A randomized controlled 1-year study of daily deferiprone plus twice weekly desferrioxamine compared with daily deferiprone monotherapy in patients with thalassemia major

Yesim Aydinok, Zulal Ulger, Deniz Nart, Aysen Terzi, Nurten Cetiner, Gareth Ellis, Arthur Zimmermann, Chantal Manz

From the Ege University, Faculty of Medicine, Department of Paediatric Haematology, Izmir, Turkey (YA, AT); Ege University, Faculty of Medicine, Department of Cardiology, Izmir, Turkey (ZU), Ege University, Faculty of Medicine, Department of Pathology, Izmir, Turkey (DN), Ege University, Faculty of Medicine, Research Laboratory, Izmir, Turkey (NC); Royal Free Hospital, Haematology Department, Hampstead, London, United Kingdom (GE); University of Berne, Institute of Pathology, Switzerland (AZ), Clinical R & D, Lipomed AG, Arlesheim, Switzerland (CM).

Manuscript received February 21, 2007.
Manuscript accepted August 16, 2007.

Correspondence:
Yesim Aydinok, Ege University Faculty of Medicine, Department of Pediatric Hematology 35100 Bornova, Izmir, Turkey.
E-mail: yesim.aydinok@ege.edu.tr

ABSTRACT

Background and Objectives

The aim of this prospective, randomized, 1-year study was to compare the efficacy and safety of oral deferiprone (DFP) with those of combinations of parenteral desferrioxamine (DFO) with oral DFP.

Design and Methods

A total of 24 patients with thalassemia major were randomized to receive one of the following two treatments; DFP given at a daily dose of 75 mg/kg in combination with DFO (40-50 mg/kg twice weekly) (n=12) or as single agent (n=12). In addition, 12 patients treated with 40-50 mg/kg DFO 5 days weekly were included as a reference group without randomization. Changes in liver iron concentration (LIC) and serum ferritin (SF) were assessed; total iron excretion (TIE), urinary iron excretion (UIE) and iron balance were calculated. Cardiac function and toxicity were also examined.

Design and Methods

SF and LIC were significantly reduced after 1 year of combination therapy (p=0.01 and 0.07, respectively). A decrease of LIC was observed in all but one patient (87.5%) following the combination therapy but in only 42% of patients treated with DFP monotherapy. In the DFO reference group, a statistically significant decrease in LIC (p=0.01) associated with a substantial decrease in SF (p=0.08) was observed after 1 year. The combination regimen resulted in greater TIE compared to DFP monotherapy (p=0.08) and was the regimen associated with the highest iron balance compared to DFP monotherapy (p=0.04) or standard DFO treatment (p=0.006).

Interpretations and Conclusions

The addition of subcutaneous DFO twice weekly to oral DFP 75 mg/kg is a highly efficacious and safe chelation therapy providing superior chelation activity to that of DFP and likely has an efficacy profile comparable to that of standard DFO.

Key words: thalassemia, deferiprone, desferrioxamine, combination therapy, iron overload, iron balance, liver iron concentration

Haematologica 2007; 92:1599-1606. DOI:10.3324/haematol.11414

It has been suggested that enhanced chelation and a decrease in total body iron stores can be obtained by combining the two iron chelating drugs desferrioxamine (DFO) and deferiprone (DFP) either sequentially or simultaneously.[1-3] In principle, this can be achieved by increasing the total exposure to chelation therapy over each 24-hour period with sequential treatment,[4] or by increasing net iron excretion by a *synergistic* effect when the two drugs are given simultaneously.[1] While metabolic balance studies have shown that excretion of urinary iron may increase when DFP is added to DFO, this may not give a true representation of iron balance because at least half of total iron excretion with DFO monotherapy is through the fecal route. Iron balance can be effectively estimated by measuring changes in liver iron concentration (LIC) over time, as LIC is precisely related to body iron stores.[5] Although there have been many observations on the combined use of these chelators showing decreases in serum ferritin (SF),[3,6-13] relatively few studies have examined changes in LIC.[12,14] Some randomized trials compared the effectiveness of either DFP monotherapy[15-17] or combination therapy[6] with that of standard DFO therapy, but the effect on LIC was not compared in patients receiving either combination treatment or DFP monotherapy in a randomized, prospective study. In the present study, the efficacy (assessed by reductions in LIC and SF, and the net iron balance) and safety of combination treatment with parenteral DFO and oral DFP were compared to those of oral DFP monotherapy in a randomized, controlled 1-year study.

Design and Methods

Patients

A total of 24 patients with thalassemia major were randomized to receive one of the following two treatments; DFP (500 mg tablets of deferiprone, Lipomed AG, Switzerland) given at a daily dose of 75 mg/kg in combination with DFO (Desferal®, Novartis, Switzerland; 40-50 mg/kg twice weekly) or as a single agent. Two of the three daily DFP doses were administered simultaneously with the DFO infusion. The randomization sequence was generated by the Department of Mathematical Statistics at the University of Berne, Switzerland according to local policy. Upon central registration of a subject by the investigator, the study co-ordinator assigned the intervention according to the randomization sequence and after eligibility control without concealing the sequence prior to allocation. This study was approved by the Institutional Review Board of the Turkish Ministry of Health and the local ethics committee and all patients treated in this study gave written informed consent prior to inclusion. In addition, 12 patients treated with 40-50 mg/kg DFO 5 days weekly were selected, without randomization, as a reference group. These selected patients were highly compliant to DFO treatment. They were not randomized into the trial, but gave their consent to be treated 5 days weekly with DFO and underwent all the evaluations set out in the study design.

