Variability in the Bioavailability of Phenytoin Capsules in Males and Females

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Purpose. To determine inter-lot and intra-subject variability in the bioavailability of the 100 mg extended phenytoin sodium capsules. In addition, to determine the effect of gender and menstrual cycle on phenytoin bioavailability.

Methods. Three different lots of extended phenytoin sodium capsules were given to 12 healthy male and 12 healthy female subjects in a crossover fashion. One of the lots was also given a second time to each subject. Plasma phenytoin was determined, using an HPLC assay, in samples collected over a 73-hr period after each dose.

Results. The mean Cmax for the four administrations ranged from 1.71–1.79 μg/ml and mean AUC(0–∞) values from ranged 53.0–54.1 μg$_*$hr/ml. The elimination half-life was 3 hr shorter, and the AUC(0–∞) adjusted for the mg/kg dose was 30% lower for females. Average bioequivalence was demonstrated between the three lots for both Cmax and AUC(0–∞) based on the BE limit of 80–125%. Further, all confidence intervals of AUC(0–∞) fell within the limit of 90–111%. There were no differences in the confidence limits for Cmax and AUC(0–∞) determined separately for males and females. Also, there was no difference in the mean Cmax or AUC(0–∞) for females when analyzed as a function of the week of their menstrual cycle. Individual bioequivalence was demonstrated between three lots of phenytoin using the constant-scaled method, but not the reference-scaled method.

Conclusions. There was very little difference in the bioavailability of the three lots of phenytoin. Females exhibited a lower AUC(0–∞) than males after adjustment of dose for body weight, but their inclusion in the study did not affect the assessment of bioequivalence. When dose was not adjusted for body weight, no difference in AUC(0–∞) was seen between males and females.

KEY WORDS: phenytoin; bioavailability; human; pharmacokinetics; gender; menstrual cycle.

INTRODUCTION

Phenytoin has a narrow therapeutic index and exhibits marked nonlinear pharmacokinetics in the usual dose range. As a result, recommendations have been given to utilize a more rigorous approval criterion for generic versions of extended phenytoin sodium capsules. For example, it has been suggested that the bioequivalence limit of the observed 90% confidence interval for the ratio of the geometric means of AUC be narrowed to 90–111% as opposed to the usual 80–125% (1,2). A more restrictive bioequivalence limit could reduce the possibility that differences seen after a single-dose study would be magnified after repeated doses because of nonlinear disposition kinetics. However, before it was possible to consider more restrictive criteria, it was necessary to determine the inherent variability in the bioavailability of the drug. Thus, this study was conducted to determine the relative bioavailability of three different lots of innovator product (Dilantin Kapseals), as well as the intra-subject variability when the same lot of innovator product was given on two different occasions. In addition, while studies have shown that males and females can differ in terms of pharmacokinetic properties, there is a paucity of data on the effect of gender on bioequivalence determinations. This study utilized an equal number of males and females to provide information on gender differences in bioequivalence, if any. In addition, the effect of the menstrual cycle on bioequivalence determinations was also examined.

EXPERIMENTAL

Dosage Forms

The dosage forms were three different lots of 100 mg extended phenytoin sodium capsules (Dilantin Kapseals, Parke-Davis): Product 1 (Lot 02293FA, Exp. 9/95); Product 2 (Lot 01993FA, Exp. 9/95); Product 3 (Lot 055D3FA, Exp. 11/95) and Product 4, which was the same as Product 1. The three lots were studied prior to their expiration dates.

Clinical Protocol

A four-way single dose crossover bioequivalence study was conducted in 12 male and 12 female healthy volunteers between the age of 22 and 28 yr. The research followed the tenets of the Declaration of Helsinki promulgated in 1964, and was approved by the Institutional Review Board of the University of Tennessee and the Risk Involving Human Subjects Committee of the FDA. All subjects provided written informed consent. All subjects were evaluated with a medical history and tests for clinical chemistry, complete blood count, urinalysis and ECG prior to entering the study. Three males and three females were randomly assigned to each of four treatment groups, and each group received the four doses in a different sequence. Seven days elapsed between each dose. On each of the four dosing days the subjects reported to the clinical laboratory in the morning after an overnight fast and received 120 ml of water. One hour later, each subject received a 100-mg extended phenytoin sodium capsules with 180-ml of room temperature water. Two hours after dosing an additional 120-ml of water was given to maintain hydration of the subjects. No food was permitted until a standard lunch was served five hours after dosing. Eight-ml blood samples were obtained before dosing and 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 25, 33, 49 and 73 hours after dosing. Samples were collected by venipuncture or indwelling catheter into heparinized evacu-

