperscript{22} fibrosis was graded 0 to 6. For the Laennec system,\textsuperscript{23} a minor modification of the META VIR system,\textsuperscript{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.\textsuperscript{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 1. Summary of clinical features

<table>
<thead>
<tr>
<th>Feature</th>
<th>All patients</th>
<th>HCV− patients</th>
<th>HCV+ patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>56</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Female-male ratio, no.</td>
<td>21:35</td>
<td>5:6</td>
<td>16:29</td>
</tr>
<tr>
<td>Age at first biopsy, y, mean (range)</td>
<td>21.4 (12-39)</td>
<td>17.7</td>
<td>22.3</td>
</tr>
<tr>
<td>Duration between biopsies, y, mean (range)</td>
<td>3.3 ± 0.9 (12-4.9)</td>
<td>3.5 ± 0.9</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>Duration of deferiprone therapy before final biopsy, y, mean (range)</td>
<td>3.1 ± 1.0 (0.7-4.6)</td>
<td>3.4 ± 1.1</td>
<td>3.0 ± 1.0</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.
Analysis of interval changes of fibrosis score within pairs of biopsies demonstrated that changes were found in a minority of patients and, when present, were usually of only one grade (Table 3). For example, in patients without hepatitis C, Laennec scores did not increase or decrease by more than one level in any of the 11 patients. An increase of greater than one level was found in only 1 of 45 patients with hepatitis C. Worsening of fibrosis did not occur significantly more often in the HCV⁺ group. Improvement in fibrosis score occurred as often as worsening in both HCV⁺ and HCV⁻ patients. Histologic cirrhosis, as determined by at least 2 pathologists, was not present in any of the entry biopsies. Among the final biopsies, only one HCV⁺ patient had cirrhosis; that patient had moderate fibrosis on the entry biopsy.

All biopsies had at least 2 portal tracts. However, 25 biopsies from 22 patients were flagged by at least 2 pathologists during the scoring process as being inadequate for assessment of fibrosis. When these 22 patients were removed from the data set, the prevalence of fibrosis was unchanged and there was still no statistically significant interval change (Table 2). When the 25 patients having at least one biopsy with fewer than 6 portal tracts were removed from the data set, the results were unchanged.

Necroinflammation was more prevalent in HCV⁺ patients both at entry and in final biopsies (Table 4). This effect was reflected in higher serum ALT levels in the HCV⁺ patients (Table 4).

### Discussion

Iron chelation therapy with DFO can prevent or ameliorate iron-induced organ damage and prolong survival in patients with transfusion-dependent thalassemia. However, because of the cumbersome method of administration of DFO, a substantial proportion of patients do not comply with chelation therapy and are at risk of organ damage and early death. Deferiprone, an orally active iron chelator and superoxide radical scavenger, is presently the only alternative for patients unable or unwilling to use deferoxamine. Clinical studies have indicated that deferiprone has a favorable benefit-to-risk ratio in the treatment of iron overload in patients with transfusion-dependent thalassemia.

Subjects with thalassemia often develop liver fibrosis. This complication is known to depend particularly on the presence of viral hepatitis but also on hepatic iron concentration, the distribution of iron within the liver, and age. Thus, while iron chelation therapy with DFO can prevent or improve liver fibrosis in some patients, fibrosis may still occur because of inadequate chelation or chronic hepatitis C.

Olivieri et al. raised concerns about the possibility of hepatic fibrosis induced by deferiprone. The authors examined interval biopsies from 14 patients treated for a median of 2.3 years with deferiprone. Progression of fibrosis was described in 5 patients, 4 of whom had anti-HCV antibodies. No progression of fibrosis was seen in 12 subjects treated with DFO, 5 of whom were anti-HCV⁻.

In contrast, studies from several institutions as well as studies that include some patients from the present study have not found evidence of hepatic fibrosis induced by deferiprone.

The present study was undertaken retrospectively to address the issue of possible deferiprone-induced hepatic fibrosis as part of a larger overall study of the safety of the chelator in 187 subjects. This series of 112 liver biopsies from 56 patients represents the largest collection of biopsies yet reported from patients receiving deferiprone. Our histologic review does not reveal any evidence of deferiprone-induced hepatic fibrosis after a median of 3.5 years of therapy (mean, 3.1 years). Among the 11 HCV-seronegative subjects, the mean fibrosis score was unchanged. Among the 45 HCV-seropositive subjects, the small increase in fibrosis score was not statistically significant. As expected, the HCV-seropositive subjects had more necroinflammatory activity and higher ALT levels. No patient developed clinical portal hypertension during the trial.

