

The New England Journal of Medicine

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VOLUME 339

AUGUST 13, 1998

NUMBER 7



LONG-TERM SAFETY AND EFFECTIVENESS OF IRON-CHELATION THERAPY WITH DEFERIPRONE FOR THALASSEMIA MAJOR

NANCY F. OLIVIERI, M.D., GARY M. BRITTENHAM, M.D., CHRISTINE E. MCLAREN, PH.D.,
DOUGLAS M. TEMPLETON, PH.D., M.D., ROSS G. CAMERON, M.D., PH.D., ROBERT A. MCCLELLAND, PH.D.,
ALASTAIR D. BURT, M.D., AND KENNETH A. FLEMING, D.PHIL., M.B., CH.B.

ABSTRACT

Background Deferiprone is an orally active iron-chelation agent that is being evaluated as a treatment for iron overload in thalassemia major. Studies in an animal model showed that prolonged treatment is associated with a decline in the effectiveness of deferiprone and exacerbation of hepatic fibrosis.

Methods Hepatic iron stores were determined yearly by chemical analysis of liver-biopsy specimens, magnetic susceptometry, or both. Three hepatopathologists who were unaware of the patients' clinical status, the time at which the specimens were obtained, and the iron content of the specimens examined 72 biopsy specimens from 19 patients treated with deferiprone for more than one year. For comparison, 48 liver-biopsy specimens obtained from 20 patients treated with parenteral deferoxamine for more than one year were similarly reviewed.

Results Of the 19 patients treated with deferiprone, 18 had received the drug continuously for a mean (\pm SE) of 4.6 ± 0.3 years. At the final analysis, 7 of the 18 had hepatic iron concentrations of at least $80 \mu\text{mol}$ per gram of liver, wet weight (the value above which there is an increased risk of cardiac disease and early death in patients with thalassemia major). Of 19 patients in whom multiple biopsies were performed over a period of more than one year, 14 could be evaluated for progression of hepatic fibrosis; of the 20 deferoxamine-treated patients, 12 could be evaluated for progression. Five deferiprone-treated patients had progression of fibrosis, as compared with none of those given deferoxamine ($P=0.04$). By the life-table method, we estimated that the median time to progression of fibrosis was 3.2 years in deferiprone-treated patients. After adjustment for the initial hepatic iron concentration, the estimated odds of progression of fibrosis increased by a factor of 5.8 (95 percent confidence interval, 1.1 to 29.6) with each additional year of deferiprone treatment.

Conclusions Deferiprone does not adequately control body iron burden in patients with thalassemia and may worsen hepatic fibrosis. (N Engl J Med 1998;339:417-23.)

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TRANSFUSIONS and iron-chelation therapy have dramatically improved the lives of patients with thalassemia major.¹ Transfusions can prevent death and promote normal development, but the iron in the transfused red cells accumulates and eventually damages the liver, heart, and other organs. Deferoxamine, the only iron-chelating agent approved for clinical use, prolongs survival and ameliorates iron-induced organ damage.^{2,3} Unfortunately, to be effective, treatment with deferoxamine requires prolonged parenteral infusion, making compliance difficult. Considerable effort has been devoted to finding alternative treatments.⁴ One candidate, deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one, or LI), was initially evaluated with an indirect indicator of therapeutic effectiveness — the serum ferritin concentration.⁵⁻¹¹ Subsequently, a direct quantitative assessment of body iron burden demonstrated a favorable effect of deferiprone on iron balance.¹² Recognized adverse effects of deferiprone include embryotoxicity, teratogenicity, arthritis, severe neutropenia, and agranulocytosis. Recently, concern about long-term treatment with deferiprone was aroused by studies of an animal model of iron overload¹³ in which long-term treatment with a closely related compound, 1,2-diethyl-3-hydroxypyridin-4-one, was associated with a loss of effectiveness and an exacerbation of hepatic fibrosis.

