Transient Steady-State Analysis: Application in the Determination of the Relative Formation and Elimination Clearances of Two Major Carbamazepine Metabolites in Humans

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INTRODUCTION

Carbamazepine (5-carbamyl-5H-dibenzo[b,f]azepine, CBZ), an anticonvulsant, is used in the treatment of epilepsy (1) and is the drug of choice for trigeminal neuralgia (2). Several metabolites of CBZ have been identified (3), the most important being carbamazepine-10,11-epoxide (CBZE), a pharmacologically active metabolite that is equipotent to CBZ in animal models of epilepsy (4). CBZE is converted almost completely to *trans*-10,11-dihydroxy-10,11-dihydrocarbamazepine (CBZD), which is excreted in the urine mainly in the unconjugated form (5).

During maintenance therapy, CBZ has been reported to induce its own metabolism, a phenomenon characterized by an increase in clearance with time (6,7). Since one of its metabolic routes involves the formation of an active metabolite, examining the contribution of the epoxide–diol pathway to the overall clearance of CBZ becomes particularly important.

This study in 10 healthy volunteers focuses on the estimation of the relative formation and elimination clearances of CBZE and CBZD using two approaches: (a) transient steady-state (concentration-ratio) analysis at maximum concentration of the metabolite, which is compared to (b) noncompartmental (area-ratio) method. Both methods were validated under single-dose and multiple-dose conditions.

MATERIALS AND METHODS

Study Design and Clinical Procedure

Ten healthy male volunteers, with mean ± SD ages and weights of 26.5 ± 4.1 years and 77.5 ± 9.5 kg, respectively, received a 200-mg single oral dose of CBZ as Tegretol chewable tablets (CT). This comprised the single-dose (baseline) phase. Blood samples were collected predose and at 2, 4, 6, 8, 10, 12, 14, 16, 20, 24, 29, 34, 39, 48, 72, 96, and 168 hr postdose for the 200-mg single oral dose. A week later they were randomly assigned, in crossover, to either Treatment A, 4 × 100-mg chewable Tegretol tablets once a day; or Treatment B, ½ × 100-mg chewable Tegretol (50 mg) every hr for 4 doses twice daily. The total daily dose for both treatments was 400 mg, and dosing continued for 21 days for each leg. Morning predose blood samples were obtained on days 18 through 20 of the first leg and on days 46 through 48 of the second leg, to confirm steady-state conditions. During the last dosing interval of the multiple-dosing phase, blood samples were collected predose and every 2 hr for 24 hr, followed by samples drawn at 29, 34, 39, 48, 72, 96, and 168 hr postdose. Plasma was harvested and maintained at −20°C until analysis.

Analytical Procedure

Plasma concentrations of CBZ, CBZE, and CBZD were determined using a sensitive microbore HPLC method developed in our laboratory (8) with the following modifications. The volume of plasma samples analyzed was 0.5 mL and the volumes of 0.2 *M* phosphate buffer (pH 11.2) and 5% t-butyl alcohol in chloroform were scaled to 1 and 10 mL, respectively. The concentration ranges of the standard curves in the single-dose phase were 0.02 to 5.0 μg/mL for CBZ, 0.01 to 1.0 μg/mL for CBZE, and 0.01 to 2.0 μg/mL for CBZD. For the multiple-dose phase, the standard curves ranged from 0.50 to 12.0 μg/mL for CBZ and 0.20 to 8.0 μg/mL for both CBZE and CBZD.

Data Analysis

Plasma data for CBZ, CBZE, and CBZD from the single-dose phase and Treatment A of the multiple-dose phase were analyzed using the model shown in Fig. 1. The clearance of CBZ through the epoxide–diol route was isolated from all other pathways. Therefore, f_m is the fraction of an absorbed dose of CBZ eliminated via epoxidation; CL is the total clearance of CBZ; f_mCL is therefore the metabolic clearance of CBZ via the epoxide–diol route and is equal to the formation clearance of CBZE; CL_m is the total clearance of CBZE and is equal to the formation clearance of CBZD; CL_d is the total clearance of CBZD; C_Z, C_E, and C_D are the plasma concentrations of CBZ, CBZE, and CBZD; and V_E and V_D are the volumes of distribution of CBZE and CBZD.

The rates of change of amounts of CBZE and CBZD in the body are expressed by the following differential equations:

$$V_E dC_E/dt = f_m CL \cdot C_Z - CL_m \cdot C_E \quad (1)$$

$$V_D dC_D/dt = CL_m \cdot C_E - CL_d \cdot C_D \quad (2)$$

The relative formation and elimination clearances of CBZE and CBZD are calculated using two methods of analysis.