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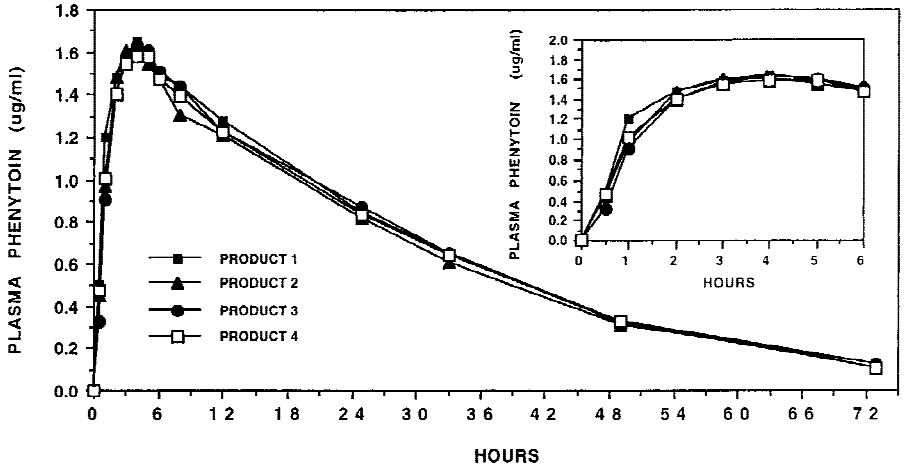

Fig. 1. Mean phenytoin plasma concentrations in 24 subjects. Insert represents mean data during the initial 6 hr after dosing.

ated tubes. Plasma was separated by centrifugation at 4°C with storage of plasma in glass vials at –20°C until analyzed.

Plasma Analysis

Phenytoin concentrations were determined by HPLC after extraction of 1 ml of plasma with 8 ml of methylene chloride. Plasma standards were prepared from drug-free human plasma fortified with 0.5 ml aliquots of phenytoin in 0.002 N sodium hydroxide to contain 0.052, 0.104, 0.259, 0.519, 1.04 and 2.59 μg/ml of phenytoin. Hexobarbital was used as the internal standard, and was added to the plasma prior to extraction as 0.5 ml aliquots containing 10 μg/ml in 1% methanol. Quality control fortified samples were also prepared to contain 0.074, 0.74 and 1.48 μg/ml of phenytoin. A standard curve in duplicate and the controls in triplicate were assayed each day that subject samples were analyzed. The HPLC system (Waters Associates) consisted of a M6000 pump (1.2 ml/min); a WISP 710B autosampler (40 μl injection); a variable wavelength detector (205 nm and 0.05 AUFS); and a Novapak C18 column (150 cm × 3.9 mm, 10 micron). The mobile phase contained 25:75 acetonitrile:pH 4.4, 0.05 M phosphate buffer; with one bottle of PIC-B8, Low UV (Waters Associates) per liter. The run time between injections was 17 min.

Pharmacokinetic and Statistical Analysis

The maximum plasma concentration (Cmax) and time to reach the maximum concentration (Tmax) were determined by inspection. The AUC to infinite time (AUC0–∞) was calculated using standard methods (3).

To determine average bioequivalence, the statistical analysis was performed using the GLM procedure from the SAS statistical package on a VAX 8000 computer. The two, one-sided tests (4) were carried out by computing 90% confidence intervals for Cmax and AUC(0–∞) using natural log (ln)-transformed data. Each of the four doses was treated as though they represented four different products, even though Product 1 and Product 4 were from the same lot.

