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Carbamazepine pharmacokinetics are not affected by zonisamide: in vitro mechanistic study and in vivo clinical study in epileptic patients

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Abstract

Carbamazepine is metabolized by CYP3A4 and several other cytochrome P450 enzymes. The potential effects of zonisamide on carbamazepine pharmacokinetics (PK) have not been well characterized, with contradictory literature reports. Hence, an in vitro study was designed to evaluate the cytochrome P450 inhibition spectrum of zonisamide using human liver microsomes. Further, an in vivo steady-state study was performed to measure the effect of zonisamide on carbamazepine PK in epileptic patients, and monitor zonisamide PK.

In vitro human liver microsomes were incubated with zonisamide (200, 600 or 1000 μM) in the presence of appropriate probe substrates to assess selected cytochrome P450 activities. In vivo, the effect of zonisamide, up to 400 mg/day, on the steady-state PK of carbamazepine and carbamazepine-epoxide (CBZ-E) was studied in 18 epileptic patients.

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In vitro, zonisamide did not inhibit CYP1A2 and 2D6, and only weakly inhibited CYP2A6, 2C9, 2C19, and 2E1. The estimated K_i for zonisamide inhibition of CYP3A4 was 1076 μM, 12 times higher than typical unbound therapeutic serum zonisamide concentrations. In vivo, no statistically significant differences were observed for mean C_{max}, T_{max}, and $AUC_{0–12}$ of total and free carbamazepine and CBZ-E measured before and after zonisamide administration (300–400 mg/day for 14 days). However, CBZ-E renal clearance was significantly ($p < 0.05$) reduced by zonisamide. The observed mean zonisamide $t_{1/2}$ (36.3 h), relative to approximately 65 h reported in subjects on zonisamide monotherapy, reflects known CYP3A4 induction by carbamazepine. Based on the lack of clinically relevant in vitro and in vivo effects, adjustment of carbamazepine dosing should not be required with concomitant zonisamide administration.

Keywords: Zonisamide; Carbamazepine; Human liver microsomes; Pharmacokinetics; Epilepsy; Drug interactions

1. Introduction

Zonisamide, 1,2-benzisoxazole-3-methanesulfonamide, is a novel anticonvulsant that is chemically classified as a sulfonamide derivative and is structurally and mechanistically different from other anti-epileptic drugs. During its clinical evaluation in patients treated with carbamazepine, apparently contradictory results were obtained regarding the effects of zonisamide on the pharmacokinetics (PK) of carbamazepine (Hachad et al., 2002). For example, Sackellares et al. (1985) reported a rise in average carbamazepine concentrations following the initiation of zonisamide therapy and Minami et al. (1994) observed in 16 pediatric patients that the addition of increasing doses of zonisamide resulted in a parallel increase in the carbamazepine-epoxide (CBZ-E)/carbamazepine ratio. This observation contradicts that of Shinoda et al. (1996) who observed a significant decrease in CBZ-E/carbamazepine ratio in plasma samples associated with a small increase in the carbamazepine plasma concentration-to-dose ratio. On the other hand, a series of studies have indicated that zonisamide, at doses up to 600 mg daily, had no effect on carbamazepine and CBZ-E plasma levels and that it can be introduced safely without dosage adjustment of concomitant anti-epileptic medications (Browne et al., 1986; Miura, 2000; Schmidt et al., 1993).

The notion that zonisamide can potentially increase carbamazepine levels rests on the fact that carbamazepine and zonisamide are both metabolized appreciably by CYP3A4 (Kerr et al., 1994; Nakasa et al., 1993,1998; Pelkonen et al., 2001). However, there is no direct evidence that zonisamide can inhibit that enzyme at any concentration. Recent studies also indicate that other enzymes (CYP2C8, CYP2B6, CYP2E1, CYP1A2 and CYP2A6) are involved in carbamazepine metabolism, in particular in the 3- and 2-hydroxylation pathways (Pearce et al., 2002), and it is theoretically possible that zonisamide could inhibit some of these enzymes. Based on this body of literature, two studies were undertaken:

(1) An investigation of the inhibition spectrum of zonisamide toward several cytochrome P450 enzymes (CYP3A4, CYP2D6, CYP1A2, CYP2C9, CYP2C19, CYP2E1 and CYP2A6) in human liver microsomes; this study included an estimation of its K_i toward CYP3A4-mediated 10-hydroxy-*R*-warfarin metabolism, as well as its K_m for CYP3A4.
(2) A measure of the clinical effect of the addition of zonisamide on carbamazepine PK under steady-state conditions in epileptic patients.

