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Safety profile of the oral iron chelator deferiprone: a multicentre study

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Summary. In previous trials, the orally active iron chelator deferiprone (L1) has been associated with sporadic agranulocytosis, milder forms of neutropenia and other side-effects. To determine the incidence of these events, we performed a multicentre prospective study of the chelator. Blood counts were performed weekly, and confirmed neutropenia mandated discontinuation of therapy. Among 187 patients with thalassaemia major, the incidence of agranulocytosis (neutrophils $<0{\cdot}5\times10^9$/l) was 0·6/100 patient–years, and the incidence of milder forms of neutropenia (neutrophils $0{\cdot}5$–$1{\cdot}5\times10^9$/l) was 5·4/100 patient–years. All cases of neutropenia resolved after interruption of therapy. Neutropenia occurred predominantly in non-splenectomized patients. Nausea and/or vomiting occurred early in therapy, was usually transient and caused discontinuation of deferiprone in three patients. Mild to moderate joint pain and/or swelling did not require permanent cessation of deferiprone and occurred more commonly in patients with higher ferritin levels. Mean alanine transaminase (ALT) levels rose during therapy. Increased ALT levels were generally transient and occurred more commonly in patients with hepatitis C. Persistent changes in immunological studies were infrequent, although sporadic abnormalities occurred commonly. Mean zinc levels decreased during therapy. Ferritin levels did not change in the overall group but decreased in those patients with baseline levels $>2500\,\mu$g/l. This study characterized the safety profile of deferiprone, and, under the specific conditions of monitoring, demonstrated that agranulocytosis is less common than previously predicted.

Keywords: thalassaemia, iron overload, chelation, deferiprone, safety.

Iron overload is a predictable and life-threatening complication of thalassaemia major and other haematological disorders whose successful management depends on the regular administration of red blood cell transfusions (Cohen, 1987). The iron chelator desferrioxamine was first introduced more than 30 years ago (Sephton-Smith, 1962; Moeschlin & Schnider, 1963) and is still the only chelator approved for regular use in North America and, until recently, in Europe. Desferrioxamine reduces excessive iron, prevents iron-induced cardiac disease and improves survival in chronically transfused patients (Zurlo *et al*, 1989; Brittenham *et al*, 1994; Gabutti & Borgna-Pignatti, 1994; Olivieri *et al*, 1994; Olivieri & Brittenham, 1997). However, successful therapy with desferrioxamine requires prolonged subcutaneous or intravenous infusions of the chelator at least 4 or 5 d each week (Gabutti & Piga, 1996), and compliance with this regimen has proven to be difficult for many patients as demonstrated by the continued development of iron-induced cardiac dysrhythmias and heart failure (Brittenham *et al*, 1994; Olivieri *et al*, 1994; Gabutti & Piga, 1996). In addition, desferrioxamine may cause serious side-effects, such as impaired vision, hearing loss, bone changes, growth retardation and pulmonary toxicity (Olivieri *et al*, 1986; DeVirgiliis *et al*, 1988; Porter & Huehns, 1989; Freedman *et al*, 1990; Brill *et al*, 1991).

After two decades of seeking an orally active chelating agent as an alternative to desferrioxamine, only one drug, deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one), has succeeded in entering extensive clinical trials. In these trials, deferiprone has decreased or stabilized the level of iron overload in most but not all patients with thalassaemia major (Kontoghiorghes *et al*, 1990; Tondury *et al*, 1990; Agarwal *et al*, 1992; Al-Refaie *et al*, 1992, 1995; Olivieri *et al*, 1995, 1998; Hoffbrand *et al*, 1998). Adverse events