To determine individual bioequivalence, the statistical analysis was carried out using the criterion described in the FDA's draft guidance (5). Because Product 1 was the same as Product 4, data for these 'products' were used to provide estimates for the reference mean and intra-subject variance using a method of moments (6,7) as well as the restricted maximum likelihood (REML) method (5). The intra-subject variability of Products 2 and 3 was calculated only by the REML method. The 95% upper confidence bound was computed using a non-bootstrap procedure (8–10). Both constant-scaled and reference-scaled methods were used. Individual bioequivalence is established for a ln-transformed bioavailability measure if the 95% upper confidence bound is less than or equal to 2.4948, an individual bioequivalence limit specified in the draft guidance.

Table I. Mean (n = 24) Phenytoin Bioavailability Parameters[a]

Parameter	Product 1[b]	Product 2	Product 3	Product 4[b]
Cmax (μg/ml)	1.79 (22)	1.73 (19)	1.76 (22)	1.71 (21)
Tmax (hr)	3.6 (64)	4.3 (105)	3.9 (43)	4.0 (56)
AUC(0-∞) (μg × hr/ml)	54.1 (59)	53.0 (73)	53.6 (57)	53.0 (66)
Half life (hr)	15.5 (37)	15.5 (61)	15.5 (42)	14.9 (49)

[a] CV% in ( ).
[b] Product 1 and Product 4 are the same lot given on two occasions.

Table II. Effect of Gender on Phenytoin Bioavailability[a]

Parameter	Mean of four doses[b] Males	Females	p value
Weight (kg)	78.0 (10)	60.7 (10)	<0.01
Cmax (μg/ml)	1.65 (19)	1.76 (21)	>0.05
AUC(0-∞) (μg × hr/ml)	48.9 (29)	45.1 (31)	>0.05
Tmax (hr)	4.0 (39)	3.1 (38)	<0.01
HALF LIFE (hr)	15.7 (31)	12.6 (24)	<0.01
AUC(0-∞) (normalized for mg/kg dose)	41.5 (31)	29.1 (24)	<0.01

[a] CV% in ( ).
[b] N = 11 Males, N = 12 females.

Table III. Effect of Gender on Bioequivalence Confidence Limits

	Confidence limits		
	Male	Female	Combined
Ln Cmax product comparisons			
2 vs 1	88–108%	89–107%	90–104%
3 vs 1	85–104%	86–103%	92–105%
4 vs 1	83–103%	85–101%	89–102%
2 vs 3	93–115%	95–114%	92–106%
2 vs 4	95–117%	96–115%	92–105%
4 vs 3	89–110%	90–108%	91–104%
Ln AUC(0-∞) product comparisons			
2 vs 1	89–102%	88–101%	90–99%
3 vs 1	93–107%	93–106%	95–104%
4 vs 1	92–105%	89–102%	92–101%
2 vs 3	89–102%	89–101%	91–100%
2 vs 4	91–103%	93–106%	93–103%
3 vs 4	92–105%	90–103%	93–102%

RESULTS AND DISCUSSION

All 24 subjects successfully completed the study. No significant adverse effects were reported, although a majority of the subjects complained of a headache on at least one occasion. No significant clinical abnormalities were found in the post-study clinical evaluations. The standard curves exhibited good linearity ($r^2 \geq 0.99$). The precision for the assay of the standards and controls was also good, with relative standard deviations of 5–13% and 10–11% for the standards and controls, respectively. The retention times for the internal standard and phenytoin were approximately 7 min and 9 min, respectively.

Mean plasma concentration-time profiles for the four doses are shown in Figure 1, and the mean bioavailability measures are summarized in Table I. The differences between the three lots were not significant ($p < 0.05$), and except for Tmax, were less than 5%.

Table II provides a comparison of the male and female data. One male was excluded from this analysis because his AUC's were four times the group mean, and his estimated half life ranged from 38–55 hr. The only abnormality that could be identified in this subject was a viral infection that was treated with amoxicillin two weeks prior to the first dose. An alternative explanation for this outlier may be the effect of genetic polymorphism of cytochrome P450 2C9 and/or 2C19 on the metabolism of phenytoin (11). As shown in Table II, the mean AUC(0–∞) for the males and females were similar. However, when the AUC(0–∞) was normalized for the mg/kg phenytoin dose, the mean AUC(0–∞) for the females was about 30% lower than for the males. The lower AUC(0–∞) in the females was in part the result of the more rapid elimination, although possible differences in the volume of distribution or fraction of dose absorbed cannot be ruled out. These data suggest that dosing regimens for phenytoin do not need to be adjusted based on gender since the generally smaller weight of females, which would tend to produce higher levels, is counterbalanced by the more rapid elimination in this gender.