All patients received regular blood transfusions at 2-4 weekly intervals to maintain hemoglobin levels above 9 g/dL and all had been treated with DFO prior to the commencement of the study. Splenectomy was considered when blood consumption required to maintain pretransfusional hemoglobin levels above 9 g/dL was greater than 200 mL/kg/year of packed red blood cells. The proportion of splenectomized patients was 7/12 (58%), 5/12 (42%) and 8/12 (67%) in the DFP monotherapy, DFP and DFO combination therapy and DFO monotherapy arms, respectively. Iron-overloaded thalassemic patients at least 4 years old were eligible for inclusion in the study. Exclusion criteria were lack of compliance, known toxicity or intolerance preventing therapy with DFO and DFP, neutropenia (neutrophils $<1.5\times10^9$/L), thrombocytopenia (platelets $<100\times10^9$/L), renal, hepatic or decompensated heart failure, active viral illness being treated with interferon-α/ribavirin, repeated *Yersinia* infections, HIV–positivity, pregnancy or nursing, and patients of reproductive age not taking adequate contraceptive precautions. There were no statistically significant differences between the characteristics of the patients enrolled in the DFP monotherapy or combined treatment arms (Table 1). The patients in the DFO reference arm were known to have excellent compliance to DFO therapy, resulting in a lower body iron burden as assessed by SF and LIC. For this reason, the patients of this group were not included in the direct statistical comparison with those of the other two treatment groups.

Methods

Changes in LIC and SF were considered as the primary efficacy end-points, but total iron excretion (TIE), urinary iron excretion (UIE), iron balance, cardiac function (determined by echocardiography) and toxicity were also assessed. Baseline investigations were completed within 4 weeks prior to the study. All patients had a wash-out period of 2 weeks without any iron chelating medication before initiating the study treatment. Full blood counts and white cell differentials were assessed weekly for the first 8 weeks and fortnightly thereafter. SF and liver enzymes were measured at 3-monthly intervals. LIC was assessed in biopsies prior to the study and after 1 year of treatment. Duplicate samples for LIC assessment obtained from one pass of a Menghini type biopsy needle were measured in two laboratories, i.e. locally at Ege University Faculty of Medicine in Izmir as well as at the Royal Free Hospital in London. Liver biopsy samples of less than 0.5 mg dry weight were excluded from analysis. Fresh samples measured in Izmir were stored frozen at -80 °C prior to analysis. Iron was measured by inductively coupled plasma - atomic emission spectrophotometry (ICP-AES) using a Jobin Yvon JY spectrophotometer.[18] Samples evaluated in London were fixed in 10% neutral buffered formalin solution and then embedded in paraffin according to the method of Villeneuve *et al.*[19] LIC was measured colori-

metrically using bathophenanthroline sulphonate according to an adapted validated method of Torrance and Bothwell.[20] A linear regression analysis comparing the two methods indicated higher values for the locally assessed samples than for those evaluated in London. Although there was a very significant correlation between LIC values measured by the two methods (baseline rho=0.6, p=0.002 and final rho=0.8, p<0.001), the coefficient variation was high and normalization to the London method widened the error. Therefore, only local data were considered for further analyses. The histology of liver biopsy samples, including fibrosis scoring and histology activity index (HAI) grading was assessed according to Ishak *et al.*[21] Liver iron was scored according to Sciot *et al.*[22] All slides were examined by the same observer (*DN*), who had no information on the patient, his/her clinical status or treatment arm. The inter-observer reproducibility of Ishak's fibrosis staging was confirmed by another pathologist (*AZ*) who repeated the assessment in a blinded way without access to any information on the patient. At quarterly intervals, prior to transfusion, urine was collected for 24 hours for evaluation of UIE. For patients receiving combination therapy, urine was collected on 2 days, during one day of DFP monotherapy and on the second day of the combination treatment. UIE was measured using ICP-AES. The average UIE of quarterly measurements was calculated for each patient. The proportion of urinary and fecal iron excretion induced by DFO and DFP monotherapies and the effect of the combination regimen on the route of iron excretion were estimated from TIE. TIE was calculated using the following formula TIE=iron transfused during study period (mg) + [(LIC at T_o-LIC at T_{1y}) × 10^6 × body weight in kg]/number of days on treatment between biopsies, as previously described by Angelucci *et al.*[5] Iron intake was calculated from the number of transfusions received during the study period considering the measured hematocrit resulting in an average iron content of 154 mg per transfusion unit. Viral serology and auditory and visual functions were assessed at 6-monthly intervals. Audiological evaluation consisted of determination of pure tone air and bone conduction thresholds of 125-10,000 Hz using an Inter Acoustic A30 or AC5 applying standard specifications.[23] Visual acuity was tested using a Snellen chart; fundoscopic examination was performed by the same ophthalmologist using indirect ophthalmoscopy and electro-oculographic (EOG) testing was carried out and recorded in accordance with the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV). Cardiac function was monitored at 6-monthly intervals by echocardiography, measuring left ventricular ejection fraction (LVEF) and fractionated shortening (FS) according to the method of Teichholz. Holter monitoring was performed at the start of the study and after 1 year of treatment. National Cancer Institute expanded common toxicity criteria for adverse events (NCI CTCAE v3.0) were used to evaluate the adverse events encountered during the study period.[41] The patients' compliance and tolerance to treatment and quality of life were also assessed at 3-monthly intervals. Compliance was assessed by drug accounting at each visit (by counting the returned empty blisters of deferiprone and used vials of desferrioxamine) as well as by a study-specific designed questionnaire completed by the patients and/or their legal representative/guardian at quarterly intervals. The same questionnaire also served for the assessment of tolerance to treatment and quality of life.

