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Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with β-thalassaemia major: application of SQUID biomagnetic liver susceptometry

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Summary. In this non-randomized prospective study, liver and spleen iron concentrations were monitored annually over a 4-year period by non-invasive Superconducting Quantum Interference Device biomagnetometry in 54 β-thalassaemia major patients (age, 7–22 years) receiving treatment with deferiprone (75 mg/kg/d). Median liver iron concentrations increased significantly from 1456 to 2029 and 2449 $\mu g/g_{liver}$ at baseline, after 2·0 and 3·2 years respectively. Another group of 51 thalassaemic patients (aged 4–34 years) who received desferrioxamine s.c. for 1·9 years increased their liver iron concentration from 1076 to 1260 $\mu g/g_{liver}$. Taking into account the increase of the daily iron input from transfusions of 3·6 mg/d, caused by weight gain in 67% of the patients treated with deferiprone, a larger total body iron elimination rate was achieved after 2 years than at baseline. A negative ferritin change was observed in 51% of the patients. In 15 non-splenectomized patients, liver iron significantly increased from 1260 to 1937 $\mu g/g_{liver}$ ($P < 0{\cdot}01$), but serum ferritin remained stable at 2100 μg/l, as did the spleen iron concentration at 1200 $\mu g/g_{spleen}$. A two-compartment model may predict an average chelation efficacy for desferrioxamine and deferiprone, with a saturation effect of the latter, for a certain chelation and transfusion regimen by a single liver iron quantification.

Keywords: thalassaemia, SQUID, iron, deferiprone, desferrioxamine.

In thalassaemia patients, the progressive iron accumulation, if not treated with adequate chelation therapy, leads to severe clinical complications and death during the second decade of life (Gabutti & Piga, 1996). Slow subcutaneous infusion by desferrioxamine (Desferal®, DFO) is the first choice approach for iron chelation. The dosage, typically 10–50 mg/kg/d, is adjusted to counter-balance the accumulation of iron from transfusion (Porter, 2001). Unfortunately, a number of patients fail to maintain satisfactory compliance with this treatment.

The need for a bioavailable oral iron chelator led to the discovery of deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one, DFP, previously named CP20, L1) (Hider *et al*, 1982). This compound has been studied extensively and several clinical trials have been reported (Kontoghiorghes *et al*, 1987, 1990a; Olivieri *et al*, 1990). Moreover, the desirable properties of this molecule have stimulated research on closely related iron chelators (Porter *et al*, 1989a; Liu *et al*, 1999).

Early short-term trials with deferiprone 75 mg/kg/24 h indicated that it possessed a similar efficacy to that of 30–40 mg/kg/24 h desferrioxamine in promoting iron excretion in iron-loaded patients (Kontoghiorghes *et al*, 1990a; Olivieri *et al*, 1990; Töndury *et al*, 1990; Al-Refaie *et al*, 1992). This claim was based on urinary iron excretion (UIE) and serum ferritin (SF) measurements. In contrast to

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desferrioxamine, faecal iron excretion was initially reported to be negligible with DFP (Kontoghiorghes *et al*, 1990b; Olivieri *et al*, 1990). However, subsequent studies reported appreciable amounts of iron in the stools (up to 33%) (Collins *et al*, 1994; Grady *et al*, 1996).

Longer-term trials (1–4 years) led to the observation of a more complex pattern of response to DFP. The most promising results were reported from severely iron-overloaded patients (Agarwal *et al*, 1992; Al-Refaie *et al*, 1995a,b). Typically, patients receiving DFO, with liver iron concentrations (LIC) >4470 $\mu g/g_{w.w.}$ (w.w. = wet weight) in biopsies had a 50% decrease of LIC as measured by biomagnetic liver susceptometry after 3 years of treatment with DFP (Olivieri *et al*, 1995). In a more recent study of 26 patients treated with DFP for 40 months, no significant change was found in SF or in UIE (Hoffbrand *et al*, 1998). In all these studies, DFP was applied to less compliant thalassaemic patients with more severe iron overload, as characterized by a mean ferritin value SF >3000 μg/l (Hoffbrand *et al*, 1998; Mazza *et al*, 1998; Taher *et al*, 1999). In most studies, the chelation effectiveness of DFP was estimated by comparing SF and UIE values with the initial values following DFO treatment. The only randomized prospective trial comparing DFO and DFP (Olivieri & Brittenham, 1997) reported a significant increase of LIC in patients treated with DFP, whereas patients under DFO treatment remained stable. These patients had remarkably low iron stores of about 2400 $\mu g/g_{w.w.}$.

In long-term studies of DFP treatment, the possibility of developing liver fibrosis has become a major concern (Olivieri *et al*, 1998; Piga *et al*, 1998; Stella *et al*, 1998; Töndury *et al*, 1998) and a loss of efficacy has been claimed (Matsui *et al*, 1991; Töndury *et al*, 1998; Taher *et al*, 1999).

The efficacy of iron chelation therapy is usually evaluated by measuring the UIE or changes in SF (Al-Refaie *et al*, 1995a,b,c; Kersten *et al*, 1996; Hoffbrand *et al*, 1998). However, these parameters give no direct information on the amount of, and changes in, body storage iron under therapy, especially in severely iron-loaded patients (Brittenham *et al*, 1993; Fischer *et al*, 1999). Direct assessment of tissue iron is crucial in the evaluation of the efficacy of an iron-chelating agent. The non-invasive method of Superconducting Quantum Interference Device biomagnetic liver susceptometry (SQUID-BLS) has been used to evaluate efficacy in only two centres (Nielsen *et al*, 1995; Olivieri *et al*, 1995). This method precisely monitors liver iron concentrations in iron-loaded patients and, in principle, can be used to determine the long-term efficacy of iron-depletion therapy (Fischer, 1998).

In this non-randomized, non-stratified prospective study, SQUID-BLS has been used in two thalassaemia major groups receiving chelation therapy with either DFP or DFO. We tried to investigate, describe and possibly predict the efficacy of the two chelators under a certain chelation and transfusion treatment regimen with the possibility of extending the findings to other chelator or treatment regimens, rather than directly comparing DFP with DFO under the same conditions.

PATIENTS AND METHODS

Patients from Turin with β-thalassaemia major ($n = 54$, aged 7–22 years) were followed annually by SQUID biomagnetometry over a 4-year period (1994/95–1999) at the Hamburg Biosusceptometer facility. Patients participated either in a programme of compassionate or experimental (Cohen *et al*, 2000) use of DFP. Further patient characteristics were as follows: mean age 16·9 ± 3·5 years, 29% were less than 16 years of age, 50% were female, 91% of patients were hepatitis C virus (HCV) positive, 32 patients were initially splenectomized and five patients received a splenectomy during the trial period (see also Table I). All patients under DFP treatment gave their free and informed consent in writing to enter the trial. The programme was approved by the ethical committee of the University of Turin.

In order to extend the investigation of efficacy to another chelator, a group of patients with β-thalassaemia major ($n = 51$, aged 4–34 years) under chelation treatment with s.c. DFO (34·2 ± 4·6 mg/kg/d) was evaluated. These patients were monitored routinely every 1–2 years by SQUID biomagnetometry for iron overload. Further patient characteristics were as follows: mean age was 16·6 ± 7·3 years, 47% were less than 16 years of age, 65% were female, 65% of the patients were HCV positive, 33 patients were initially splenectomized and one patient had a splenectomy during the observation period. Vitamin C (25–100 mg/d) was prescribed for 44 of 51 patients receiving DFO.

