UGT1A6 genotype-related pharmacokinetics of deferiprone (L1) in healthy volunteers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- UGT1A6 has been proposed as the predominant isoform responsible for the glucuronidation of deferiprone.
- UGT1A6*2 allele has been associated with the altered enzyme activity.

WHAT THIS STUDY ADDS

- There is no statistically significant effect of UGT1A6 genotype on the single-dose pharmacokinetics of deferiprone in healthy volunteers.
- Gender influences serum pharmacokinetics of deferiprone.
- Body iron stores reflected by serum ferritin levels may have an influence on the extent of extravascular deferiprone distribution.

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AIMS

To examine the effects of UGT1A6 polymorphisms on the pharmacokinetics of deferiprone in healthy volunteers.

METHODS

Twenty-two healthy volunteers were enrolled and grouped according to UGT1A6 genotype. After an overnight fast, the subjects received a single oral dose of 25 mg kg⁻¹ deferiprone. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after dosing. Urine output was collected at 0, 0–2, 2–4, 4–8, 8–12 and 12–24 h. Deferiprone (L1) and deferiprone-glucuronide (L1G) concentrations in serum and urine were determined using a validated high-performance liquid chromatography method. UGT1A6 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism analysis.

RESULTS

No statistically significant differences in any pharmacokinetic parameters of either deferiprone or deferiprone-glucuronide among the genotype groups were noted. Likewise, there were no statistically significant differences in 24-h urinary deferiprone and deferiprone-glucuronide excretion among the genotype groups. Significant differences between men and women were found in AUC_{0–∞}, V_d/F, and CL/F of deferiprone. Gender differences in 24-h urinary deferiprone and its metabolite excretion, however, failed to reach statistical significance. The V_d/F of deferiprone was found to correlate significantly with serum ferritin ($r_s = 0.665$; $P = 0.001$).

CONCLUSION

The studied single nucleotide polymorphisms in UGT1A6 do not appear to exert statistically significant effects on the single-dose pharmacokinetics of deferiprone. Gender appears to influence the serum pharmacokinetics of deferiprone, but not urinary excretion of deferiprone and its metabolite. Body iron stores may have an influence on the extent of extravascular deferiprone distribution.

Introduction

Deferiprone (1,2 dimethyl-3-hydroxypyrid-4-one, also known as L1; Figure 1) is an orally active iron chelator, which has been widely used in the treatment of iron overload diseases and has shown its potential in the treatment of non-iron loading conditions, such as anaemic rheumatoid arthritis and malaria [1, 2]. Moreover, deferiprone remains the only lifesaving drug for β -thalassaemia patients in developing countries, where the cost and availability of both deferoxamine and deferasirox are out of reach. Deferiprone chelates iron in a bidentate manner and the deferiprone–iron complexes (3 : 1 molar ratio) formed are excreted in the urine together with the parent compound and gluruconide metabolite. The lack of chelating activity of deferiprone-glucuronide accounts for >85% of the administered dose [3]. Variations in the efficacy of deferiprone have been observed in several studies, and differences in the rate of glucuronidation have been considered as a potential cause of variability [4–6].

A recent study using human liver microsomes has indicated that UGT1A6 is the predominant UGT isoform involved in the glucuronidation of deferiprone [7]. Three most common coding single nucleotide polymorphisms (SNPs) (Ser7Ala, Thr181Ala and Arg184Ser) have been identified in a single allele *UGT1A6*2* and the encoded proteins have been shown to metabolize β -blockers in 28–69% of the wild-type *UGT1A6*1* [8]. Indeed, the *UGT1A6*2* allele is common in the population. The frequency of *UGT1A6*2* allele in the Japanese population (frequency of 0.213) was comparable to that in Whites (frequency of 0.274) and African-Americans (frequency of 0.243) [9, 10].

In the present study, the *UGT1A6* genotype-related pharmacokinetics of deferiprone was investigated in healthy volunteers in order to focus on the effect of *UGT1A6* polymorphisms on deferiprone pharmacokinetics in the absence of iron loading conditions.

