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Chapter 3

The Effect of Genetic Polymorphisms in UGT2B15 on the Pharmacokinetic Profile of Sipoglitazar, a Novel Anti-diabetic Agent

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Summary

Purpose

Sipoglitazar was a novel, azolealkanoic acid derivative that possesses selective activity for the peroxisome proliferator-activated receptors (PPAR) PPARγ, PPARα, and PPARδ. The compound undergoes phase II biotransformation by conjugation catalyzed by UDP-glucuronosyltransferase (UGT); the aim of this analysis was to explore the influence of genetic polymorphism in UGT on the pharmacokinetics of sipoglitazar.

Methods

Three preliminary phase I clinical pharmacology studies were conducted in tandem in healthy human subjects. Genotyping was undertaken in a total of 82 subjects in the phase I program for the purpose of genotyping UGT polymorphisms. Plasma samples were collected for up to 48 hours post dose to characterize the pharmacokinetic profile following a single oral dose of the drug.

Results

Plasma concentrations of sipoglitazar and the distribution of dose-normalized individual values for area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) before any stratification were considerably skewed with a multi-modal distribution. The proportion of variability in $AUC_{0-\infty}$ explained by UGT2B15 was 66.7% ($P<0.0001$); the addition of other genetic or demographic factors was not statistically significant. Subjects homozygous for the UGT2B15 D85Y variant (UGT2B15 *2/*2) were exposed to greater plasma concentrations of sipoglitazar compared with subjects homozygous for the wild-type allele UGT2B15 *1/*1 (3.26 times higher) or heterozygous allele UGT2B15 *1/*2 (2.16 times higher).

Conclusions

These results indicate that sipoglitazar clearance is substantially modified by UGT2B15 enzyme variants, with higher exposure observed in the UGT2B15 *2/*2 genotype group.

Introduction

Sipoglitazar, a novel, orally-available, azolealkanoic acid derivative, has selective peroxisome proliferator-activated receptor (PPAR) agonist activities for PPARγ, PPARα, and PPARδ. As such, sipoglitazar was developed to improve peripheral insulin sensitivity, normalize circulating lipid profiles, and reduce body weight in patients with metabolic syndrome and type 2 diabetes mellitus (T2DM). A preliminary phase I program of clinical pharmacology studies was conducted in healthy human subjects to examine the pharmacokinetics, safety, and tolerability of sipoglitazar as single and multiple doses.

Many drugs are subject to phase II biotransformation processes, by which the parent compound or its intermediate metabolites are conjugated and subsequently excreted from the body as water soluble products such as glucuronides [1]. Pharmacogenetic variation has been identified for glucuronidation by uridine 5'-diphospate-glucuronosyltransferases (UGTs), specifically for the isoforms, UGT1A1, UGT1A7, UGT1A9, UGT2B7, and UGT2B15 [1]. However, clinical relevance for polymorphism in UGTs has currently only been identified for a few drugs, primarily catalyzed by UGT1A1. The anticancer drug, irinotecan, includes a label recommendation to lower the starting dose for subjects with the homozygous allele UGT1A1*28/*28 and nilotinib, carrying a label warning of increased risk of hyperbilirubinemia for subjects genotyped as UGT1A1*28 [2].

Preclinical studies of sipoglitazar metabolism conducted *in vitro* using human and animal liver microsome preparations suggest that enzymatic glucuronidation is central to its biotransformation [3]. Sipoglitazar is relatively stable in the absence of the UGT co-substrate, uridine diphosphoglucuronic acid (UDP), whereas the parent compound is susceptible to conjugation by the active enzymes UGT1A1, UGT1A3, UGT1A6, and UGT2B15. The principal metabolite of sipoglitazar is the the dealkylated derivative M-I. The potency of metabolite M-I relative to that of the parent sipoglitazar was 33%, 37%, and 17% for PPARγ, PPARα, and PPARδ, respectively. The metabolite is generated *in vitro* by the action predominantly of cytochrome P450 (CYP) 2C8 on the glucuronide intermediates. It is therefore hypothesized that initially sipoglitazar is metabolized to the glucuronide conjugate, sipoglitazar-Glu, by UDP glucuronyl transferase and secondly sipoglitazar-Glu is metabolized to M-I by dealkylation by CYP2C8 and deconjugation. Therefore, due to its

unique metabolic formation, the metabolite M-I was considered to be a marker for the level of metabolic activity of UGT.

On the basis of the *in vitro* findings, UGTs are hypothesized to play an important role in the disposition of sipoglitazar, and it is therefore postulated that abnormalities in the gene encoding UDP-glucuronosyl transferase may alter the rate of clearance of sipoglitazar from the body. Therefore, during the first human studies of sipoglitazar, pharmacogenetic investigation of relevant drug metabolizing enzymes was focused on UGT genetic polymorphisms.

