The Relative Bioavailability and *In Vivo-In Vitro* Correlations for Four Marketed Carbamazepine Tablets

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Purpose. To determine if three marketed generic carbamazepine tablets were bioequivalent to the innovator formulation, as well as to each other. In addition, to examine *in vivo-in vitro* relationships among the four formulations.

Methods. Each formulation was given as a single dose to 18 healthy male and female subjects using a crossover design. Blood samples were collected for 169 hr. Carbamazepine was assayed by HPLC with UV detection.

Results. *In vivo* fraction absorbed plots indicated that the three generic formulations were absorbed more rapidly than the innovator product, and the mean time of maximum plasma concentration was 6–7 hr sooner for the generic formulations. The mean maximum plasma concentration ranged from 17–19 percent higher for the generic products compared to the innovator, and the 90% confidence limits for Cmax data ranged from 111% to 126%. The mean AUC(0−∞) for the generic products ranged from 101–104% compared to the innovator, and the confidence limits for AUC ranged from 97–108%.

Conclusions. The generic products were all more rapidly absorbed than the innovator, but simulations of steady-state concentrations indicated that it would be unlikely that these differences would have any significant clinical effect. An excellent association was seen between the Cmax and the percent of drug dissolved *in vitro*. The correlation was used to accurately predict the Cmax of four other 200 mg tablets evaluated in an earlier study.

KEY WORDS: carbamazepine; human; bioavailability: gender, dissolution.

INTRODUCTION

An earlier study (1) showed that three different lots of a marketed generic carbamazepine 200 mg tablet were not bioequivalent to the innovator formulation, nor were they bioequivalent to each other. Bioinequivalence of the products was due to post-approval, manufacturer initiated changes that had not been approved by the U.S. Food and Drug Administration. All FDA approved generic carbamazepine tablet products are required to demonstrate bioequivalence to the innovator tablet in a single-dose study conducted in healthy human volunteers. However, because the innovator product was being reformulated while the generic products were being developed, all of the generic products have not been compared to the same formulation of the innovator product. The new innovator formulation has been shown to be approximately 6% more bioavailable than the old formulation in terms of AUC (2). Accordingly, the interchangeability of generic products with each other should be considered. Even though each FDA approved generic product is tested versus the innovator, a direct comparison of different generic products is not required by the FDA. The present study compared three generic carbamazepine tablet formulations to each other, as well as to the innovator product.

METHODS

Subjects

Twenty healthy, non-smoking human subjects, 16 males and 4 females, were enrolled in the study. They ranged in age from 22–36 yr and weighed 50–98 kg. All subjects had normal clinical chemistry laboratory values, including reticulocyte count and serum iron. The research followed the tenets of the Declaration of Helsinki promulgated in 1964, and was approved by the University Institutional Review Board and the Risk Involving Human Subject Committee of the FDA. All subjects provided written informed consent.

Carbamazepine Products

Three marketed lots of 200 mg generic carbamazepine tablets and one marketed lot of the innovator product were obtained through a local hospital pharmacy: Innovator product—Geigy, Lot 1T151342, Exp. 9/97 (Product 1); Inwood, Lot 2L023, Exp. 12/94 (Product 2); Sidmak, Lot 1122961, Exp. 1/95 (Product 3); and Purepac, Lot 019B3, Exp. 2/95 (Product 4).

Clinical Protocol

The subjects did not ingest any drugs for 21 days and avoided alcohol for 48 hr prior to each dose of carbamazepine. The twenty subjects were randomly divided into four groups of 5 subjects each. Subjects received the four products in the following four sequences: Sequence 1—Products 1, 2, 4, 3 (Subjects 1–5); Sequence 2—Products 2, 3, 1, 4 (Subjects 6–10); Sequence 3—Products 3, 4, 2, 1 (Subjects 11–15); Sequence 4—Products 4, 1, 3, 2 (Subjects 16–20). After an overnight fast, each subject received a single 200 mg tablet along with 240 ml water at room temperature. No food was permitted until a standard meal was served 4 hr after dosing. Ten milliliter blood samples were obtained through a heparin lock or direct venipuncture just before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 25, 49, 73, 97, 121 and 169 hr after dosing. The blood was centrifuged and the plasma fraction stored at −20°C until assayed. A 21-day washout period was used between doses.