Materials and methods

Reagents and standard

Deferiprone reference standard was kindly provided by the Government Pharmaceutical Organization (Bangkok,

Figure 1

Chemical structure of deferiprone

Thailand). *Escherichia coli* β -glucuronidase type IX-A was purchased from Sigma-Aldrich (St Louis, MO, USA). HEPES was purchased from Fluka (Buchs, Switzerland). Ethylenediamine tetraacetate (EDTA) and sodium dihydrogen phosphate were purchased from E. Merck (Darmstadt, Germany). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Labscan Analytical Sciences (Bangkok, Thailand). All other chemicals were commercially purchased and were of analytical reagent grade.

Subjects

Seventy-one healthy volunteers participated in genotyping. Following the determination of *UGT1A6* genotype, 22 healthy volunteers (10 women and 12 men) were enrolled and classified into three groups according to *UGT1A6* genotype as follows: group I, *UGT1A6*1/*1* ($n = 10$); group II, *UGT1A6*1/*2* ($n = 8$); and group III, *UGT1A6*2/*2* ($n = 4$). The sample size calculation was based on hypothesized differences of 25% in mean deferiprone $AUC_{0-\infty}$ values between the three genotype groups. A study by Stobie *et al.* [11] was taken as a reference for the $AUC_{0-\infty}$ of deferiprone, as the respective study was the only published study with pharmacokinetics in healthy volunteers. A minimum of three subjects in each group is adequate to achieve 80% power with a 5% significance. All subjects were required not to take any prescription or nonprescription medication a week before and throughout the study. They were instructed to abstain from alcohol and caffeine-containing beverages for at least 24 h before the study and during the study period. The complete blood count, iron profiles [serum iron (SI), total iron bind capacity (TIBC), serum ferritin (SF)], renal and liver function tests were performed prior to the study. The subjects were informed about the experimental procedure and purpose of the study, and then written informed consent was obtained from all subjects. The study protocol was approved by the committee on human rights to researches involving human subjects, Ramathibodi Hospital, Mahidol University (Bangkok, Thailand).

Pharmacokinetic study

After an overnight fast, the subjects received a single oral dose of 25 mg kg^{-1} deferiprone (Government Pharmaceutical Organization, Bangkok, Thailand). Standardized meals were served 2 and 8 h after dosing. Through the venous catheter, samples of 5 ml blood were collected before dosage and at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after dosing. Samples were centrifuged, and the separated serum samples were stored at $-20^{\circ}C$. Urine output was pooled and collected at 0 (baseline), 0–2, 2–4, 4–8, 8–12 and 12–24 h after dosing; urine was refrigerated and 10 ml of each collection was frozen prior to analysis.

Genotyping of *UGT1A6*2*

DNA was extracted from venous blood of each subject by a salting-out process (Puregene; Gentra Systems,

Minneapolis, MN, USA). The wild-type allele *UGT1A6*1* and mutant allele *UGT1A6*2* (Ser7Ala, Thr181Ala and Arg184Ser) were determined by a polymerase chain reaction-restriction fragment length polymorphism method as described elsewhere [12, 13].

Determination of deferiprone and deferiprone-glucuronide

Serum samples were deproteinized by ultrafiltration with the Amicon Centrifree micropartition device (Millipore Corp., Bedford, MA, USA) at 2300 *g* for 30 min, and 20 μ l of ultrafiltrate was injected into the HPLC system for the determination of nonconjugate forms of deferiprone. Determination of total deferiprone was done after converting the glucuronide to parent deferiprone by *E. coli* β -glucuronidase type IX-A; 480 μ l of serum was mixed with 20 μ l of β -glucuronidase (12 500 U ml^{-1} in 1.0 M HEPES buffer pH 7) and then incubated at 37°C for 16 h. After the reaction, serum samples were deproteinized and injected into HPLC as described above.