1,2-dimethyl-3-hydroxypyridin-4-one (DFP) was supplied as 0·5 g tablets from Apotex Inc. (Weston, Ontario, Canada). A standard dose of 75 mg/kg/d in three divided doses was adopted. Compliance was controlled by the Medication Event Monitoring System (MEMS®) (Olivieri *et al*, 1991). Before treatment with DFP, patients were treated with 35·3 ± 9·3 mg/kg/d s.c. DFO and vitamin C. The patient's nominal chelator dosage was multiplied by his/her compliance in order to obtain a mean chelation dose for comparison with other patients and for further calculations.

Haematological and biochemical parameters. Serum ferritin was determined by a fluoroimmuno assay (Autodelfia, Wallac Oy, Finland). Mean SF and alanine aminotransferase (ALT) was derived from at least three values within two transfusion intervals that were nearest to the SQUID-BLS measurement. Increased values during infection and/or occasionally elevated ALT levels were not considered.

The mean daily iron input from transfusion, K_{in} [mg Fe/d], was calculated according to accepted international guidelines from the amount of blood transfused between two SQUID-BLS measurements (Thalassemia International Federation, 2000).

Measurement of liver iron concentration (LIC) and estimation of total body iron stores. Liver iron concentrations were measured using a SQUID biosusceptometer (BTi, San Diego, CA, USA) (Paulson *et al*, 1991; Fischer, 1998). As this technique relies only on the magnetic properties of haemosiderin and ferritin iron, spleen iron concentration was also measured (Engelhardt *et al*, 1995). Assuming 80% of storage iron to be present in the liver and spleen of

Table I. Monitoring of iron stores by biomagnetic liver susceptometry: results after desferrioxamine treatment (baseline) and annually during 4 years of continuous deferiprone treatment in 54 patients (mean age 16·9 ± 3·5 years) and during 1·9 years of continuous desferrioxamine treatment in 51 patients (mean age 16·6 ± 7·3 years) with β-thalassaemia major.

	Patients on deferiprone (months after start of treatment)					Patients on desferrioxamine (months of monitoring)	
	Baseline	13·1 ± 1·9	23·9 ± 2·8	39·2 ± 4·1	48·5 ± 3·3	Baseline	23·0 ± 8·3
Number of patients (total/splenectomized)	54/32	54/34	54/37	45/32	10/10	51/33	51/34
Body weight (kg)	45 ± 13	50* ± 13	52* ± 13	54* ± 12	55* ± 7	40 ± 14	44* ± 13
Compliance†	0·88 [0·12]	0·98* [0·00]	0·98* [0·00]	0·96* [0·07]	0·97 [0·08]	0·94 [0·11]	0·90 [0·14]
Mean chelator dose rate (mg/kg/d)‡§	29·4 ± 8·2	72·3 ± 4·1	72·8 ± 2·7	69·7* ± 4·9	69·4 ± 5·5	36·2 ± 6·2	29·7 ± 4·6
Daily iron input from transfusion (mg/d)	20·8 ± 4·8	23·5* ± 4·5	24·4* ± 5·3	25·1* ± 4·5	24·8 ± 3·1	16·5 ± 4·9	18·9* ± 4·7
SF† (μg/l)	1897 [885]	2041 [838]	2116 [1402]	2235 [1466]	2519 [2582]	1422 [795]	1631 [951]
Ratio ferritin : LIC† (μg/l : $\mu g/g_{liver}$)	1·37 [0·68]	1·06* [0·66]	1·04* [0·81]	0·90* [0·56]	0·90* [0·55]	1·30 [0·88]	1·28 [0·91]
LIC by SQUID-BLS† ($\mu g/g_{liver}$)	1456 [898]	1873* [728]	2029* [912]	2449* [1022]	3064 [2040]	1076 [567]	1260 [770]
Liver volume (ml)	1472 ± 335	1569* ± 376	1727* ± 375	1705* ± 326	1732* ± 317	1265 ± 417	1387* ± 398
Total body iron stores† (mg)	2860 [1912]	3611* [1836]	4201* [1961]	4680* [2384]	5259 [5359]	1845 [1394]	2162 [1176]
TBIE rate (mg/d)	20·8 ± 4·8	21·5 ± 5·7	22·9* ± 6·5	23·8* ± 5·7	23·1 ± 5·2	16·5 ± 4·9	18·5 ± 4·9
Specific chelator dose rate† (mmol/d/g-Fe)‡	0·62 [0·39]	2·5 [1·1]	2·3* [1·2]	1·9* [1·0]	1·6 [1·6]	1·09 [0·97]	0·90 [0·51]
Molar efficiency (%)‡¶	19·6 ± 6·8	4·6 ± 0·9	4·6 ± 1·0	4·9 ± 1·4	4·5 ± 0·9	15·3 ± 3·8	17·6 ± 4·8
TBIE rate constant† k_{el} (%/d)	0·66 [0·43]	0·57* [0·30]	0·57 [0·38]	0·46* [0·29]	0·43 [0·44]	0·87 [0·48]	0·87 [0·43]

*Significance was tested versus baseline or initial values, respectively, at a level of $P < 0·01$ by paired T-test (means) or paired Wilcoxon-test (medians).
‡Significance tested at a level of $P < 0·01$ versus values under DFP treatment after 13·1 months.
†Results given as mean ± SD or median [interquartile range].
§Mean chelator dose rate: prescribed chelator dose rate normalized to compliance.
¶Molar efficiency = TBIE rate (mmol/d) / chelator dose (mmol/d).
BMI, body mass index; LIC, liver iron concentration; SQUID-BLS, Superconducting Quantum Interference Device biomagnetic liver susceptometry; TBIE, total body iron elimination.

iron-overloaded patients, the main total body iron stores could be estimated from liver and spleen volumes and biomagnetically-determined iron concentrations (Fischer *et al*, 1999). Liver and spleen volumes were measured using bedside sonographic imaging and cross-laser alignment of an ultrasound probe (Leung *et al*, 1986). Total body iron elimination (TBIE) rates were estimated from the daily iron input from transfusions and the difference of iron stores in a certain treatment interval (Fischer *et al*, 1999).

While the assessment of the chelator efficacy, according to the difference between initial and final total body iron stores (or LIC), had to assume a constant daily iron input within the chelation treatment interval, the TBIE rate also took into account a variable daily iron input from transfusions.

Model interpretation of chelator efficacy. The total body iron store during a certain treatment interval can be interpreted in the framework of a two-compartment model (Fischer *et al*, 1999). The transfused iron K_{in} and, to a lesser extent, the absorbed iron accumulate in the body iron store $U_S(t)$. Iron stores can change dramatically over long treatment intervals (> 1 year), giving rise to different iron concentrations in the chelatable iron pool $U_{CIP}(t)$ which is assumed to be in fast exchange with the iron store itself, i.e. $U_{CIP}(t) = \text{const} \cdot U_S(t)$. It can be shown that the time-dependent body iron store $U_S(t)$ is described by a non-linear 1st order differential equation (1):

$$dU_s/dt = K_{in} - k_{el} \cdot U_s(t) \quad (1)$$

where the TBIE rate constant k_{el} [d^{-1}] (= chelation probability) is a function of the mean chelator dose rate K_{ch} and the chelatable iron pool U_{CIP}. Assuming a saturation function (Gold, 1977: Hill equation) for the iron elimination rate constant, as in equation (2), results in a non-linear differential equation (1) with an approximate solution (Euler–Cauchy method) for the iron store $U_S(t)$.

$$k_{el} = k_{el}^0 + k_{el}^{max}/[1 + (M_{ch}/C_{ch})^n] \quad (2)$$

Thus, the efficacy of a chelator can be described by the iron elimination rate constant k_{el} [%/d] with a maximum saturation rate constant k_{el}^{max}, the specific chelator dose rate $C_{ch} = K_{ch}/U_S$ [mmol/d/g-Fe] and its dose constant M_{ch} at half-maximum elimination rate. The value for the basal elimination rate constant k_{el}^0 is 0.06%/d (Fischer *et al*, 1999).