Statistical analyses were performed using non-parametric tests in SPSS 1.0 for Windows. All evaluations were performed on an intention-to-treat basis. For continuous variables descriptive statistics were obtained and comparisons between treatment arms were made using the corresponding non-parametric approaches (Mann-Whitney test, Wilcoxon signed ranks test) when appropriate. Spearman's correlation coefficient was used to measure how variables are related.

Table 1. Characteristics of patients at the start of the study.

Chelation Regimen	*Age (years)*	*Pretransfusional Hb (g/dl)*	*Blood Consumption (mL/kg/year)*	*Baseline Ferritin (μg/L)*	*Baseline LIC (mg/g dw)*
DFP (n=12)	15.9±4.2 (9-23)	8.9±0.5 (8.2-9.7)	156±45 (80-255)	4070±3223 (1014-10859)	30.7±10.6 (13.5-48.7)
DFP+DFO (n=12)	16.6±4.8 (9-23)	8.6±0.5 (7.7-9.3)	145±20 (106-167)	4453±2858 (570-9508)	27.0±13.4 (11.0-53.4)
*p**	0.74	0.32	0.57	1.0	0.39
DFO (n=12)	15.0±6.4 (5.0-24.5)	9.2±0.5 (8.2-10.0)	166±44 (114-237)	1900±1358 (972-6028)	10.7±4.7 (5.0-19.4)
*p***	0.93	0.12	0.63	0.09	<0.001
*p****	0.52	0.01	0.31	0.07	0.001

*LIC, liver iron concentration; dw, dry weight; Mann-Whitney-U test: *, DFP vs DFP+DFO **, DFP vs DFO; ***, DFP+DFO vs DFO. Demographic data are shown as mean ± SD and ranges are indicated in brackets.*

Results

Eight of the 12 patients in the combination treatment arm and all patients in the DFP and DFO arms were eligible for assessment of efficacy and safety. In total, four patients treated with combination therapy dropped out of the study: two patients withdrew consent just after enrol-lment because of refusal of DFO therapy, one died from arrhythmia-induced congestive heart failure at the beginning of the study, and one developed agranulocytosis at week 14. The mean DFP (78.2±1.4 mg/kg/day) and DFO (43.8±2.8 mg/kg × 2 days/week) doses given to patients receiving combination therapy were comparable to the mean doses given to patients receiving DFP monotherapy (78.2±2.6 mg/kg/day) or DFO monotherapy (41.3±6.9 mg/kg × 5 days/week) (p=0.82 and p=0.26, respectively).

Efficacy

The majority of patients in both treatment arms receiving DFP showed a decrease in SF after 1 year which was statistically significant in those treated with the combination regimen (p=0.01) (Figure 1, Table 2). LIC was reduced in all but one patient (87.5%) receiving combination therapy (p=0.07) (Table 2, Figure 2B) whereas only 42% of patients treated with DFP monotherapy showed a decrease in LIC after 1 year (Figure 2A). LIC did not change in 8% and increased in 50% of the patients on DFP treatment (Figure 2A). In the DFO-treated reference group, two liver biopsies were not assessable due to low sample weight (<0.5 mg). A statistically significant decrease in LIC (p=0.03), associated with a substantial decrease in SF (p=0.08), was observed in the DFO group after 1 year (Table 2). LIC decreased in all but one patient in whom LIC was below 7 mg/g dw at study start and after 12 months of DFO therapy. The mean hemoglobin level increased significantly in the group treated with combination therapy from 8.6±0.6 to 9.2±0.4 mg/dL (p=0.02), but not in the other two treatment arms. However, the blood consumption and pre-transfusional hemoglobin levels did not vary in any of the treatment groups before and during the study (*data not shown*). Under the conditions of this trial, monotherapy with DFP had the least effect on TIE. The addition of subcutaneous DFO twice weekly to daily DFP therapy resulted in greater iron excretion (p=0.08) than that produced by DFP monotherapy, and in a significantly higher ratio of iron excretion to iron intake (net iron balance) compared to that achieved with DFP monotherapy (p=0.04) or standard DFO therapy given subcutaneously 5 days a week (p=0.006) (Table 3). The mean UIE on days of combination therapy (0.88±0.32 mg/kg/day) was significantly higher than on days of DFP monotherapy (0.38±0.22 mg/kg/day) (p=0.01) indicating an additive effect on UIE by simultaneous administration of both drugs (*data not shown*). However, it has been shown that only 42% of estimated TIE was achieved by the urinary route in patients given DFO monotherapy whereas iron excretion caused by DFP occurred exclusively via the urinary route (100%). The addition of subcutaneous DFO twice weekly to daily DFP therapy diverted on average 10% of total iron to the bile and feces whereas the remaining 90% was excreted via urine (Figure 3).

Safety

Neutropenia and agranulocytosis. Neutropenia was observed in two non-splenectomized patients, one of whom was receiving combination therapy while the other was treated with DFP as a single agent. One of these patients was neutropenic twice at weeks 4 and 10 before she developed agranulocytosis week 14. This patient fully recovered within 11 days following empirical antibiotic therapy as well as granulocyte-colony stimulating factor therapy. Although neutropenia was observed in three non-splenectomized patients treated with DFO in the reference arm, and occurred in two of them repeatedly, these patients did not develop agranulocytosis.