The urine samples were filtered through a 0.2- μ m cellulose filter membrane to remove any particulate material. For the determination of nonconjugate forms of deferiprone in urine, 100- μ l aliquots of filtered urine samples from 0 to 12 h and 12–24 h were diluted, respectively, twofold and 1.5-fold with 1.0 M HEPES buffer, pH 7, and 20 μ l of each sample was injected into HPLC. To determine the total deferiprone in urine, 100- μ l aliquots of filtered urine were diluted with 660 μ l 1.0 M HEPES buffer, pH 7, with the exception of the urine sample from the 12–24 h interval, then mixed with 240 μ l of β -glucuronidase (12 500 U ml^{-1} in 1.0 M HEPES buffer, pH 7) and incubated at 37°C for 16 h. After the reaction, 20 μ l of each sample was injected into HPLC.

The stock standard solutions of deferiprone were prepared in methanol at a concentration of 1000 $\mu\text{g ml}^{-1}$. The working standard solutions of lower concentration were prepared by serial dilution of the stock standard solutions with mobile phase. Calibration standards for serum and urine assay were prepared by spiking the appropriate amount of the working standard solutions into serum and urine to give final concentrations over the range of 1–50 $\mu\text{g ml}^{-1}$ and 5–200 $\mu\text{g ml}^{-1}$, respectively.

Nonconjugate forms of deferiprone and total deferiprone (nonconjugate and conjugate forms of deferiprone) in serum and urine were assayed by reversed-phase HPLC with a UV detector. The separation was performed under isocratic conditions on a Eclipse® XDB-C18 column (5 μ m, 150 $\times$ 4.6 mm i.d., Agilent Technologies, Waldborn, Germany) with a guard column in a Waters 2695 separations module with autosampler (Waters, Milford, MA, USA). The eluent was monitored by Waters 2487 dual-wavelength absorbance detector (Waters) at 280 nm. The mobile phase consisted of 10 mM sodium dihydrogen phosphate and 2 mM EDTA, pH 3, adjusted with phosphoric acid and methanol at the volume ratio of 93 : 7 buffer to

methanol. The flow rate was 1.0 ml min^{-1} . Peak areas were integrated with Millenium 3.2 software (Waters). The retention time for deferiprone was approximately 3.6 min. The concentrations of nonconjugated deferiprone and total deferiprone were determined by interpolation to the standard reference calibration curve. The concentration of deferiprone-glucuronide was obtained by the difference between total deferiprone and nonconjugated deferiprone and was presented as deferiprone equivalence.

The precision and accuracy were assessed at three different concentrations (1, 15 and 50 $\mu\text{g ml}^{-1}$ for serum assay; 5, 100 and 200 $\mu\text{g ml}^{-1}$ for urine assay). The intraday coefficients of variation of the serum and urine assays were 1.34–2.46% and 0.86–1.96%, respectively. The interday coefficients of variation of the serum and urine assays were 1.34–9.72% and 0.88–2.91%, respectively. The accuracy of the serum and urine assays, determined by recovery analysis, ranged from 92.2% to 96.6% and from 107% to 110%, respectively. The lower limits of detection were 0.84 $\mu\text{g ml}^{-1}$ and 8.36 $\mu\text{g ml}^{-1}$ (before 10-fold dilution) for serum and urine assays, respectively.

Determination of iron profiles

SI and TIBC were determined by a method recommended by the International Council for Standardization in Haematology [14]. Transferrin saturation was calculated from SI and TIBC. SF was determined by use of a ferritin Diametra kit (Diametra, Milan, Italy).