Plasma Assay

Carbamazepine (Sigma Chemical, Inc.) plasma concentrations were assayed using a modification of the HPLC method with UV detection based on the work of Riad and Sawchuck (3). A 0.5 ml plasma sample was adjusted to pH 11 and extracted with 10 ml of 1.5% isoamyl alcohol in chloroform. Cyheptamide

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(Alltech/Applied Science, Inc.) was employed as the internal standard. Samples of 10,11-epoxide carbamazepine (Alltech/Applied Science, Inc.) were also chromatographed to test for potential interference from this metabolite. For each set of subjects samples a calibration curve was prepared consisting of six standard concentrations ranging from 0.05 to 4.03 ug/ml. These calibrators were extracted and assayed in duplicate. In addition each analytical run contained high, medium and low controls which were extracted and assayed in quadruplicate. Calibration curves were linear over the assay range and controls were within 95% nominal with CV's ranging from 2% for the high control to 13% for the low control. Recovery of carbamazepine and internal standard was 91% and 82%, respectively. The limit of quantitation was 0.05 ug/ml. No stability problem was found for carbamazepine in plasma stored at −20°C for 90 days or in plasma extracts stored at ambient temperature for 20 hr.

Data Analysis

The area under the plasma concentration-time curve to 169 hr (AUC (0–169)) was determined by the trapezoidal rule, and the AUC (0–∞) was determined by the sum of the AUC (0–169) and the last log-linear concentration divided by the terminal disposition rate constant (λ) obtained from a least-squares analysis of the terminal log-linear concentration-time data (4). The fraction absorbed calculations employed the Wagner-Nelson method (5), applied to the individual carbamazepine plasma concentration-time data. The time of maximum plasma concentration (Tmax) and maximum plasma concentration (Cmax) were determined by inspection. The statistical analysis employed a SAS program (SAS Institute Inc., Cary, NC) with a general linear model for treatment, period, sequence and subject (sequence) effects.

Steady-state simulations for 200 mg doses given every 12 hr were generated by superposition (6), using simulated single-dose data with an elimination half life of 40 hr or 12 hr. The latter value is based on reported half lives of 12–17 hr after autoinduction in patients receiving chronic therapy with carbamazepine (7). The simulated single-dose data were obtained using Stella II software (High Performance Systems, Hanover, NH). The mean plasma carbamazepine concentration-time profiles for Products 1 and 4 were first deconvoluted to obtain fraction absorbed (FA) versus time data. These FA data were then used as input rates for a one-compartment pharmacokinetic model with first-order elimination (T1/2 = 40 hr). The volume of distribution was fixed at 70 L and 74 L for Products 1 and 4 respectively. These values were selected to provide simulated plasma concentration-time profiles that best matched the experimental profiles, but they do not necessarily reflect actual volumes of distribution. The elimination half life was then reduced to 12 hr in the model, without changing the input rates and volumes of distribution, and the Stella II software was used to generate new single-dose profiles for Products 1 and 4. Finally superposition was used to simulate steady-state profiles for Products 1 and 4 with each half life.

Dissolution Testing

The *in vitro* dissolution testing employed the USP paddle method at 75 rpm with 900 ml of water containing 1% sodium lauryl sulfate (8). Six tablets of each product were tested, and samples of the dissolution media were removed at 5, 10, 15, 20, 30, 60, 90 and 120 min.