Transient Steady-State Analysis

This approach assumes a one-compartment model for

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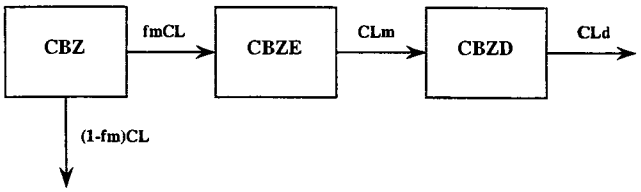

Fig. 1. Diagram representing the pharmacokinetic model.

the metabolites under study. When a metabolite plasma concentration is at maximum, a transient steady state occurs where the rate of change in concentration is equal to zero. Therefore, Eqs. (1) and (2) yield

$$\{C_E/C_Z\}t_{me} = \frac{f_m \cdot CL}{CL_m} \quad (3)$$

$$\{C_D/C_E\}t_{md} = \frac{CL_m}{CL_d} \quad (4)$$

Here t_{me} is the time to peak for CBZE levels and t_{md} is that for CBZD levels, obtained directly from the plasma concentration–time data. Therefore, the relative clearances are given by a concentration ratio of the metabolite to its precursor, at the metabolite's time to peak (9).

Noncompartmental Analysis

This method assumes that concentration-independent (linear) kinetics of CBZ and its metabolites are obeyed. Equations (1) and (2) are integrated from 0 to τ, where $\tau = \infty$ in the single-dose phase and τ = 24 hr for the multiple-dose phase. The following relative clearances are then calculated:

$$\frac{AUC_e}{AUC_z} = \frac{f_m CL}{CL_m} \quad (5)$$

$$\frac{AUC_d}{AUC_e} = \frac{CL_m}{CL_d} \quad (6)$$

AUC_z, AUC_e, and AUC_d are the areas under the plasma concentration–time curves for CBZ, CBZE, and unconjugated CBZD calculated using linear trapezoidal integration (10). The area ratios therefore provide estimates of the relative clearances of the metabolites.

Agreement between the concentration ratio and the corresponding area ratios as estimators of the relative formation and elimination clearance of CBZE [Eqs. (3) and (5)] and CBZD [Eqs. (4) and (6)] is examined for both the single- and the multiple-dose phases, using orthogonal regression analysis.

RESULTS

The concentration–time profiles for CBZ, CBZE, and unconjugated CBZD in plasma of the 10 subjects, following a 200-mg single oral dose, showed rapid absorption of the parent drug, followed by a slow elimination characterized by an average half-life of approximately 40 hr. Times to peak for CBZE ranged from 16 to 39 hr. Plasma concentrations at 168 hr were still measurable in the majority of the subjects, averaging 0.013 mg/L. These relatively late times to peak, as well as those for CBZD (range, 29 to 72 hr) reflect the long half-life of the parent drug under baseline conditions.

Attainment of steady state during the multiple-dosing phase was examined by comparing predose plasma levels of CBZ, CBZE, and unconjugated CBZD for both treatments using analysis of variance. Analysis of the predose levels for CBZ, CBZE, and unconjugated CBZD, on days 18–20 of the first leg and days 46–48 of the second leg of the induction phase, showed no significant differences ($P > 0.05$), indicating that steady state was achieved.

Although noncompartmental analysis was possible for both Treatment A and Treatment B data of the multiple-dose phase, the flat concentration–time profile for all three compounds in the Treatment B phase made the assignment of times to peak difficult. Thus, concentration ratios (transient steady-state analysis) as well as area ratios (noncompartmental analysis) were calculated for the single-dose and Treatment A data of the multiple-dose phase. The results are shown in Table I. Figures 2 and 3 portray the agreement of the results of the concentration- and area-ratio analyses for CBZE and CBZD, respectively. Both the single- and the multiple-dose data are plotted on the same graph. The agreement between methods was examined by orthogonal regression. The equations representing the regression lines are

$$\{C_E/C_Z\}t_{me} = 0.985 \frac{AUC_e}{AUC_z} + 0.0035 \qquad (r^2 = 0.93)$$

$$\{C_D/C_E\}t_{md} = 0.871 \frac{AUC_d}{AUC_e} + 0.071 \qquad (r^2 = 0.87)$$

Table I shows that there was no significant difference in both methods during either the single- or the multiple-dose phases for CBZE and CBZD (Student paired t test, $P > 0.05$). The means (SD) of the relative clearances of CBZE as well as CBZD were compared for the induced and baseline phases. These ratios were significantly higher in the induced phase, for both CBZE and CBZD, than the corresponding baseline values (Student paired t test, $P < 0.05$).