From the Departments of Medicine and Pediatrics (N.F.O.), Laboratory Medicine and Pathobiology (D.M.T., R.G.C.), and Chemistry (R.A.M.), University of Toronto, Toronto; the Departments of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York (G.M.B.); the Division of Epidemiology, Department of Medicine, University of California, Irvine (C.E.M.); the University Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom (A.D.B.); and the Department of Pathology, Nuffield Department of Pathology and Bacteriology, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom (K.A.F.). Address reprint requests to Dr. Olivieri at the Hemoglobinopathy Program, Hospital for Sick Children, 555 University Ave., Toronto, ON M5G 1X8, Canada.

To determine whether the effects of deferiprone are sustained during long-term therapy, we measured hepatic iron during continued treatment of patients in whom body iron had been measured during short-term therapy.¹² To assess whether long-term therapy was associated with progression of hepatic fibrosis, a panel of hepatopathologists evaluated the liver-biopsy specimens obtained during this trial.

METHODS

Patients

Of 21 previously studied patients who received deferiprone for a mean (\pm SE) of 3.1 ± 0.3 years,¹² 19 continued to receive deferiprone at a dose of 75 mg per kilogram of body weight per day, while undergoing repeated biopsies for hepatic iron measurements. Long-term effectiveness could be evaluated in 18 patients, who had received the drug continuously for 4.6 ± 0.3 years; 1 patient had stopped taking deferiprone shortly after the previous analysis, but the results of biopsies up to the discontinuation of therapy are included in the histologic analysis. Beginning in year 3 of the seven-year study, hepatic iron was measured by magnetic susceptometry *in vivo*.¹⁴ Because this technique does not provide histologic information, the follow-up period for effectiveness (range, 2 to 7 years) was longer than that for histologic analysis (range, 2 to 6 years).

Patients received regular transfusions. The objective of transfusion was to maintain the hemoglobin concentration above 9.5 g per deciliter. From November 1989 to November 1993, deferiprone was synthesized at the University of Toronto and encapsulated by NovaPharm Pharmaceuticals (Toronto). After November 1993, Apotex (Weston, Ont., Canada) supplied deferiprone tablets. The equivalence of the two formulations was not evaluated.

Body iron was evaluated in tissue obtained at biopsy, as described previously, and by magnetic susceptometry (Biomagnetic Technologies, San Diego, Calif.).^{14,15} The magnetic measurements have been validated previously.¹⁴ We converted the concentration of iron in dried samples to a wet weight, assuming a liver water content of 70 percent; chemical and magnetic values were used interchangeably.¹²

In the original trial design, each biopsy specimen, obtained primarily to monitor therapeutic effectiveness, was histologically reviewed, but serial biopsy specimens were not prospectively compared. Concern about hepatotoxicity^{13,16} prompted a retrospective review of these results and of those from a comparison group of 20 deferoxamine-treated patients. The comparison group included all patients eight years of age or older for whom the results of two or more biopsies performed one year apart during continuous deferoxamine treatment were available.

Efficacy Monitoring

A hepatic iron concentration of less than 80 μ mol per gram of liver, wet weight, was considered to indicate effective iron-chelation therapy, and a concentration of 80 μ mol or more per gram of liver, wet weight, was considered to indicate ineffective therapy.¹² These criteria, derived from a long-term trial in deferoxamine-treated patients,¹⁷ were used to evaluate the short-term effectiveness of deferiprone in our previous study¹² and were applied in an identical manner in the present long-term study. Similarly, a serum ferritin concentration of less than 2500 μ g per liter was considered to indicate effective iron-chelation therapy, and higher values ineffective therapy.^{12,18}

Histologic Evaluation

An independent initial review of the biopsy specimens was carried out by the two study investigators who are hepatopathologists and a consultant. Before this review, the 72 biopsy slides were randomly arranged and each slide was assigned a unique number. Each pathologist graded the findings according to the system summarized in Table 1.¹⁹ Each was unaware of the patients' clin-