In Vivo-In Vitro Relationships

The relationship between the *in vitro* dissolution data and the *in vivo* pharmacokinetic data was examined by plotting the percent of drug dissolved (FD) after 30, 60 and 120 min versus the percent absorbed data (FA) at 30, 60 and 120 min after dosing. These data were also compared to a previously reported FA versus FD plots for a different lot of Product 1 (1). Plots were also constructed for the mean Cmax and the mean Tmax versus mean percent dissolved at 5, 10, 15, 20 and 30 min.

RESULTS AND DISCUSSION

Eighteen subjects successfully completed all four phases of the study. Two subjects, one male and one female, withdrew from the study after the second phase. The male subject experienced dizziness, nausea and vomiting for an extended period of time, beginning approximately 12 hr after the second dose. The dizziness did not completely subside for approximately two weeks. The female subject reported extreme fatigue following the second dose and decided to withdraw from the study. It was not readily apparent that these adverse reactions were drug related.

Statistical analysis was performed on the data from the 18 subjects who completed all four phases. Mean carbamazepine plasma concentrations through 49 hr are illustrated in Figure 1. Plasma carbamazepine concentrations were essentially identical among the four products from 49 to 169 hr. Mean concentrations at these sampling times ranged from 1.20–1.28 μg/ml at 49 hr and 0.16–0.17 μg/ml at 169 hr. Statistically significant differences ($p < 0.05$) were detected among the four products at every sampling time from 0.5 through 25 hr. Mean pharmacokinetic metrics are summarized in Table I. Relatively small intersubject

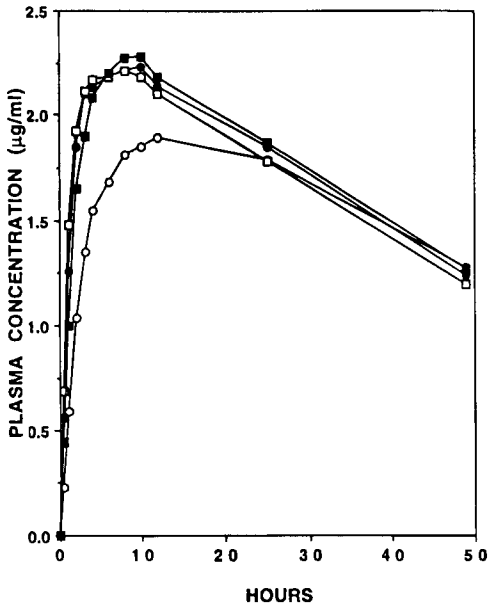

Fig. 1. Mean carbamazepine plasma concentrations. (○, Product 1; ●, Product 2; □, Product 3; ■, Product 4).

Table I. Mean Carbamazepine Pharmacokinetic Metrics[a]

Parameter	Product 1	Product 2	Product 3	Product 4
C_{max} (μg/ml)	1.95(16)	2.32(15)	2.30(21)	2.34(15)
T_{max} (hr)	13.8(45)	7.1(43)	7.1(42)	8.0(31)
AUC (0–169) (μg*hr/ml)	147.1(17)	153.0(18)	148.9(18)	152.8(16)
AUC (0–∞) (μg*hr/ml)	157.4(18)	163.3(19)	159.2(19)	162.9(17)
λ (hr-1)	0.0178(19)	0.0180(21)	0.0178(21)	0.0181(22)

[a] % Relative Standard Deviation, CV% in ( ).