There is no reason to believe that there was bias in selection of patients who were biopsied. The initial biopsies were obtained 2 to 3 years prior to the first suggestion that there might be drug hepatotoxicity. The various reasons that biopsies were performed do not suggest that the biopsied subgroup would differ from the whole group in the susceptibility to drug-induced hepatic fibrosis. The availability of subsequent biopsies in 56 of the 58 subjects who underwent initial biopsy also makes a selection bias unlikely.

In this study, the initial biopsy served as a control for each patient. We did not have a group of iron-overloaded patients untreated with deferiprone as further controls, but historical data place our findings in context. The anticipated increase in fibrosis in HCV⁺ patients without thalassemia has been estimated to be 0.1 to 0.133 fibrosis units per year. The observed progression rate in our series calculated by the Poyrand method is on the order of 0.03 fibrosis units per year, indicating that these patients did not show progression of fibrosis in excess of that expected for HCV⁺ individuals.

### Table 2. Hepatic fibrosis in paired biopsies from deferiprone-treated patients

<table>
<thead>
<tr>
<th>Criteria</th>
<th>HCV⁻ (n = 11)</th>
<th>HCV⁺ (n = 45)</th>
<th>P for HCV⁻ versus HCV⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry</td>
<td>Final</td>
<td>Entry</td>
<td>Final</td>
</tr>
<tr>
<td>Mean Laennec grade, all pathologists</td>
<td>0.71 ± 0.65</td>
<td>0.70 ± 0.53</td>
<td>0.91 (n = 11)</td>
</tr>
<tr>
<td>Mean Ishak grade, all pathologists</td>
<td>1.12 ± 1.07</td>
<td>0.97 ± 0.84</td>
<td>0.42 (n = 11)</td>
</tr>
<tr>
<td>Mean Ishak grade after removing &quot;inadequate&quot; biopsies</td>
<td>1.06 ± 1.29</td>
<td>1.11 ± 1.09</td>
<td>.74 (n = 6)</td>
</tr>
<tr>
<td>Mean Ishak grade after removing biopsies with fewer than 6 portal tracts</td>
<td>1.47 ± 1.15</td>
<td>1.53 ± 0.80</td>
<td>.75 (n = 5)</td>
</tr>
<tr>
<td>Percentage of patients with mean Ishak grade greater than 2</td>
<td>18.2</td>
<td>9.1</td>
<td>1.0 (n = 11)</td>
</tr>
</tbody>
</table>

Data are derived from the mean grade for the 3 pathologists. The t-test was used except in the last row, where Fisher exact test was used.

### Table 3. Interval changes in hepatic fibrosis scores using various criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>HCV⁻ (n = 11)</th>
<th>HCV⁺ (n = 45)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<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>1</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

*Proportion with worse fibrosis HCV⁻ versus HCV⁺ (Fisher exact test, 2 sided).
† Interval changes of 1-2, 3-4, and 5-6 ignored, as employed in Olivieri et al.¹⁰
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, no information was available at that time regarding the HCV status of the patients. Other studies in patients treated with deferoxamine 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. Our finding of no progression of fibrosis while on deferoxamine is in agreement with these smaller studies. The differing conclusion of Olivieri et al may be related to the small number of patients in their study and to the difficulties of grading fibrosis in the small biopsies. 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 grading of hepatic copper with specimen quality. In the overall analysis, all biopsies were graded. The results were unchanged when a more conservative limit of at least 6 portal tracts was adopted or when otherwise deficient biopsies were excluded. The 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


### Table 4. Hepatic necroinflammatory activity and serum alanine aminotransferase

<table>
<thead>
<tr>
<th></th>
<th>HCV- patients</th>
<th>HCV+ patients</th>
<th>P for HCV- versus HCV+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entry</td>
<td>Final</td>
<td>P</td>
</tr>
<tr>
<td>Mean Ishak activity grade, all pathologists*</td>
<td>0.42 ± 0.70</td>
<td>0.55 ± 0.58</td>
<td>.60 (n = 11)</td>
</tr>
<tr>
<td>Serum ALT (IU/L)†</td>
<td>16.5 ± 6.4</td>
<td>24.5 ± 13.3</td>
<td>.09</td>
</tr>
</tbody>
</table>

Notes: 
*Data are generated from the mean grade for the 3 pathologists. 
†Single determination closest to time of biopsy. Normal, below 40 IU/L.
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