2. Materials and methods

2.1. In vitro study in human liver microsomes

2.1.1. Substrates, metabolites and other chemicals

Racemic warfarin was obtained from Sigma Biochemicals and resolved as described by West et al. (1961). 6-Hydroxy-*R*-warfarin, 10-hydroxy-*R*-warfarin and (*S*)-mephenytoin were synthesized according to methods described previously (Hermodson et al., 1971; Lawrence et al., 1990; Wienkers et al., 1996). 4′-Hydroxy-mephenytoin was obtained from Research Biochemicals International (Natick, MA). Diazomethane was generated from Diazald (Aldrich Chemical Co.) according to the manufacturer's directions. Zonisamide and its metabolite

SMAP [2-(sulfamoylacetyl)-phenol] were supplied by Dainippon Pharmaceutical Co. Ltd., Osaka, Japan. Other chemicals were obtained from Sigma Chemicals.

2.1.2. Preparation of liver microsomes

Whole human livers were obtained through the Solid Organ Transplant Program at the University of Washington Medical Center and Northwest Organ Procurement Agency (Seattle, WA). Microsomes were prepared by differential centrifugation (Thummel et al., 1993) and suspended in potassium dihydrogen phosphate buffer (100 mM) pH 7.4 with 1 mM EDTA and stored in aliquots at −70 °C until utilization. Protein content was determined by the Lowry assay (Lowry et al., 1951) and total cytochrome P450 content was determined from the reduced minus oxidized carbon monoxide difference spectra (Estabrook et al., 1972). Incubations for each CYP isoform were performed using human liver microsomes from three different livers chosen to represent a range of activities, but all with sufficient activity to catalyze the specific reaction.

2.1.3. Inhibition of CYP-specific biotransformation rates by zonisamide

Various in vitro CYP-specific biotransformations were performed in the presence of 0, 200, 600, or 1000 μM zonisamide ([μM] = [μg/mL] × 4.71). The seven metabolic pathways (and CYPs) that were monitored were (*R*)-warfarin 6-hydroxylase for CYP1A2 (Bush et al., 1983), coumarin 7-hydroxylase for CYP2A6 (Miles et al., 1990), (*S*)-warfarin 7-hydroxylase for CYP2C9 (Rettie et al., 1992), (*S*)-mephenytoin 4′-hydroxylase for CYP2C19 (Goldstein et al., 1994; Wrighton et al., 1993), dextromethorphan *o*-demethylation for CYP2D6, (Dayer et al., 1989; Ducharme et al., 1996; Kerry et al., 1994), *p*-nitrophenol hydroxylase for CYP2E1 (Tassaneeyakul et al., 1993), and (*R*)-warfarin 10-hydroxylase for CYP3A4 (Bush et al., 1983; Lawrence et al., 1990); the assays for metabolic products were performed as described previously (Erickson et al., 1999). Data analysis was performed by comparison of the metabolite production rates from probe compounds in the presence of zonisamide to those observed in incubations containing no zonisamide. For CYP3A4 inhibition, K_i was calculated using a Dixon plot; the concentrations tested were 100, 300 or 900 μM for (*R*)-warfarin and 0, 0.75, 1.5, 2.5 and 3.5 mM for zonisamide.

2.1.4. Estimation of K_m for the formation of 2-sulfamoylacetylphenol (SMAP)

The formation of SMAP from zonisamide was measured based on the method of Nakasa et al. (1993), using final concentrations of zonisamide of 150, 300, 450, 700, 1200 and 2000 μM. SMAP concentrations were calculated by comparison to a standard curve extracted from inactivated microsomes. Data were analyzed graphically using Lineweaver-Burk plots and fitted using WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC).

2.2. In vivo study in epileptic patients

The study was performed in accordance with the Declaration of Helsinki and its amendments and in compliance with the guidelines of Good Clinical Practice. The study protocol and informed consent form were approved by investigational review boards before recruitment of any patients. Written informed consent was obtained from all subjects before entry into the study.

2.2.1. Population and study design

The study was a three-center, steady-state, open-label, one-sequence drug interaction trial in otherwise healthy male or female volunteers (18–55 years-old) with a seizure disorder controlled by carbamazepine alone, at a stable dose for at least four weeks prior to initial study dosing. A total of 20 epileptic patients were to be enrolled in the study. Patients were to receive gradual dose titration of zonisamide to 400 mg/day (200 mg twice daily), while maintaining their stable carbamazepine dose throughout the study. The study was implemented in three stages: a pre-zonisamide period from Days −7 through −1 (Period 0), a zonisamide dose titration period from Days 1 through 35 (Period 1), and a post-zonisamide period thereafter until study conclusion on Day 49 (Period 2).