Pharmacokinetic analysis

The pharmacokinetic parameters of deferiprone and its glucuronide were determined by noncompartmental methods with the aid of PK Solutions 2.0 software (Summit Research Service, Montrose, CO, USA). Peak serum concentrations (C_{max}) and the time to reach peak concentrations (t_{max}) of deferiprone and its glucuronide were determined by observation of individual concentration–time data. A terminal disposition rate constant (λ_z) was calculated by linear least-squares regression of the log-transformed serum concentration–time data. The terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The area under the curve for the time at which the final measurable concentration (C_{last}) was obtained ($\text{AUC}_{0-\text{last}}$) was calculated by the trapezoidal rule. The AUC from the final time point to time infinity ($\text{AUC}_{\text{last}-\infty}$) was calculated as the ratio of the C_{last} to λ_z . The AUC from time 0 to infinity ($\text{AUC}_{0-\infty}$) was calculated by addition of $\text{AUC}_{0-\text{last}}$ and $\text{AUC}_{\text{last}-\infty}$. The apparent oral clearance (CL/F) of deferiprone was calculated as the ratio of administered dose to $\text{AUC}_{0-\infty}$.

Statistical analysis

The data were expressed as median [95% confidence interval (CI)] unless otherwise indicated. The pharmacokinetic parameters of deferiprone and its glucuronide among the genotype groups were compared by Kruskal–Wallis test. When statistically significant differences were found by

Table 1

Genotype and allele frequencies of *UGT1A6* in healthy volunteers

Genotype	Healthy volunteers (n = 71) Observed (95% CI) (%)	Expected (%)
<i>UGT1A6</i> *1/*1	67.61 (56.07, 77.35)	65.59
<i>UGT1A6</i> *1/*2	26.76 (17.85, 38.05)	30.80
<i>UGT1A6</i> *2/*2	5.63 (2.21, 13.60)	3.62

Kruskal–Wallis test, a nonparametric multiple-comparison test was applied to examine all possible pairwise comparisons. Mann–Whitney *U*-test was applied to compare the pharmacokinetic parameters of both deferiprone and its glucuronide between genders. The relationship of iron profiles vs. pharmacokinetic parameters of deferiprone and its glucuronide was evaluated by the Spearman rank correlation coefficient. χ^2 test was applied to test the deviation of allele and genotype frequency distributions from Hardy–Weinberg equilibrium. Statistical analysis was performed with SPSS software (version 11.5; SPSS Inc., Chicago, IL, USA). *P*-values <0.05 were considered to be significant.

Results

UGT1A6 allele and genotype frequencies in healthy volunteers

Of the 71 healthy volunteers, there were four homozygote variants (*UGT1A6**2/*2), 19 heterozygotes (*UGT1A6**1/*2) and 48 wild-type (*UGT1A6**1/*1). The distribution of genotype frequencies was in Hardy–Weinberg equilibrium ($P > 0.05$, χ^2 test, Table 1). The allele frequency of *UGT1A6**2 in Thai healthy volunteers (0.19) was slightly lower than that reported in the Japanese, White and African-American populations (0.213, 0.274 and 0.243, respectively) [9, 12].

Pharmacokinetics of deferiprone and its glucuronide in healthy volunteers, according to UGT1A6 genotype

Complete deferiprone and its glucuronide pharmacokinetic data following oral administration were available for 22 healthy volunteers. There were no significant differences in age, body weight, iron profiles or biochemical and haematological characteristics among genotype groups (Table 2). Serum profiles of deferiprone and its glucuronide according to genotype groups are shown in Figure 2. Serum concentration of deferiprone reached its peak within an hour of deferiprone ingestion and was comparable among the genotype groups. $C_{\max}$ and $AUC_{0-\infty}$ of deferiprone median values were 24% and 38% higher, respectively, in the *UGT1A6**1/*1 group, and were 35% and 24% higher, respectively, in the *2/*2 group compared with

those in the *1/*2 group (Table 3). However, none of the differences in pharmacokinetic parameters of deferiprone among the genotype groups was statistically significant. Interestingly, $C_{\max}$ and $t_{\max}$ of deferiprone-glucuronide were similar among the genotype groups. The median $AUC_{0-\infty}$ of the deferiprone-glucuronide value was slightly higher (16% and 21%) in the *UGT1A6**1/*1 and *2/*2 groups, respectively, compared with that in the *1/*2 group, but again the differences were not significant. Similarly, the $AUC_{0-\infty}$ ratio of deferiprone-glucuronide to deferiprone was also comparable among the genotype groups.

