Deferiprone versus Deferoxamine in Patients with Thalassemia Major: A Randomized Clinical Trial

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ABSTRACT: Deferiprone has been suggested as an effective oral chelation therapy for thalassemia major. To assess its clinical efficacy, we compared deferiprone with deferoxamine in a large multicenter randomized clinical trial. One-hundred forty-four consecutive patients with thalassemia major and serum ferritin between 1500 and 3000 ng/ml were randomly assigned to deferiprone (75 mg/kg/day) ($n = 71$) or deferoxamine (50 mg/kg/day) ($n = 73$) for 1 year. The main measure of efficacy was the reduction of serum ferritin. Liver and heart iron contents were assessed by magnetic resonance. Liver iron content and fibrosis stage variations were assessed on liver biopsy by the Ishak score in all patients willing to undergo liver biopsy before and after treatment. The mean serum ferritin reduction was 222 ± 783 ng/ml in the deferiprone and 232 ± 619 ng/ml in the deferoxamine group ($P = 0.81$). No difference in the reduction of liver and heart iron content was found by magnetic resonance between the two groups. Thirty-six patients accepted to undergo repeat liver biopsy: 21 in the deferiprone and 15 in the deferoxamine group. Their mean reduction of liver iron content was 1022 ± 3511 μg/g of dry liver and 350 ± 524, respectively ($P = 0.4$). No difference in variation of the Ishak fibrosis stage was observed between the two groups. Treatment was discontinued because of reversible side effects in 5 patients in the deferiprone group (3 hypertransamin/asemia and 2 leukocytopenia) and in none in the deferoxamine group. These findings suggest that deferiprone may be as effective as deferoxamine in the treatment of thalassemia major with few mild and reversible side effects.   © 2002 Elsevier Science (USA)

Key Words: L1 therapy; oral chelation; randomized clinical trial; chelation therapy; L1 efficacy; thalassemia major management.

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INTRODUCTION

Prognosis of patients with thalassemia major has dramatically improved in the past two decades as a consequence of improvement in transfusional and chelation therapy (1, 2). Deferoxamine B mesylate (DF), is widely accepted as the standard chelation therapy (2). However it requires overnight subcutaneous infusion and is associated with serious side-effects (3–11). For these reasons, several oral iron chelators have been studied. Among these, deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, also called L1) appears to be promising. In the only randomized clinical trial so far reported, including 20 patients, L1 proved to have the same chelating effect as subcutaneous DF (12). However several other uncontrolled and small studies reported contrasting results which are difficult to interpret because of different patients selection and different length of follow-up (13–26). We therefore carried out a randomized clinical trial comparing L1 with subcutaneous DF.

METHODS

Patients

All the patients with thalassemia major consecutively observed at the participating centers between September 1994 and October 1997 were considered eligible for the trial if they had a serum ferritin concentration equal to or lower than 3000 ng/ml. Before the trial all patients were treated by deferoxamine B mesylate therapy at dosage of 50 mg/kg given subcutaneously during a 12-h period usually overnight for 5 days a week.

We decided to exclude patients with greater serum ferritin concentrations to minimize the inclusion of patients with a serious risk of multiple organ damage from iron overload. The diagnosis of thalassemia major was based on accepted clinical and molecular criteria (27, 28).

The exclusion criteria were (a) known intolerance to one of the trial treatments; (b) presence of rheumatoid factor; (c) serum antinuclear-autoantibody (ANA); (d) platelet count $<100,000/$mm^3 or leukocyte $<3000/$mm^3; (e) severe liver damage indicated by ascites; (f) clinical evidence of heart failure; (g) sepsis; and (h) α-interferon treatment (29). Eligibility and exclusion criteria were checked at each participating center in the outpatient or day-hospital section where the patients were also seen throughout the whole follow-up period.

Interventions

The trial treatments were given according to following schedule: deferiprone, 75 mg/kg divided in three daily doses administered as 500 mg pills every 8 h; deferoxamine B mesylate, 50 mg/kg given subcutaneously during a 12-h period, usually overnight for 5 days a week. Deferiprone was obtained by Inselspital (Berne, Switzerland), Lipomed (Basel, Switzerland), CIPLA Ltd. (India) and Apotex (Toronto, Canada). The purity of the drug was assessed in random samples throughout the study at the School of Life, Basic Medical and Health Sciences–King's College London—by Professor R. C. Hider Laboratory, and always exceeded 98%.

The planned duration of treatment was 1 year. It was established that at the end of the trial each patient had to continue chelation therapy by the conventional therapy (i.e., DF) until the study analysis was completed. We decided to assess the treatment efficacy over a 1-year period, considering it unlikely that a clinically significant iron overload would develop in this relatively short time in patients treated with the experimental treatment with this baseline serum ferritin levels, even if L1 was less effective than DF.

Compliance with the trial treatment was assessed by counting the pills in each returned bag of deferiprone and by assessing the total dose of deferoxamine B mesylate consumed each week. Compliance was also checked by interviewing the patient relatives.

Standard transfusional therapy was aimed at maintaining the hemoglobin blood concentration $\geq$9.5 g/dl.

Objectives

The study objective was to compare the two treatments in the reduction of iron overload or to prevent its increase.