Methods

Subjects

Three phase I studies, referred to as Studies EC001, EC002, and EC003, were undertaken, all in the United Kingdom. A summary of the demographic and genotype information across the studies is described in Table 1.

Table 1 Demographic and genotype information for subjects with known UGT2B15 genotype included in the phase I trials (n=82)

Genotype	UGT2B15*1/*1	UGT2B15*1/*2	UGT2B15*2/*2
Number of subjects	19	41	22
Proportion	23%	50%	27%
Age (years) mean ± SD[a]	34.1 ± 12.9	31.6 ± 9.7	32.6 ± 11.0
Age elderly cohort (years) mean ± SD	72 ± 5.7[b]	71.7 ± 3.8[c]	77[d]
Height (cm) mean ± SD	173.7 ± 7.3	171.9 ± 11.3	172.8 ± 8.4
Weight (kg) mean ± SD	71.4 ± 6.5	70.0 ± 10.1	72.4 ± 12.3
BMI (kg/m^2) mean ± SD	23.7 ± 2.3	23.7 ± 3.0	25.0 ± 2.8
Sex (male/female)	15/4	25/16	15/7
Race (Caucasian/Asian/Mixed race)	17/1/1	40/1/0	22/0//0

a Excluding elderly cohort. b n=2. c n=9. d n=1.
BMI=body mass index.

Table 2 shows the genotype information by dose group for all studies.

Table 2 Genotype information for UGT2B15 by dose group for all studies

Dose (mg)	0.2 (n=5)	0.4 (n=3)	1 (n=5)	2[a] (n=3)	4[a] (n=3)	8[a] (n=4)	16[a] (n=16)	32[a] (n=8)	64[a] (n=35)	Total (n=82)
Genotype										
UGT2B15*1/*1	1	1	2	0	1	2	5	0	7	19
UGT2B15*1/*2	3	2	2	2	0	0	5	5	22	41
UGT2B15*2/*2	1	0	1	1	2	2	6	3	6	22

[a] In EC001 the same subjects received two different doses of sipoglitazar, the number included in each dose represents the first dose received for all subjects

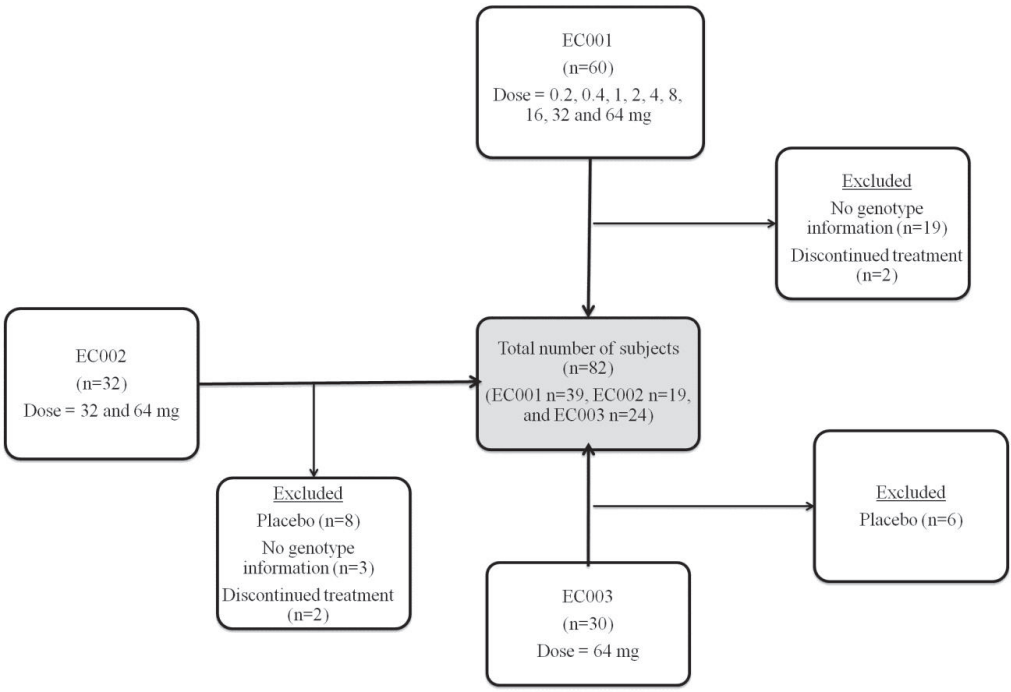
Study EC001 was a double-blind, placebo-controlled, cross-over study. A total of 60 healthy male and female subjects aged 18 to 55 years took part, of whom, 58 completed the investigation. In the ascending dose part of the study, 48 subjects received one dose of placebo and two single doses of sipoglitazar