variabilities were observed for each of the four products, with relative standard deviations (CV's) of 21% or less for Cmax and AUC. The ANOVA CV%, which includes true within-subject variability, within formulation variability, analytical variability and random variability, was also low: 9.8% for Cmax and 7.2% for AUC(0–∞). Confidence intervals (90%) around ratios (test/reference) of least squares means derived from log-transformed AUC and Cmax data are presented in Table II. The least-squares mean ratio of maximum plasma concentrations ranged from 1.17 to 1.19 for the generic products compared to the innovator product. The upper confidence limit for Cmax for Product 4 (126%) was slightly outside the current FDA acceptance criterion of 80– 125%. The least-square mean ratio for AUC(0–∞) for the generic products ranged from 1.01–1.04 compared to the innovator, and the confidence limits ranged from 97–108%, which were within the FDA's limit for bioequivalence. When the three generic formulations were compared, the confidence limits for Cmax and AUC(0–∞) were all well within the range of 80–125%, regardless of which generic was used as the reference. The mean time of maximum plasma concentration was also 6–7 hr sooner for the generic formulations ($p < 0.001$), suggesting more rapid absorption of the three generic products. Figure 2 illustrates a plot of mean percent absorbed and percent dissolved versus time for the four products. Dissolution rates during the first 30 min and in vivo absorption rates of each generic product exceeded those of the innovator ($p < 0.001$). There was no significant difference ($p > 0.05$) in the mean terminal dispositon rate constant (λ) among the four study phases indicating that, as expected, autoinduction did not occur.

Since the upper confidence limit for Cmax for Product 4 was 126%, simulations were conducted to determine how differences between this product and the reference might translate to differences during chronic administration. The single-dose carbamazepine concentration-time profiles predicted with the Stella II software were in excellent agreement with the observed experimental data. A plot of observed versus predicted plasma concentrations for Products 1 and 4 were linear (slope = 0.99, $r^2 > 0.99$). The maximum concentration in the mean plasma concentration-time curve for Products 1 and 4 differed by 17%. Simulations were then conducted using an assumed elimination half life of 12 hr and predicted a 23% difference in the maximum concentrations for Products 1 and 4 after a single-dose. When the single-dose data were projected to steady-state the difference in the maximum of the mean concentration-time data decreased to 8% and 9% for the 40 hr and 12 hr elimination half life simulations, respectively. Thus the Cmax differences seen among the four products after a single dose would be expected to decrease with multiple dosing, even if a reduction in the elimination half life due to autoinduction does occur.

This study, initiated by FDA, was designed to determine if three different generic carbamazepine formulations were bioequivalent to each other. The products were comparable, even though two different reference formulations had been employed in the bioequivalence studies conducted by the three firms. Thus concerns regarding the interchangeability of generic dosage forms were not warranted for these dosage forms.

The influence of gender was also examined. The mean Cmax and AUC(0–∞) were 25% and 14% higher, respectively for the four females ($p < 0.01$). However the mean apparent half life was 8 hr shorter for the females ($p < 0.01$). When

Table II. Statistical Analysis Ratio and 90% Confidence Limits (Two, One-Sided Test)

Parameter	Product comparison	LS mean ratio	90% Confidence interval
C_{max}	2 vrs 1	1.19	113% to 125%
	3 vrs 1	1.17	111% to 123%
	4 vrs 1	1.19	114% to 126%
AUC (0–∞)	2 vrs 1	1.04	100% to 108%
	3 vrs 1	1.01	97% to 105%
	4 vrs 1	1.03	99% to 108%

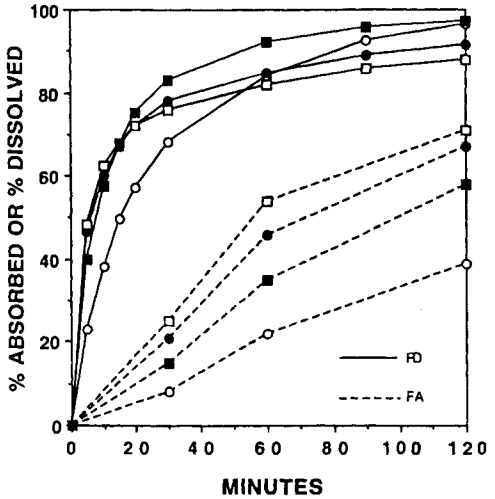

Fig. 2. Percent dissolution (_____) or percent absorbed (----) versus time profiles for four different 200 mg carbamazepine tablet Products. (○, Product 1; ●, Product 2; □, Product 3; ■, Product 4).

the AUC(0–∞) values were normalized for body weight, the AUC(0–∞) for the females was 19% lower ($p < 0.01$) than for the males. When the data were normalized for both weight and elimination rate constant, the AUC(0–∞) for the two groups differed by less than 1% ($p > 0.05$).