In 1st order approximation, equation (1) can be solved analytically by assuming constant elimination rate constants within 1-year DFP treatment intervals. These elimination rate constants were fitted by equation (2) by calculating an averaged specific chelator dose rate C_{ch} from the initial and final iron store of the respective interval. Assuming steady state conditions: $dU_S/dt = K_{in} - k_{el} \cdot U_S = 0$, a TBIE rate constant was also derived for treatment with DFO at baseline.

Statistical methods. The skewness and kurtosis were calculated for all parameters. For those parameters with an absolute skewness > 0·5 or an absolute kurtosis > 1·0 (e.g. SF, LIC), a median value and the interquartile range was calculated instead of the arithmetic mean and its standard deviation. Consequently, the U-test or paired Wilcoxon-test was used for testing significant differences between groups instead of the paired T-test. Paired statistical tests take into account different numbers of patients, especially after 4 years of DFP treatment. Linear correlation was performed only after logarithmic transformation of the observables with skewed distributions. For statistical parameters and significance tests, the respective built-in functions of EXCEL 97 (Microsoft Corp., Seattle, WA, USA) and STATISTICA, version 6·0 (StatSoft Inc. 1998, Tulsa, OK, USA) were used. Special fit procedures based on the least chi-squares method (Marquardt algorithm) were written in Microsoft Visual Basic and HP-Basic for Windows.

RESULTS

Fifty-four patients with β-thalassaemia major were monitored non-invasively by SQUID BLS for iron store changes at baseline, under s.c. desferrioxamine chelation therapy before beginning the clinical trial with deferiprone, and after 1·1, 2·0, 3·2 and 4·0 years (see Table I). At baseline, the median LIC was 1456 μg/g_{liver}, with an interquartile range of 1070–1968 μg/g_{liver}, and a total range of 460–4040 μg/g_{liver}. Typical examples of changes in LIC in response to the underlying iron transfusion rate and chelation dose are presented for three patients in Fig 1. One patient exhibited increased iron levels, one displayed virtually no change and one displayed a negative iron balance.

No severe side effects were observed in this group of patients during 2–4 years of DFP treatment as reported by Cohen *et al* (2000). In the third year, five patients discontinued the chelation treatment with DFP, two of them for reasons of discomfort associated with the weekly white blood count controls, two for low efficacy of the chelation treatment by DFP, and one patient for experiencing a decrease of LIC from 1462 to 341 μg/g_{liver}. SQUID measurement could not be performed for four patients in the third year. After 4 years, only 10 out of 45 patients had a fifth SQUID measurement performed.

As many of the patients were children or adolescents, they gained height and weight during the study (Table I), and this was associated with increased daily iron input from transfusions. The LIC and the total body iron store increased significantly. In the group of 51 patients receiving only DFO treatment, apart from growth-related parameters [body mass index (BMI), liver volume], no significant changes were observed. Daily iron input of 18·9 mg/d from transfusions was in equilibrium with the TBIE rate of 18·5 mg/d.

A poor correlation between SF and LIC after logarithmic transformation was observed at baseline (R = 0·45), after 1·1 years (R = 0·49), 2·0 years (R = 0·49) and 3·2 years (R = 0·62). The LIC increased steadily under deferiprone, whereas the mean SF values were found to be constant, at 1900–2200 μg/l, with no significant difference between baseline and final ferritin values (Table I).

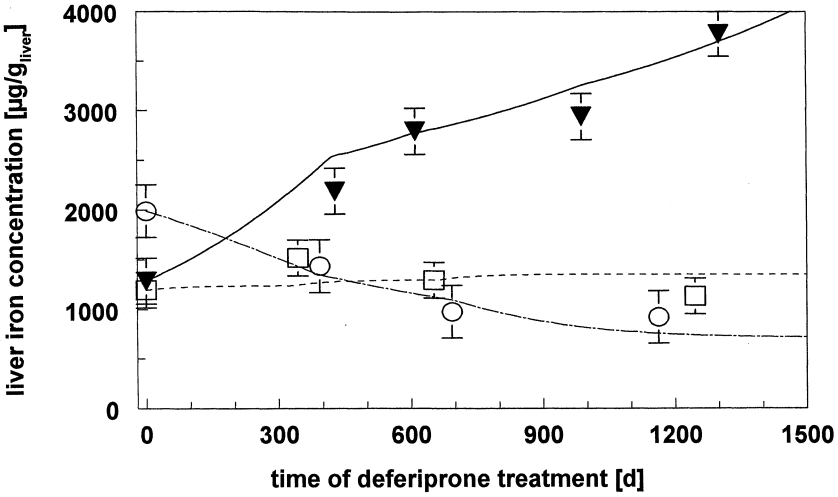

Fig 1. LICs ± methodological errors in patients with β-thalassaemia major with typical positive (▼), zero (□) and negative (○) iron balance under treatment with deferiprone. Curves are non-linear model fits adjusted for initial iron stores, annual transfusion and mean chelator dose rates.

As shown in Fig 2, the relative change of LIC after 2·0 years of treatment with DFP, with respect to the baseline LIC, appeared to depend significantly on the initial LIC ($R = 0{\cdot}66$, $P < 10^{-3}$). However, this extrapolation could not be derived from a similar comparison with SF values ($R = 0{\cdot}28$, $P > 0{\cdot}01$; data not shown).

The relative change of mean annual SF values versus the relative change of total body iron stores after 2 years of treatment with DFP is presented in Fig 3. The rectangle indicates 16 patients out of 54 with negative or zero relative change of body iron store (error of iron store difference < 14%). Although there was a significant linear correlation between these two determinations of iron balance ($R = 0{\cdot}56$, $P < 0{\cdot}001$), a negative ferritin change did not implicate a negative total body iron balance. This trend became even clearer after 3·2 years of DFP treatment ($R = 0{\cdot}64$, data not shown). A positive ferritin change indicated a positive iron balance.

In the group of 51 patients under continuous DFO a similar correlation was observed ($R = 0{\cdot}60$, $P < 0{\cdot}001$, data not shown). However, there were fewer patients with a negative ferritin change and a positive iron balance.

The different response of SF and LIC was clearly shown by its ratio (Table I), which decreased significantly from 1·37 under DFO treatment to 0·9 after 3·2 years of DFP treatment. In 15 of 17 non-splenectomized patients, no significant change was observed by the paired Wilcoxon-test (Table II), either in spleen iron ($P > 0{\cdot}7$) or in SF ($P > 0{\cdot}6$) concentrations, whereas LICs increased significantly ($P < 0{\cdot}01$).