Outcomes

The main measure of the treatment efficacy was the difference between the serum ferritin concentration before and after 1 year of treatment. Secondary efficacy measures were (a) variation of liver iron content (LIC) measured as μg/gram of dry weight in patients willing to undergo liver biopsy prior and after the treatment period; (b) variation of liver and heart iron content estimated by NMR performed by a 0.5-T superconducting unit (Vectra, General Eletric Medical Systems, Paris, France) using 0.24-cm^2 operator-defined regions of interest (ROIs) and expressed as average intensity signal ratio (ISR) (30–33). (NMR was performed at the Institute of Radiology, University of Palermo for all patients); (c) heart function as assessed by the following parameters recorded on heart ultrasonography: left ventricular ejection fraction (LVEF), left ventricular shortening fraction (LVSF) and the ratio of the right ventricle telediastolic to the telesystolic area (mm^3) (RVDSR); (d) variation in 24-h urinary iron excretion (UIE) measured during treatment.

A liver biopsy before and after the treatment was performed in all the patients who accepted it, according to a standard technique, to assess liver iron content and fibrosis. Biopsies were blindly examined under code by two independent observers experienced in liver histology, unaware of the type of treatment and of timing of biopsies. Liver inflammation and fibrosis were rated according to the Ishak scoring system (34). Interobserver agreement beyond chance for the fibrosis score was assessed by the weighted kappa statistic (35).

Liver iron content was assessed by atomic spectrophotometry on liver biopsy and expressed as amount of iron in μg/g dry liver weight. All patients were seen once or twice a month in the outpatient or day-hospital section of each participating center, according to the transfusional requirement. Clinical and biochemical assessment was repeated monthly, according to a prefixed data form.

Adverse Events

Any potential adverse event was recorded and the relationship with the trial treatment was care-fully investigated. Variation in liver fibrosis was investigated in all patients accepting to undergo liver biopsy before and after the trial treatment.

If an increase of ferritin levels more than 1000 ng/ml, confirmed by two determinations apart with respect to the previous values was detected during the study period, the treatment was stopped and the alternative therapy was started.

Sample Size

The sample size estimate was based on the expected mean reduction in serum ferritin concentration at the end of 1 year of treatment. Based on previous experience at the coordinating center we knew that in patients with initial serum ferritin below 3000 ng/ml and treated with subcutaneous DF, the mean reduction in serum ferritin after one year of therapy was 250 ng/ml with a standard deviation of 65 ng/ml. We assumed that a difference higher than 30 ng/ml with respect to this expected ferritin reduction with DF, would be clinically significant. Therefore we calculated that to detect a 30 ng/ml difference (i.e., from 250 to 220), 70 patients should be included per group (two sided test; $\alpha = 0.05$; $\beta = 0.80$).

Randomization

The randomization was based on a computer generated random list in permuted blocks of 10. The *randomization sequence* was generated at the Biometrics Institute of the University of Milano. To ensure *allocation concealment,* treatment was assigned by telephone contact of each participating center with a physician (FP) of the coordinating center who kept the randomization sequence, but was not otherwise involved in the study. *Treatment assignment* was done when the inclusion and exclusion criteria per each consecutively observed patient were verified and treatment was started within the following 24 h.

Assessments of Outcome

Because of the modality of administration of deferoxamine B mesylate, a double blind design was considered unethical. However all the outcomes assessments (determination of serum fer-

ritin concentration, urinary iron excretion, liver iron content and fibrosis on liver biopsy, liver and heart iron content estimated by MRI, heart function on ultrasound) were done under code by physicians blinded to the trial treatment. Also the statistical analysis was performed under code at the Biometrics Institute of the University of Milano, by a biostatistician (A.M.) blinded to the trial treatment.

Statistical Methods

Means are reported with standard deviation (SD); proportions and differences between proportions are reported with 95% confidence intervals (CI). The statistical analysis was based on the intention to treat principle. Continue scale values were compared between the two study groups by paired t test or two-sample t test with equal variances, as appropriate, by using a logarithmic transformation whenever this improved the approximation to normal distribution. Differences in proportions observed on contingency tables were assessed by χ^2 analysis. A multiple linear regression analysis by a step-wise backward procedure was planned to identify potential confounding factors affecting the mean serum ferritin reduction at the end of the treatment period. The following set of variables to be included in the multivariable analysis was defined a priori: sex, age, splenectomy, total number of blood units transfused in the last 12 months before randomization, initial serum ferritin concentration, 24-h urinary iron excretion before randomization, cirrhosis, HBsAg, anti-HCV, diabetes, left ventricular ejection fraction, endocrine dysfunctions, number of transfusions during the study period, and trial treatment. All statistical analyses were performed by STATA 6 (1999 STATA Corp.).

Ethics

The study protocol conformed to the ethical guidelines of Declaration of Helsinki (36) and was approved by the local ethics committee for human investigations. The patients gave their written informed consent to participate in the study.
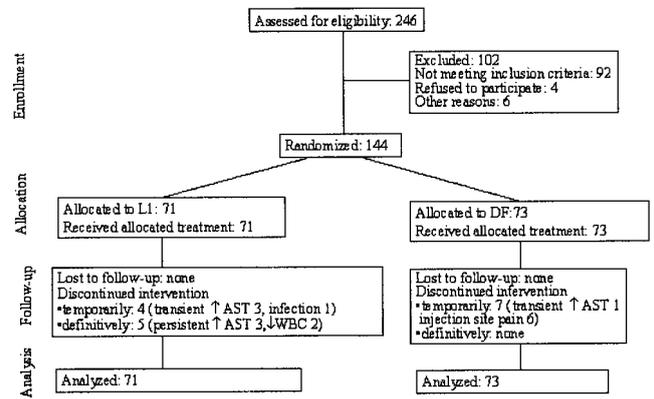

FIG. 1. Trial profile.