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associated with deferiprone in clinical trials have included agranulocytosis, arthropathy, gastrointestinal symptoms, increased ALT levels and progression of hepatic fibrosis (Hoffbrand *et al*, 1989; Bartlett *et al*, 1990; Agarwal *et al*, 1992; Al-Refaie *et al*, 1992, 1994a, 1995; Berkovitch *et al*, 1994; Olivieri *et al*, 1998). However, a clear safety profile has not emerged from these studies because of the small number of patients, variations in study design and differences in drug formulation. To overcome these difficulties, we conducted a multicentre study of a single formulation of deferiprone in patients with thalassaemia major. We designed the study to determine primarily the incidence of agranulocytosis and other serious adverse events. As discussed below, the present study was not designed to address prospectively the concern regarding hepatic fibrosis that was raised after the completion of the trial (Olivieri *et al*, 1998). In this largest prospective study of iron chelation therapy to date, we also assessed the effectiveness of deferiprone, using serum ferritin levels to measure the severity of iron overload. A preliminary report has been published previously (Cohen *et al*, 1998).

PATIENTS AND METHODS

Patients

Investigators in three thalassaemia centres in Italy (Cagliari, Ferrara, Torino) and one centre in the USA (Philadelphia) enrolled 187 patients with thalassaemia major in the study. The major elements of the inclusion criteria were: (1) transfusion-dependent thalassaemia; (2) age ≥ 10 years; (3) serum ferritin level $> 2000\ \mu g/l$ or liver iron concentration > 4 mg/g dry weight; and (4) an inability to use desferrioxamine or an unwillingness to continue the use of the chelator despite medical advice. Exclusion criteria included: (1) a neutrophil count $< 2{\cdot}0 \times 10^9/l$ or a platelet count $< 100 \times 10^9/l$ during the past two years; (2) cardiac disease requiring medication; (3) hepatic or renal failure; (4) use of other investigational drugs and/or drugs known to cause neutropenia; (5) arthropathy; or (6) pregnancy or plans to become pregnant during the study period. Patients with cirrhosis or chronic active hepatitis on liver biopsy were also excluded, but liver biopsy was not a prerequisite for study entry except for the evaluation of hepatic iron concentration in patients with serum ferritin levels $< 2000\ \mu g/l$.

Study design

The trial was a prospective open-label 1-year study of deferiprone. The primary objective was to determine the incidence of agranulocytosis and other severe adverse events. The secondary objective was to determine the efficacy of deferiprone in the treatment of iron overload as assessed by serum ferritin level.

Deferiprone was manufactured by Apotex (Toronto), the study sponsor, as 500-mg scored compressed film-coated tablets. Both the raw material and the finished dosage form were manufactured in Canada under Good Manufacturing Practices (GMP). A Data and Safety Monitoring Committee reviewed reports of serious adverse events such as agranulocytosis and other cases of neutropenia.

Deferiprone therapy

The dose of deferiprone was 25 mg/kg body weight (bw), administered three times a day for a total daily dose of 75 mg/kg bw. Each dose was rounded down to the nearest half tablet (250 mg) as required. The total daily dose was adjusted quarterly in accordance with changes in body weight. Patients received a new supply of tablets every 4 weeks, and they returned all unused tablets at the same time. Each centre maintained detailed drug accountability logs. Adherence to the treatment regimen was evaluated monthly as the percentage of prescribed tablets that were not returned.

Definitions and monitoring

A complete blood count (CBC) and white cell differential count were measured weekly (maximum 10-d interval), and each patient was questioned about the occurrence of any adverse events or the use of concurrent medications during the preceding week. If patients developed signs of infection, the CBC was determined twice weekly or more frequently as clinically indicated. If the neutrophils fell to $< 1{\cdot}5 \times 10^9/l$, therapy with deferiprone was interrupted, and a CBC with a manual differential count was repeated the next day. If the second neutrophil count was $\geq 1{\cdot}5 \times 10^9/l$, deferiprone therapy was reinstituted. If the two consecutive neutrophil counts were $< 1{\cdot}5 \times 10^9/l$, the patient was considered to have confirmed neutropenia and deferiprone therapy was discontinued. A confirmed neutrophil count $< 0{\cdot}5 \times 10^9/l$ was defined as agranulocytosis.