Transaminase levels. Transient fluctuations in serum alanine aminotransferase (ALT) levels were observed in patients treated with DFP either alone or in combination with DFO (*data not shown*). The mean baseline ALT value was higher than the upper normal limit in patients in these

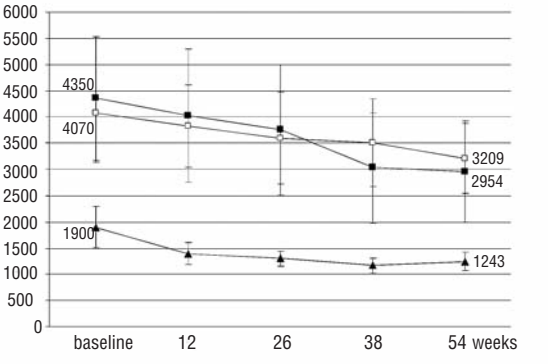

Figure 1. Effects of different chelation regimens (J DFP+DFO, k DFP, G DFO) on serum ferritin levels. Mean SF levels ± standard errors (SE) at the start of the study and after 3, 6, 9 and 12 months of therapy with DFP (12 patients), the combination of DFP and DFO (8 patients) or DFO (12 patients). Mean SF levels decreased steadily during the 12 months of therapy in all treatment arms; the change was statistically significant (*, Wilcoxon paired test: $p \leq 0.01$) in the combination treatment arm.

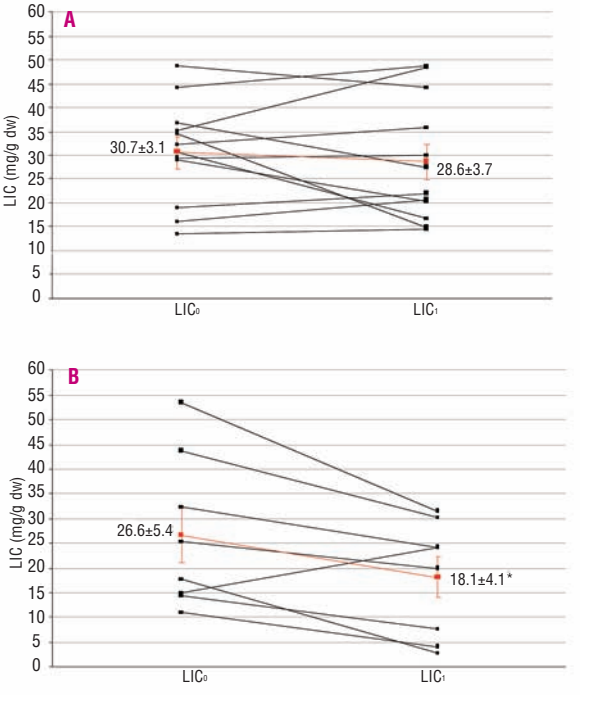

Figure 2. A. Effects of DFP therapy on LIC after 12 months. B. Effects of combination therapy on LIC after 12 months. Individual LIC values (black lines) and mean ± standard errors (SE) values (red line) at the start of the study and after 12 months of treatment with DFP (A) or a combination of DFP and DFO (B) in 12 and 8 patients, respectively. The mean LIC value did not change significantly in patients treated with DFP, but decreased significantly (*Wilcoxon's paired test: p=0.01) in patients receiving the combination therapy.

Table 2. Changes in serum ferritin (SF) levels and liver iron concentration (LIC) after 1 year of treatment with different iron chelation regimens.

Chelation regimens	Basal SF (μg/L)	Final SF (μg/L)	Absolute change of SF	% change of SF	p*
DFP (n=12)	4070±3223 (1014-10859)	3209±2279 (870-8100)	-861	-21	0.10
DFP+DFO (n=8)	4350±3342 (570-9508)	2954±2765 (69-6825)	-1396	-32	0.01
DFO (n=12)	1900±1358 (972-6028)	1243±610 (264-2337)	-657	-34.5	0.08
Chelation regimens	**Basal LIC (mg/g/ d.w.)**	**Final LIC (mg/g/ d.w.)**	**Absolute change of LIC**	**% Change of LIC**	**p***
DFP (n=12)	30.7±10.6 (13.5-48.7)	28.6±12.8 (14.5-48.6)	-2.1	-7	0.61
DFP+DFO (n=8)	26.6±15.4 (11.0-53.4)	18.1±11.6 (2.7-31.5)	-8.5	-32	0.07
DFO (n=12)	10.7±4.7 (5.0-19.4)	6.2±2.7 (1.6-11.6)	-4.5	-42	0.03

*d.w.: dry weight; *: Wilcoxon's paired test. Initial and final mean ± SD values of SF and LIC with ranges in brackets as well as the corresponding absolute changes and percentage changes are indicated.*

two treatment groups, but normalized towards the end of study. The overall decrease in ALT between the start and the end of the study was significant in patients on combination therapy (p=0.04). Patients treated with DFO showed no change in serum ALT levels during the study. *Liver histopathology*. The dual histological assessment of stage of liver fibrosis showed very small inter-observer variation and there was a good correlation between two independent readings (*DN vs AZ*: rho=0.8, p<0.0001). While the fibrosis score did not change significantly after 1 year in patients in any of the treatment arms, the histology activity index (HAI) and liver iron score decreased substantially in patients receiving combination treatment (p=0.07 and p=0.06, respectively), but not in those given either of the monotherapies (Figure 4).

Cardiac function. With the exception of one patient who died from cardiac failure on day 7 just after enrollment into the study, none of the patients experienced clinical heart failure or arrhythmia during the study. LVEF and FS values remained within normal ranges in all patients. However, it is noteworthy that LVEF and FS had decreased significantly (p=0.03 and p=0.04, respectively) among patients in the DFO reference group by the end of study, although values were still within the normal range (Table 4).

Auditory and visual functions. Audiometric assessments did not indicate any significant hearing loss among patients at screening or after 1 year in all treatment groups. Visual acuity was found to be affected at baseline in one patient (0.5/0.6) only, in whom measurement of visual evoked potentials (VEP) confirmed this finding with apparently delayed latency (146/168 msec). There were no pathologi-

Table 3. Iron balance associated with the different chelation regimens.