The zonisamide dosage titration schedule consisted of the following four regimens: 100 mg (a.m. only) of zonisamide on Days 1–3; 200 mg/day (100 mg BID) on Days 4–10; 300 mg (100 mg a.m. and 200 mg p.m.) on Days 11–17 and 400 mg/day (200 mg BID) from Day 18 to Day 34. Subjects received a final 200 mg dose

of zonisamide on the morning of Day 35. If a patient was unable to tolerate the maximum dose of 200 mg BID, the patient was given zonisamide at the previously tolerated dosage level and needed to be stabilized at this dose level for a minimum of 14 Days prior to the determination of the PK profile on Day 35. The BID dosing of zonisamide was timed to coincide with the patient's BID carbamazepine dosing (approximately 8 a.m. and 8 p.m.).

2.2.2. Study procedures

Three PK profiles were performed. Profiles 1 and 2 were taken on Days −7 and −1, respectively, to assess PK parameters of carbamazepine administered alone. Profile 3 was performed on Day 35 to assess PK of zonisamide and carbamazepine taken in combination. Plasma samples for measurement of free and total carbamazepine and free and total carbamazepine-10, 11-epoxide (CBZ-E) were collected within 15 min prior to morning dosing on Day −7 and −1, and carbamazepine and CBZ-E plasma and zonisamide serum PK samples were collected within 15 min prior to morning dose on Day 35. Additional carbamazepine and CBZ-E plasma samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h postdose on Days −7, −1, and 35. Three 4 h urine samples for CBZ-E levels were collected throughout the postdose PK blood sampling on Days −7, −1, and 35. Zonisamide predose serum samples were collected on Days 1, 33, 34, and 35. Additional samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h postdose on Day 35. Zonisamide serum samples were also collected at 24, 36, and 72 h and 7, 10, and 14 Days subsequent to the Day 35 final zonisamide dose.

2.2.3. Safety

Safety was monitored throughout the study by assessing adverse events (AEs), laboratory tests (hematology, biochemistry, urinalysis), physical examinations, vital signs, and concomitant medications. Women of childbearing potential were to agree to use a medically acceptable barrier method of contraception (excepting oral/implanted contraceptives) throughout the study duration, and serum pregnancy tests were performed at screening and at termination.

2.2.4. Analytic methods

2.2.4.1. Carbamazepine and carbamazepine-10,11-epoxide (CBZ-E) measurement in plasma. Total and free (unbound) plasma concentrations of carbamazepine and CBZ-E were determined using a validated high performance liquid chromatography method (HPLC) with ultraviolet (UV) detection at PPD Development (Middleton, WI). The lower limits of quantitation were 0.05 and 0.10 μg/mL for total carbamazepine and CBZ-E, and free carbamazepine and CBZ-E, respectively. All calibration curves had a correlation coefficient greater than 0.996.

2.2.4.2. CBZ-E measurement in urine. Urine concentrations of CBZ-E were determined using a validated HPLC with UV detection at PPD Development (Middleton, WI). The lower limit of quantitation for CBZ-E was established at 0.10 μg/mL using 1.00 mL urine samples. All calibration curves had a correlation coefficient greater than 0.999.

2.2.4.3. Zonisamide measurement. Serum concentrations of zonisamide were determined using a validated HPLC method with ultraviolet detection at Kansas City Analytical Services Inc. (Shawnee, KS). *N*,*N*-dimethylzonisamide was added as an internal standard to the serum samples, the mixtures of which were subject to liquid–liquid extraction. The extracts were then injected onto the HPLC–UV system for quantitation. The lower limit of quantitation for zonisamide was established at 0.5 μg/mL. In-study calibration curves contained eight standards ranging from 0.5 to 64.0 μg/mL. All calibration curves had a correlation coefficient equal to 0.999. Samples quantified below the lowest standard were reported as below the quantifiable limit (BQL).

2.2.5. Pharmacokinetic and statistical analysis

The PK analysis included the calculation of the following parameters for free and total carbamazepine and CBZ-E on Days −7, −1, and 35 and for zonisamide on Day 35: T_{max} – the time to maximum observed plasma/serum concentration for a subject; C_{max} – the maximum observed plasma/serum concentration for a subject; C_{min} – the minimum observed plasma/serum concentration for a subject; $AUC_{0–12}$ – the area under the concentration versus time curve from the time of dosing to 12 h postdose, calculated using the linear-log trapezoidal method; and CL/F – the apparent oral clearance, calculated as dose/AUC.