For patients with confirmed neutropenia, the neutrophil count was measured daily until it exceeded $2{\cdot}0 \times 10^9/l$. Resolution of neutropenia was defined as two consecutive neutrophil counts $> 2{\cdot}0 \times 10^9/l$, measured at least 3 d apart.

At the time the study was initiated, a platelet count $< 100 \times 10^9/l$ was considered an adverse haematological event requiring discontinuation of therapy. After 3 months, the threshold was reduced to $50 \times 10^9/l$ because platelet counts of $50–100 \times 10^9/l$ were not uncommon in the study population and because this level was not considered to pose a significant risk.

Antinuclear antibody (ANA) titres, rheumatoid factors (RF), anti-double-stranded DNA (anti-dsDNA) and antihistone antibodies (AHA) were obtained at baseline and every 3 months. Serum ALT levels to assess liver function were obtained on a similar schedule. At baseline and every 6 months, B lymphocytes, T lymphocytes, T-lymphocyte subsets and serum zinc levels were measured, and patients underwent a detailed rheumatological assessment. Serum ferritin levels to assess the degree of iron overload were measured at baseline and every 3 months. All baseline values were single measurements.

Statistical analyses

The statistical significance of changes from baseline for ALT level, B and T lymphocytes and T-lymphocyte subsets, plasma zinc concentration and serum ferritin level was determined by a repeated measures analysis of variance, using the mixed model approach. The PROC MIXED program from SAS (SAS Institute, Cary, NC, USA) was used for these

analyses. The model contained 'visit' as the repeated measure and 'patient' as the random factor. The covariance structure was autoregressive of order 1. If 'visit' was shown to be statistically significant ($P<0{\cdot}05$), the CONTRAST statements comparing the means from various visits to that at baseline were examined to determine the significance of change from baseline. For ALT and serum ferritin levels, the analyses were conducted on the data set for all patients (intention-to-treat analysis) as well as the data set for completers only. In the intention-to-treat analyses, the last available data for patients who withdrew during the study were carried forward to the subsequent time points. For the analysis of zinc concentration and lymphocyte number, only the data set for completers was used.

The change in incidence of ALT levels greater than twice the upper limit of the reference range was analysed by logistic regression analysis. The difference in incidence of ALT levels greater than twice the upper limit of the reference range in patients who were seropositive and seronegative for hepatitis C was analysed by the chi-squared test. The significance of the association between arthropathy and serum ferritin level was determined by the Mantel-Haenszel chi-squared test. The trend of serum ferritin level in each patient was determined by simple linear regression. The Fisher's exact test was used to evaluate the difference in incidence of neutropenia between splenectomized and non-splenectomized patients. The significance of the differences in the neutrophil count between splenectomized and non-splenectomized patients at baseline and during the study was determined by analysis of covariance, using the mixed model approach. The model contained 'spleen status' as the fixed factor and 'patient' as the random factor. The covariance structure used was autoregressive of order 1.

All statistical tests were two-sided with a type I error (α) of 0·05.

Informed consent

The study, designed in accordance with the standards of Good Clinical Practice, was approved by the institutional review board of each of the participating centres. Informed consent was obtained from all patients and/or their parents.

RESULTS

Study patients

One hundred and eighty-seven patients with thalassaemia major, ranging in age from 10 to 41 years (mean 18·4 years), were enrolled in the trial. Seventy-four patients (40%) had previously undergone splenectomy, and 142 (76%) were seropositive for hepatitis C. Regular chelation therapy with desferrioxamine was not prescribed for six patients at the time of study entry. The mean prescribed dose of desferrioxamine in the remaining 181 patients was 40 mg/kg bw, with a mean frequency of 6·5 prescribed infusions per week.

