Determination of the Relative Formation and Elimination Clearance of Two Major Carbamazepine Metabolites in Humans: A Comparison Between Traditional and Pooled Sample Analysis

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INTRODUCTION

Carbamazepine (5-carbamyl-5H-dibenzo[*b,f*]azepine, Tegretol, CBZ) is an anticonvulsant used in the treatment of epilepsy and trigeminal neuralgia (1,2). It is extensively metabolized (3,4), a major biotransformation pathway being epoxidation of the double bond at the 10,11 position followed by hydration to the *trans*-10,11-dihydroxy-10,11-dihydrocarbamazepine (CBZD) which is excreted in the urine, mainly in the unconjugated form (5). This route is especially important, as carbamazepine-10,11-epoxide (CBZE) is pharmacologically equipotent to CBZ in animal models of epilepsy (4,6).

Interindividual differences in drug metabolism play an important role in affecting therapeutic response. Since one metabolic pathway of CBZ involves the formation of an active metablite, the contribution of this pathway to the overall clearance becomes particularly important. In addition, little information concerning the formation clearances of carbamazepine metabolites in the noninduced state in humans exists, making comparisons with the corresponding values in induced patients difficult. This study in normal volunteers focuses on the estimation of (a) the average plasma concentrations and areas-under-the curve for CBZ, CBZE, and CBZD over a dosing interval and (b) the baseline or noninduced relative formation and elimination clearance of CBZE and CBZD.

MATERIALS AND METHODS

Study Design and Clinical Procedure

Ten normal healthy male volunteers, average age 38.9 years and average weight 76.9 kg, received 400-mg single oral doses of carbamazepine in a 2 × 2 balanced crossover bioequivalency study which utilized Tegretol as the reference formulation. Blood samples (n = 14) were collected before medication and at 1, 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 168, and 216 hr postdose. Heparinized plasma was harvested, frozen immediately, and stored at −20°C until analysis.

Analytical Procedure

In the first phase of sample analysis, plasma samples from all 10 subjects were analyzed for CBZ and CBZE. The analytical procedure allows for simultaneous quantitation of the drug and its metabolite by HPLC (7).

The second phase of the analysis was undertaken following modification of the analytical method to allow for the analysis of CBZD. This procedure utilizes microbore HPLC and has been validated for accuracy and precision (8). Because of a limitation in sample volume, plasma samples from only 3 of the 10 subjects were analyzed for CBZ, CBZE, and CBZD simultaneously.

The third phase involved the analysis of plasma from all 10 subjects, with samples from each subject pooled into a single aliquot for analysis. Each aliquot represents a combination of specified volumes of all the plasma samples (n = 14) from a given study subject. The concentrations of drug and metabolites in this single sample reflect the corresponding time-averaged concentrations of CBZ, CBZE, and CBZD in the original set of samples, which were taken over a 216-hr period.

Pooled-Sample Technique

The theoretical basis of the pooled-sample technique has been reported elsewhere (9). Briefly, the volumes of the samples comprising the pooled plasma sample are selected to represent the corresponding sampling intervals. Areas under the curve over the sampling period can therefore be obtained when the concentration of drug in the pooled sample is multiplied by the overall time interval, e.g., 216 hr. An example of the calculation of the volumes of each sample to be used in the total sample pool is provided in the Appendix.

Data Analysis

Agreement Between Methods. Corresponding areas under the curve for CBZ and CBZE were calculated and compared for all 10 subjects using linear trapezoidal integration (10) and the pooled-sample technique. Also, both integrals were compared for 3 of the 10 subjects whose CBZD plasma concentration–time profiles were also determined prior to pooling. Orthogonal regression was implemented to examine the agreement between methods.

Determination of Relative Clearances. To calculate the formation and elimination clearances for CBZE and CBZD, the clearance of CBZ through the epoxide pathway (fmCl) is isolated from the sum of all other clearance pathways (Clo) as shown in Fig. 1. The relative formation and elimination

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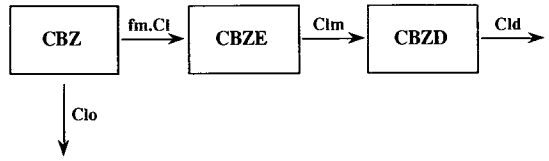

Fig. 1. A diagram representing the pharmacokinetic model: fmCL is the clearance of CBZ through the epoxide pathway, Clo is the sum of all other clearance pathways for CBZ, Clm is the elimination clearance of CBZE, and Cld is the elimination clearance of CBZD.

clearances were calculated for CBZE [Eq. (1)] and for CBZD [Eq. (2)] as quotients of areas under the curve. Therefore,

$$AUC(CBZE)/AUC(CBZ) = fmCl/Clm \quad (1)$$

and

$$AUC(CBZD)/AUC(CBZE) = Clm/Cld \quad (2)$$

where fm is the fraction of CBZ cleared through epoxide formation; Cl is the total elimination clearance of CBZ; fmCl is therefore the formation clearance of CBZE; Clm is the elimination clearance of CBZE (equal to the formation clearance of CBZD, where it is assumeed (5,11) that all CBZE is converted to the *trans*-dihydrodiol); and Cld is the elimination clearance of CBZD.