Urine PK parameters for CBZ-E included: Ae_{0-12} – the cumulative amount of CBZ-E measured from urine collection during the 0–12 h interval; Fu – fraction of the administered CBZ dose excreted as CBZ-E in urine (Ae_{0-12} CBZ-E/dose CBZ); and CLr – renal clearance (Ae_{0-12}/AUC_{0-12}).

For zonisamide (Day 35), the following additional PK parameter was computed: $t_{1/2}$ – the terminal elimination half-life of zonisamide, defined as $0.693/\lambda_z$, where λ_z is the slope of the terminal elimination phase of the log concentration versus time curve.

All PK analysis were performed using non-compartmental methods with WinNonlin Professional Version 3.1 (Scientific Consulting Inc., Apex, NC). An initial analysis was performed using the observed plasma concentration, independent of the dose: for each patient, a pair-wise comparison (paired *t*-test) was used to compare the carbamazepine (total) C_{max} and AUC_{0-12} observed before (Day-1) and in combination with zonisamide (Day 35) and the 90% confidence intervals were constructed. In a further analysis, all subjects were normalized to a common dose (400 mg a.m. for carbamazepine and 200 mg a.m. for zonisamide). Statistical analysis were performed on Profile 1 (Day −7) and 2 (Day −1) of carbamazepine to test for variability and confirm steady-state. Comparisons were made of PK parameters of carbamazepine at steady-state (Days −7 and −1) and carbamazepine taken without and in combination with zonisamide (Days −1 and 35, respectively). A repeated measures analysis of variance (ANOVA) on change from baseline was performed and associated 95% confidence limits were calculated.

3. Results

3.1. In vitro effects of zonisamide on cytochromes P450

At concentrations of 200–1000 μM, zonisamide did not inhibit CYP1A2 and CYP2D6 in the three livers tested (results not shown). The effect of zonisamide on the in vitro activities of CYP2A6, CYP2C9, CYP2C19, and CYP2E1 was small and appeared to be concentration-dependent (Table 1). In general, a reduction of >25% in the activities of these enzymes was apparent only at zonisamide concentrations at least four- to six-fold higher than typical therapeutic concentrations of zonisamide. In initial studies on CYP3A4 activity using (*R*)-warfarin at a concentration of 500 μM (above its K_m), inhibition of 16.7–21.9% were observed at 1000 μM of zonisamide (Table 1). These observations were extended by further experiments using 10-hydroxylation of (*R*)-warfarin (with 100, 300 and 900 μM of (*R*)-warfarin), yielding a zonisamide K_i value of 1076 μM. Also, using three livers with varying content of CYP3A4, the K_m for the CYP3A4-mediated reduction of zonisamide to SMAP in human liver microsomes was estimated to be 312 ± 16 μM (mean ± S.D.) (Table 2).

3.2. In vivo study in epileptic patients

3.2.1. Disposition of subjects

A total of 20 patients were enrolled in the study and 18 patients completed the study. Two patients discontinued the study, one during Period 0 before zonisamide administration and one during Period 1 due to adverse events (nausea and insomnia). Of the 19 patients who received zonisamide, 12 were males and 7 were females. Mean age at baseline was 38.1 years (range 18–58 years) and mean weight was 75.7 kg (range 61.2–104.8 kg). Four patients were unable to reach or maintain the maximum titrated zonisamide dosage level of 400 mg/day and their dose of zonisamide was stabilized at 300 mg/day. All of the 18 patients who underwent Profile 3 (Day 35) had received a stable dose of carbamazepine throughout the study (range: 200–2000 mg/day in two divided doses) and had received their respective maximum tolerated zonisamide dose for a minimum of 14 days (400 mg/day for 14 patients and 300 mg/day for 4 patients). Two patients, who had serum rather than plasma samples drawn on Day 35, were excluded from the analysis of total carbamazepine and CBZ-E.