Drug exposure

One hundred and sixty-two patients completed the 1-year study. Two patients withdrew, two were removed because of protocol violations, and 21 stopped therapy after the occurrence of adverse events described previously (Cohen *et al*, 1998). The mean number of prescribed days of deferiprone for the entire study population was 325, yielding a cumulative total of 168 patient–years of drug exposure. The mean total daily dose was 73 mg/kg bw. Adherence to therapy, as assessed by pill counts, was 93 ± 8% (range 40–100%).

Urinary changes

Reddish discoloration of the urine, the most commonly reported event (74 patients), began as early as the first week of treatment. The finding persisted or recurred sporadically throughout the study period. The change in urine colour was not accompanied by dysuria, increased frequency or other urinary symptoms.

Gastrointestinal symptoms

Nausea and/or vomiting (45 patients) occurred most commonly during the first few weeks of therapy, were usually transient, were mild to moderate in severity and generally required no alteration in treatment. However, nausea caused interruption of therapy in four patients (2%) and subsequent discontinuation in three. Abdominal pain (26 patients) was mild to moderate in severity and resolved without discontinuation of therapy.

Arthropathy

Twenty patients reported joint pain, two reported joint swelling and two reported both symptoms. In 14 of these patients, the complaints were judged to be possibly related to the study drug. The onset of joint symptoms varied from 7 to 361 days after study entry, and all episodes were considered mild or moderate in severity. The median time to resolution was 12 days. Joint symptoms persisted for ≥ 3 weeks in five patients. Therapy with deferiprone was discontinued temporarily in four patients and the symptoms resolved within 13 days. All four patients restarted therapy with deferiprone; in one patient, mild arthralgia recurred but required no change in treatment. None of these 24 patients had abnormal joint findings on rheumatological assessment at baseline, at 6 months, or at 12 months. The frequency of reported arthropathy increased with the level of iron overload ($P=0{\cdot}03$). Arthropathy was reported in 9% of patients with baseline ferritin levels less than 2500 μg/l, 15% of patients with baseline ferritin levels of 2500–5000 μg/l, and 26% of patients with baseline ferritin levels greater than 5000 μg/l.

Alanine transaminase (ALT) levels

ALT values in individual patients fluctuated throughout the trial, with declines from baseline of up to 369 U/l and increases from baseline of up to 564 U/l. For patients who completed the study, the mean ALT was significantly higher than the baseline level of 59 ± 65 U/l at 3 months (76 ± 72 U/l; $P=0{\cdot}007$) and at 6 months (78 ± 81 U/l; $P=0{\cdot}002$). For all patients (intention-to-treat analysis), the mean ALT was significantly higher than the baseline level of 61 ± 63 U/l at 3 months (81 ± 73 U/l; $P=0{\cdot}0001$),

Table I. Number of patients with ALT levels > 2× upper limit of normal, according to hepatitis C serostatus.

Hepatitis C serostatus	Baseline	3 months	6 months	9 months	Termination
Negative	4	3	6	6	6
Positive	39	43	38	41	43
Total	43	46	44	47	49

Table II. Number of patients with positive tests for antinuclear antibody (ANA), anti-double-stranded DNA (anti-dsDNA), rheumatoid factor (RF) and antihistone antibody (AHA).

	Positive at baseline and negative at termination	Positive at baseline and at termination	New and transient positive	New and persistent positive	New positive at completion only
ANA	1	0	2	0	1
Anti-dsDNA	3	1	3	3	2
RF	6	12	26	3	6
AHA	0	1	2	0	1

6 months (81 ± 79 U/l; $P = 0{\cdot}0005$) and 9 months (76 ± 63 U/l; $P = 0{\cdot}014$). The proportion of patients with ALT levels greater than twice the upper limit of the reference range did not differ at each quarterly assessment ($P = 0{\cdot}43$; Table I). ALT levels greater than twice the upper limit of the reference range were present in 43 patients (23%) at baseline and in 55 additional patients (29%) on at least one measurement during the study. Eighty-three of these 98 patients (85%) were seropositive for hepatitis C, compared with 59 of the 89 patients (66%) with ALT levels less than twice the upper limit of the reference range ($P = 0{\cdot}003$).