RESULTS

Participant Flow and Recruitment

From September 1994 to October 1997, 246 patients with thalassemia major were consecutively observed at the 15 participating centers. Among these patients 92 were not eligible because of serum ferritin concentration above 3000 ng/ml. Among the 154 eligible patients 3 were excluded because of ongoing α-interferon treatment, 3 because of rheumatoid factor positivity and 4 because of unwillingness to participate in the study. The remaining 144 patients were included: 71 were randomly assigned to L1 and 73 to DF. None of the patients was lost to follow-up (Fig. 1).

Eleven patients in the L1 group and 7 in the DF group with initial serum ferritin concentration over 3000 ng/ml were erroneously randomized. Individual data of these patients are shown in Table 5, and separate results are also reported for them.

Baseline Data

Clinically relevant patient characteristics at enrollment and corresponding values at the end of the one-year study period are shown in Tables 1–3.

Numbers Analyzed

All 71 patients randomized to L1 and 73 to DF were included in the analysis according to the "intention to treat" principle.

TABLE 1

Patients' Characteristics at Baseline

Characteristics	L1 group (*n* = 71)	L1 group with liver biopsy (*n* = 21)	DF group (*n* = 73)	DF group with liver biopsy (*n* = 15)
Age, years[a]	20 ± 5.3	19 ± 3.1	21 ± 4.2	20 ± 4
Sex (M/F)[a]	37/34	11/10	34/39	7/8
Total blood transfusions in the year before randomization, ml (mean ± SD)[a]	8,302 ± 541	8,000 ± 430	8,965 ± 278	8,700 ± 650
Total blood transfusions during the study, ml (mean ± SD)	10,142 ± 1071	8,985 ± 1694	9,181 ± 227	8,380 ± 1043
Mean serum ferritin in the year before randomization, ng/ml (mean ± SD)	2,159 ± 668	2,300 ± 590	2,074 ± 608	2,200 ± 650
Liver iron concentration, μg/gr dry liver (mean ± SD)	—	3,363 ± 5,490[b]	—	3,516 ± 2,974
HbsAg positive[a]	—	—	2	—
Anti-HCV positive[a]	59	16	65	11
Cirrhosis[a]	2	1	7	0
Splenectomy[a]	27	8	27	7
Diabetes[a]	1	—	2	—
Hypogonadism[a]	26	10	29	7
Hypothyroidism[a]	15	7	11	3
Hypoparathyroidism[a]	1	—	4	1

Note. Data are numbers of patients unless otherwise stated.

[a] Variables included in the multiple linear regression analysis of confounding factors for the treatment effect.

[b] Liver iron concentration was measured in 20 patients of the L1 group because of insufficient material in one patient.

Treatment

Fifty-five patients per each trial group took the prescribed dose of the trial treatment during the whole study period; four patients in the L1 group and seven in the DF group took a reduced does because of low compliance. Twenty-four patients in the L1 group were not willing to switch to the conventional DF treatment at the end of the study period. After the recent report of a study suggesting that L1 may increase the risk of liver fibrosis (23), the patients who were still willing to continue L1 treatment were asked to undergo a second liver biopsy before continuing treatment.

Long-term variation of liver iron content and fibrosis of all these patients is reported (Tables 3 and 4; Figs. 2 and 3).

Adverse Events

In the L1 group, 24 patients developed side effects requiring temporary dose reduction in 3 (nausea) and temporary treatment withdrawal in 4 (transient hypertransaminasemia 3, infection 1). Five patients were definitively withdrawn from treatment because of recurrence of hypertransaminasemia (>2 times the pretreatment values) (*n* = 3, anti-HCV positive 1) or leukocytopenia (*n* = 2) even at reduced doses of the study drug. Mild hypertransaminasemia, spontaneously recovering developed in 10 other patients and mild joint pain in two. Overall, 14 of the 16 patients who developed hypertransaminasemia, were anti-HCV positive. Adverse events occurred in 11 DF treated patients: temporary dose reduction was needed in 6 patients because of pain and erythema at the injection site and in one because of transient hypertransaminasemia. Two patients developed infections (*Yersinia enterocolitica*) and two ototoxicity, requiring temporary treatment withdrawal. All of these patients continued on DF after temporary dosage reduction or temporary treatment withdrawal.

One patient per group was withdrawn from the trial treatment because of an increase of serum ferritin of more than 1000 ng/ml during the first 6

TABLE 2

Relevant Blood and Biochemistry Parameters at Baseline and at the End of Treatment in the 144 Patients Included in the Study