DISCUSSION

The single-dose phase of the study furnished the data used to examine the baseline (noninduced) clearances of CBZ and its metabolites. In addition, the multiple-dose phase provided data representing the induced clearances of

Table I. Summary of the Relative Formation and Elimination Clearances of CBZE and CBZD Using Transient Steady-State and Noncompartmental Analyses

	Conc. ratio	Area ratio
	CBZE/CBZ	
Single dose (baseline)	0.0671 (0.0095)*,**	0.0645 (0.011)*,**
Multiple dose (induced)	0.132 (0.018)*,**	0.130 (0.020)*,**
	CBZD/CBZE	
Single dose (baseline)	1.13 (0.14)*,**	1.23 (0.24)*,**
Multiple dose (induced)	1.81 (0.29)*,**	1.89 (0.32)*,**

* Concentration and area ratios not significantly different from one another (Student paired t test, $P > 0.05$).

** The induced ratios significantly different from the corresponding baseline ratios (Student paired t test, $P < 0.05$).

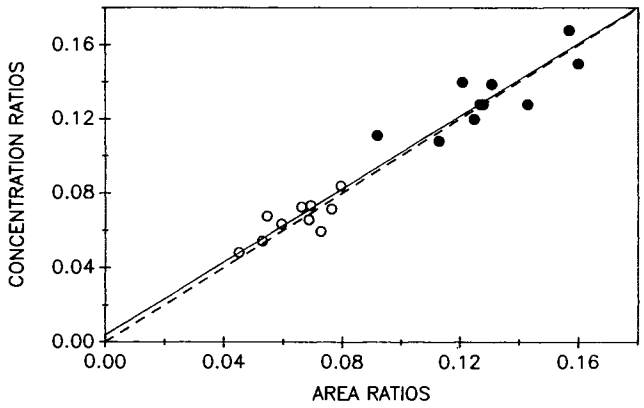

Fig. 2. Agreement between the transient steady-state (concentration-ratio) and the noncompartmental (area-ratio) analyses on the relative formation and elimination clearances of CBZE. (○) Single-dose (baseline) phase; (●) multiple-dose (induced) phase. (——) Orthogonal regression line; (---) line of identity.

CBZ and CBZE. Data from both phases were analyzed using two different methods.

The transient steady-state analysis involves a calculation of the plasma concentration ratio of CBZE to CBZ at the time of maximum concentration of CBZE (t_{me}) as well as the ratio of plasma concentrations of CBZD to CBZE at the time of maximum concentration of CBZD (t_{md}). This analysis assumes a one-compartment model for CBZE and CBZD. The noncompartmental analysis, however, assumes only that concentration-independent (linear) kinetics of CBZ and its metabolites are obeyed. Linear trapezoidal integration was used here to calculate areas under the plasma concentration–time profiles of CBZ, CBZE, and CBZD.

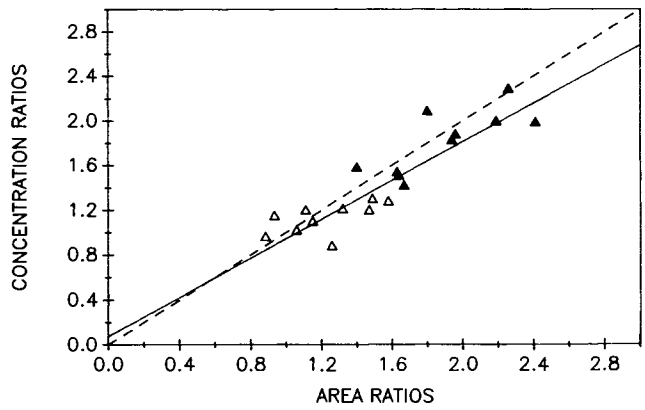

Fig. 3. Agreement between the transient steady-state (concentration-ratio) and the noncompartmental (area-ratio) analyses of the relative formation and elimination clearances of CBZD. (△) Single-dose (baseline) phase; (▲) multiple-dose (induced) phase. (——) Orthogonal regression line; (---) line of identity.

Figures 2 and 3 show the agreement of both approaches for CBZE relative to CBZ (Fig. 2) and CBZD relative to CBZE (Fig. 3). This agreement provides support for the assumption of a one-compartment model for CBZE and CBZD in the transient steady state approach. The data in Table I show that there was no significant difference between methods during either the baseline or the induced phases of the study. Also, a comparison of the relative formation and elimination clearances of CBZE and CBZD before and during induction suggests that not only is the epoxidation of CBZ induced, but also the hydrolysis of the epoxide to the *trans*-diol.

In conclusion, where the one-compartment model can be assumed for metabolites, information concerning the relative formation and elimination clearances of these metabolites may be obtained by two methods of analyses: the transient steady-state (concentration-ratio) method and the noncompartmental (area-ratio) technique. The former may be particularly useful where it is not possible to characterize the total area under the curve for a long-half-lived metabolite.

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