Four patients, all seropositive for hepatitis C, interrupted therapy with deferiprone as a result of increased ALT levels. The ALT levels subsequently returned to baseline values. One of the four patients discontinued therapy without rechallenge. Upon reintroduction of deferiprone in the other three patients, ALT levels rose again and treatment was discontinued in one patient.

Agranulocytosis and other neutropenias

Agranulocytosis (neutrophils $< 0{\cdot}5 \times 10^9$/l) occurred in 1 out of 187 patients (0·5%). The overall incidence was 0·6/100 patient–years of therapy. Less severe forms of confirmed neutropenia (neutrophils $0{\cdot}5$–$1{\cdot}5 \times 10^9$/l) occurred in nine patients (4·8%), giving an incidence of 5·4/100 patient–years of treatment. All cases of agranulocytosis and neutropenia resolved after discontinuation of deferiprone. The clinical courses of affected patients have been described previously (Cohen *et al*, 1998).

The incidence of neutropenia in splenectomized patients was 1·6/100 patient–years and the incidence in non-splenectomized patients was 8·7/100 patient–years ($P = 0{\cdot}09$). The mean neutrophil count at baseline in non-splenectomized patients ($4{\cdot}0 \pm 1{\cdot}5 \times 10^9$/l) was significantly lower than in splenectomized patients ($6{\cdot}3 \pm 3{\cdot}1 \times 10^9$/l; $P = 0{\cdot}0001$) and remained so throughout the study (Fig 1).

Immunological studies

The number of B lymphocytes and $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes, measured by flow cytometry at 6 months and at the completion of the study period, did not differ from the baseline values ($P > 0{\cdot}05$). Twenty-four patients (12·8%) had positive results for ANA, anti-dsDNA, RF or AHA at baseline (Table II), and results remained positive in 14 patients at completion of the study. Transiently positive results during therapy with deferiprone were common, occurring in 33

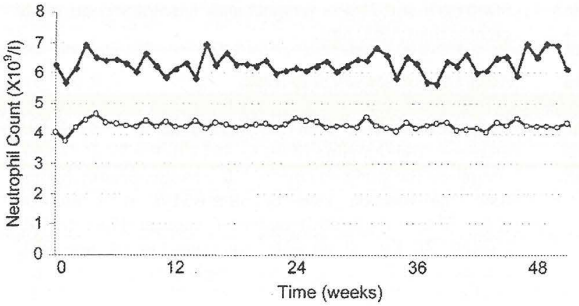

Fig 1. Mean weekly neutrophil count in 74 splenectomized and 113 non-splenectomized patients. The mean neutrophil count in non-splenectomized patients was significantly lower than in splenectomized patients ($P = 0{\cdot}0001$) from study entry to completion.

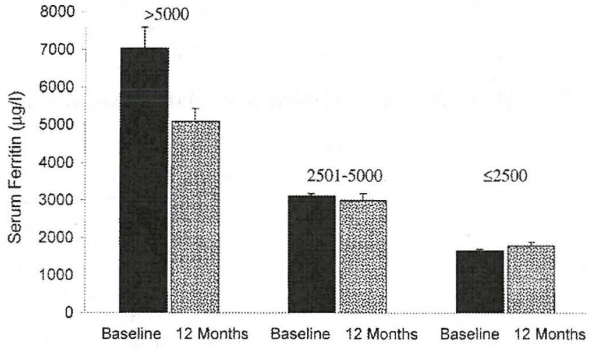

Fig 2. Mean serum ferritin levels at baseline and at 12 months according to ferritin levels at study entry.

patients (17·6%). Six patients (3·2%) who had negative studies for ANA, anti-dsDNA, RF or AHA at baseline subsequently developed a new antibody that persisted on the next measurement. In addition, ten patients (5·3%) had a first positive antibody at the final assessment.