RESULTS

Figures 2A and B portray the agreement between the results of the sample-pooling and trapezoidal integration techniques for CBZ and CBZE, respectively. Orthogonal regression was used to examine the agreement between the areas under the curve obtained using the pooled method and the trapezoidal rule. The equations for the regression lines are given by

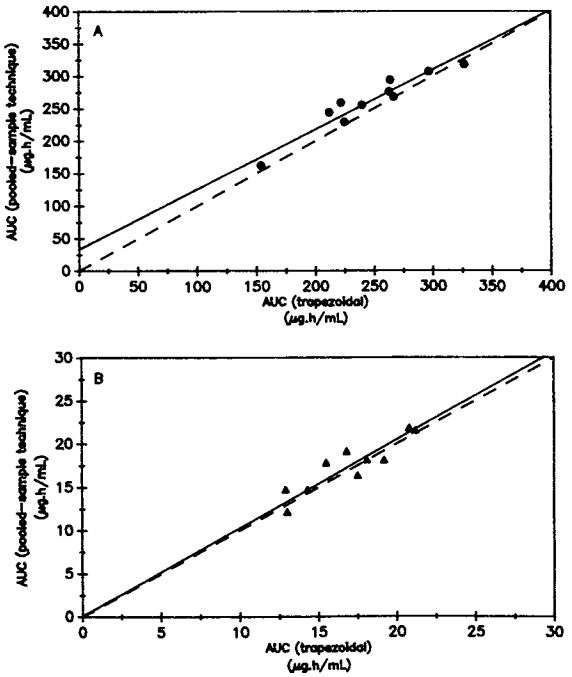

Fig. 2. Agreement between the results of the sample-pooling and trapezoidal integration techniques: (A) CBZ and (B) CBZE. (——) Orthogonal regression line; (---) line of identity.

$$AUC(CBZ)_{pool} = 0.922\ AUC(CBZ)_{trap} + 33.4 \quad (r^2 = 0.91)$$

$$AUC(CBZE)_{pool} = 1.02\ AUC(CBZE)_{trap} + 0.104 \quad (r^2 = 0.81)$$

To estimate the relative formation and elimination clearances of CBZE and CBZD, the corresponding quotients of the areas under the curve were compared using Eqs. (1) and (2), respectively. These results are given in Table I.

DISCUSSION

In this work, areas under the curve for CBZ, CBZE, and CBZD were calculated and compared using both linear trapezoidal integration and pooled-sample techniques. A major advantage of the latter is that it requires the analysis of only one pooled sample to characterize a time-averaged concentration or an AUC over a specified time interval. This advantage would be significant where the analysis of samples is particularly labor intensive. This approach is also applicable when it is difficult to monitor accurately low concentrations of drug and/or metabolite because of limitations in assay sensitivity. The analysis of a pooled sample with a total volume of 5 to 10 ml of blood or plasma rather than a series of samples with volumes of 1 ml or less may thus be considered, allowing a larger amount of drug and/or metabolite to be carried through a single analysis. The pooled-sample technique would also minimize the difficulty encountered with assays which exhibit nonlinear standard curves, as the average concentration of the pooled sample would fall into a narrower range than the individual samples.

The sample-pooling method therefore has potential application in therapeutic drug monitoring, bioavailability studies, and determining individual drug clearances (9). It has been used in quantitating low concentrations of cyclosporine metabolites in blood (12) and as a means of reducing the volume of blood samples harvested in children (13). Although the pooled-sample technique fails to characterize the shape of the plasma concentration–time profile, it provides an estimate of the average plasma level over a fixed time interval, and this may be useful in defining therapeutic ranges for drug monitoring.

In single-dose studies, since the AUC used for clearance (or bioequivalence) calculations results from a single determination of the average concentration in the pooled sample, sampling over a period long enough to include virtually all of the AUC is required. At steady state, however, using a pooled sample consisting of samples drawn over a dosing interval would furnish enough data for clearance (or bioequivalence) estimates.

Table I. Summary of the Relative Clearances for CBZE and CBZD

	Trapezoidal integration (mean ± SD)	Pooling technique (mean ± SD)
fmCl/Clm	0.0698 ± 0.0123* (n = 10)	0.0677 ± 0.0115* (n = 10)
Clm/Cld	1.20 ± 0.035* (n = 3)	1.13 ± 0.150* (n = 3)
Clm/Cld		1.12 ± 0.223 (n = 10)

* No significant difference between methods (paired t test, $P > 0.05$).

In the present study, where sampling occurred over a 216-hr period, the contribution of the terminal AUC was negligible. The close agreement observed between both methods lends support to the estimation of the relative formation and elimination clearances of CBZ metabolites based upon the ratio of their AUCs or time-averaged concentrations.

In conclusion, the results of this study provide support for the use of a pooled-sample technique in determining mean plasma levels to estimate the area under the curve over a specified time interval. Relative clearances associated with the disposition of carbamazepine and its metabolites calculated with this technique compare closely with those determined by trapezoidal integration.

APPENDIX

Table AI. An Example of the Calculation of the Volumes of Each Sample to Be Used in the Total Sample Pool

Sample No.	Sampling time (hr)	Δt (hr)	Sample volume (μl)	Total (μl)
1	0	1	5	5
2	1	1	5 + 5	10
3	2	2	5 + 10	15
4	4	2	10 + 10	20
5	6	2	10 + 10	20
6	8	4	10 + 20	30
7	12	12	20 + 60	80
8	24	8	60 + 40	100
9	32	16	40 + 80	120
10	48	24	80 + 120	200
11	72	24	120 + 120	240
12	96	72	120 + 360	480
13	168	48	360 + 240	600
14	216		240	240
			Pooled Sample Volume = 2160 μl	

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