3.2.2. Carbamazepine and CBZ-E pharmacokinetics

Summary statistics (before dose-normalization) of total CBZ C_{max} and AUC_{0-12} pre- (Day −1) and post- (Day 35) zonisamide administration are presented in Table 3, together with the geometric mean ratios and the associated 90% confidence interval values. There were no statistically significant differences between the two periods of treatment. Pharmacokinetic parameters

Table 1
Effect of zonisamide on the activity of CYP2A6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4

	Liver #143	Control (%)	Liver #144	Control (%)	Liver #148	Control (%)
Effect of zonisamide (ZNS) on CYP2A6 activity as measured by 7-hydroxylation of coumarin[a]						
Control	219 ± 4	100.0	1022 ± 31	100.0	602 ± 14	100.0
200 μM ZNS	201 ± 2	91.8	913 ± 12	89.3	599 ± 68	99.5
600 μM ZNS	179 ± 7	81.7	781 ± 25	76.4	482 ± 15	80.1
1000 μM ZNS	150 ± 5	68.5	729 ± 32	71.3	394 ± 38	65.4
Effect of zonisamide (ZNS) on CYP2C9 activity as measured by 7-hydroxylation of (*S*)-warfarin[b]						
Control	6.42	100.0	3.11	100.0	5.84	100.0
200 μM ZNS	5.80	90.3	2.66	85.5	5.69	97.4
600 μM ZNS	4.87	75.9	2.28	73.3	4.70	80.5
1000 μM ZNS	4.36	67.9	2.34	75.2	4.32	74.0
Effect of zonisamide (ZNS) on CYP2C19 activity as measured by 4′-hydroxylation of (*S*)-mephenytoin[c]						
Control	76.29	100.0	18.45	100.0	10.60	100.0
200 μM ZNS	72.44	95.0	17.88	96.9	9.75	92.0
600 μM ZNS	56.29	73.8	14.03	76.0	6.74	63.6
1000 μM ZNS	45.92	60.2	12.39	67.2	5.42	51.1
Effect of zonisamide (ZNS) on CYP2E1 activity as measured by hydroxylation of *p*-nitrophenol[d]						
Control	1.923	100.0	1.476	100.0	1.266	100.0
200 μM ZNS	1.618	84.1	1.076	72.9	1.131	89.3
600 μM ZNS	1.382	71.9	0.922	62.5	0.933	73.7
1000 μM ZNS	1.252	65.1	0.878	59.5	0.871	68.8
Effect of zonisamide (ZNS) on CYP3A4 activity as measured by 10-hydroxylation of (*R*)-warfarin[e]						
Control	94.4	100.0	486.9	100.0	316.5	100.0
200 μM ZNS	101.1	107.1	479.7	98.5	298.2	94.2
600 μM ZNS	82.6	87.5	412	84.6	261.2	82.5
1000 μM ZNS	75	79.4	380.3	78.1	263.8	83.3

Results are expressed as pmol/nmol/min. [a]Mean coefficient of variation in triplicate incubations = 4.02%. Mean coefficient of variation in duplicate incubations = [b]5.71%, [c]3.62%, [d]4.44%, and [e]3.27%.

(dose-normalized to 400 mg a.m.) for total and free carbamazepine and CBZ-E pre (Days −7 and −1) and post-treatment with zonisamide (Day 35) are summarized in Table 4 and the mean plasma concentration versus time profiles are shown in Fig. 1 (upper panel: carbamazepine and lower panel: CBZ-E).

Table 2
Zonisamide K_m and V_{max} values for SMAP formation, as calculated from the Lineweaver–Burk plot and using WinNonlin estimation program

Lineweaver–Burk			WinNonLin			
Liver	K_m[a]	V_{max}[b]	K_m[a]	CV (%)	V_{max}[b]	CV (%)
HL143	291	0.9	297	5.2	0.9	1.5
HL154	342	2.6	329	10	2.6	3.2
HL144	227	3.1	311	14.8	3.4	4.7
Mean	287	2.2	312	NA	2.3	NA
S.D.	58	1.2	16	NA	1.3	NA

[a] K_m expressed as μM.
[b] V_{max} expressed as nmol/min/nmolP450.

The mean C_{min} values for total and free carbamazepine fluctuated less than 10% between Days −7 and −1 and there were no statistically significant differences in total and free carbamazepine between Days −7 and −1 for C_{max}, $AUC_{0–12}$, and CL/F, demonstrating the achievement of steady-state. The statistical comparison between Days −1 and 35 showed that the total and free carbamazepine pharmacokinetic parameters, namely C_{min}, C_{max}, $AUC_{0–12}$, and CL/F, were essentially unchanged after the addition of zonisamide (Table 4). To assess the effect of the addition of zonisamide on carbamazepine disposition more fully, the 90% confidence intervals of the population geometric mean ratio of Day 35 to −1 for C_{max} of total and free carbamazepine (97.6–119.9% total; 96.3–116.2% free) and the corresponding values for