Chelation regimens	Iron intake (mg/kg/day)	Iron excretion (mg/kg/day)	Iron excretion/ intake ratio
DFP (n=12)	0.30±0.06	0.36±0.27	1.23±1.08
DFP+DFO (n=8)	0.26±0.07	0.57±0.14	2.37±1.07
p*	0.24	0.08	0.04
DFO (n=12)	0.34±0.14	0.49±0.25	1.39±0.71
p**	0.71	0.27	0.36
p***	0.24	0.62	0.006

*Mann-Whitney-U test: *DFP vs DFP+DFO; **DFP vs DFO; ***DFP+DFO vs DFO. Iron intake means the iron transfused during the study period. Total iron excretion was calculated from the change in LIC according to Angelucci et al.[42] The ratio between total iron excretion and iron intake indicates the net iron balance. All values are shown as mean±SD.*

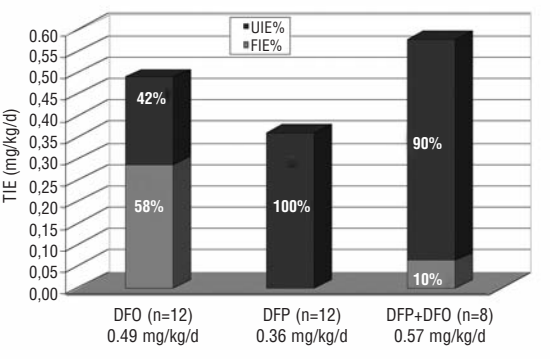

Figure 3. The proportions of urinary (UIE) and fecal (FIE) iron excretion in relation to daily total iron excretion (TIE) associated with the different chelation regimens. FIE was calculated by subtracting UIE from TIE, all indicated as mean values in mg/kg/day for each chelation regimen.

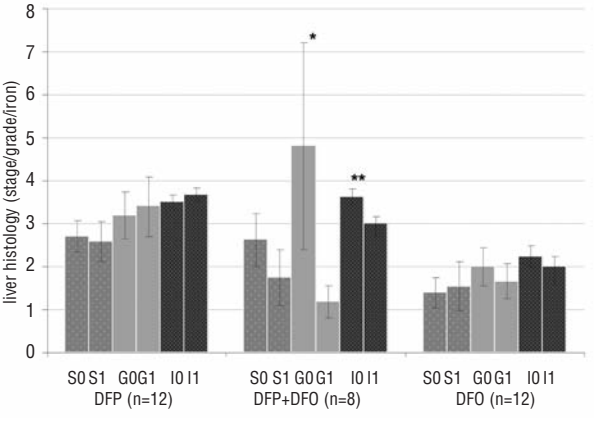

Figure 4 (right). Fibrosis (stage) (S), histology activity index (grade) (G), and iron (grade) scores (I) at the start of the study (S0, G0, I0) and after 12 months (S1, G1, I1). The fibrosis stage (according to Ishak), HAI grade (according to Ishak) and iron grade score (according to Sciot) were determined in liver biopsies at the start and at the end of study. The mean values ± standard errors (SE) are shown for each treatment regimen. There were no significant changes in fibrosis stage (S) in any of the treatment arms. HAI (G) and iron grades (I) were reduced substantially in patients treated for 12 months with DFP and DFO (Wilcoxon paired test: *p=0.07 and **p=0.06, respectively).

cal changes at fundus examinations and all patients had a normal EOG pattern at baseline and at the end of the study.

Other adverse events. Mild nausea was initially observed in five patients treated with either combination therapy or DFP monotherapy and resolved within a few weeks without antiemetic treatment. One patient on combination therapy suffered from grade 2 arthralgia which was controlled by short-term anti-inflammatory therapy. Aseptic meningitis not associated with neutropenia developed in one patient receiving DFP monotherapy in week 45. One patient experienced an acute cerebellar syndrome with symptoms of dizziness, tinnitus and truncal ataxia during the follow-up period with DFP monotherapy. Bilateral slowness of conduction in VEP was also detected. Although the symptoms and findings were probably related to the sequelae of infection, DFP therapy was discontinued and the symptoms resolved gradually after cessation of DFP. Mild local reactions were observed in several patients treated with DFO.

Compliance and quality of life. Compliance was generally excellent during the entire study period. There was only one patient in the DFP treatment arm who missed more than one chelation dose per week because of problems with swallowing. Quality of life was assessed by a non-validated study-designed questionnaire which was filled out by parents and patients at the start of the study and at quarterly intervals during the study: 67% of patients treated with the combination regimen and 64% of patients receiving DFP alone, but only 20% patients treated with DFO alone described an improvement of quality of life. The majority of patients had no problems with the intake and swallowing of the deferiprone tablets, the parenteral use of DFO or inserting a needle.

Table 4. Cardiac functions at the start of the study and after 1 year.