(3-(3-ethoxy-1-{4-[(2-phenyl-1,3-thiazol-4-yl)methoxy]benzyl}-1*H*-pyrazol-4-yl)propanoic acid, also known as TAK-654), Takeda Pharmaceutical Company Limited, Osaka, Japan, at doses of 0.2, 0.4, 1, 2, 4, 8, 16, 32 and 64 mg. Study EC002 was a double-blind, placebo-controlled, parallel groups study. A total of 32 healthy male and female subjects aged 18 to 55 years took part, of whom, 30 completed the investigation. An equal number of male and female subjects who had been allocated to the active treatment received either a single dose 32 or 64 mg of sipoglitazar. Study EC003 was a double-blind, placebo-controlled, parallel groups study. A total of 30 healthy male and female subjects took part; 15 subjects aged 18 to 45 years (young cohort) and 15 subjects aged ≥65 years (elderly cohort), received a single 64mg dose of sipoglitazar.

Samples were collected for PK at the following time points in all studies: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h postdose.

Figure 1 shows the disposition of subjects who were included in the analysis. The total number of subjects who received an active dose of sipoglitazar and for whom genotype information was collected, was 82 (EC001 n=39, EC002 n=19, and EC003 n=24).

Figure 1 Flow chart of subjects included in the analysis, EC001, EC002, EC003 Phase 1 double-blind, placebo controlled studies (see Subjects section for details)



Ethical Considerations

All three studies were conducted in accordance with the Declaration of Helsinki (Edinburgh 2000). Written approval was obtained from the relevant local independent ethics committee before the start of each study and for the amendments made to the protocol.

In the case of Study EC001, 39 subjects were traced retrospectively and provided consent so that samples might be taken for genotyping. In Study EC002, consent was prospectively obtained for limited CYP genotyping only: 23 subjects (19 who received sipoglitazar and 4 who received placebo) were later traced and provided consent to allow UGT genotyping also. In study EC003, *CYP2C8* and *UGT2B15* genotyping were incorporated into the original protocol, and consent for all study procedures and analyses was obtained from every subject prospectively.

Analytical methods

At each specified time point, plasma samples were collected into sodium heparin–containing tubes. The tubes were inverted gently in order to dissolve the heparin and they were placed on ice until processing. The plasma was separated in a refrigerated centrifuge within 60 minutes of collection at approximately 1500 g for 10 minutes.

Sipoglitazar was analyzed in plasma by a validated liquid chromatography method with tandem mass spectrometry (HPLC/MS/MS) in selected reaction monitoring mode using turbo ionspray. The method was validated by Covance Laboratories Ltd (Harrogate, United Kingdom).

Briefly, plasma (0.25 mL), was mixed with internal standard (25 µL, 1 µg/ml sipoglitazar-d5, 5 µg/mL M-I-d5), 0.05 M phosphate buffer (0.75 mL, pH 3) and diethyl ether (3 mL). The organic layer was evaporated under nitrogen at 40°C and reconstituted in methanol:water:acetic acid (50:50:0.4 v/v/v). Following mixing and centrifugation, the supernatant fraction (5 µ L) was injected onto a Xetarra RP18, 5 µm, 150 x 2.1 mm (i.d.) HPLC column (Waters, Milford, MA) at 40°C. The mobile phase was water:acetic acid (100:0.2 v/v) and methanol:acetic acid (100:0.2 v/v) at a gradient of 40/60 from 0 to 3 min after injection, 20:80 from 3 to 4.5 min and 40:60 for 0.1 min. The mass spectrometer was operated under the following conditions: ionspray voltage; 5200V, heated capillary temperature; 425°C, auxillary gas flow; 8000 cc/min and nebuliser pressure; 15 psi.

The validated calibration range for sipoglitazar in human plasma was from 1 to 2500 ng/mL. Quality control samples were prepared in control human plasma with concentrations of sipoglitazar as follows: 3 (low), 50 (mid), and 1750 (high) ng/mL. For samples with concentrations higher than the assay range, a validated dilution (with human plasma) procedure was adopted. In study EC001, EC002 and EC003 assay precision for sipoglitazar was ≤9.1%, ≤9.4%, ≤7.1% and accuracy was 94.0-110.0%, 93.6-106.0% and 96.4-105.9%, respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined study-by-study using noncompartmental analysis at Covance Clinical Research Unit Limited, Harrogate, United Kingdom, using WinNonlin Version 3.2 (Study EC001), at Medeval Limited, Manchester, United Kingdom,

using WinNonlin Version 4.0 (Study EC002), and at Data Magik Limited, Salisbury, United Kingdom, using procedures implemented in SAS Version 8.2 (Study EC003).