Parameter	L1 group ($n = 71$)		DF group ($n = 73$)	
	Baseline	After treatment	Baseline	After treatment
Hemoglobin (g/dl)	10.3 ± 1.2	10.0 ± 0.9*	10.2 ± 1.4	10.0 ± 1.0
Leucocytes ($\times 10^9$/L)	9.4 ± 4.8	11.0 ± 7.9*	8.9 ± 5.8	9.1 ± 7.4
Platelets ($\times 10^9$/L)	369 ± 170	396 ± 184	344 ± 135	358 ± 147
Glucose (mg/dl)	98 ± 16	95 ± 17	97 ± 36	97 ± 18
Urea (mg/dl)	31 ± 10	30 ± 11	31 ± 10	32 ± 9
Creatinine (mg/dl)	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
Uric acid (mg/dl)	4.1 ± 1.2	4.2 ± 1.3	4.1 ± 1.3	4.3 ± 1.1
AST (U/L)	37 ± 24	55 ± 112	33 ± 23	34 ± 24
Anti-HCV positive	37 ± 25	59 ± 122	35 ± 24	36 ± 24
Anti-HCV negative	30 ± 17	30 ± 18	22 ± 12	22 ± 5
ALT (U/L)	58 ± 61	80 ± 125	50 ± 47	48 ± 46
Anti-HCV positive	61 ± 60	86 ± 135	53 ± 48	51 ± 47
Anti-HCV negative	29 ± 27	41 ± 38	26 ± 31	24 ± 12
γGT (U/L)	21 ± 14	35 ± 31**	21 ± 16	25 ± 24
Anti-HCV positive	23 ± 17	38 ± 33	22 ± 16	25 ± 25
Anti-HCV negative	12 ± 7	18 ± 9	12 ± 4	12 ± 5
Bilirubin (mg/dl)	1.4 ± 0.8	1.2 ± 0.6	1.4 ± 0.6	1.5 ± 0.7
Prothrombin activity (%)	84 ± 14	91 ± 11**	81 ± 13	85 ± 12
Albumin (g/dl)	4.5 ± 0.5	4.5 ± 0.9	4.5 ± 0.5	4.5 ± 0.9
γ-Globulins (g/dl)	2.3 ± 1.0	2.2 ± 0.9	2.4 ± 1.1	2.3 ± 1.1
Iron (μg/dl)	214 ± 49	216 ± 64	207 ± 49	201 ± 48
Transferrin (mg/dl)	215 ± 70	223 ± 76	215 ± 87	213 ± 91

Note. Data are means $\pm$ standard deviations. No significant differences were found at the end of treatment compared with baseline (paired t test) unless otherwise specified.

  * $P < 0.05$ compared with baseline (paired t test).

  ** $P < 0.01$ compared with baseline (paired t test).

months of trial treatment. Four patients in the L1 group and seven in the DF group took a reduced dose because of low compliance.

Outcomes

Mean *serum ferritin* concentration before and after one year of treatment are reported in Table 3 and Figs. 4–7. The mean reduction in serum ferritin concentration was 222 ± 783 ng/ml in the L1 group and 232 ± 619 ng/ml in the DF group ($P = 0.81$) (Table 3). Corresponding results in patients with baseline serum ferritin equal or lower than 3000 ng/ml ($n = 126$) and above this value ($n = 18$) are reported in Tables 4 and 5, respectively.

Secondary Measures of Treatment Efficacy

NMR assessment of liver and heart iron content are reported in Table 3. A statistically signif-

icant increase in ISR was found after both treatments for all the NMR measurements, suggesting that a significant reduction in the iron content in the heart and in the liver was associated with the two trial treatments (Tables 3 and 4). This increase was not statistically significant for the liver in the L1 group (Tables 3 and 4) although the change in liver ISR after treatment was not significantly different between the two study groups (Tables 3 and 4).

Assessment of heart function by ultrasound did not show appreciable variation with either treatment after the study period (Table 3). The values of other clinically relevant parameters at the end of treatment are reported in Table 2. It is important to note that a slight but not statistically significant increase of transaminases was found after L1 treatment greater in anti-HCV positive patients; γGT significantly increased in

TABLE 3

Summary of Treatment Efficacy Assessment

Measures of treatment efficacy	L1 group (*n* = 71)			DF group (*n* = 73)		
	Baseline	End of treatment	Difference[a]	Baseline	End of treatment	Difference[a]
Serum ferritin (ng/ml)	2283 ± 754[g]	2061 ± 853*	222 ± 783	2019 ± 678	1787 ± 893*	232 ± 619
Liver iron concentration (μg/g/dry weight)[b]	3363 ± 5490	2341 ± 2197	1022 ± 3511	3516 ± 2974	3166 ± 2519	350 ± 524
Anti-HCV positive	3651 ± 5928	2506 ± 2321	1145 ± 3812	3483 ± 3049	3184 ± 2604	299 ± 487
Anti-HCV negative[c]	1731 ± 708	1353 ± 626	378 ± 192	3718 ± 3560	3046 ± 2734	672 ± 825
Urinary iron excretion (mg/24 h)	11.4 ± 8.5[g]	15.8 ± 10.9*	−4.4 ± 13.2	15.7 ± 12.8	19.9 ± 13.6**	−4.2 ± 12.5
Liver NMR[d]	0.83 ± 0.32	0.89 ± 0.26	−0.06 ± 0.38	0.85 ± 0.36	0.98 ± 0.35**	−0.13 ± 0.28
Heart septum NMR[d]	1.06 ± 0.20	1.18 ± 0.30*	−0.12 ± 0.32	0.98 ± 0.26	1.12 ± 0.29**	−0.14 ± 0.30
Left ventricular NMR[d]	1.02 ± 0.26	1.23 ± 0.46**	−0.21 ± 0.46	0.99 ± 0.27	1.12 ± 0.25*	−0.13 ± 0.31
Right ventricular NMR[d]	0.99 ± 0.24	1.22 ± 0.50**	−0.23 ± 0.50	0.97 ± 0.32	1.16 ± 0.32*	−0.19 ± 0.48
Left ventricular EF (%)[e]	63 ± 6[g]	63 ± 6	0 ± 8	62 ± 7	61 ± 7	1 ± 6
Left ventricular SF (%)[e]	41 ± 11	40 ± 8	1 ± 10	40 ± 12	38 ± 8	2 ± 9
Right ventricular area ratio[f]	1.9 ± 0.28	2.04 ± 0.32	−0.14 ± 0.42	1.9 ± 0.28	2.0 ± 0.18	0.1 ± 0.28

Note. Data are means ± standard deviations.

[a] Values at randomization minus values at the end of treatment. Differences were not statistically different between the two study groups (two-sample *t* test with equal variances).