Chelation therapy	*Initial EF (%)*	*Final EF (%)*	*Absolute change of EF*	*% Change of EF*	*p**
DFP (n=12)	72.5±7.4	67.4±9.8	-5.1	-7.0	0.30
DFP+DFO (n=8)	71.7±8.6	72.6±6.6	0.9	1.2	0.88
DFO (n=12)	74.0±10.9	67.4±8.0	-6.6	-8.9	0.03
Chelation therapy	*Initial FS (%)*	*Final FS (%)*	*Absolute change of FS*	*% Change of FS*	*p**
DFP (n=12)	41.7±6.6	37.3±7.1	-4.4	-10.5	0.26
DFP+DFO (n=8)	41.5±7.0	41.2±5.1	-0.3	-0.7	0.94
DFO (n=12)	43.5±9.6	37.6±6.3	-5.9	-13.5	0.04

**: Wilcoxon's paired test. Initial and final mean ± SD values of left ventricular ejection fraction (EF, normal value >55%) and fractionated shortening (FS, normal value >28%) as well as the corresponding absolute changes and percentage changes are indicated.*

Discussion

Although the prognosis of patients with thalassemia major was greatly improved with the introduction of DFO chelation therapy three decades ago, clinical experience indicates that patients' adherence to parenteral DFO treatment is poor in a clinically significant subset of patients, thus resulting in inadequate chelation and eventually complications of iron toxicity in non-compliant patients.[24] Considerable efforts have been devoted to finding an orally active chelator as an alternative to DFO and clinical trials with DFP started in the 1980s.[25-29] Initial clinical studies revealed that DFP was capable of decreasing SF levels and the decline was significant, particularly when pre-treatment ferritin values were high.[30,37] However, in a multicenter, prospective study, 25% of patients receiving DFP at a dose of 75 mg/kg/day during an observation period of 4 years discontinued therapy because of insufficient long-term effectiveness of the chosen dose, as assessed by SF levels and/or LIC.[31] In a 3-year large-scale study performed in Italy, SF decreased in 61%, remained stable in 21% and increased in 18% of patients.[32] In the present study, a reduction of SF was observed in 67% of subjects, whereas 33% of the patients randomized to DFP arm were considered unresponsive, as defined by a stabilization or increase of SF levels. By contrast, a statistically significant decrease in SF was achieved by the combination regimen, with all but one patient showing a clear decrease in SF levels. Similar results were previously obtained in several non-randomized trials investigating the combination regimen.[3,7-11,13] There are a few randomized, prospective trials indicating that combination therapy with DFP and DFO is at least as effective as DFO in reducing SF after 12 months of treatment.[6,37] This study is the first confirming that the combination regimen is superior to DFP monotherapy in lowering SF. These results contrast with those of another randomized trial, in which SF increased in the DFP treatment arm and stabilized in the combination treatment arm;[33] however, it should be considered that the observation period of 6 months was rather short. LIC has been described as an important criterion for assessing the efficacy of chelation therapy based on net iron balance.[34] However, only a few studies have analyzed LIC in patients receiving DFP-containing regimens. Olivieri and Brittenham[35] initially reported that DFP therapy decreased mean LIC significantly after a mean of 3.1 years follow-up ($p<0.005$) and this decrease was maintained after a mean of 4.6 years of DFP therapy ($p=0.07$).[35,36] The same authors later reported significant increases in LIC in patients treated in a randomized study with DFP ($p<0.01$), while no significant change of LIC was observed in patients maintained on DFO.[15] In a randomized 1-year study by Maggio *et al.*,[16] no significant change in LIC was seen in patients assigned to either DFP or DFO. Furthermore, Pennell *et al.*[17] reported that the reduction in LIC did not differ significantly between patients receiving higher doses of DFP (100 mg/kg/day) or standard DFO, although the reduction in LIC was significant only in the DFO arm after 1 year. In the current study, 87.5% of patients randomized to receive the

combination treatment had a decrease in LIC, only 42% of patients given DFP monotherapy had decreases, which were statistically insignificant, in LIC. A significant decrease in LIC was also recorded (p=0.03) in our previous study in which we investigated the sequential therapy of DFP (4 days a week) and DFO (2 days a week) in thalassemia patients over a period of 6 months.[12] The randomized study by Galanello *et al*.[42] also revealed that alternating use of DFP 5 days a week and DFO twice weekly had comparable efficacy to daily DFO monotherapy in controlling body iron. These results differ from data published earlier by Balveer *et al*.[8] and Ha *et al*.;[37] neither of these groups observed a significant drop in LIC measured in liver biopsies of patients treated with a combination regimen of daily DFP and twice weekly DFO after a period of 1 year. The present randomized study shows that the chosen combination regimen was more effective than DFP monotherapy at reducing liver iron. Although the reference group receiving standard DFO regimen consisted of patients with considerably lower initial mean LIC than the patients receiving the combination therapy (p=0.001), the combination regimen seemed to be as effective as DFO standard therapy in reducing liver iron.

It was first reported in 1998 that the simultaneous use of DFP and DFO had an additive effect on daily UIE.[3] In this study, the addition of subcutaneous DFO twice weekly to daily DFP not only resulted in an increase of UIE, but also induced some fecal iron excretion. The combination regimen was superior and more efficient in achieving a negative iron balance than DFP. However, the TIE achieved by the combination therapy was not additive if compared to that of DFO-induced iron excretion since a large proportion of iron was just diverted from the feces to the urine. The net iron balance was significantly better in patients on combination therapy than on DFO monotherapy.