Genotyping

Venous blood for genotyping was taken into an EDTA tube and the whole blood sample (4 ml) stored at -20°C pending shipping in the frozen state (-20°C) to the site of analysis.

Genotyping was performed by DxS, Manchester, United Kingdom. DNA was prepared from whole blood samples by the AGOWA/Hamilton automated extraction system (Bonaduz, Switzerland). Real time polymerase chain reaction (PCR) methods using the Stratagene Mx4000 (La Jolla, California, USA) and appropriately designed primers allele-specific at the 3′nucleotide end (Amplification Refractory Mutation System strategy [4]) were used to determine UGT1A6*2 (T181A, R184S), UGT1A7*2 (N129K, R131K), UGT1A7*3 (N129K, R131K, W208R), and UGT2B15*2 (D85Y), and the products of UGT1A1*28 (promoter $A(TA)_6TAA$ to $A(TA)_7TAA$) were analyzed according to their relative capillary electrophoretic mobility using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Warrington, United Kingdom). Similar real time PCR methods were used to determine CYP2C8*3 and CYP2C8*4 (A1196G and C792G, respectively).

To confirm the results in the two subjects in whom there appeared to be disconnect between genotype and sipoglitazar pharmacokinetic phenotype, all six exons plus nearby intronic regions of UGT2B15 were amplified and the fragments sequenced in forward and reverse directions using Big-Dye Terminators (Applied Biosystems). The reaction products were purified by gel exclusion chromatography and analyzed using an ABI Prism 3100 Genetic Analyzer.

Statistical methods

A dose proportionality assessment was performed on area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) by combining data from all three studies. The analysis was performed on the log transformed parameter, $AUC_{0-\infty}$ (dose range 0.2 to 64mg). For subjects who received two different doses of sipoglitazar, the $AUC_{0-\infty}$ from both doses was included in the analysis. The power model was used for analysis, $\ln(AUC_{0-\infty}) = a + b*\ln(dose) + error$ where a is the intercept and b is the dose-proportionality coefficient. For dose proportionality the slope of the regression line (b) = 1 and for dose independence b

= 0. The degree of proportionality was assessed using the value of b and the associated 95% confidence interval (CI). If the 95% CI for the slope of the regression line was close to unity, the relationship between dose and the pharmacokinetic parameter was concluded to be dose proportional for the dose range studied.

Analysis of variance (ANOVA) models were used to explore the effects on dose-normalized $AUC_{0-\infty}$ using log transformed data. For each UGT enzyme, a one-way ANOVA model with the genotype enzyme as a fixed effect factor was used to evaluate differences between levels of each UGT enzyme. Then separate ANOVA models, including UGT2B15 as a fixed effect factor and other covariates, were also produced. The coefficient of determination (R-square) from these models was used to estimate the proportion of variance accounted for by each statistical model. SAS version 9.1.3 (SAS Institute Inc. Cary, NC, USA) was used to produce all the analyses.

Results

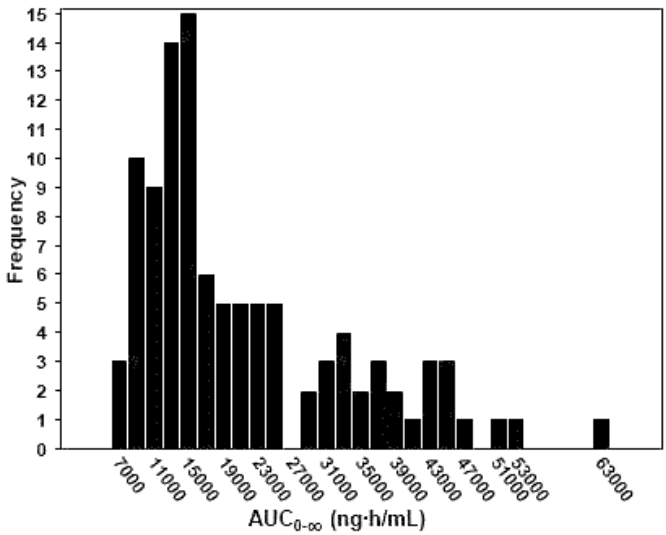
Pharmacokinetic results

A program of three phase I clinical pharmacology studies was undertaken in tandem to investigate the pharmacokinetics of single and repeated oral doses of sipoglitazar administered to male and female healthy subjects. In male subjects, single oral doses of sipoglitazar, 0.2 to 64 mg, were well tolerated, as were single oral doses of 16 to 64 mg in female subjects. Sipoglitazar was rapidly absorbed with a maximum observed plasma concentration occurring 0.6 to 1 h postdose across the dose levels investigated. Plasma concentrations of sipoglitazar declined with bi-phasic kinetics and with a terminal elimination half-life ($T_{1/2}$) of approximately 3 to 5 h. Statistical analysis of $AUC_{0-\infty}$, revealed dose proportionality across the dose range with a slope and 95% CI of 0.99 (0.92 - 1.05). The plasma exposure of the major metabolite M-I was approximately 10% of the parent, with a $T_{1/2}$ of approximately 6 to 7 h. After correcting for body weight, plasma concentrations of sipoglitazar were only slightly greater in female (n=27) subjects than in male subjects (n=55). Exposures to sipoglitazar and M-I metabolite were somewhat increased in elderly subjects (>65 years) compared with younger subjects.