[b] Liver iron concentration was measured in 20 and 15 patients in L1 and DF treatment groups.

[c] All patients anti-HCV negative had baseline ferritin levels lower or equal to 3000 ng/ml.

[d] NMR, nuclear magnetic resonance. Values are expressed as intensity signal ratios.

[e] EF, ejection fraction on ultrasonography.

[f] Teledyastolic/telesystolic area on ultrasonography.

[g] Variables included in the multiple linear regression analysis of confounding factors for the treatment effect.

* $P < 0.05$ compared with baseline (paired *t* test).

** $P < 0.01$ compared with baseline (paired *t* test).

anti-HCV positive patients (Table 2). Comparable results were observed in the 126 patients with baseline serum ferritin equal or lower than 3000 ng/ml.

Analysis of Confounding Factors

The multiple regression analysis performed to assess whether potential confounding factors (variables included are reported in the methods and corresponding values in Tables 1–3) might have affected the study results, confirmed that the type of the trial treatment as well as the baseline value of serum ferritin concentration, did not have any independent association with the size of serum ferritin reduction at the end of the study. The only two variables independently associated with a higher serum ferritin reduction were female sex ($P = 0.036$) and the number of blood units transfused in the year before randomization ($P = 0.033$).

Liver Iron Content (LIC)

Altogether 36 patients gave consent to undergo liver biopsy before and after treatment, 21 in the L1 group and 15 in the DF group (Table 1). In the L1 group the difference of liver iron content (LIC) between the two biopsies was assessed in 20 of them because one biopsy in one patient in the L1 group provided insufficient material. The mean follow-up until the second liver biopsy was 30 ± 2.4 months for L1 and 34 ± 6.7 for DF group, respectively. The distribution values of LIC before and after treatment is shown on Fig. 2. All the patients were still continuing the trial treatment at the time of the second biopsy. The mean values of LIC before and after treatment are reported in Table 3. The mean difference in liver iron content from before to after treatment was 1022 ± 3511 (median 317; range 1590 to 15570) in the

TABLE 4

Summary of Treatment Efficacy Assessment in 126 Patients with Baseline Serum Ferritin Lower or Equal to 3000 ng/ml

Measures of treatment efficacy	L1 group ($n = 60$)			DF group ($n = 66$)		
	Baseline	End of treatment	Difference[a]	Baseline	End of treatment	Difference[a]
Serum ferritin (ng/ml)	2036 ± 463	1894 ± 760	142 ± 750	1877 ± 533	1678 ± 800**	198 ± 561
Liver iron concentration (μg/g/dry weight)[b]	3290 ± 5706	2191 ± 2229	1099 ± 3697	3500 ± 3232	3263 ± 2826	237 ± 506
Anti-HCV positive	3602 ± 6232	2358 ± 2408	1244 ± 4057	3457 ± 3369	3307 ± 2987	150 ± 433
Anti-HCV negative	1731 ± 708	1353 ± 626	378 ± 192	3718 ± 3560	3046 ± 2734	672 ± 825
Urinary iron excretion (mg/24 h)	11.6 ± 7.7	16.0 ± 11.3*	−4.5 ± 12.7	15.5 ± 13.1	19.8 ± 14.1*	−4.3 ± 12.8
Liver NMR[c]	0.83 ± 0.21	0.90 ± 0.26	−0.07 ± 0.38	0.87 ± 0.34	1.02 ± 0.33**	−0.15 ± 0.27
Heart septum NMR[c]	1.08 ± 0.19	1.19 ± 0.31*	−0.11 ± 0.33	0.96 ± 0.26	1.09 ± 0.28**	−0.13 ± 0.31
Left ventricular NMR[c]	1.02 ± 0.23	1.23 ± 0.40*	−0.21 ± 0.47	0.97 ± 0.27	1.07 ± 0.18	−0.10 ± 0.30
Right ventricular NMR[c]	0.99 ± 0.22	1.20 ± 0.50*	−0.21 ± 0.48	0.93 ± 0.30	1.13 ± 0.31*	−20 ± 49
Left ventricular EF (%)[d]	63 ± 7	62 ± 7	1 ± 8	63 ± 7	62 ± 7	1 ± 5
Left ventricular SF (%)[d]	42 ± 12	41 ± 8	1 ± 11	40 ± 12	38 ± 8	2 ± 8
Right ventricular area ratio[e]	1.9 ± 0.29	2.1 ± 0.29	−0.2 ± 0.39	1.9 ± 0.28	2 ± 0.26	0.1 ± 0.28

Note. Data are means ± standard deviations.

[a] Values at randomization minus values at the end of treatment. Differences were not statistically different between the two study groups (two-sample *t* test with equal variances).

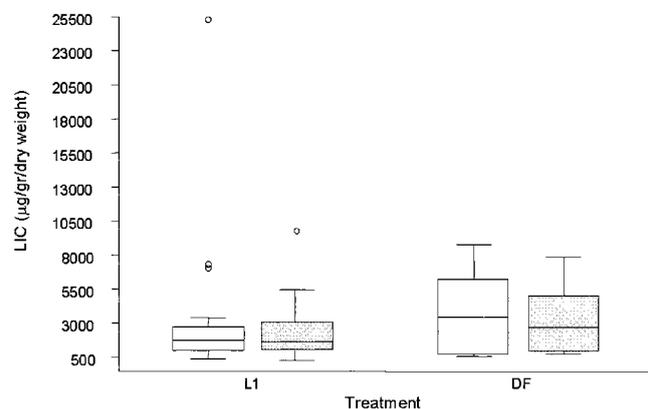[b] Liver iron concentration was measured in 20 and 15 patients in L1 and DF treatment groups.