In addition to the change of iron stores, TBIE rates also took into account the daily iron input rate from transfusion rates, increasing from 20·8 to 25·1 mg/d (Table I). Out of 54 patients, 36 had higher TBIE rates than under baseline DFO treatment. In Fig 4, the TBIE rates for the two patient groups under continuous 2-year treatment with DFP and DFO are compared in relation to their mean chelator dose rates. Patients treated with DFP had significantly higher mean rates (22·9 ± 6·5 mg/d) than patients treated with DFO (18·5 ± 4·9 mg/d). The mean molar efficiencies may be calculated for DFO and DFP from the slopes of the straight lines in Fig 4, of 9·2 ± 0·2 and 2·5 ± 0·1 (mg/d)/(mmol/d) respectively. In addition, the 16 patients with negative iron store balance under DFP showed considerably

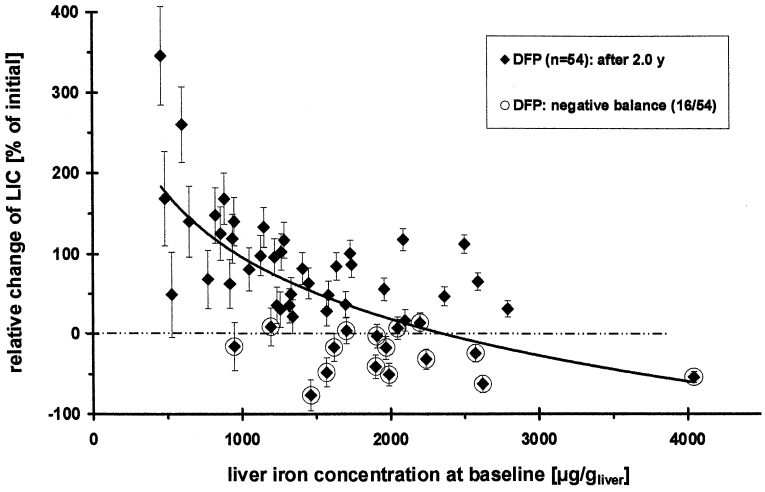

Fig 2. The relative change in LIC after 2 years (y) of deferiprone (DFP) treatment in relation to the LIC at baseline ($R = 0{\cdot}66$, $P < 10^{-3}$).

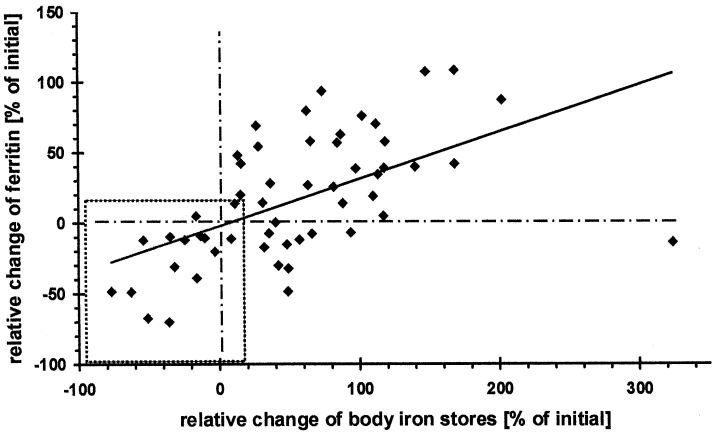

Fig 3. Correlation of relative SF levels with total body iron store changes as indicators of iron balance after 2 years of treatment with deferiprone (DFP: R = 0·56, $P < 0·001$). The rectangle indicates the patients with a negative iron balance (± uncertainty range of 14%).

Table II. Monitored parameters at baseline [desferrioxamine (DFO) treatment] and after 3 years of continuous deferiprone (DFP) treatment in 15 non-splenectomized patients with β-thalassaemia major.

Months after start of DFP	Liver iron by BLS ($\mu g/g_{liver}$)	Liver volume (ml)	Spleen iron ($\mu g/g_{spleen}$)	Spleen volume (ml)	Serum ferritin (μg/l)
Baseline DFO	1260 [910]	1345 ± 293	1280 [547]	353 ± 147	1880 [865]
13·4 ± 2·0	1750 [581]	1455 ± 252	1227 [444]	376 ± 134	1908 [629]
23·4 ± 2·0	1937 [706]	1670 ± 428	1208 [642]	420 ± 124	2103 [749]
38·1 ± 4·8	2371 [755]	1617 ± 348	1264 [631]	424 ± 165	2198 [593]
*P**	0·006	0·008	0·73	0·048	0·65

Results given as median [interquartile range].

*Comparing baseline results with results after 2 years of deferiprone treatment by paired T-test (means) or paired Wilcoxon-test (medians).

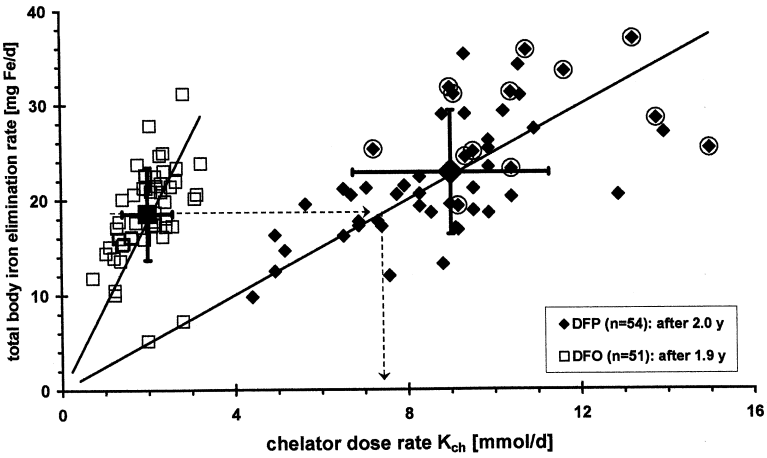

Fig 4. TBIE rates in relation to mean chelator dose rates, K_{ch}, under continuous treatment with desferrioxamine (DFO) and deferiprone (DFP) for 2 years (y). Large solid symbols with error bars represent mean values ± SD, the circled symbols indicate 16/54 patients with negative iron balance after 2 years of DFP treatment, and slopes of the straight lines indicate molar efficiencies.

higher chelator rates and chelated iron more efficiently under DFP than under baseline DFO ($P < 0·001$, paired Wilcoxon test).