Table 3
Summary satistics of total CBZ C_{max} and $AUC_{0–12}$ with (Day 35) and without ZNS (Day −1)

Summary statistics	C_{max} (μg/mL)			AUC_{0-12} (μg h/mL)		
	Day −1	Day 35	Ratio Day 35/Day −1	Day −1	Day 35	Ratio Day 35/Day −1
Mean	9.2	10.0	1.10	99.9	104.3	1.05
S.D.	2.6	2.9	0.25	27.2	31.4	0.19
Paired *t*-test		$p = 0.14$ (NS)			$p = 0.32$ (NS)	
Geometric mean	8.9	9.6	1.08	96.7	99.49	1.03
90% Confidence interval			0.98–1.20			0.95–1.12

Non-dose-normalized, $N = 16$.

$AUC_{0–12}$ (95.0–112.3% total; 93.3–110.3% free) were calculated and fell within the generally accepted limits for bioequivalence (80–125%).

No statistically significant differences were observed for any of the plasma PK parameters for either total or free CBZ-E. ANOVA performed on the urinary parameters calculated for CBZ-E showed only a statistically significant reduction ($p < 0.05$) in renal clearance from baseline (Days −7 and −1) to Day 35 (Table 5).

3.2.3. Zonisamide pharmacokinetics

The calculated PK measures of zonisamide exposure at steady-state, normalized to a 200 mg a.m. dose, included C_{max} (21.4 ± 8.6 μg/mL–100.8 μM), $AUC_{0–12}$ (230.5 ± 93.4 μg h/mL), CL/F (975.9 ± 334.9 mL/h) and $t_{1/2}$ (36.3 ± 10.6 h).

3.2.4. Adverse events

A total of 15 patients (78.9%) reported treatment-emergent AEs (9 during carbamazepine monotherapy and 15 during carbamazepine + zonisamide); nine patients (47.4%) reported AEs considered by the investigator to be related to study drugs. The most frequently reported AEs during carbamazepine monotherapy prior to Day 1 (pre-zonisamide period) were headache (15.8% of patients) and rhinitis (10.5%). The most frequently reported AEs during combined carbamazepine and zonisamide were

Table 4
Steady-state PK parameters of total and free carbamazepine and CBZ-E (normalized to 400 mg dose) given alone (Day −7 and −1) and with ZNS (Day 35)

	Day −7	Day −1	Day 35
Total carbamazepine			
$AUC_{0–12\,h}$ (μg h/mL)	109.9 (66.0)	107.8 (66.6)	107.1 (48.2)
C_{max} (μg/mL)	10.3 (6.2)	10.2 (6.5)	10.8 (6.1)
C_{min} (μg/mL)	8.3 (4.8)	8.0 (4.9)	7.3 (3.9)
CL/F (mL/h)	4626.9 (2255.4)	4704.8 (2134.8)	4470.9 (1908.9)
Total CBZ-E			
$AUC_{0–12\,h}$ (μg h/mL)	13.9 (6.1)	14.3 (7.9)	16.3 (6.5)
C_{max} (μg/mL)	1.3 (0.6)	1.4 (0.8)	1.8 (1.4)
T_{max} (hr)	5.7 (3.4)	4.6 (3.7)	5.0 (3.6)
Free carbamazepine			
$AUC_{0–12\,h}$ (μg h/mL)	34.1 (22.6)	33.3 (23.3)	32.6 (16.7)
C_{max} (μg/mL)	3.2 (2.0)	3.1 (2.2)	3.3 (2.0)
C_{min} (μg/mL)	2.5 (1.6)	2.4 (1.6)	2.2 (1.3)
CL/F (mL/h)	14989.5 (6937.1)	15460.0 (6771.1)	15062.2 (6405.5)
Free CBZ-E			
$AUC_{0–12\,h}$ (μg h/mL)	8.3 (3.6)	8.4 (4.9)	9.2 (4.1)
C_{max} (μg/mL)	0.8 (0.3)	0.8 (0.4)	1.0 (0.9)
T_{max} (h)	6.4 (3.6)	4.4 (3.5)	5.7 (3.5)

Results expressed as means (S.D.), $N = 16$.

dyspepsia (36.8% of patients), nausea and tiredness (26.3%), and somnolence (21.1%). Most AEs were mild or moderate in severity except for two AEs that were reported as severe (anxiety and depression, the latter of which was a serious adverse event) in one patient; however, these events were assessed by the investigator as not related to the study drugs.