Clinical experience has shown that the most serious side effect of DFP is agranulocytosis, which occurs in approximately 0.5% of patients and is more frequently observed in the first months of therapy as well as in patients with an intact spleen.[31,32,38] In concordance with these published results, the patient who developed agranulocytosis in this study had not been splenectomized and the event occurred just 3 months after starting the combination therapy. This study also confirmed that neutropenia is a common finding in chelated patients with an intact spleen since neutropenia was also observed in patients receiving standard DFO chelation. However, in agreement with the literature, none of the patients treated with DFO in this trial developed agranulocytosis. Transient fluctuating increases in serum ALT levels were observed throughout the study in patients receiving either DFP monotherapy or combination therapy. Previously, changes in ALT were attributed to concomitant hepatitis C virus (HCV) infection[38] although more recent studies have revealed that fluctuations in ALT occur regardless of hepatitis C status.[7,31] In the present study none of the patients suffered from HCV infection, but mean ALT levels were higher than the upper normal limit in both treatment groups at the beginning of the study. The ALT levels normalized by the end of 1 year of treatment. Substantial decreases in histological activity index and liver iron grades were also accompanied by ALT normalization in patients receiving combination treatment. In agreement with the literature,[7,31] nausea was the most prominent side effect observed, occurring in one in four patients during the first few weeks of therapy with DFP-containing regimens. However, neither antiemetic therapy nor cessation of DFP therapy was necessary and the nausea was transient, i.e. it did not reappear during the course of the study. In one patient suffering from arthralgia of her left knee, the symptoms were relieved by short-term anti-inflammatory therapy and temporary discontinuation of DFP for a few days. An association of joint problems with higher SF levels has been proposed by some investigators.[38,39] Interestingly, the only patient with joint symptoms presented with the highest pre-study LIC value (53.4 mg/g dw) of all patients in this study. A retrospective study showed significantly less myocardial iron overload and improved LVEF in thalassemia patients treated continuously with DFP than in those chelated with DFO.[40] In accordance with these data, a recent randomized 1-year study reported that myocardial T2* improved faster and was associated with an increase in LVEF in thalassemia patients treated with a higher dose of DFP (average dose: 92 mg/kg/day) than in those given a relatively low dose of DFO (average dose: 43 mg/kg/day for 5.7 days/week corresponding to 35 mg/kg/day for 7 days/week).[17] In our study, initial LVEF values were within the normal range in both groups of patients, receiving either DFP monotherapy or combination therapy and the mean LVEF values did not change significantly after 1 year of observation. However, LVEF and FS decreased among the patients in the DFO reference group by the end of study, although not below the lower normal range. Compliance to oral DFP is generally better than compliance to parenteral DFO therapy.[6,15] Compliance and tolerance to chelation therapy were excellent in all patients in all three treatment arms during this study. Only one patient treated with the combination regimen was withdrawn from the study because of an adverse event (agranulocytosis) related to the study drug (DFP).

In summary, this is the first randomized, controlled study comparing changes in LIC and thus TIE in patients treated with DFP as monotherapy or in combination with DFO. It was demonstrated that the combination of daily DFP and twice weekly DFO at standard doses is a highly efficacious and safe chelation therapy for patients with thalassemia major. The chelation potency of the combination therapy is superior to the chelation activity of DFP and the regimen is likely to have an efficacy profile comparable to that of standard DFO.

Authors' Contributions

YA, PI: contributions to conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the version to be published; AT: acquisition of

data, drafting the article, final approval of the version to be published; DN: acquisition of data, revising it critically for important intellectual content, final approval of the version to be published; NC: acquisition of data, revising it critically for important intellectual content, final approval of the version to be published; GE: acquisition of data, revising it critically for important intellectual content, final approval of the version to be published; AZ: acquisition of data, revising it critically for important intellectual content, final approval of the version to be published; CM: contributions to conception and designof the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the version to be published.

Conflict of Interest

This clinical study was sponsored by the pharmaceutical company Lipomed AG, Switzerland, but no grants or any financial support apart from providing the study medication and part of laboratory equipment were obtained for conducting this trial.

The authors reported no potential conflicts of interest.