The distribution of sipoglitazar plasma $AUC_{0-\infty}$ values for all subjects who took at least one dose of sipoglitazar (n=82) in the phase I program, normalized to a dose of 64 mg, is shown

in Figure 2. The distribution of $AUC_{0-\infty}$ values was notably skewed with an apparently multi-modal disposition suggesting the existence of a number of potential subpopulations.

Figure 2 Histogram plot of dose-normalized AUC0-∞ values for sipoglitazar in all phase I studies. AUC0-∞ Area under the plasma concentration– time curve from time 0 to infinity



Impact of UGT polymorphisms on pharmacokinetic parameters

In vitro studies conducted prior to human dosing predicted a central role for glucuronidation in the in vivo biological transformation of sipoglitazar. Following the analysis of the results from the first two phase I studies and the potential existence of subpopulations, a total of 62 subjects who took part in these studies (Studies EC001 and EC002) were retrospectively traced for variants of UGT1A1, UGT1A6, UGT1A7, and UGT2B15. Fifty-eight of these subjects had taken active drug. Based on the results, subjects who took part in the third phase I study (Study EC003) gave consent prospectively for genotyping for UGT2B15 and CYP2C8 only.

The majority of the subjects who took part in these studies were classified as Caucasians (Table 1). The proportion of participants shown to have UGT2B15*1/*1, UGT2B15*1/*2,

and UGT2B15*2/*2 genotypes was consistent with the literature for subjects having this ethnic background [5].

ANOVA was used to examine the influence of the four UGT enzymes on sipoglitazar $AUC_{0-\infty}$. Initially, separate one-way ANOVAs were performed for each enzyme in turn for subjects taking part in the first two studies (in whom all four enzymes were genotyped). The proportion of $AUC_{0-\infty}$ variance explained by UGT2B15 was 71% ($P < 0.0001$). Addition of the other genotype enzymes in turn, to this model, achieved no increase in the proportion of $AUC_{0-\infty}$ variance explained in the model (Table 3). The proportion of $AUC_{0-\infty}$ variance explained by UGT2B15 when this was tested across all three studies was 66.7% ($P < 0.0001$). Addition of demographic factors age, gender, and body weight did not explain additional $AUC_{0-\infty}$ variance with statistical significance (Table 3). No relationship between sipoglitazar $AUC_{0-\infty}$ and variants of glucuronosyltransferase genes other than UGT2B15 was evident on inspection.

Table 3 ANOVA results for sipoglitazar to determine the effect of UGT2B15 on dose-normalized $AUC_{0-\infty}$ (log transformed)

Effect of UGT2B15 and other genotypes (Studies EC001 and EC002; n=58)			
Source	**df**	**P value**	**R-square**
Model UGT2B15 alone			
UGT2B15	2	<0.0001	0.709
Model with UGT2B15 plus			
+UGT1A6	3	0.6505	0.718
+UGT1A1	3	0.8458	0.713
+UGT1A7	5	0.5438	0.731
+UGT1A6+UGT1A1+UGT1A7	-	-	0.737
Effect of UGT2B15 and demographic factors (Studies EC001, EC002, EC003; n=82)			
Source	**df**	***P* value**	**R-square**
Model UGT2B15 alone			
UGT2B15	2	<0.0001	0.666
Model with UGT2B15 plus			
+Gender	3	0.0464	0.683
+Age	3	0.0703	0.680
+BMI	5	0.8737	0.666

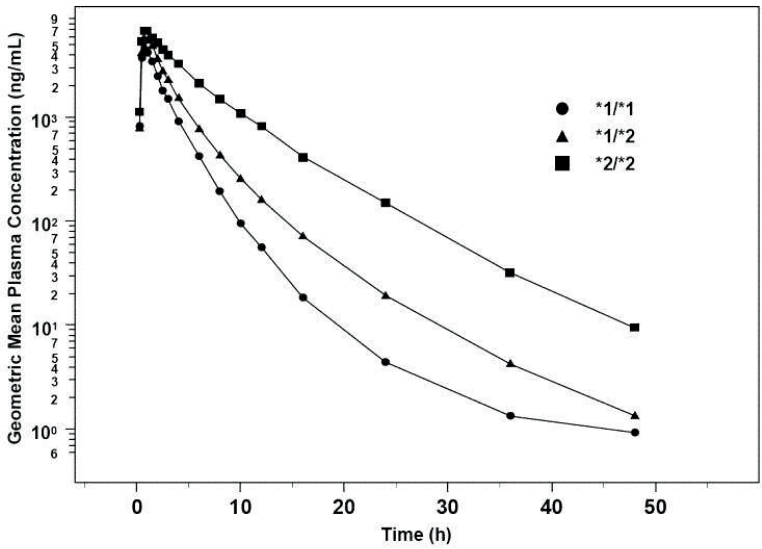
ANOVA, analysis of variance; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; BMI=body mass index.