TABLE 1. SYSTEM OF EVALUATING HEPATIC-BIOPSY SPECIMENS FOR ARCHITECTURAL CHANGES, FIBROSIS, AND CIRRHOSIS.*

FINDING	SCORE†
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas, with occasional portal-to-portal bridging	3
Fibrous expansion of portal areas, with marked portal-to-portal bridging as well as portal-to-central bridging	4
Marked portal-to-portal bridging as well as portal-to-central bridging, with occasional nodules (incomplete cirrhosis)	5
Probable or definite cirrhosis	6

*The criteria were obtained, with modifications, from Ishak et al.¹⁹ The following additional features were noted but not scored: intra-acinar fibrosis, perivenular ("chicken wire") fibrosis, and phlebosclerosis of terminal hepatic venules.

†The maximal score is 6.

ical status, the date each sample was obtained, and the hepatic iron content of each biopsy specimen. After the completion of the initial review, the two study investigators conducted a consensus review, in which all biopsy specimens were examined jointly, after standards regarding sample adequacy and definitions of progression and regression of fibrosis had been agreed on. The results of this evaluation were subsequently reviewed with the consultant, and a final decision was made with regard to the adequacy and stage of each biopsy specimen.

Clinically significant progression of fibrosis was considered to have occurred if there was a change in the histologic score from 0 (no fibrosis) to 1 or greater, from 1 or 2 to 3 or greater, or from 3 or 4 to 5 or 6. Changes in the score from 1 to 2 or from 3 to 4 were not considered clinically significant. Similarly, a change in the score from 5 (incomplete cirrhosis) to 6 (probable or definite cirrhosis) was not considered clinically significant; hence, patients whose initial biopsy specimen showed cirrhosis could not be evaluated for progression of fibrosis. A biopsy specimen was considered adequate if two or more portal tracts were present. This was considered the absolute minimum necessary for the assessment of fibrosis and cirrhosis, since the ability to identify these processes varies considerably depending on the size of the biopsy specimen.

Monitoring of Toxicity and Compliance

Other types of safety monitoring in this study have been described previously.¹² Sexually active patients were asked to use reliable methods of contraception. We assessed compliance by monitoring the frequency with which pill bottles were opened, using bottles with microprocessors in the caps.^{12,20}

Statistical Analysis

Data are presented as means \pm SE. Medians and ranges are given for continuous variables, and proportions are given for dichotomous variables. Pretreatment variables were compared between treatment groups by the Mann-Whitney test for continuous variables and by the Fisher-Irwin exact test for dichotomous variables.²¹ The Wilcoxon signed-rank test was used to compare pretreatment and post-treatment values for continuous variables and to assess whether there was a change in compliance during the last two years of deferiprone therapy. The Kaplan-Meier product-limit

method was used to estimate the probability that each patient would not have progression of fibrosis for a specified period. The log-rank test was used to compare differences in the length of time to the progression of fibrosis in the treatment groups.²² Because the only patients with progression of fibrosis were in the deferiprone group, it was not possible to estimate the risk or odds of progression to fibrosis on the basis of the type of chelating therapy, the dichotomous predictor variable. Thus, multivariate logistic-regression models were formed to examine the relation between the dependent variable, progression of hepatic fibrosis, and predictor variables, including the duration of deferiprone therapy, age at initial biopsy, sex, the presence of antibody to hepatitis C virus, initial hepatic iron concentration, and the amount of blood transfused.²³ Stepwise analysis and an analysis of all possible subgroups were performed to choose the most parsimonious model with statistically significant predictors. All tests were two-tailed; a P value of 0.05 was considered to indicate statistical significance. The BMDP (BMDP Statistical Software, Los Angeles) and S-PLUS (Statistical Sciences, version 3.3 for Windows, Seattle) statistical computer packages were used for computations.