[c] NMR, nuclear magnetic resonance. Values are expressed as intensity signal ratios.

[d] EF, ejection fraction on ultrasonography.

[e] Teledyastolic/telesystolic area on ultrasonography.

* $P < 0.05$ compared with baseline (paired *t* test).

** $P < 0.01$ compared with baseline (paired *t* test).


FIG. 2. Liver iron content (LIC) before (□) and after (▨) treatment. The mean follow-up until the second liver biopsy was 30 ± 2.4 months for L1 and 34 ± 6.7 for DF group, respectively. The lines emerging from the box indicate the upper and lower values. The upper values is defined as the largest data point greater than or equal to $x_{[75]} + 1.5x$ interquartile range; the lower value is defined as the smallest data point greater than or equal to $x_{[25]} − 1.5x$ interquartile range. Observed circles more extreme than the upper and lower values, if any, are referred to as *outside values* and are individually plotted.

L1 group and 350 ± 524 (median 272; range 405 to 1256) in the DF group ($P = 0.44$; $t = 0.77$) (Table 3). Relevant values according to the HCV status are reported in Table 3.

Liver Fibrosis

Liver biopsies from the 21 in the L1 (16 anti-HCV positive) (Table 1) and 15 in the DF group (11 anti-HCV positive) (Table 1) who accepted to undergo repeat biopsy, were blindly assessed under code for the degree of liver fibrosis by two independent observers. The interobserver agreement beyond chance as assessed by the *k*-weighted statistic was 0.59 ($P = 0.001$).

The mean of fibrosis scores before treatment were 2.1 ± 1.3 (median 2, range 1.5–2.7) in the L1 group and 2.2 ± 1.3 (median 2, range 1.5–3.05) in the DF group ($P = 0.77$). The corresponding values after treatment were not different from those before ($P = 0.84$; $t = −0.20$): 2.1 ± 1.5 (median 2, range 1.4–2.8) in the L1 group and 2.2 ± 1.2 (median 2, range 1.5–2.9) in the DF

L1 DF
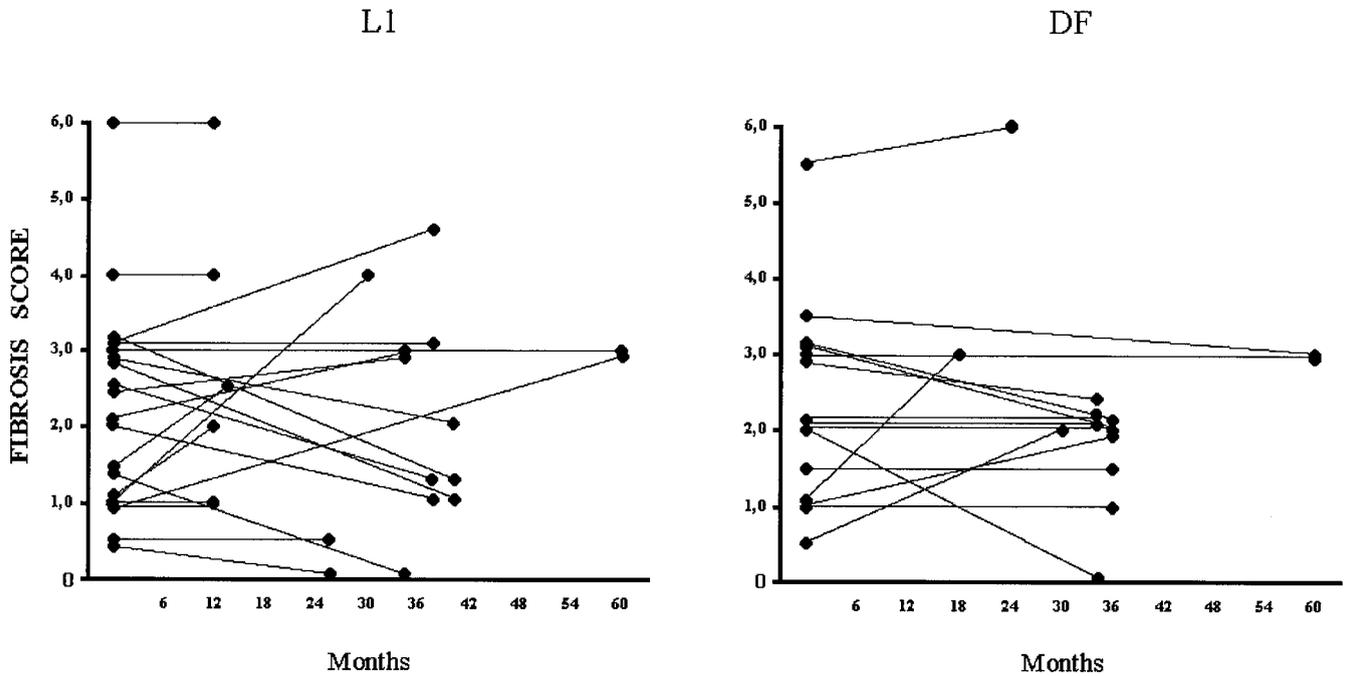
FIG. 3. Baseline and follow-up values of liver fibrosis score, represented as the mean value of the two independent observations performed by two independent observers unaware of the type of treatment and of timing of biopsies, in the 21 L1 and 15 DF patients who underwent repeat liver biopsy after treatment.

group (Fig. 3). Fibrosis score increased in 7 out of 21 patients in the L1 group and in 4 of 15 patients in DF group (Fig. 3). Among these patients 6 were anti-HCV positive in the L1 group and 4 in the DF group, respectively. Their mean initial vs final LIC values were 2360 ± 2313 (median 2084; range 506 to 7290) vs 2095 ± 1746 (median 2000; range 262 to 4800) in the L1 and 2270 ± 3173 (median 709; range 632 to 7030) vs $2318 \pm$ 2737 (median 1031; range 790 to 6419) in the DF group. One in the L1 and 1 in the DF group, respectively discontinued treatment because of side effects.