Impact of UGT2B15 polymorphisms on pharmacokinetic parameters

The summary of pharmacokinetic parameters by UGT2B15 genotype for sipoglitazar and its main metabolite M-I are shown in Table 4. Figure 3 shows the corresponding plasma concentration-time profiles of sipoglitazar for the three UGT2B15 genotypes. Sipoglitazar $AUC_{0-\infty}$ was increased by approximately two- to three-fold in subjects with UGT2B15*2/*2 genotype as compared with subjects with genotype UGT2B15*1/*2 or UGT2B15*1/*1.

Parent to metabolite ratios for AUC (AUC-MR) were calculated with respect to UGT2B15 genotype and were found to vary across the UGT2B15*1/*1 (22%) and UGT2B15*1/*2 (13%) or UGT2B15*2/*2 (5%) genotype groups. The geometric mean AUC for sipoglitazar increased by approximately 51% from UGT2B15*1/*1 to UGT2B15*1/*2 groups.

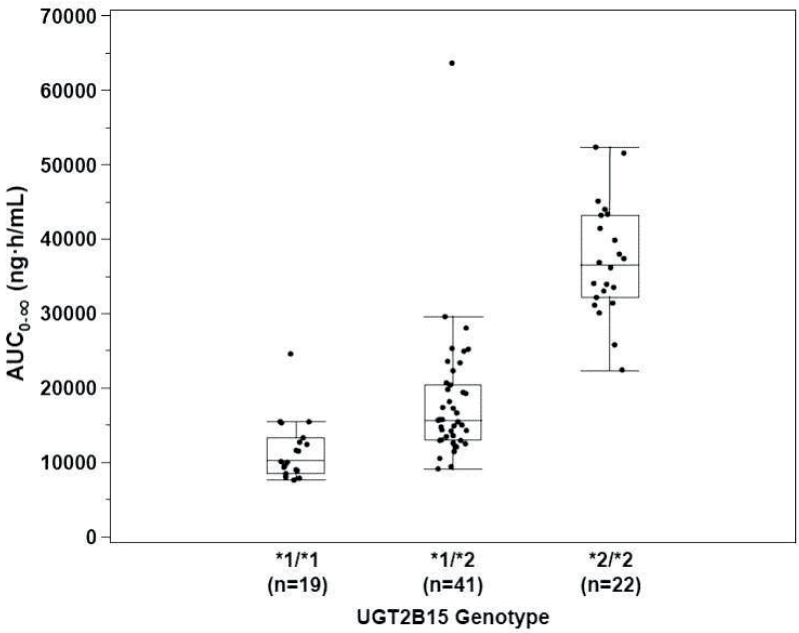
Figure 3 Plasma concentration–time profile dose-normalized (sipoglitazar, single 64 mg dose) by genotype group



$T_{1/2}$ values were comparable between the genotype groups for both sipoglitazar and M-I; however, the concentration 24 h postdose (C24) for sipoglitazar was approximately 52 and 21 times higher in UGT2B15*2/*2 as compared with that of UGT2B15*1/*2 or UGT2B15*1/*1, respectively. The M-I C24 in UGT2B15*2/*2 was approximately double that of UGT2B15*1/*2 or UGT2B15*1/*1.

The 25th and 75th percentile distributions show overlap between the UGT2B15*1/*1 and UGT2B15*1/*2 genotype (Figure 4); however, two outlier subjects (>1.5 times the interquartile range) were observed in the UGT2B15*1/*2 and UGT2B15*1/*1 groups. The UGT2B15*1/*2 and UGT2B15*1/*1 status of the outliers was later confirmed by direct gene sequencing.