The specific chelator dose rate was calculated from the mean chelator dose rate divided by the mean iron store of the respective treatment interval (Fig 5). Thus, patients with large iron stores and/or lower chelator dose had low specific chelator dose rates. Elimination rate constants were calculated for the baseline DFO treatment, for the successive DFP treatment intervals after 1·1, 2·0, 3·2 years and for the group of thalassaemic patients after 1·9 years of continuous DFO treatment. For the baseline DFO treatment regimen, steady-state conditions were assumed ($dU_S/dt = 0$, see equation 1). This enabled the calculation of the TBIE rate constant from the baseline iron store value only. The TBIE rate constants for the patients at baseline DFO treatment

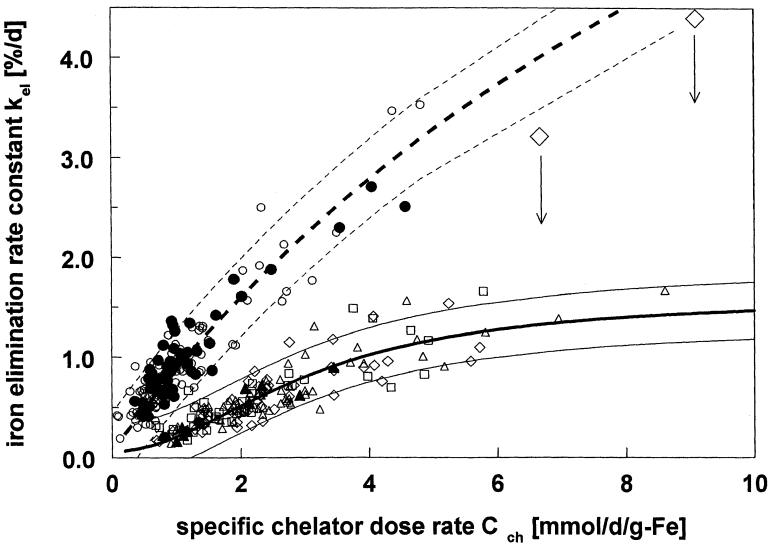

Fig 5. TBIE rate constants for thalassaemic patients at baseline and under continuous desferrioxamine treatment (DFO: ○ and ●). Patients at baseline DFO were continuously treated for 1·1, 2·0, 3·2 and 4·0 years with deferiprone (DFP: △, ◇, □ and ▲). Saturation functions of the specific chelator dose rates were fitted to DFO (dashed lines) and DFP (solid lines) data with 90% prediction range respectively.

and for those 51 patients under continuous DFO treatment were not significantly different (Fig 5). There was also no significant difference between the TBIE rate constants after 1·1, 2·0, 3·2 and 4·0 years of DFP treatment (paired Wilcoxon-test). Thus, all TBIE rate constants were fitted by the saturation function of equation (2), resulting in a maximum saturation rate constant, k_{el}^{max}, of 12·2 ± 1·7%/d and in a specific chelator dose rate for half-maximum elimination rate, M_{ch}, at 13·9 ± 2·3 mmol/d/g-Fe for DFO ($n = 1$: $R^2 = 0{\cdot}89$). For DFP the respective parameters were 1·54 ± 0·08%/d and 3·10 ± 0·17 mmol/d/g-Fe ($n = 2$: $R^2 = 0{\cdot}77$).

DISCUSSION

Patients under chelation treatment with deferiprone and desferrioxamine

The effects of deferiprone and desferrioxamine were studied in the same time interval (1994–1998) in two groups of well-treated β-thalassaemia major patients. The two groups were similar in baseline or initial values of number, age, number of splenectomized patients, compliance with DFO, LIC and other parameters (Table I). The assumption of steady-state at baseline DFO treatment may not hold for every patient in the DFP study group, therefore the group of patients under continuous DFO treatment was monitored for iron overload. The most striking differences were seen in the daily iron input rates from transfusion, which increased significantly under DFP treatment because of weight gain, with important consequences for the evaluation of the iron balance. The age-adjusted higher weight gain in DFP patients may be a result of a drug-specific stimulation of appetite.

Iron stores

Disregarding the systematic uncertainty in the liver iron fraction of about 10% (Fischer *et al*, 1999), the relative error of total body iron stores was determined mainly by the inaccuracy of LIC by biomagnetic liver susceptometry (mean error, 200 ± 65 $\mu g/g_{liver}$) and that of the mean liver volume, estimated from at least three repeated scans (mean error, 185 ± 102 ml). Then, the resulting mean relative error of the iron store determination was 10%. Consequently, iron store differences were inaccurate to about 14% (e.g. in Fig 3).

Iron balance

The LIC indicates a negative or positive relative iron store change. Although organ volumes vary significantly in thalassaemic patients (Fischer *et al*, 1999), a negative or positive iron balance can be quantified in a straightforward manner from the difference between the iron stores before and after a certain chelation treatment interval in relation to the baseline value. Interestingly, as shown in Fig 2, the iron balance also depends on the initial LIC as previously reported (Olivieri *et al*, 1995; Del Vecchio *et al*, 2000).

A superior indicator of iron balance is the TBIE rate, which also takes into account the differing daily iron input rates from transfusions. Comparing the mean TBIE rates of 22·9 and 18·5 mg/d (Table I) after 2 years of chelation treatment indicated that deferiprone and desferrioxamine had similar efficacy in most patients. If the TBIE rates after 2 years of treatment with DFP were compared with the baseline TBIE rate (20·8 mg/d), 67% of the patients would have at least baseline TBIE rates.

The TBIE rate was derived without any model assumptions from the transfusional iron input and the observed differences in iron stores. On average, the iron store difference per day and the daily iron input rate from transfusions contributed 6% and 94%, respectively, to the TBIE rate. Thus, it would be difficult to estimate the iron balance from the UIE, which will mainly be determined by the 94% of iron transfused.

Long-term efficacy

This should be derived from treatment periods lasting for at least 2 years. Very few studies have been performed with a reasonably large number of patients over such a time period. In most studies, the mean initial SF level or LIC were higher than the median SF value (1897 μg/l) or the median LIC (1456 $\mu g/g_{liver}$) of this study (Al-Refaie *et al*, 1995a,b; Olivieri *et al*, 1995; Hoffbrand *et al*, 1998; Taher *et al*, 2001). This could possibly explain why some of these studies have reported encouraging results whereas discouraging results (mean LIC, 2370 $\mu g/g_{liver}$) were reported by Olivieri & Brittenham, 1997). Direct comparison with some of the earlier studies is difficult as iron store assessments at baseline were performed by liver biopsies and thereafter by SQUID liver susceptometry (Olivieri *et al*, 1995; Diav-Citrin *et al*, 1999). Indeed, possible overestimation of the LIC on liver biopsy (Ambu *et al*, 1995) may lead to overestimation of chelator efficacy.

SF is not useful for the purpose of efficacy evaluation as is clearly demonstrated in this study (Fig 3) and as previously reported by Hoffbrand *et al* (1998), when relatively large liver iron estimations (based on biopsies) were obtained after 40 months of DFP treatment. Moreover, SF is not only a poor predictor of iron stores (Brittenham *et al*, 1993; Fischer *et al*, 1999), but also fluctuates considerably during treatment (Nielsen *et al*, 1995). Nevertheless, SF changes may preferentially indicate changes in the reticulo-endothelial iron stores. Together with the observation of constant spleen iron concentrations during DFP treatment, this may reflect different chelatable iron pools for DFP and DFO.

A direct comparison of the chelation efficacy of DFO and DFP has been performed in only a few studies (Olivieri *et al*, 1997; Taher *et al*, 2001). In a prospective randomized trial, Olivieri *et al* (1997) found an increase in mean liver iron stores of 1·5-fold and 1·1-fold over a treatment period of 33 months with DFP and DFO respectively. A similar result was found in this study over a treatment period of 24 (39) months with a 1·2(1·7)-fold and 1·1-fold increase of LIC for DFP and DFO respectively (Table I). However, such an approach does not take into account a possible small change in the amount of transfused iron and its resulting large impact on iron stores.

Molar efficiencies were calculated from the TBIE rate and the mean chelator dose rate for DFO and DFP (Fig 4 and Table I). Based on the changes in total body iron stores of thalassaemia major patients (2000 $\mu g/g_{liver}$), the same mean molar efficiency was recently reported for DFO (Brittenham *et al*, 1998). A similar ratio of molar efficiency of 3 : 1 was found by Bergeron *et al* (1992) for the two chelators in Cebus monkeys.