Cumulative urinary excretion profiles of deferiprone and its glucuronide are shown in Figure 3. The *UGT1A6**1/*2 group demonstrated a statistically insignificant tendency towards higher 24-h urinary excretion of nonconjugated deferiprone and lower 24-h urinary deferiprone-glucuronide excretion compared with the *1/*1 and *2/*2 groups. The 24-h urinary nonconjugated deferiprone excretion in the *1/*1, *1/*2 and *2/*2 groups was 39.2 mg (95% CI 11.4, 61.8), 58.2 mg (95% CI 17.0, 116) and 43.1 mg (95% CI 22.1, 63.0), respectively, representing 2.4%, 4.3% and 2.9% of total dose, respectively (Figure 4a). The 24-h urinary deferiprone-glucuronide excretion in the *1/*1, *1/*2 and *2/*2 groups were 1032 mg (95% CI 932, 1359), 751 mg (95% CI 579, 1182) and 903 mg (95% CI 609, 1260), representing 71.2%, 53.9% and 66.4% of total dose, respectively (Figure 4b).

Pharmacokinetics of deferiprone and its glucuronide in healthy volunteers, according to gender

The male and female groups were comparable with respect to $C_{\max}$, $t_{\max}$ and $t_{1/2}$ of deferiprone. Likewise, all pharmacokinetic parameters of deferiprone-glucuronide were similar between male and female groups. However, there were significant differences in $AUC_{0-\infty}$, V_d/F and CL/F of deferiprone between male and female groups. Median values of $AUC_{0-\infty}$ and CL/F of deferiprone in the female group were 40% higher ($P < 0.05$) and 37% lower ($P < 0.01$), respectively, than that in the male group. The median V_d/F value of deferiprone in the female group was 30% lower ($P < 0.01$) than that in the male group (Table 4). As regards urinary excretion, no statistically significant differences in 24-h urinary deferiprone and its metabolite excretion were noted between male and female groups. The possibility of a gender–genotype interaction could not be directly assessed due to the lack of female subjects with *UGT1A6**2/*2 genotype.

Correlation between iron status and the pharmacokinetic parameters of deferiprone

The V_d/F of deferiprone was found to correlate significantly with SF, with r_s value of 0.665 ($P = 0.001$) (Figure 5). No correlation was observed between the pharmacokinetic parameters of deferiprone-glucuronide and iron profiles.

Table 2

Clinical characteristics of healthy volunteers according to UGT1A6 genotype

Characteristics	Genotype <i>UGT1A6</i> *1/*1	<i>UGT1A6</i> *1/*2	<i>UGT1A6</i> *2/*2
Subjects (M/F)	5/5	4/4	3/1
Age (years)	24 (23, 26)	24.5 (22, 37)	25.5 (23, 30)
Weight (kg)	56.3 (53, 69.5)	52.5 (47, 63)	49 (45, 58)
Hb (120–160 g l ⁻¹)	13.6 (13.1, 15.8)	13.9 (11.6, 16.6)	14.0 (12.8, 15.3)
HCT (36–46%)	39.1 (37.3, 44.2)	39.8 (34.3, 47.9)	40.1 (36.7, 43.6)
ALP (40–117 U l ⁻¹)	65.5 (50, 87)	69.5 (45, 96)	58.5 (57, 96)
AST (0–37 U l ⁻¹)	19 (15, 24)	20.5 (13, 30)	17 (15, 21)
ALT (0–40 U l ⁻¹)	37 (24, 44)	33.5 (28, 81)	29 (25, 33)
TP (64–82 g l ⁻¹)	79.6 (72.2, 84.5)	74.5 (68.9, 89.0)	75.0 (70.6, 82.7)
Albumin (42–54 g l ⁻¹)	49.6 (43.4, 52.3)	46.5 (41.2, 51.9)	47.6 (41.7, 51.3)
Total cholesterol (3.0–5.5 mmol l ⁻¹)	5.3 (4.0, 6.1)	5.2 (4.2, 5.7)	5.3 (4.6, 5.9)
Serum iron (8–24 μmol l ⁻¹)	20.9 (15.5, 27.2)	23.7 (15.0, 27.6)	19.0 (15.6, 24.5)
Transferrin saturation (20–50%)	32.3 (24.7, 39.8)	34.3 (15.4, 40.0)	34.4 (24.6, 35.4)
Serum ferritin (50–350 μg l ⁻¹)	92.2 (5.0, 198.8)	45.4 (5.0, 273.8)	79.1 (5.0, 185.2)
Serum creatinine (50–120 μmol l ⁻¹)	71 (58, 101)	60.5 (38, 92)	71 (35, 98)
TB (0–17.1 μmol l ⁻¹)	13.9 (11.1, 18.9)	15.8 (7.2, 18.6)	16.3 (12.9, 19.5)
DB (0–5 μmol l ⁻¹)	4.2 (3.2, 5.1)	4.3 (1.9, 5.0)	3.9 (3.8, 4.7)