The study was approved by the human subjects committee of the Hospital for Sick Children, Toronto, and the Health Protection Branch of Health Canada. Written informed consent was obtained from each patient or the patients' parents.

RESULTS

Effectiveness of Deferiprone

Among the 18 patients in whom the effectiveness of deferiprone could be evaluated, the mean (±SE) hepatic iron concentration decreased from 88.7±12.1 to 65.5±7.9 μmol per gram of liver, wet weight (normal value, about 1.6), after a mean of 4.6±0.3 years of therapy (range, 2 to 7); this decrease of 23.2±10.9 μmol of iron per gram of liver, wet weight, was not significant (P=0.07). Initial and final hepatic iron concentrations are shown in Figure 1. In seven patients, hepatic iron concentrations at the end of treatment met or exceeded the threshold value of 80 μmol per gram of liver, wet weight, which is associated with an increased risk of cardiac disease and early death.¹⁷ The serum ferritin concentration decreased from 4455±841 μg per liter at the beginning of treatment to 2831±491 μg per liter at the end of treatment. Expressed logarithmically, this decrease was significant (P=0.03). In nine of the patients, the serum ferritin concentration exceeded 2500 μg per liter, the threshold used to distinguish effective from ineffective chelation therapy.^{12,18}

Data on compliance were available for all 18 patients for the last two full years of treatment. The median rate of compliance each year was 98 percent, with ranges of 90 to 100 percent for the penultimate year and 87 to 100 percent for the final year (P=0.70).

Histologic Analysis

Of the 72 biopsy specimens available from the patients treated with deferiprone, 17 (24 percent) were judged inadequate for evaluation. Histologic changes could not be evaluated in five patients: two did not have two adequate biopsy specimens that had been obtained at least one year apart, and three had cirrhosis at the initial evaluation. Thus, a total of 55 biopsy specimens from 14 patients were examined (Table 2).

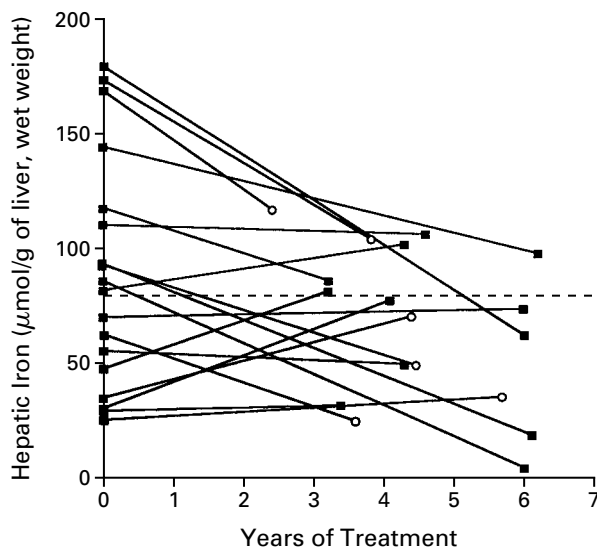


Figure 1. Initial and Final Hepatic Iron Concentrations in 18 Patients with Thalassemia Major Treated with Deferiprone.

The dashed line indicates the value of 80 μmol of iron per gram of liver tissue, wet weight, above which there is an increased risk of cardiac disease and early death due to iron loading during long-term treatment with deferoxamine.¹⁷ Squares indicate concentrations determined by liver biopsy, and circles concentrations determined by magnetic susceptometry.

TABLE 2. CHARACTERISTICS OF THE 14 DEFERIPRONE-TREATED PATIENTS AND THE 12 DEFEROXAMINE-TREATED PATIENTS IN WHOM PROGRESSION OF HEPATIC FIBROSIS COULD BE EVALUATED.