Derivations from model interpretation

The striking parameter of the assumed two-compartment model is its iron elimination rate constant (equations 1 and 2). This model allows for the time-dependent description of the change of iron stores and, for the first time, a theoretically derived quantitative formulation of the efficacy of a chelator. If it is assumed that a chelatable iron pool (Hershko & Rachmilewitz, 1978; Octave *et al*, 1983) is in equilibrium with the iron storage pool, the respective iron elimination rate constant k_{el} will be inversely proportional to the iron store U_S (Fischer *et al*, 1999). In 1st order approximation, the TBIE rate can be theoretically derived from equation (1) as $k_{el} \cdot U_S$, which would result in a nearly constant rate for a given chelator dose. This may explain why many investigators have not observed any significant change in UIE (Kersten *et al*, 1996; Hoffbrand *et al*, 1998).

The existence of a limited chelatable iron pool leads to a 'bottleneck' effect (Gold, 1977), which can be considered as a saturation function of the elimination rate constant. Currently, there is no method capable of quantifying the chelatable iron pool, and therefore a specific chelator dose rate C_{ch} has been introduced as an approximate representation (Fischer *et al*, 1999). The basic concept is similar to the therapeutic index of Porter *et al* (1989b), which relates the chelator dose with the iron store, i.e. the SF concentration.

For the involvement of more than one substrate molecule, as in the case of DFP, a more complex function has to be assumed for the chelation process. Taking into account the different number of iron binding ligands of the two chelators (DFO:Fe ratio = 1 : 1, $n = 1$; DFP:Fe ratio = 3 : 1, $n = 1, 2, 3$) in equation (2), acceptable fit parameter errors and an optimum coefficient of determination ($R^2 = 0.77$) resulted in $n = 2$ for chelation with DFP.

Describing the mean efficacy of a chelation treatment with DFP or DFO by the iron elimination rate constant as derived from Fig 5, will result in the calculation of a maximum daily iron input rate from transfusion for a given chelator dose and total body iron store (see equation 1). This practical approach has been plotted for various chelator doses and initial total body iron stores for a chelator treatment with DFP (Fig 6A) and DFO (Fig 6B) and may be used to predict the probable outcome of a particular chelation treatment.

For a typical DFP dose of 75 mg/kg/d in a 50 kg patient corresponding with 9 mmol/d (see dashed line in Fig 6A), only patients with a daily iron input rate from transfusion (and absorption) of less than about 18–22 mg/d are predicted to achieve a negative iron balance. Patients with iron stores between 2000 and 5000 mg are predicted to tolerate transfusion rates up to 23 mg/d (Fig 6A).

For a typical mean DFO dose of 30 mg/kg/d in a 50 kg patient corresponding with 2·3 mmol/d (see dashed line in Fig 6B), only patients with a daily iron input rate from transfusion (and absorption) of less than about 22–27 mg/d are predicted to achieve a negative iron balance. Patients with iron stores below 4000 mg are predicted to run into positive iron balance with such a transfusion rate (Fig 6B).

Conclusion

Every new oral iron chelator must be demonstrated to possess the capability of depleting excess iron stores in iron-loaded patients, as has been shown for desferrioxamine (Cohen *et al*, 1984). A TBIE similar to that of DFO was achieved in 67% of thalassaemia major patients ($n = 54$) after 2 years, while LIC or the total body iron stores increased in 72%, mainly caused by an increase of transfused iron (17%) due to weight gain. These results

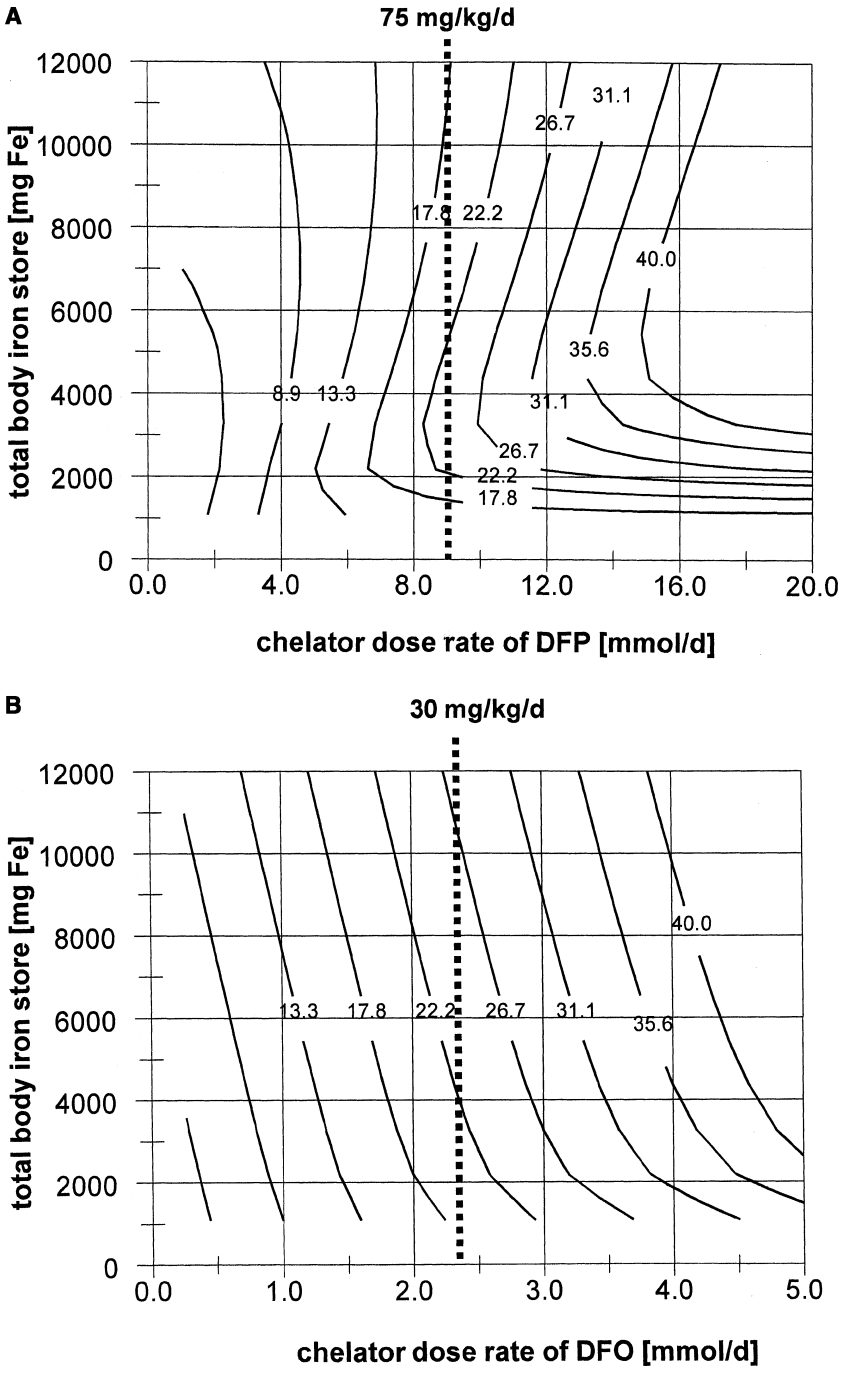

Fig 6. Contour plots for achieving equilibrium between daily iron input from transfusions and TBIE under chelator treatment with deferiprone (DFP: A) and desferrioxamine (DFO: B). A negative iron balance is achieved if the intersection between a mean chelator dose rate and a measured total body iron store is *left* of the isoline of daily iron input from transfusion (mg/d) (see text for further explanation).

indicate that the standard dose of 75 mg/kg/d would be sufficient to induce a negative iron balance in a large proportion of thalassaemic patients on long-term iron chelation. Thus, these results shed new light on the controversy of DFP efficacy (Pippard & Weatherall, 2000).