Hb, Haemoglobin; HCT, haematocrit; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; TB, total bilirubin; DB, direct bilirubin. Data are presented as median (95% CI).

Figure 2

Time course of serum concentration of deferiprone (a) and deferiprone-glucuronide (b) in *UGT1A6**1/*1 subjects ($n = 10$) (●), *UGT1A6**1/*2 subjects ($n = 8$) (■), and *UGT1A6**2/*2 subjects ($n = 4$) (▲). Data are presented as mean $\pm$ SD

Similarly, neither the urinary excretion of nonconjugated deferiprone nor its glucuronide correlated significantly with iron profiles.

Discussion

The results of the present study indicate statistically insignificant changes in deferiprone pharmacokinetic parameters in the *UGT1A6**1/*2 group. Likewise, the differences in 24-h urinary deferiprone and its glucuronide excretion among genotype groups failed to reach statistical significance. Nevertheless, this study provides the evidence of a meaningful effect of gender-related differences in deferiprone pharmacokinetics. Significant gender-related changes, however, were observed in serum pharmacokinetic parameters, but not in urinary excretion levels (Table 4). The intergender differences in serum pharmacokinetic parameters of deferiprone may be due to *UGT1A6* content or activity variation by gender. Previous *in vivo* studies of drugs metabolized by *UGT* have demonstrated faster clearance in men compared with women [15, 16]. Using human liver microsomes, Court *et al.* have demonstrated that men have 50% higher levels of *UGT1A6* activity than do women, as the *UGT1A6* protein content in male was greater than in female liver microsomes [17]. Unfortunately, it was not possible to evaluate the gender-genotype interaction in this study due to the lack of female subjects with *UGT1A6**2/*2 genotype. Using the available data, deferiprone pharmacokinetic parameters were compared between genotype groups in men, but no statistically significant difference was noted (data not shown).

Table 3

Pharmacokinetic parameters of deferiprone and deferiprone-glucuronide in healthy volunteers according to *UGT1A6* genotype