CHARACTERISTIC	DEFERIPRONE GROUP (N=14)	DEFEROXAMINE GROUP (N=12)	P VALUE
Progression of hepatic fibrosis — no. (%)	5 (36)	0	0.04
Age at initial biopsy — yr			
Median	18.2	13.9	0.1
Range	10.5–23.7	8.7–31.5	
Male sex — no. (%)	3 (21)	6 (50)	0.2
Antibody to hepatitis C — no. (%)	6 (43)	5 (42)	1.0
Initial hepatic iron concentration — μmol/g of liver, wet weight			
Median	80.9	35.2	0.01
Range	24.2–180.1	10.8–226.4	
Duration of treatment — yr*			
Median	2.3	3.2	0.4
Range	1.3–4.0	1.3–4.3	
Amount of blood transfused — ml packed cells/kg/yr*			
Median	77	82	0.5
Range	56–114	57–109	

*The period under consideration is the interval between the initial biopsy and the final biopsy. Data were available for 13 patients in the deferiprone group and 10 in the deferoxamine group.

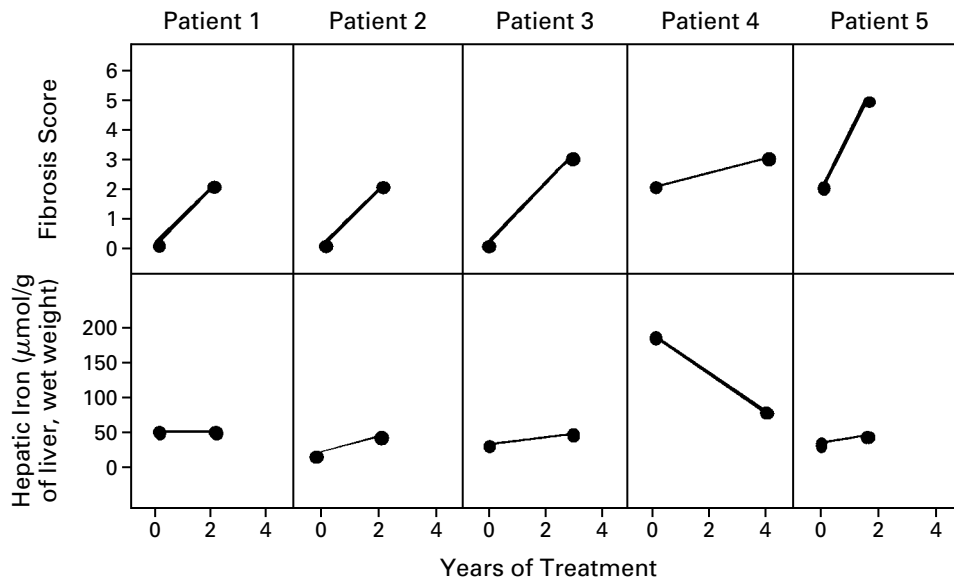


Figure 2. Changes in Histologic Findings and Hepatic Iron Concentrations in the Five Deferiprone-Treated Patients with Progression of Hepatic Fibrosis.

The worst possible fibrosis score is 6; the staging system is described in Table 1.

Five patients treated with deferiprone had evidence of progression of fibrosis. The estimated median time to progression was 3.2 years. Figure 2 shows the initial and final histologic stages, hepatic iron concentrations, and duration of therapy for these five patients. In four patients, hepatic iron concentrations stabilized during therapy; in the fifth, the iron concentration decreased substantially. Figure 3 shows representative photomicrographs of liver specimens from the initial and final biopsies in these patients. In one other patient, there was an improvement in the histologic stage over a period of 3.5 years.

Table 3 shows that deferiprone-treated patients with progression of fibrosis were older than those without progression ($P=0.03$). There were no other significant differences (sex, prevalence of hepatitis C infection, initial hepatic iron concentration, or the duration of therapy or amount of blood transfused between the initial biopsy and the final biopsy) between the two groups.