Zinc levels

The plasma zinc levels at baseline (14·4 ± 2·3 µmol/l) decreased to 13·9 ± 2·3 µmol/l at 6 months ($P = 0·013$) and to 13·0 ± 2·1 µmol/l at 12 months ($P = 0·0001$). Thirteen of the 171 patients with normal levels at baseline had values below the reference range at 6 months only, 24 at study completion only, and seven at both 6 months and study completion.

Efficacy

In the 162 patients who completed 1 year of therapy, the mean serum ferritin levels at baseline and at 12 months were 2579 ± 1777 µg/l and 2452 ± 1452 µg/l respectively ($P = 0·26$). The values at baseline and termination for all patients who received deferiprone, whether or not they completed the study (intention-to-treat analysis), were 2696 ± 1877 µg/l and 2633 ± 1815 µg/l respectively ($P = 0·58$).

Baseline serum ferritin levels were stratified using a previously defined threshold for development of cardiac disease (Olivieri *et al*, 1994). For patients with baseline ferritin levels ≤ 2500 µg/l who completed 1 year of therapy ($n = 99$), the mean ferritin levels at baseline and at 12 months did not differ ($P = 0·28$; Fig 2). For patients with baseline ferritin levels > 2500 µg/l who completed 1 year of therapy ($n = 63$), the mean ferritin level declined from baseline to 12 months ($P = 0·001$). A subanalysis of patients with baseline ferritin levels > 5000 µg/l ($n = 15$) demonstrated a decline in ferritin level from 7042 ± 2143 µg/l at baseline to 5094 ± 1334 µg/l at 12 months ($P = 0·0008$). Simple linear regression indicated a negative slope in 57% of the 162 patients who completed the study.

Therapy with deferiprone was interrupted temporarily in six patients when their ferritin levels fell below 500 µg/l and was reinstituted when the ferritin level exceeded this threshold. The mean time of interruption was 62 ± 20 d.

DISCUSSION

This multicentre study was designed to characterize prospectively the incidence of adverse events during therapy with deferiprone and the relationship of these events to the chelator. In particular, enrolment of 187 patients and the weekly monitoring of blood counts enabled this study to determine, for the first time, the incidence of agranulocytosis, the most serious side-effect associated with deferiprone. This 1-year trial, completed before the question of progression of hepatic fibrosis during therapy with deferiprone arose (Olivieri *et al*, 1998), did not require liver biopsies and therefore cannot address this issue. Additional studies to answer this question are presently under way.

The most frequently reported adverse event in this study was a reddish discoloration of the urine. The colour was attributed to the excretion of the iron–deferiprone complex, which is a chromophore (Kontoghiorghes *et al*, 1987). No patient discontinued therapy with deferiprone because of the change in urine colour. Gastrointestinal symptoms, the second most commonly reported adverse event, usually occurred during the first weeks of therapy and resolved without altering the dose of deferiprone. These findings are similar to those reported by other investigators (Al-Refaie *et al*, 1992, 1995).

In previous studies, the frequency of arthralgia and arthritis during therapy with deferiprone has varied from 14% to 38% (Agarwal *et al*, 1992; Olivieri *et al*, 1995). The International Study Group on Oral Iron Chelators (ISGOIC) found joint pain or stiffness in 20% of patients in their combined data pool (Al-Refaie *et al*, 1995). In these studies, knee pain was the most common complaint, sometimes accompanied by joint swelling and loss of motion. Magnetic resonance imaging and synovial biopsies have not established a pathological basis for the joint abnormalities, and serological studies have failed to detect evidence of autoimmune disease in most patients (Berkovitch *et al*, 1994; Hoffbrand *et al*, 1998). In the present study, the frequency of arthropathy (13%) was less than previously reported. Four patients temporarily interrupted therapy because of joint pain, but no patient discontinued treatment for this reason.

A previous report suggested that deferiprone-associated arthropathy is more frequent in patients who are more

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