The difference between the two chelators DFO and DFP is clearly demonstrated by a saturation effect of the model-derived TBIE rate constant of 1·6%/d, at a specific DFP chelator dose rate of 3 mmol/d/g-Fe, which corresponds to liver iron concentrations below 1000–2000 $\mu g/g_{liver}$ at the standard dose of 75 mg/kg/d. The model parameters for DFO and DFP (Fig 6A and B) enable the prediction of an average chelation efficacy for a certain chelation treatment regimen with the knowledge of the total body iron store, the daily iron input from transfusions and the mean chelator dose rate. Periodic monitoring of iron overload by biomagnetic liver susceptometry over an observation period of at least 2 years is the only sufficiently precise non-invasive

method to assess the efficacy of a chelator in thalassaemic patients. Our results confirm that SF changes alone are unable to provide quantitative information on the efficacy of a chelator, and that they may be influenced preferentially by RES iron store changes. Deducing the efficacy of a chelator only from the change of LIC (LIC) or total body iron stores could be misleading. This would imply that all other parameters influencing LIC, such as the transfusion rate, should be accurately monitored and taken into account.

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REFERENCES

Agarwal, M.B., Gupte, S.S., Viswanathan, C., Vasandani, D., Ramanathan, J., Desai, N., Puniyani, R.R. & Chhablani, A.T. (1992) Long-term assessment of efficacy and safety of L1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. *British Journal of Haematology*, **82**, 460–466.

Al-Refaie, F.N., Wonke, B., Hoffbrand, A.V., Wickens, D.G., Nortey, P. & Kontoghiorghes, G.J. (1992) Efficacy and possible adverse effects of the oral iron chelator 1,2- dimethyl-3-hydroxypyrid-4-one (L1) in thalassemia major. *Blood*, **80**, 593–599.

Al-Refaie, F.N., De Silva, C.E., Wonke, B. & Hoffbrand, A.V. (1995a) Changes in transferrin saturation after treatment with the oral iron chelator deferiprone in patients with iron overload. *Journal of Clinical Pathology*, **48**, 110–114.

Al-Refaie, F.N., Hershko, C., Hoffbrand, A.V., Kosaryan, M., Olivieri, N.F., Tondury, P. & Wonke, B. (1995b) Results of long-term deferiprone (L1) therapy: a report by the International Study Group on Oral Iron Chelators. *British Journal of Haematology*, **91**, 224–229.

Al-Refaie, F.N., Sheppard, L.N., Nortey, P., Wonke, B. & Hoffbrand, A.V. (1995c) Pharmacokinetics of the oral iron chelator deferiprone (L1) in patients with iron overload. *British Journal of Haematology*, **89**, 403–408.

Ambu, R., Crisponi, G., Sciot, R., Van Eyken, P., Parodo, G., Iannelli, S., Marongiu, F., Silvagni, R., Nurchi, V. & Costa, V. (1995) Uneven hepatic iron and phosphorus distribution in beta-thalassemia. *Journal of Hepatology*, **23**, 544–549.

Bergeron, R.J., Streiff, R.R., Wiegand, J., Luchetta, G., Creary, E.A. & Peter, H.H. (1992) A comparison of the iron-clearing properties of 1,2-dimethyl-3- hydroxypyrid-4-one, 1,2-diethyl-3-hydroxypyrid-4-one, and deferoxamine. *Blood*, **79**, 1882–1890.

Brittenham, G.M., Cohen, A.R., McLaren, C.E., Martin, M.B., Griffith, P.M., Nienhuis, A.W., Young, N.S., Allen, C.J., Farrell, D.E. & Harris, J.W. (1993) Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *American Journal of Hematology*, **42**, 81–85.

Brittenham, G.M., Taylor, C.M., Angelucci, E. & Olivieri, N.F. (1998) Long-term evaluation of the efficiency of iron chelation in thalassemia major with balance studies using hepatic iron concentration to determine body iron stores. *Blood*, **92**, 38b.

Cohen, A., Martin, M. & Schwartz, E. (1984) Depletion of excessive liver iron stores with desferrioxamine. *British Journal of Haematology*, **58**, 369–373.

Cohen, A.R., Galanello, R., Piga, A., Dipalma, A., Vullo, C. & Tricta, F. (2000) Safety profile of the oral iron chelator deferiprone: a multicentre study. *British Journal of Haematology*, **108**, 305–312.

Collins, A.F., Fassos, F.F., Stobie, S., Lewis, N., Shaw, D., Fry, M., Templeton, D.M., McClelland, R.A., Koren, G. & Olivieri, N.F. (1994) Iron-balance and dose–response studies of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in iron-loaded patients with sickle cell disease. *Blood*, **83**, 2329–2333.

Del Vecchio, G.C., Crollo, E., Schettini, F., Fischer, R. & De Mattia, D. (2000) Factors influencing effectiveness of deferiprone in a thalassaemia major clinical setting. *Acta Haematologica*, **104**, 99–102.

Diav-Citrin, O., Atanackovic, G. & Koren, G. (1999) An investigation into variability in the therapeutic response to deferiprone in patients with thalassemia major. *Therapeutic Drug Monitoring*, **21**, 74–81.

Engelhardt, R., Tiemann, C.D., Janka, G.E., Nielsen, P., Gabbe, E.E. & Fischer, R. (1995) Biomagnetic susceptibility measurements of liver and spleen in thalassemic patients. In: *Biomagnetism: Fundamental Research and Clinical Applications* (ed. by C. Baumgartner et al), pp. 789–792. Elsevier-IOS, Amsterdam.

Fischer, R. (1998) Liver iron susceptometry. In: *Magnetism in Medicine – a Handbook* (ed. by W. Andrä & H. Nowak), pp. 286–301. Wiley-VCH, Berlin.

Fischer, R., Tiemann, C.D., Engelhardt, R., Nielsen, P., Dürken, M., Gabbe, E.E. & Janka, G.E. (1999) Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptometry. *American Journal of Hematology*, **60**, 289–299.

Gabutti, V. & Piga, A. (1996) Results of long-term iron-chelating therapy. *Acta Haematologica*, **95**, 26–36.

Gold, H.J. (1977) *Mathematical Modeling of Biological Systems – an Introductory Guidebook*. Wiley, New York, pp. 178–185.

Grady, R.W., Hilgartner, M.W. & Giardina, P.J. (1996) Deferiprone: its efficacy relative to that of desferal. *Blood*, **88**, 310a.

Hershko, C. & Rachmilewitz, E.A. (1978) Iron chelation in thalassemia: mechanism of desferrioxamine action. *Israel Journal of Medical Sciences*, **14**, 1111–1115.

Hider, R.C., Kontoghiorghes, G.J. & Silver, J. (1982) UK patent (GB-2118176).