Of the 48 biopsy specimens available from the 20 patients treated with deferoxamine, 8 (17 percent) were judged inadequate for evaluation. Histologic changes could not be evaluated in eight patients: six did not have two adequate biopsy specimens that had been obtained at least one year apart, and two had cirrhosis at the initial evaluation. Thus, a total of 31 biopsy specimens from 12 patients were examined (Table 2). None of the specimens showed evidence of progression of fibrosis. In one patient, there was an improvement in the histologic stage over a two-year period.

After adjustment for initial hepatic iron concentrations, multivariate logistic-regression analysis showed that the estimated odds of progression to fibrosis increased by a factor of 5.8 (95 percent confidence interval, 1.1 to 29.6) with each additional year of deferiprone treatment. The deferiprone-treated patients had a significantly higher mean initial hepatic iron concentration than the deferoxamine-treated patients ($P=0.01$) (Table 2), but no significant differences between the two groups were identified with respect to age at initial biopsy, sex, prevalence of hepatitis C infection, or the duration of therapy or amount of blood transfused between the initial biopsy and the final biopsy.

Other Adverse Effects

Deferiprone therapy was not associated with clinically significant hematologic changes, as evidenced by regular blood counts. No characteristic abnormalities in liver function were observed, although many patients had the small elevations in serum aminotransferase concentrations that are commonly found during iron overload. In no patient did heart disease requiring medication develop during the study.

DISCUSSION

These results indicate that deferiprone is not an effective means of iron-chelation therapy in patients with thalassemia major and may be associated with worsening of hepatic fibrosis, even in patients whose hepatic iron concentrations have stabilized or decreased. After a mean of 4.6 years of deferiprone