Figure 4 Box plot of relationship between genotype and exposure for sipoglitazar. UGT Uridine 5′ -diphosphate-glucuronosyltransferase



Based on the results of the two outlier subjects, it was thought probable, then, that variations in CYP2C8 activity could contribute to the variation in sipoglitazar exposure observed in these subjects. To formally exclude an important influence of CYP2C8 variants on exposure to sipoglitazar, CYP2C8 genotype samples were collected in subjects who received active compound in the third clinical study (EC003). Exposure values for sipoglitazar were clearly correlated with UGT2B15 genotypes when data from all three studies were combined (Figure

4, n=82); however, no relationship was observed between sipoglitazar exposure and CYP2C8 genotypes *1/*1, *1/*3, or *3/*3 when this data was explored in study EC003 (Figure 5, n=24). In addition, the two outlier subjects observed in Figure 4 did not correlate with the outlier subjects observed in Figure 5.

Figure. 5 Box plot of relationship between the cytochrome P450 2C8 (CYP2C8) genotype and exposure for sipoglitazar (study EC003 only)

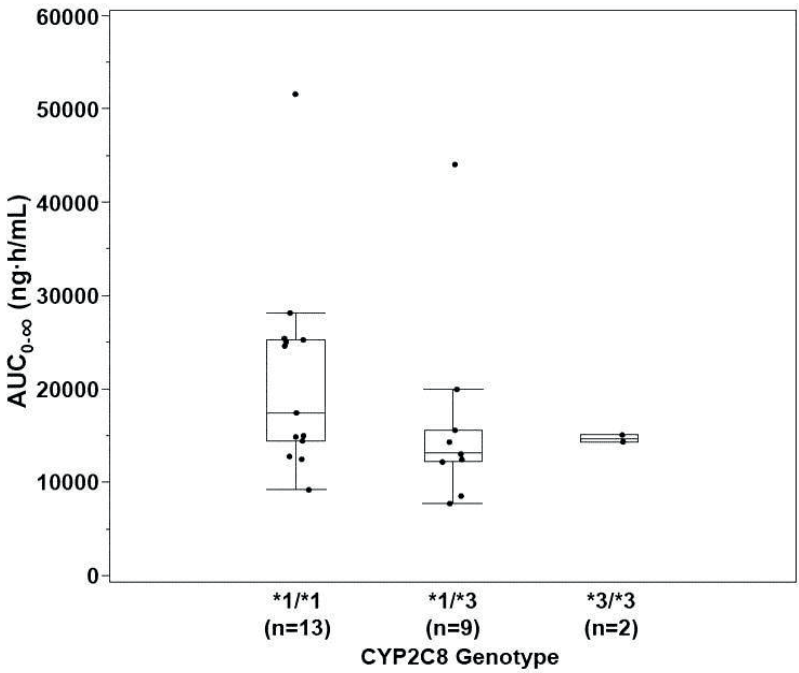


Table 4 Dose-normalized pharmacokinetic parameters (GM (%CV)) of sipoglitazar and metabolite M-I

Genotype	UGT2B15*1/*1 (n=19)	UGT2B15*1/*2 (n=41)	UGT2B15*2/*2 (n=22)	GM ratio 90% CI *2/*2 vs *1/*1	GM ratio 90% CI *2/*2 vs *1/*2
Sipoglitazar					
Cmax (ng/mL)	4,695 (39.5)	6,283 (30.1)	7,169 (24.0)	1.53 (1.30, 1.79)	1.14 (0.99, 1.30)
C24 (ng/mL)[a]	2.52 (0–29.3)	6.37 (0–275)	131.6 (48–354)	-	-
$AUC_{0-\infty}$ (ng·h/mL)	11,194 (30.7)	16,910 (36.9)	36,476 (20.9)	3.26 (2.77, 3.83)	2.16 (1.88, 2.47)
$T_{1/2}$ (h)[b]	3.82 (52.6)	3.29 (36.6)	5.05 (11.0)	1.32 (1.10, 1.59)	1.53 (1.31, 1.79)
$AUC_{0-\infty}$ MR	0.22 (27.5)	0.13 (35.2)	0.05 (19.4)	0.25 (0.21, 0.29)	0.41 (0.36, 0.47)
M-I					
Cmax (ng/mL)	591.4 (29.4)	468.2 (27.1)	233.5 (24.1)	0.39 (0.34, 0.45)	0.50 (0.44, 0.56)
C24 (ng/mL)[a]	8.08 (0–24.5)	7.48 (0–25.6)	15.9 (0–34.72)	-	-
$AUC_{0-\infty}$ (ng·h/mL)	2,484 (24.5)	2,223 (26.6)	1,986 (27.1)	0.80 (0.70, 0.92)	0.89 (0.80, 1.00)
$T_{1/2}$ (h)[b]	7.30 (51.8)	6.42 (36.5)	7.85 (17.7)	1.08 (0.89, 1.29)	1.22 (1.05, 1.43)

$AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; C24, concentration at 24 hours; CI, confidence interval; Cmax, maximum observed plasma concentration; CV, coefficient of variation; GM, geometric mean; $T_{1/2}$, terminal elimination half-life; MR, metabolite ratio.