In Vitro-In Vivo Relationships

As shown in Figure 2, the USP requirement that carbamazepine tablets must be not less than 75% dissolved in 60 min was met, as was a proposed specification (10) that 40–70% should be dissolved in 15 min. Figure 3 illustrates an attempt at a Level A, 1:1 *in vivo-in vitro* correlation, which is thought to be the most useful relationship for predicting *in vivo* performance from dissolution data (10). However, even though all four products demonstrate a linear relationship between the percent dissolved and the percent absorbed, no single relationship could be employed to predict the bioavailability of all four products. Because fraction absorbed/fraction dissolved data had been previously reported for a different lot of Product 1 (1), the agreement between data from the present and previous studies was assessed. Results presented in Figure 3 suggest a good linear relationship ($r2 \geq 0.96$). However, an attempted prediction of the percent absorbed in the first study from the correlation obtained in the present study resulted in at least a 25% overestimation. Thus, this 1:1 correlation is not useful in predicting across different formulations, nor is it accurate in predicting across different groups of subjects. Figures 4 and 5 illustrate a Level C correlation (9), which involves a plot of *in vitro* dissolution data at a certain time, versus Tmax and Cmax. These correlations suggest that the percent dissolved at 15 min appears to be the best predictor of the in vivo data. However, such a correlation ($r^2 \geq 0.98$) could be misleading because the dissolution and *in vivo* parameters for the three generic formulations were closely grouped together (11). Since the mean AUC's for all four products were essentially the same, no correlation was possible between dissolution rate and AUC. The relationship shown in Figure 5 was also employed to predict the Cmax values from the 15 min dissolution data for the four products studied earlier (1). Results presented in Table III suggest an agreement between predicted and observed Cmax values. Such a prediction would require similar disposition metrics (volume of distribution and clearance) in each subject group. AUC(0–∞), Cmax and λ for two lots of the innovator product differed by <9% for the two studies, possibly indicating

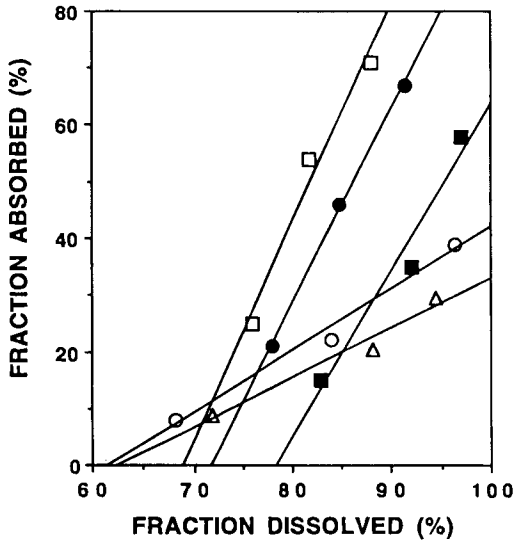

Fig. 3. Relationship between the mean percent absorbed *in vivo* and the percent dissolved *in vitro* at 30, 60 and 90 or 120 min for two lots of the innovator 200mg carbamazepine tablet and three generic tablets. (○, product 1, present study, $R^2 = 0.98$, $y = -67.1 + 1.09x$; △, Product 1, previous study, $R^2 = 0.97$, $y = -54.4 + 0.87x$; ●, Product 2; $R^2 = 0.99$, $y = -246 + 3.43x$; □, Product 3, $R^2 = 0.97$, $y = -261 + 3.79x$; ■, Product 4, $R^2 = 0.95$, $y = -235 + 2.98x$).