DISCUSSION

Interpretation and overall evidence. This is the first large randomized clinical trial comparing L1
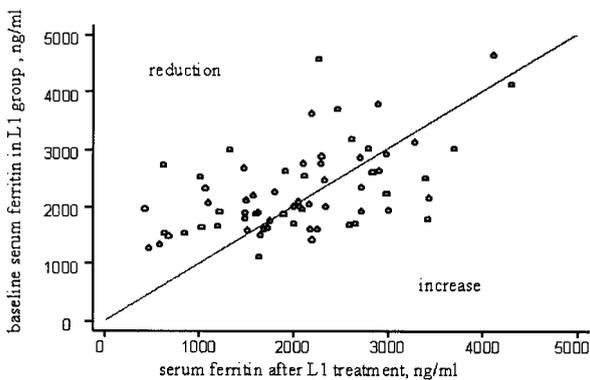
FIG. 4. Baseline and end of treatment values of serum ferritin in the 71 patients in L1 group. The diagonal line denotes equivalence values. Values above the equivalence line indicate reduction and those below the line indicate increase of ferritin after treatment.
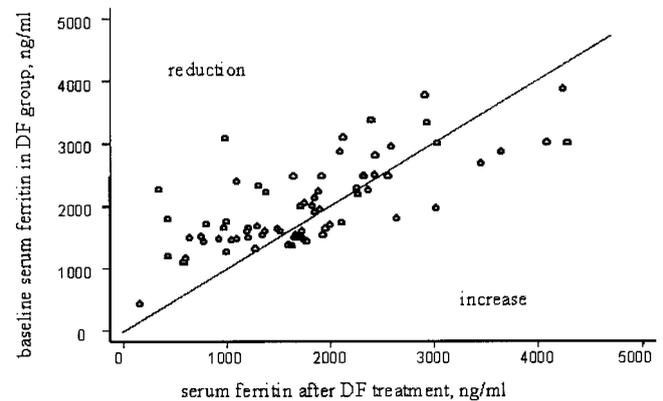
FIG. 5. Baseline and end of treatment values of serum ferritin in the 73 patients in DF group. The diagonal line denotes equivalence values. Values above the equivalence line indicate reduction and those below the line indicate increase of ferritin after treatment.
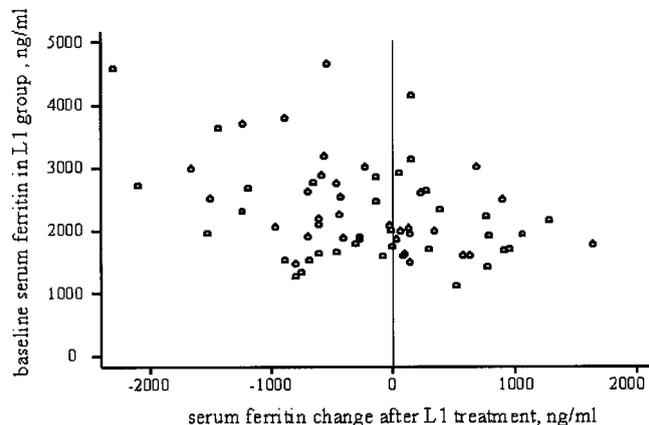
FIG. 6. Serum ferritin change from baseline to end of treatment according to baseline values in the 71 patients in L1 group. The vertical line indicates no change.

and DF for iron chelation in β-thalassemia. The results show that the two treatments cause a similar reduction in serum ferritin over one year, in patients with relatively low serum ferritin before treatment (Table 3, Figs. 4–7).

The study showed also several other indicators of a similar chelating effect of the two trial treatments. The most important is the mean reduction in the liver iron content. Although only 36/144 patients gave consent to undergo liver biopsy before and after treatment, the clinical characteristics of these 36 patients were comparable to the whole trial population and within the two treatment subgroups. The presence of not high LIC levels in both treated groups (Tables 1, 3, and 4) could be due to the reason that the selected subjects having relatively low serum ferritin levels, according to the main inclusion criteria, are representative of a well-chelated patients group in which beneficial effects of subcutaneous deferoxamine on iron loading within the liver has been shown (37, 38).

It is worth to note that although the liver iron overload was largely below the value previously suggested as a threshold for predicting a beneficial effect of L1 (14), the observed reduction of liver iron content might be clinically remarkable over a longer period of treatment. The comparability of the reduction of liver iron content achieved with the two treatments was also confirmed by the comparable increase of liver ISR assessed by NMR in the two treatment groups

(Table 3). We previously showed a correlation in thalassemia major patients between liver ISR, assessed by the same NRM equipment used in this study, and Liver Iron Concentration (30).

The comparable increase of heart ISR on NMR (in the whole heart as well as in the left and in the right ventricles separately) as well as the lack of significant variations of the heart function we found in the two trial treatment groups, also adds to the evidence that the two treatments have similar effects on the overall iron overload.