Parameters	<i>UGT1A6</i> *1/*1	<i>UGT1A6</i> *1/*2	<i>UGT1A6</i> *2/*2
Subjects (M/F)	5/5	4/4	3/1
Deferiprone			
C _{max} (µg ml ⁻¹)	24.6 (15.3, 31.1)	19.8 (14.9, 30.7)	26.7 (17.6, 38.6)
t _{max} (min)	45.0 (30.0, 90.0)	30.0 (30.0, 60.0)	37.5 (15.0, 120.0)
t _{1/2} (min)	128 (101, 154)	122 (95.8, 139)	121.8 (110, 129)
AUC _{0-∞} (min µg ⁻¹ ml ⁻¹)	4300 (2643, 5356)	3110 (2464, 4712)	3868 (3017, 4007)
V _d /F (l)	74.2 (55.3, 89.4)	76.2 (45.3, 115.7)	64.2 (49.5, 69.8)
CL/F (ml min ⁻¹)	369 (280, 514)	441 (265, 609)	353 (312, 402)
Deferiprone-glucuronide			
C _{max} (µg ml ⁻¹)	14.4 (11.5, 17.8)	14.9 (8.7, 23.8)	15.9 (12.2, 20.2)
t _{max} (min)	120 (90.0, 180)	120 (60.0, 180)	120 (90.0, 180)
t _{1/2} (min)	135 (95.7, 144)	119 (85.3, 134)	123 (99.3, 183)
AUC _{0-∞} (min µg ⁻¹ ml ⁻¹)	4471 (2758, 4958)	3853 (2722, 6196)	4677 (3171, 7073)
AUC ratio of deferiprone-glucuronide to deferiprone	0.99 (0.87, 1.31)	1.07 (0.71, 2.48)	1.31 (0.79, 1.89)

C_{max}, Maximum serum drug concentration; t_{max}, time to reach maximum serum drug concentration; t_{1/2}, elimination half-life; AUC_{0-∞}, area under the serum concentration–time curve from time zero to infinity; V_d/F, apparent volume of distribution; CL/F, apparent clearance. Data are given as median (95% CI).

Figure 3

Cumulative urinary nonconjugated deferiprone (a) and deferiprone-glucuronide (b) excretion in *UGT1A6**1/*1 subjects (*n*=10) (●), *UGT1A6**1/*2 subjects (*n*=8) (■), and *UGT1A6**2/*2 subjects (*n*=4) (▲). Data are presented as mean ± SD

Figure 4

Scatter plots of urinary nonconjugated deferiprone (a) and deferiprone-glucuronide (b) excretion in healthy volunteers according to *UGT1A6* genotype. Bars represent median values

Table 4

Pharmacokinetic parameters of deferiprone and deferiprone-glucuronide in healthy volunteers according to gender

Parameters	Males (n = 12)	Females (n = 10)
Deferiprone		
C_{max} ($\mu\text{g ml}^{-1}$)	19.9 (16.2, 22.7)	27.4 (17.1, 38.0)
T_{max} (min)	37.5 (30, 60)	37.5 (30, 60)
Terminal $elt_{1/2}$ (min)	125 (113, 144)	115 (98.8, 142)
$AUC_{0-\infty}$ (min $\mu\text{g}^{-1} \text{ml}^{-1}$)	3218 (2643, 4000)	4499 (3047, 5356)*
V_d/F (l)	80.7 (69.8, 105)	56.2 (46.2, 69.4)**
CL/F (ml min $^{-1}$)	473 (375, 536)	299 (265, 410)**
Deferiprone-glucuronide		
C_{max} ($\mu\text{g ml}^{-1}$)	16.2 (13.5, 19.3)	13.0 (10.2, 16.6)
T_{max} (min)	120 (90, 120)	120 (90, 180)
$elt_{1/2}$ (min)	133 (112, 138)	117 (85.3, 141)
$AUC_{0-\infty}$ (min $\mu\text{g}^{-1} \text{ml}^{-1}$)	4770 (3508, 6112)	3743 (2822, 4576)
24 h urinary excretion		
Deferiprone (mg)	39.6 (22.1, 61.8)	52.9 (14.5, 72.2)
(% dose)	2.6 (2.0, 3.8)	3.5 (1.2, 5.0)
Deferiprone-glucuronide (mg)	1093 (760, 1314)	840 (609, 1072)
(% dose)	72.6 (50.7, 81.3)	59.9 (48.7, 78.0)

*P-value < 0.05; **P-value < 0.01. Data are given as median (95% CI).

Figure 5

Correlation between V_d/F of deferiprone and serum ferritin concentration

Similarly, no significant difference in deferiprone pharmacokinetic parameters was observed between $UGT1A6^{*1/*1}$ and $*1/*2$ groups in women (data not shown). Therefore, it seems unlikely that there is an interaction between gender and $UGT1A6$ genotype on pharmacokinetics of deferiprone. Further study is required to support this possibility.