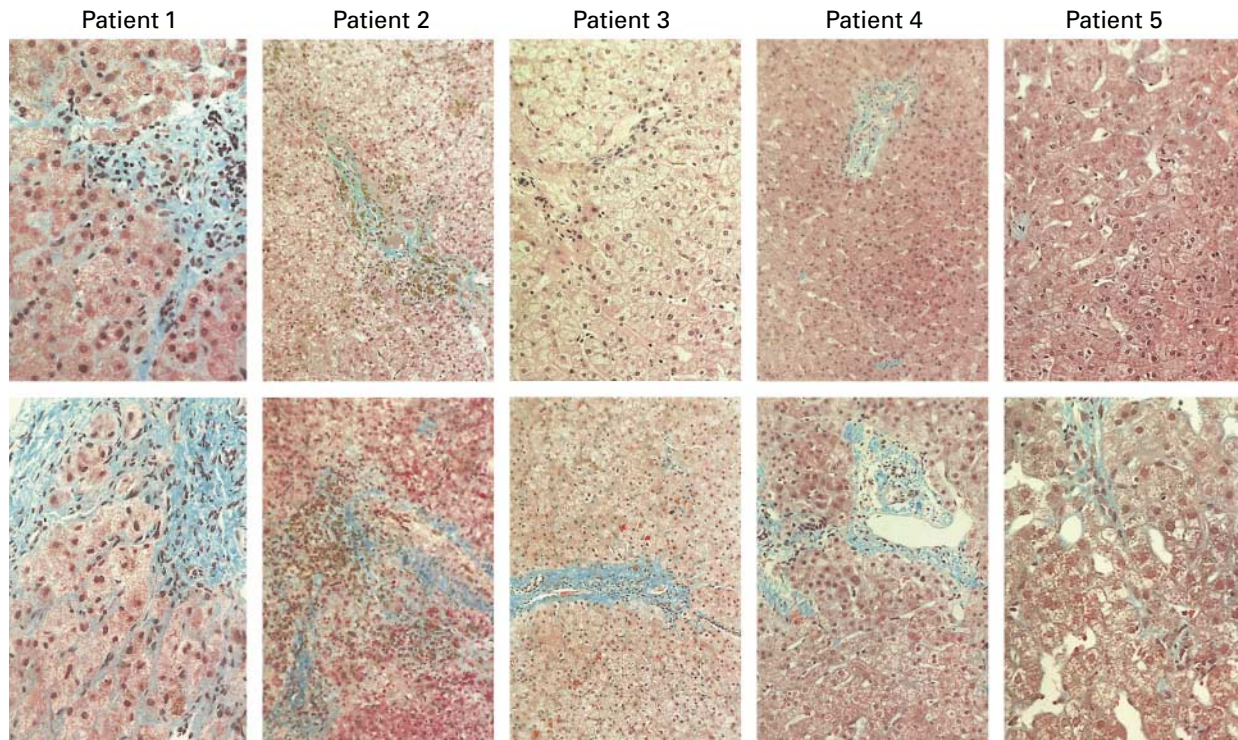


Figure 3. Changes in Histologic Findings in the Five Deferiprone-Treated Patients with Progression of Hepatic Fibrosis. The top panels show the initial biopsy specimens, and the bottom panels the last biopsy specimens that could be evaluated (Masson's trichrome, $\times 100$).

therapy, body iron burden was at concentrations associated with a greatly increased risk of cardiac disease and early death¹⁷ in 7 of 18 patients (39 percent). Other investigators have recently reported that hepatic iron exceeded this threshold in 58 percent of patients who were treated with deferiprone for one to four years.²⁴ In our patients, differences in objectively determined rates of compliance or the rate of iron loading could not account for the lack of effectiveness of deferiprone.

The results of our review of liver-biopsy specimens suggest that extended deferiprone therapy may be associated with a worsening of hepatic fibrosis. Fibrosis progressed in 5 of the 14 patients (36 percent) in whom it could be evaluated, despite the stabilization of or a marked reduction in hepatic iron concentrations in all 5 patients. The estimated median time to progression of fibrosis was 3.2 years, and after adjustment for initial hepatic iron concentrations, the estimated odds of progression of fibrosis increased by a factor of 5.8 (95 percent confidence interval, 1.1 to 29.6) with each additional year of deferiprone treatment.

The worsening of hepatic fibrosis in deferiprone-treated patients is in contrast to the arrest of fibrosis regularly observed with deferoxamine therapy. In a

TABLE 3. CHARACTERISTICS OF THE PATIENTS WITH PROGRESSION OF HEPATIC FIBROSIS DURING TREATMENT WITH DEFERIPRONE AND THOSE WITHOUT PROGRESSION.

CHARACTERISTIC	PROGRESSION OF HEPATIC FIBROSIS (N=5)	NO PROGRESSION OF HEPATIC FIBROSIS (N=9)	P VALUE
Age at initial biopsy — yr			
Median	21	16	0.03
Range	18–24	10–22	
Male sex — no. (%)	2 (40)	1 (11)	0.50
Antibody to hepatitis C — no. (%)	4 (80)	2 (22)	0.09
Initial hepatic iron concentration — $\mu\text{mol/g}$ of liver, wet weight			
Median	29.6	93.6	0.07
Range	24.2–180.1	55.4–174.2	
Duration of treatment — yr*			
Median	2.1	2.4	0.80
Range	1.6–4.0	1.3–3.4	
Amount of blood transfused — ml packed cells/kg/yr*			
Median	77	77	0.60
Range	56–89	64–114	

*The period under consideration is the interval between the initial biopsy and the final biopsy. Data were available for five patients with progression of hepatic fibrosis and eight with no progression.

seminal study, long-term therapy with deferoxamine halted the progression of hepatic fibrosis in patients with thalassemia major.²⁵⁻²⁷ In these patients, progression was arrested despite a regimen of deferoxamine (0.5 g per day intramuscularly, six days per week)²⁵ now considered suboptimal because it merely stabilizes, rather than reduces, hepatic iron concentrations. Moreover, fibrosis was halted despite a final mean hepatic iron concentration (139 μmol per gram of liver, wet weight)²⁵ that was more than twice that in our deferiprone-treated patients with progression of fibrosis (49.5 μmol per gram of liver, wet weight). Subsequent studies²⁸ and the results in our comparison group of deferoxamine-treated patients confirm that modern regimens of parenteral deferoxamine arrest fibrosis.