Adverse events were more frequent with L1 (24/71 patients vs 11/73 patients), although the difference was almost entirely due to a moderate hypertransaminasemia which spontaneously subsided in ten patients. The recurrence of hypertransaminasemia when restarting the treatment caused a temporary treatment withdrawal in three and a definitive withdrawal in other three. Among this group of patients with hypertransaminasemia 14/16 (87%) were Anti-HCV positive. The other adverse events were few and mild, requiring a reduction of the drug dose only in 3 patients experiencing nausea. A marked leukopenia caused a definitive treatment withdrawal only in two other patients. In these two patient pre-treatment leukocyte count was achieved after stopping treatment. Therefore only in 5 of 24 patients experiencing adverse events with L1, treatment withdrawal was needed. However, all of the adverse events were reversible and not otherwise
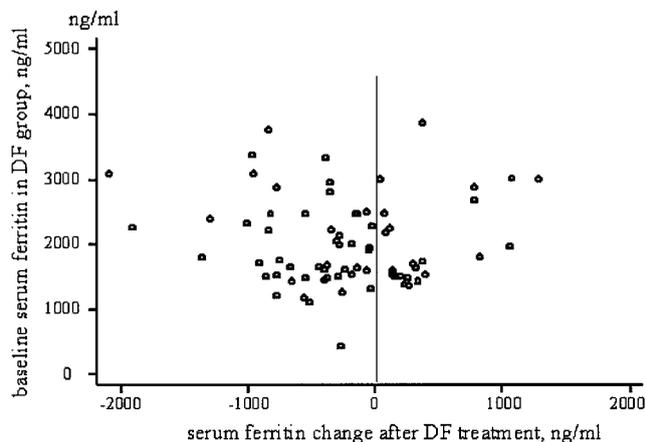
FIG. 7. Serum ferritin change from baseline to end of treatment according to baseline values in the 73 patients in DF group. The vertical line indicates no change.

TABLE 5

Individual Serum Ferritin Concentration at Baseline and at the End of the Treatment for 18 Patients with Baseline Values >3000 ng/ml

	L1 group				DF group		
Patient ID	Baseline	End of treatment	Difference	Patient ID	Baseline	End of treatment	Difference
3	3630	2190	−1440	30	3760	2920	−840
4	3700	2470	−1230	57	3010	4090	1080
70	3010	3700	690	62	3330	2940	−390
103	4650	4110	−540	64	3090	2130	−960
104	4140	4300	160	82	3370	2400	−970
105	3180	2620	−560	117	3080	990	−2090
106	3790	2900	−890	126	3870	4250	380
115	3010	2790	−220				
120	3090	2060	−1030				
124	3130	3290	160				
142	4570	2270	−2300				
Total ($n = 11$)[a]	3627 ± 610	2973 ± 777*	654 ± 848**	Total ($n = 7$)[a]	3358 ± 340	2817 ± 1131	541 ± 1028

Note. Data are ng/ml. Difference is "end of treatment" value minus baseline value.
[a] Mean ± SD.
* $P < 0.05$ compared with baseline (paired t test).
** $P = 0.81$ compared with DF group (two-sample t test with equal variances).

clinically significant. Side effects with DF were observed in 11/73 patients and the most frequent was pain and erythema at the injection site which required a reduction of the drug dose in 6 patients.

We did not find any appreciable difference in liver fibrosis with the two treatments, in the patients who gave consent for a repeat liver biopsy. Fibrosis was blindly rated by two independent observers; they rated the fibrosis according to the same score (34) used in the Olivieri's study (23) and their agreement beyond chance was satisfactory (k-weighted $= 0.59$; $P < 0.0001$) (Fig. 3).

There are several possible explanations for the different findings on the progression of liver fibrosis in our study compared with that by Olivieri and colleagues (23). The mean follow-up was 55 months in the Olivieri's study and 30 months in the L1 treated patients in the present one; the initial serum ferritin concentration and liver iron content were distinctly lower in the present study; the two studies assessed a relatively low number of patients and the differences in the results may be due to chance. There are, however several methodological differences between the two studies, that have to be considered. The present study is prospective, the treatment was assigned by randomization, the control group was a parallel one, the interobserver agreement was assessed accord-

ing to standard methodology and was satisfactory. All these methodological characteristics, were not met by the Olivieri's study, therefore making the comparability of the two studies uncertain. Our results agree with previous studies, reported by ourselves and other authors, giving liver biopsy data (either single or repeated) in patients receiving deferiprone (22, 39–42). Moreover, Cohen *et al.* (43) during a safety prospective multicenter study suggested that, although alanine transaminase (ALT) levels rose during therapy, the increase of ALT levels were generally transient and occurred more commonly in patients with virus C hepatitis. Thus, although our results concerning the risk of progression of fibrosis may not be considered conclusive, they at least suggest that whether L1 treatment is associated to such a risk remains still unsettled and that further well designed and well conducted studies to assess this specific point should be performed especially to assess if other factors as HCV or liver iron concentration could be involved.

Interpretation

This study shows that over a relatively short time period in patients with relatively low initial serum ferritin concentration, deferiprone has an

iron chelating effect not significantly different from deferoxamine. Under the conditions of this study, deferiprone proved to be satisfactorily safe and the previously reported risk of progression of liver fibrosis with this drug was not confirmed.

Generalizability

According to the eligibility and exclusion criteria used in this study and the large ratio of recruited to excluded patients, it may be expected that the observed treatment effect may be reproduced in patients with thalassemia major and serum ferritin concentration below 3000 ng/ml over a 1-year treatment period. The results in the subgroup of patients with baseline serum ferritin above 3000 ng/ml suggest that deferiprone might be beneficial also in patients with higher values of ferritin. However this should be confirmed in future RCTs.

ACKNOWLEDGMENTS

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