Studies *in vitro* and *in vivo* on $UGT1A6^{*2}$ polymorphisms have, thus far, yielded conflicting results. The initial

study by Ciotti *et al.* [8] demonstrated that recombinant $UGT1A6^{*2}$ allozyme was less active than recombinant $UGT1A6^{*1}$ allozyme. In contrast, the recent study by Krishnaswamy *et al.* [18] reported similar findings to those previously reported by Nagar *et al.* [12], who reported that the recombinant $UGT1A6^{*2}$ allozyme had lower K_m , higher V_{max} and high intrinsic clearance values compared with $UGT1A6^{*1}$ allozyme. Interestingly, both studies [12, 18] indicated that $UGT1A6^{*2}$ in the heterozygous state ($UGT1A6^{*1/*2}$) had lower activity than $UGT1A6^{*1}$ and $UGT1A6^{*2}$ in the homozygous state. However, Krishnaswamy *et al.* [18] have further found that, in contrast to the study by Nagar *et al.* [12], the $UGT1A6^{*2/*2}$ livers had significantly higher K_m values than did the $*1/*1$ livers. Furthermore, two case-control studies of colorectal adenoma [19, 20] have reported a significant impact of $UGT1A6$ polymorphisms (T181A and R184S) in modifying the influence of aspirin on adenoma risk, whereas, in contrast, a controlled trial by Hubner *et al.* [21] found that colorectal adenoma patients with variant $UGT1A6$ genotypes had a significantly reduced risk of neoplasia recurrence regardless of aspirin treatment.

The present study has found clear evidence of the association between V_d/F of deferiprone and SF ($P < 0.05$) (Figure 4), suggesting that iron may have a role in influencing deferiprone pharmacokinetics. Deferiprone can access intracellular iron pools due to its small molecular weight and lipophilic properties [22]. In pathological conditions of iron overload, transferrin is saturated with iron and therefore intracellular and plasma iron pools are available for chelation. In the case of normal physiological condition, plasma iron is bound to transferrin at 20–35% saturation and the ability of deferiprone to mobilize iron from transferrin depends on the concentration and iron saturation of both deferiprone and transferrin [23]. SF reflects the body iron stores as the synthesis of ferritin reflects the cellular iron status [24]. The level of body iron stores, thus, may have an influence on the extent of extravascular deferiprone distribution.

The lack of statistically significant effect of $UGT1A6^{*1/*2}$ genotype in deferiprone pharmacokinetics does not necessarily mean that there really is no effect. $UGT1A$ isoforms have been shown to have overlapping substrate specificity, but differ in their affinity and capacity [17, 25–27]. The contribution of other $UGT1A$ isoforms to deferiprone metabolism therefore should also be taken into consideration. On the other hand, the studied SNPs may indeed have marginal phenotypic effects on the glucuronidation rate, as speculated previously [18], and may not be strong enough to influence the pharmacokinetics of deferiprone. Several studies have been carried out using heterologous expression systems to identify conserved residues of $UGT1A6$ essential for enzyme activity [28, 29]. Further studies are needed to discover SNPs, especially those that reside within conserved regions of $UGT1A6$, amongst the population and to investigate the effects of

respective SNPs on pharmacokinetics of drugs that are substrates of UGT1A6. Lastly, inducible *UGT1A6* expression should also be considered, as this gene is known to be induced by environmental factors such as alcohol consumption and oxidative stress [30, 31].

In summary, the studied SNPs in *UGT1A6* do not appear to exert a statistically significant effect on the single-dose pharmacokinetics of deferiprone in a non-iron loading condition. Gender appears to influence the serum pharmacokinetics of deferiprone, but not urinary excretion of deferiprone and its metabolite. Furthermore, body iron stores in healthy volunteers may have an influence on the extent of extravascular deferiprone distribution.

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