[a] median and range. [b] not dose normalised.

Discussion

The investigation we report here of UGT genetic polymorphisms in human subjects dosed with the novel, nonthiazolidinedione, insulin-sensitizing agent sipoglitazar was prompted by observing considerable inter-subject variability in drug plasma concentration profiles as data emerged from an on-going trio of clinical pharmacology studies. Further inspection of the skewed distribution of sipoglitazar $AUC_{0-\infty}$ values suggested the presence of subpopulations. The results presented show a strong correlation between the genetic variants of UGT2B15 and the sipoglitazar exposure. Approximately two-thirds of the inter-subject variability in sipoglitazar plasma exposure is explained by UGT2B15 genetic variation and no relationship between sipoglitazar plasma exposure and variants of the other UGT enzymes was found.

UGTs, together with acetyltransferases, glutathione-S-transferases, and sulfotransferases, are responsible for the phase II biotransformation of many drugs. Amongst these enzyme families, UGTs are considered to show the most profound effects on drug elimination [5]. Examples where inter-patient differences in drug elimination may result from differences in glucuronidation rates and underlying UGT allelic variation include lorazepam [6], the toxic irinotecan metabolite SN-38 [7,8], and mycophenolic acid [9]. For S-oxazepam [10] and rofecoxib [11], provocative in vitro data show, respectively, that polymorphisms of UGT2B15 and UGT2B7/UGT2B15 differ in their activity with respect to drug and have not as yet been shown to cause inter-patient differences in drug exposure. Polymorphisms of UGT1A6 appear to exert little effect in practice on the rate of paracetamol elimination [12,13]. However, glururonides have been relatively under studied compared with the CYP mediated metabolism; the literature is divided upon the impact of this variant on enzyme function and further in vivo studies are necessary to evaluate the clinical significance [14].

Although the exposure was approximately two- to three-fold higher in the UGT2B15*2/*2 genotype than either UGT2B15*1/*1 or UGT2B15*1/*2, two outlier subjects genotyped as UGT2B15*1/*1 and UGT2B15*1/*2 were observed. These subjects genotyped as UGT2B15*1/*1 and UGT2B15*1/*2, but had considerably higher exposure than expected based on their genotype. Gene sequencing confirmed that heterozygosity for the D85Y mutation had been correctly identified on initial investigation and revealed no alternative unexpected genetic mutations. Our analysis showed that across the population UGT2B15 genotype could explain 66% of the variability of sipoglitazar exposure as determined by

$AUC_{0-\infty}$. Other factors such as age, body mass index or sex appeared to contribute little to explaining the additional variability or outlying subjects. This experience suggests that analysis of UGT2B15 genotype alone may not invariably predict the extent of individual exposure to sipoglitazar and as yet other unidentified factors may affect the clearance of sipoglitazar in subjects. In addition, the rates of glucuronidation are affected not only by genetically determined variation but also by age, gender, disease, diet, and other environmental influences [5]. The relationship between exposure to sipoglitazar and UGT2B15 genotype in phase I trials significantly accounts for the variability. The extensive sipoglitazar metabolizer phenotype associated with UGT2B15*1/*1 and the relatively poor metabolizer phenotype associated with UGT2B15*2/*2 genotype can be inferred from the results presented. Rates of drug metabolism were not directly measured; however, there was a significant decrease in the AUC metabolite ratios of UGT2B15*1/*1 or UGT2B15*1/*2 as compared with UGT2B15*2/*2, indicating reduced levels of metabolic activity associated with UGT2B15*2/*2. The conclusion that subjects with UGT2B15*2/*2 genotype metabolize sipoglitazar poorly is nonetheless consistent with the results of others who, using S-oxazepam as a substrate, have shown that the UGT2B15 D85Y variant is less active than the wild-type enzyme [10]. Potentially, the UGT2B15 D85Y variant could have a significant impact because of its high population frequency (approximately 50% of all alleles) [15]. Due to this frequency, approximately 22% of the Caucasian population is homozygous for this allele with a potentially significant impact on their ability to metabolize drugs and other chemicals by this pathway [5].

As predicted from the mechanism of action and from its pharmacological profile in animal models, sipoglitazar exerts little effect over blood glucose levels in healthy, nonobese human subjects with normal insulin sensitivity. However, following completion of the phase I clinical pharmacology studies described here, studies of sipoglitazar have been completed in patients with T2DM. All patients taking part in these studies were genotyped for UGT2B15 prospectively to assess the clinical relevance of variants of this enzyme [16].

In summary, it is clear that the activity of UGT2B15 transferase is important for the elimination of sipoglitazar, and that individual exposure to sipoglitazar is dependent on the differential activity of naturally occurring enzyme variants.

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