4. Discussion

4.1. Zonisamide spectrum of inhibition in human liver microsomes

In human liver microsomes from three different livers, zonisamide, at concentrations up to 1000 μM (the highest concentration tested), did not appear to

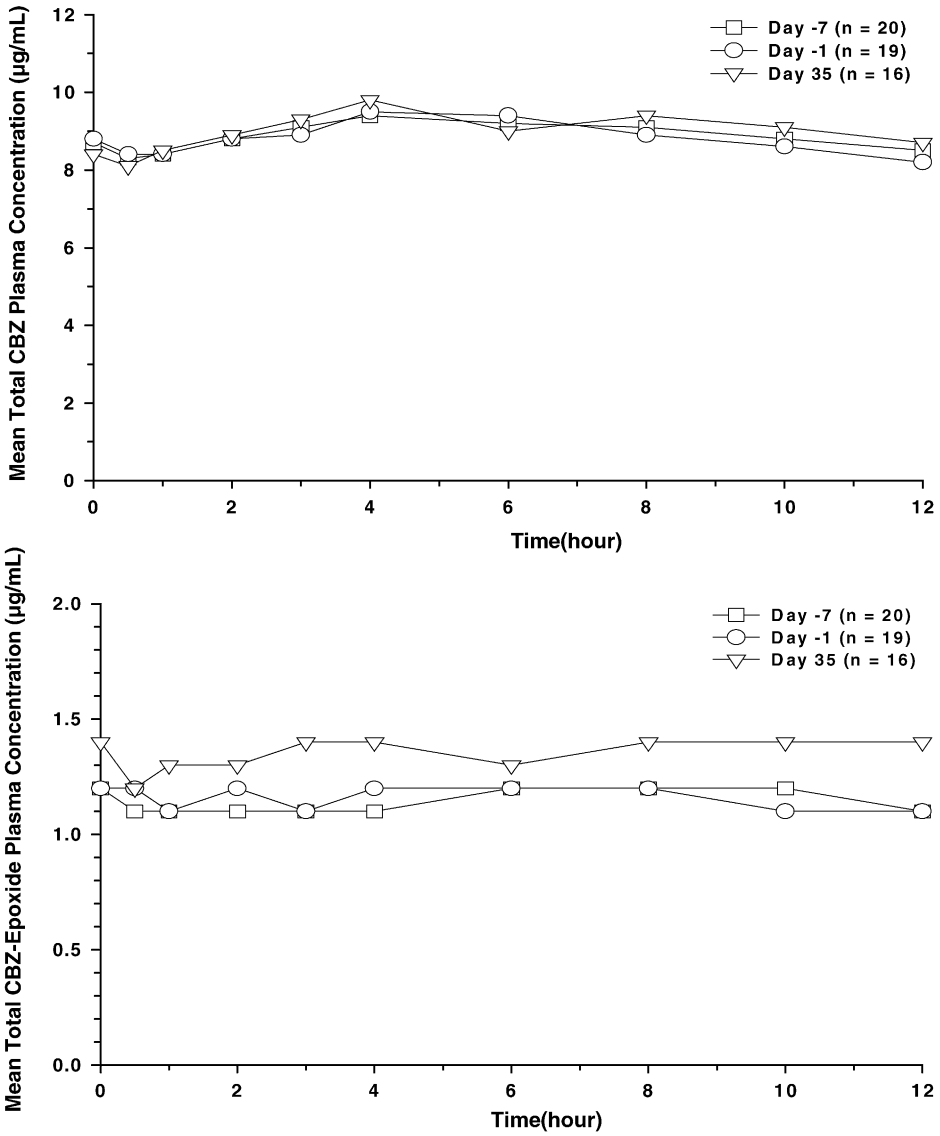

Fig. 1. Upper panel – Mean total carbamazepine plasma concentration–time profiles (dose-normalized to 400 mg a.m.) before (Day −7 and −1) and after treatment with zonisamide (Day 35). Lower panel – Mean total carbamazepine-epoxide plasma concentration–time profiles (dose normalized to 400 mg a.m. carbamazepine) before (Day −7 and −1) and after treatment with zonisamide (Day 35).