Hoffbrand, A.V., Al-Refaie, F., Davis, B., Siritanakatkul, N., Jackson, B.F., Cochrane, J., Prescott, E. & Wonke, B. (1998) Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. *Blood*, **91**, 295–300.

Kersten, M.J., Lange, R., Smeets, M.E., Vreugdenhil, G., Roozendaal, K.J., Lameijer, W. & Goudsmit, R. (1996) Long-term treatment of transfusional iron overload with the oral iron chelator deferiprone (L1): a Dutch multicenter trial. *Annals of Hematology*, **73**, 247–252.

Kontoghiorghes, G.J., Aldouri, M.A., Sheppard, L. & Hoffbrand, A.V. (1987) 1,2-Dimethyl-3-hydroxypyrid-4-one, an orally active chelator for treatment of iron overload. *Lancet*, **1**, 1294–1295.

Kontoghiorghes, G.J., Bartlett, A.N., Hoffbrand, A.V., Goddard, J.G., Sheppard, L., Barr, J. & Nortey, P. (1990a) Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1). Iron chelation and metabolic studies. *British Journal of Haematology*, **76**, 295–300.

Kontoghiorghes, G.J., Goddard, J.G., Bartlett, A.N. & Sheppard, L. (1990b) Pharmacokinetic studies in humans with the oral iron chelator 1,2- dimethyl-3-hydroxypyrid-4-one. *Clinical Pharmacology and Therapeutics*, **48**, 255–261.

Leung, N.W., Farrant, P. & Peters, T.J. (1986) Liver Volume measurement by ultrasonography in normal subjects and alcoholic patients. *Journal of Hepatology*, **2**, 157–164.

Liu, Z.D., Liu, D.Y., Lu, S.L. & Hider, R. (1999) Synthesis, physicochemical properties and biological evaluation of aromatic ester prodrugs of 1-(2′-hydroxyethyl)-2-ethyl-3-hydroxypyridin-4-one (CP102): orally active iron chelators with clinical potential. *Journal of Pharmacy and Pharmacology*, **51**, 555–564.

Matsui, D., Klein, J., Hermann, C., Grunau, V., McClelland, R., Chung, D., St-Louis, P., Olivieri, N. & Koren, G. (1991) Relationship between the pharmacokinetics and iron excretion pharmacodynamics of the new oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one in patients with thalassemia. *Clinical Pharmacology and Therapeutics*, **50**, 294–298.

Mazza, P., Amurri, B., Lazzari, G., Masi, C., Palazzo, G., Spartera, M.A., Giua, R., Sebastio, A.M., Suma, V., De Marco, S., Semeraro, F. & Moscogiuri, R. (1998) Oral iron chelating therapy. A single center interim report on deferiprone (L1) in thalassemia. *Haematologica*, **83**, 496–501.

Nielsen, P., Fischer, R., Engelhardt, R., Tondury, P., Gabbe, E.E. & Janka, G.E. (1995) Liver iron stores in patients with secondary haemosiderosis under iron chelation therapy with deferoxamine or deferiprone. *British Journal of Haematology*, **91**, 827–833.

Octave, J.N., Schneider, Y.J., Crichton, R.R. & Trouet, A. (1983) Iron mobilization from cultured hepatocytes: effect of desferrioxamine B. *Biochemical Pharmacology*, **32**, 3413–3418.

Olivieri, N.F. & Brittenham, G.M. (1997) Final results of the randomized trial of deferiprone (L1) and deferoxamine (DFO). *Blood*, **90**, 264a.

Olivieri, N.F., Koren, G., Hermann, C., Bentur, Y., Chung, D., Klein, J., St Louis, P., Freedman, M.H., McClelland, R.A. & Templeton, D.M. (1990) Comparison of oral iron chelator L1 and desferrioxamine in iron-loaded patients. *Lancet*, **1990**, 1275–1279.

Olivieri, N.F., Matsui, D., Hermann, C. & Koren, G. (1991) Compliance assessed by the Medication Event Monitoring System. *Archives of Disease in Childhood*, **66**, 1399–1402.

Olivieri, N.F., Brittenham, G.M., Matsui, D., Berkovitch, M., Blendis, L.M., Cameron, R.G., McClelland, R.A., Liu, P.P., Templeton, D.M. & Koren, G. (1995) Iron-chelation therapy with oral deferipronein patients with thalassemia major. *New England Journal of Medicine*, **332**, 918–922.

Olivieri, N.F., Brittenham, G.M., McLaren, C.E., Templeton, D.M., Cameron, R.G., McClelland, R.A., Burt, A.D. & Fleming, K.A. (1998) Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. *New England Journal of Medicine*, **339**, 417–423.

Paulson, D.N., Fagaly, R.L., Toussaint, R.M. & Fischer, R. (1991) Biomagnetic susceptometer with SQUID instrumentation. *IEEE Transactions on Magnetism*, **MAG-27**, 3249–3252.

Piga, A., Facello, S., Gaglioti, C., Pucci, A., Pietribiasi, F. & Zimmermann, A. (1998) No progression of liver fibrosis in thalassemia major during deferiprone or desferrioxamine iron chelation. *Blood*, **92**, 3065.

Pippard, M.J. & Weatherall, D.J. (2000) Oral iron chelation therapy for thalassaemia: an uncertain scene. *British Journal of Haematology*, **111**, 2–5.

Porter, J.B. (2001) Practical management of iron overload. *British Journal of Haematology*, **115**, 239–252.

Porter, J.B., Huehns, E.R. & Hider, R.C. (1989a) The development of iron chelating drugs. *Bailliere's Best Practice and Research. Clinical Haematology*, **2**, 257–292.

Porter, J.B., Jaswon, M.S., Huehns, E.R., East, C.A. & Hazell, J.W. (1989b) Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. *British Journal of Haematology*, **73**, 403–409.

Stella, M., Pinzello, G. & Maggio, A. (1998) Iron chelation with oral deferiprone in patients with thalassemia. *New England Journal of Medicine*, **339**, 1712–1714.

Taher, A., Chamoun, F.M., Koussa, S., Saad, M.A., Khoriaty, A.I., Neeman, R. & Mourad, F.H. (1999) Efficacy and side effects of deferiprone (L1) in thalassemia patients not compliant with desferrioxamine. *Acta Haematologica*, **101**, 173–177.

Taher, A., Sheikh-Taha, M., Koussa, S., Inati, A., Neeman, R. & Mourad, F. (2001) Comparison between deferoxamine and deferiprone (L1) in iron-loaded thalassemia patients. *European Journal of Haematology*, **67**, 30–34.

Thalassemia International Federation (TIF) (2000) *Guidelines for Clinical Management of Thalassemia* (ed. by N. Cappellini et al.). TIF, Nicosia, Cyprus.

Töndury, P., Kontoghiorghes, G.J., Ridolfi-Luthy, A., Hirt, A., Hoffbrand, A.V., Lottenbach, A.M., Sonderegger, T. & Wagner, H.P. (1990) L1 (1,2-dimethyl-3-hydroxypyrid-4-one) for oral iron chelation in patients with beta-thalassaemia major. *British Journal of Haematology*, **76**, 550–553.

Töndury, P., Zimmermann, A., Nielsen, P. & Hirt, A. (1998) Liver iron and fibrosis during long-term treatment with deferiprone in Swiss thalassaemic patients. *British Journal of Haematology*, **101**, 413–415.