References

1. Grady RW, Berdoukas V, Rachmilewitz E, Galanello R, Borgna-Pignatti C, Giardina PJ. Iron chelation therapy: optimizing chelation therapy: combining deferiprone and desferrioxamine. Blood 2000;96 Suppl 1:604a[abstract].
2. Breuer W, Ermers MJJ, Pootrakul P, Abramov A, Hershko C, Cabantchik ZI. Desferrioxamine chelatable iron, a component of serum non-transferrin bound iron, used for assessing chelation therapy. Blood 2001;97:792-8.
3. Wonke B, Wright C, Hoffbrand AV. Combined therapy with deferiprone and desferrioxamine. Br J Haematol 1998;103: 361-4.
4. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005;18:277-87.
5. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343:327-31.
6. Mourad FH, Hoffbrand AV, Sheikh -Taha M, Koussa S, Khoriaty AI, Taher A. Comparison between desferrioxamine and combined therapy with desferrioxamine and deferiprone in iron overloaded thalassaemia patients. Br J Haematol 2003; 12:187-9.
7. Origa R, Bina P, Agus A, Crobu G, Defraia E, Dessi C, et al. Combined therapy with deferiprone and desferrioxamine in thalassaemia major. Haematologica 2005;90: 1309-14.
8. Balveer K, Pryor K, Wonke B. Combined oral and parenteral iron chelation in β thalassaemia major. Med J Malaysia 2001; 55: 493-7.
9. Alymara V, Bourantas D, Chaidos A, Bouranta P, Gouva M, Vassou A, et al. Effectiveness and safety of combined iron-chelation therapy with deferoxamine and deferiprone. Hematol J 2004;5:475-9.
10. Kattamis A, Ladis V, Berdousi H, Kelekis NL, Alexopoulou E, Papasotiriou I, et al. Iron chelation treatment with combined therapy with deferiprone and deferioxamine: a 12-month trial. Blood Cells Mol Dis 2006;36:21-5.
11. Daar S, Pathare AV. Combined therapy with desferrioxamine and deferiprone in beta thalassemia major patients with transfusional iron overload. Ann Hematol 2006;85:315-9.
12. Aydinok Y, Nisli G, Kavakli K, Coker C, Kantar M, Cetingul N. Sequential use of deferiprone and desferrioxamine in primary school children with thalassemia major in Turkey. Acta Haematol 1999; 102:17-21.
13. Peng CT, Wu KH, Wu SF, Liang DC, Yang CP, Jang RC, et al. Deferiprone or deferoxamine vs. combination therapy in patients with β-thalassemia major: a case study in Taiwan. Hemoglobin 2006;30:125-30.
14. Francis S, McGirr M, Browne E, Berdoukas V, Lindeman R. Efficacy of combined chelation therapy with subcutaneous desferrioxamine and oral deferiprone in patients with thalassemia major. Blood 2003; 102:517a[abstract].
15. Olivieri NF, Brittenham GM. Final results of the randomized trial of deferiprone and deferoxamine. Blood 1997;90: 264a[abstract].
16. Maggio A, D'Amico G, Morabito A. Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. Blood Cells Mol Dis 2002;28:196-208.
17. Pennell DJ, Berdoukas V, Karagiorga M. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Blood 2006;107:3738-44.
18. Robbins DA. Methods for Biological Monitoring. 1988; chap 118. p. 223-7.
19. Villeneuve JP, Bilodeau M, Lepage R, Cote J, Lefebvre M. Variability in hepatic iron concentration measurement from needle-biopsy specimens. J Hepatol 1996;25:172-7.
20. Torrance JD, Bothwell TH. A simple technique for measuring storage iron concentrations in formalinised liver samples. S Afr J Med Sci 1968; 33: 9-11.
21. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-9.
22. Sciot R, Van Eyken P, Facchetti F, Callea F, Van Der Steen K, Van Dijck H, et al. Hepatocellular transferrin receptor expression in secondary siderosis. Liver 1989;9:52-61.
23. American National Standards Institute. Specifications for audiometers ANSI S36-1969. New York: American National Standards Institute. 1969.
24. Modell B, Khan M, Darlison M. Survival in β-thalassemia major in the UK: data from the UK Thalassemia Register. Lancet 2000;355:2051-2.
25. Kontoghiorghes GJ. New orally active iron chelators. Lancet 1985;1:817.
26. Kontoghiorghes GJ, Aldouri MA, Hoffbrand AV, Barr J, Wonke B, Kourouclaris T, et al. Effective chelation of iron in β thalassaemia with the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one. Br Med J (Clin Res Ed) 1987; 295:1509-12.
27. Kontoghiorghes GJ, Aldouri MA, Sheppard L, Hoffbrand AV. 1,2-Dimethyl-3-hydroxypyrid-4-one, an orally active chelator for treatment of iron overload. Lancet 1987;1:1294-5.
28. Tondury P, Kontoghiorghes GJ, Ridolfi-Luthy A, Hirt A, Hoffbrand AV, Lottenbach AM, et al. L1 (1,2-dimethyl-3-hydroxypyrid-4-one) for oral iron chelation in patients with β-thalassaemia major. Br J Haematol 1990;76:550-3.
29. Olivieri NF, Koren G, Hermann C, Bentur Y, Chung D, Klein J, et al. Comparison of oral iron chelator L1 and desferrioxamine in iron-loaded patients. Lancet 1990;336: 1275-9.
30. Hoffbrand AV, Cohen A, Hershko C. Role of deferiprone in chelation therapy for transfusional iron overload. Blood 2003; 102:17-24.
31. Cohen AR, Galanello R, Piga A, De Sanctis V, Tricta F. Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone. Blood 2003; 102: 1583-7.
32. Ceci A, Biardi P, Felisi M, Capellini MD, Carnelli V, De Sanctis V, et al. The safety and effectiveness of deferiprone in a large-scale, 3- year study in Italian patients. Br J Haematol 2002;118:330-6.
33. Gomber S, Saxena R, Madan N. Comparative efficacy of desferrioxamine, deferiprone and in combination on iron chelation in thalassemic children. Indian Ped 2004;41:21-7.
34. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassaemia. Blood 1997;89:739-61.
35. Olivieri NF, Brittenham GM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, et al. Iron chelation therapy with oral deferiprone in patients with thalassaemia major. N Engl J Med 1995; 332:918-22.
36. Olivieri NF, Brittenham GM, McLaren CE, Templeton DM, Cameron RG, McClelland RA et al. Long-term safety and effectiveness of iron chelation therapy with deferiprone for thalassaemia major. N Engl J Med 1998;339:417-23.
37. Ha SY, Chik KW, Ling, SC, Lee AC, Luk CW, Lam CW, et al. A randomized controlled study evaluating the safety and efficacy of deferiprone treatment in thalassemia major patients from Hong Kong. Hemoglobin 2006; 30:263-74.
38. Cohen AR, Galanello R, Piga A, Dipalma A,Vullo C, Tricta F. Safety profile of oral iron chelator deferiprone: a multicenter study. Br J Haematol 2000;108:305-12.
39. Agarwal MP, Gupte SS, Viswanathan C, Vasandani D, Ramanathan J, Desai N, et al. Long-term assessment of efficacy and safety of L1, an oral iron chelator, in transfusion dependent thalassemia: Indian trial. Br J Haematol 1992;82:460-6.
40. Anderson LJ, Wonke B, Prescott E, Holden S, Walker JM, Pennell DJ. Comparison of effects of oral deferiprone and subcutaneous desferrioxamine on myocardial iron concentrations and ventricular function in beta-thalassemia. Lancet 2002;360: 516-20.
41. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 3.0, DCTC, NCI, NIH, DHHS, March 31, 2003 (see http://ctep.cancer.gov), Publish date: 12 December 2003.
42. Galanello R, Kattamis A, Piga A, Fischer R, Leoni G, Ladis V, et al. A prospective randomized controlled trial on the safety and efficacy of alternating deferoxamine and deferiprone in the treatment of iron overload in patients with thalassemia. Haematologica 2006;91:1241-3.