Table 5
Urinary pharmacokinetic parameters of CBZ-E

Carbamazepine epoxide	Day −7	Day −1	Day 35
Ae (mg)	6.3 (4.9)	5.4 (3.9)	3.9 (2.6)
Fu (%)	1.4 (0.7)	1.4 (1.0)	1.0 (0.5)
CLr (mL/h)	410.4 (154.7)	366.1 (124.7)	242.0 (113.8)

Results are expressed as means (S.D.), $N = 16$.

inhibit CYP1A2, CYP2D6, or CYP3A4 activities by more than 25%. The in vitro activities of isoforms CYP2A6, CYP2C9, CYP2C19 and CYP2E1 were reduced in the presence of zonisamide in an apparently concentration-dependent manner. Nevertheless, a reduction of >25% in the enzymatic activities was observed only at concentrations more than four to six times the concentrations of zonisamide achieved therapeutically [C_{max} of 159 ± 51 μM after a single administration of 8 mg/kg/day of zonisamide (Hosada et al., 1994). The zonisamide K_i for the inhibition of CYP3A4 was estimated at 1076 μM, a value corresponding to six times the zonisamide C_{max} value (Hosada et al., 1994). Finally, using three livers with varying amounts of CYP3A4, zonisamide K_m was estimated at 312 ± 16 μM, a value consistent with that reported previously in human liver microsomes, 274 μM (Nakasa et al., 1998). The large difference between the estimated values of K_m and K_i may be due to the fact that K_m is related to CYP3A4-mediated reductive cleavage of zonisamide to SMAP whereas K_i is related to inhibition of CYP3A4-mediated oxidative metabolism of other CYP3A4 substrates. Alternatively, the difference could be due to the presence of other pathways of zonisamide metabolism catalyzed by CYP3A4.

Recent studies indicate that in addition to CYP3A4 and CYP2C8 (Kerr et al., 1994; Pelkonen et al., 2001), other enzymes (CYP2B6, CYP2E1, CYP1A2 and CYP2A6) are involved in carbamazepine metabolism (Pearce et al., 2002). Based on the zonisamide CYP3A4 K_i value determined in this study and its weak inhibitory effect toward CYP2A6 and CYP2E1, it is unlikely that zonisamide at therapeutic concentrations would inhibit carbamazepine metabolism. The effect of zonisamide on CYP2C8 and CYP2B6 was not evaluated, but any possible effect of zonisamide inhibition of these enzymes would likely not be detectable in vivo.

4.2. Effect of zonisamide on the pharmacokinetics of carbamazepine in vivo

In epileptic patients treated with carbamazepine, steady-state zonisamide at a dose up to 200 mg BID, did not statistically significantly affect the pharmacokinetics of either carbamazepine or its main active metabolite, CBZ-E. These results are consistent with a series of earlier clinical studies showing no effect of zonisamide on carbamazepine disposition in epileptic patients (Browne et al., 1986; Miura, 2000; Schmidt et al., 1993).

Although the observed small apparent increase in $AUC_{0–12}$ of CBZ-E and the associated small apparent decrease in the amount of CBZ-E excreted in urine (Ae) during zonisamide treatment were not statistically significant, the calculated renal clearance was significantly decreased ($p < 0.05$). This effect of zonisamide on CBZ-E renal clearance could perhaps explain the small increase in the CBZ-E/carbamazepine ratio observed in other studies (Minami et al., 1994). However, CBZ-E renal clearance is relatively low, with CBZ-E eliminated primarily by hydrolysis (up to 80%) to the corresponding dihydrodiol, while only a small fraction (<10%) is excreted unchanged renally (Kerr and Levy, 1989). The impact of the decrease in CBZ-E renal clearance on CBZ-E plasma levels will depend on the significance of the renal excretion pathway in different patient populations, but should be minimal in most clinical situations.

4.3. Effect of carbamazepine on the pharmacokinetics of zonisamide in vivo

During zonisamide monotherapy, or when given in the absence of concomitant medications known to induce CYP3A4 metabolism, zonisamide has a reported elimination half-life and oral clearance of about 65 h and 650 mL/h, respectively (Kochak et al., 1998).

The half-life of 36.3 h and the apparent oral clearance of 976 mL/h found in this study are consistent with previous results showing induction of zonisamide metabolism when it is co-administered with the known CYP3A4 inducer, carbamazepine (Hashimoto et al., 1994; Ojemann et al., 1986; Shinoda et al., 1996).

5. Conclusion

In human liver microsomes, zonisamide at therapeutic concentrations did not behave as an inhibitor of evaluated cytochrome P450 isozymes, including CYP3A4, the main isozyme involved in carbamazepine metabolism. In epileptic patients, zonisamide had no significant effect on plasma levels of carbamazepine and CBZ-E. Based on these findings, it appears that adjustment of carbamazepine dosing is not required with concomitant zonisamide administration.

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