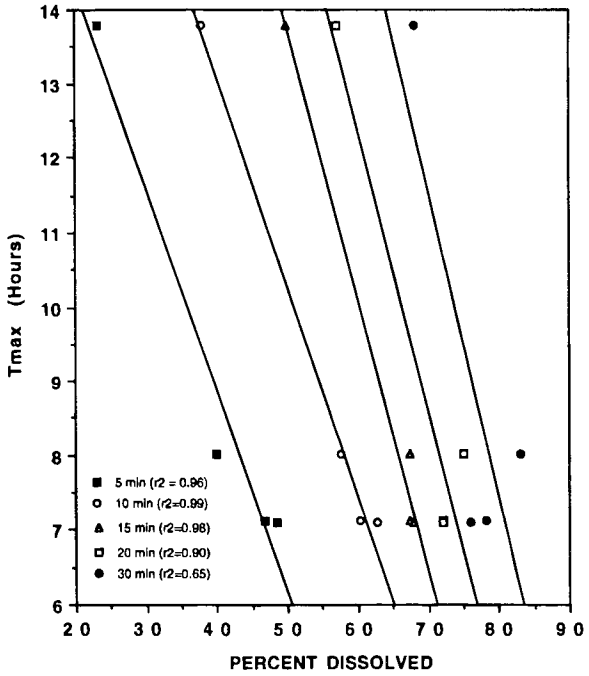

Fig. 4. Relationship between the mean Tmax and the percent dissolved *in vitro* at 5, 10, 15, 20 and 30 min.

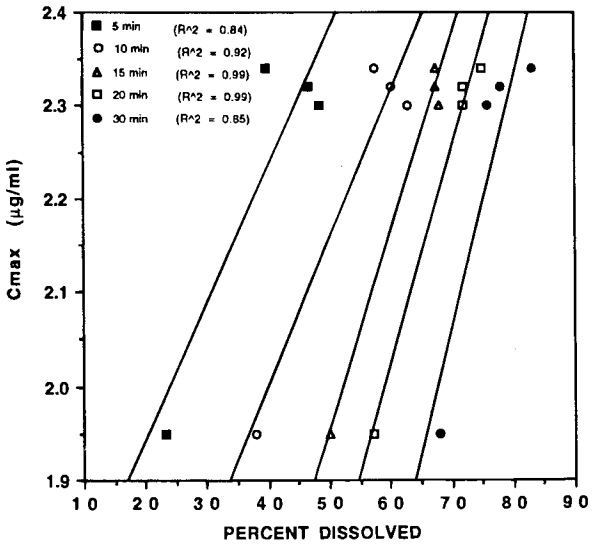

Fig. 5. Relationship between the mean Cmax and the percent dissolved *in vitro* at 5, 10, 15, 20 and 30 min.

Table III. Prediction of C_{max} from Percent Dissolved at 15 Minutes

Product[a]	Percent dissolved[a]	C_{max} Observed[a] (μg/ml)	C_{max} Predicted[b] (μg/ml)
1	53.2	1.89 μg/ml	2.02 μg/ml
2	9.5	1.15 μg/ml	1.10 μg/ml
3	82.1	2.69 μg/ml	2.62 μg/ml
4	22.0	1.40 μg/ml	1.36 μg/ml

[a] Data from Reference 1.
[b] C_{max} PREDICTED = 0.9 μg/ml + 0.021 μg/ml (% Dissolved *In vitro* at 15 min), from the regression line given in Figure 5.

such a similarity. These results clearly illustrate the importance of the recently proposed (10) 15 min sampling time for *in vitro* dissolution studies. This observed association between the *in vitro* dissolution and the Cmax values across two different studies, different lots, and different formulations adds credibility to the use of dissolution testing as a means to detect potential bioavailability problems *in vivo*.

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