Our findings virtually recapitulate those in a gerbil model of iron overload in which administration of a closely related drug (1,2-diethyl-3-hydroxypyridin-4-one) was associated with initial loss of efficacy, worsening of hepatic fibrosis, and cardiac fibrosis.¹³ There has been concern that hydroxypyridinones may exacerbate iron-related tissue damage.²⁹⁻³¹ Deferiprone is a bidentate chelator, and three molecules are needed to occupy the six coordination sites of a single atom of iron. In contrast, one molecule of the hexadentate deferoxamine binds a single atom of iron; the chelate (ferrioxamine) is virtually inert biologically. At low concentrations of deferiprone relative to the concentrations of available iron, partially bound forms of iron (bound to only one or two molecules of deferiprone) appear in which the unoccupied coordination sites remain reactive and able to catalyze the formation of hydroxyl radical or other reactive oxygen species.³² There is increasing evidence to suggest that these reactive oxygen species are involved in the pathogenesis of hepatic fibrosis. Recently, the potential cellular toxicity of deferiprone has been shown in erythrocytes³³ and cultured myocytes.³⁴

The limitations of our histopathological analysis should be emphasized: our analysis was retrospective, the number of patients studied was small, and the patients treated with deferoxamine do not constitute a true control population. Furthermore, histologic assessment was based on relatively few biopsy specimens, although this was true in both the deferiprone group and the deferoxamine group. Because this study was observational rather than randomized and the effect of hepatic fibrosis was not confirmed by challenge after discontinuation of the drug, the relation between deferiprone and fibrosis cannot be considered definite or proved. Nonetheless, we could identify no other causes of the accelerated fibrosis.

The patients with progression of fibrosis did not differ significantly from those without progression with respect to sex, prevalence of hepatitis C infection, initial hepatic iron concentrations, duration of therapy, or the rate of iron accumulation. The con-

sensus of the pathologists was that there was no difference between groups in the type of inflammatory changes. Nonetheless, we cannot rule out the possibility of an interaction between deferiprone and hepatitis C infection. The patients with progression of fibrosis were older than those without progression, and the likelihood of progression of fibrosis was greater in patients with lower hepatic iron concentrations. Because we are unable to identify definite risk factors for accelerated fibrosis, we have discontinued deferiprone therapy in all patients, including those who are unable or unwilling to use deferoxamine in standard regimens.

Despite their limitations, the results of our analysis, together with theoretical considerations and findings in animal studies, indicate that deferiprone may worsen hepatic fibrosis. Before it can be considered for clinical use, even in patients who are unwilling or unable to use deferoxamine in standard regimens, prospective clinical trials are mandatory to evaluate the possibility of irreversible hepatic damage.

Supported in part by research grants from the Medical Research Council of Canada, the Ontario Heart and Stroke Foundation, the Ontario Thalassemia Foundation, the Cooley's Anemia Foundation, Apotex, and the National Institutes of Health (DK49108, HL58203, and HL61219). Dr. Olivieri is supported in part by a Scientist Award from the Medical Research Council of Canada.

We are indebted to Professor Peter Scheuer, London, for his independent analysis of the liver-biopsy slides; to David Nathan, David Weatherall, Michael Baker, Antonio Cao, Helen Chan, John Dick, Peter Durie, Brenda Gallie, Marc Giacomelli, John Harris, David Kern, F.A. Olivieri, Robert Phillips, Eliot Phillipson, Sergio Piomelli, Alvin Zipursky, and Stanley Zlotkin for ongoing encouragement, advice, and support; and to Naomi Klein, Maria Muraca, Allyson Muroff, and Helen Schinkel for assistance in data management.

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