Table III summarizes the 90% confidence limits for Cmax and AUC(0–∞) using ln-transformed data. All of the comparisons for any lot were well within the acceptable range of 80–125%, with no apparent effect of gender on the bioavailability measures calculated without regard to body weight. The narrower confidence limits for the analysis that combined both males and females is due to the larger number of observations in the combined analysis. Based on these results, it appears feasible to tighten the bioequivalence limits to 90–111% for AUC of extended phenytoin sodium products because the intra-subject variability is small. The ANOVA CV% for Cmax and AUC(0–∞) were 14% and 11%, respectively. In half of the subjects the differences between the two replicated lots were less than 10%, and the mean difference for all 24 subjects was only 12.3%. The differences in AUC(0–∞) for the two doses of the lot that was replicated ranged from 0.4% to 34.2% for individual subjects. Thus, although the mean intra-subject variability was low, inter-occasion differences for individual subjects were sometimes substantial even when they were administered the same lot.

Table IV summarizes the data analyzed using the currently proposed individual bioequivalence criterion (5), with Products 2 and 3, separately, as the test products and Products '1' and '4' combined as the reference product. The estimated intra-subject variability for the reference is similar between the method of moments and restricted maximum likelihood method. The results indicated that all the bioavailability measures, Cmax and AUC(0–∞), for Products 2 and 3 met the individual bioequivalence limit using the constant-scaled method. The constant-scaled approach is the method of choice because the intra-subject variability of the reference (σ_{WR}) is less than the regulatory standard (σ_{W0}) in this study (5). If the reference-scaled method were used, some of the measures would exceed the limit, presumably due to the ex-

Table IV. Statistics Using an Individual Bioequivalence Criterion

	Intra-subject std.dev.[a]			95% upper confidence bound	
	Test	Reference	T/R ratio	Constant-scaled	Reference-scaled
LnCmax					
2 vs (1,4)	0.048	0.115	0.417	2.450 (pass)[b]	2.521 (fail)
3 vs (1,4)	0.043	0.115	0.374	2.424 (pass)	2.496 (fail)
Ln AUC(0-∞)					
2 vs (1,4)	0.074	0.078	0.949	2.417 (pass)	2.504 (fail)
3 vs (1,4)	0.060	0.078	0.769	2.407 (pass)	2.494 (pass)

[a] All analyses were conducted using ln-transformed data, and the standard deviation approximated the coefficient of variation (% CV) on the original scale.

[b] Compared with the bioequivalence limit of 2.4948, as specified in the FDA draft guidance.

tremely low intra-subject variability (7.8–11.5%) for the reference.

A fundamental assumption for a bioequivalence study is that the drug clearance by a subject does not change during the course of the study. One of the concerns regarding the use of females in a bioequivalence study is the possible influence of the menstrual cycle on drug clearance. In fact, there have been several reports of clearance differences in females receiving doses of phenytoin. Shavit et al. (12) measured plasma phenytoin concentrations 12 hr after a dose on days 1 or 2 and again, on day 17 in seventeen women with catamenial epilepsy and nine women with epilepsy, but without an increase in seizures during the premenstrual period. These authors observed an average 25% decrease in the phenytoin concentrations in the catamenial epilepsy patients during menstruation, and a 15% decline in the non-catamenial patients. Kumar et al. (13) reported similar observations. All of the healthy females in the present study had a normal menstrual cycle, and single doses of phenytoin were given once each week over a four-week period. Figures 2 and 3 illustrate the Cmax and AUC(0–∞) values in each of the twelve females, plotted as a function of the week of their menstrual cycle. Although three of the four doses were different lots of the product, this should not affect the analysis since the three lots were determined to be bioequivalent, as discussed above. There was only an 8% difference (week 1 <week 3) in the mean Cmax values ($p > 0.05$) and a 15% difference (week 2 > week 4) for AUC(0–∞) ($p > 0.05$). Thus, it did not appear that the menstrual cycle of the females included in this study significantly affected the bioequivalence determinations.

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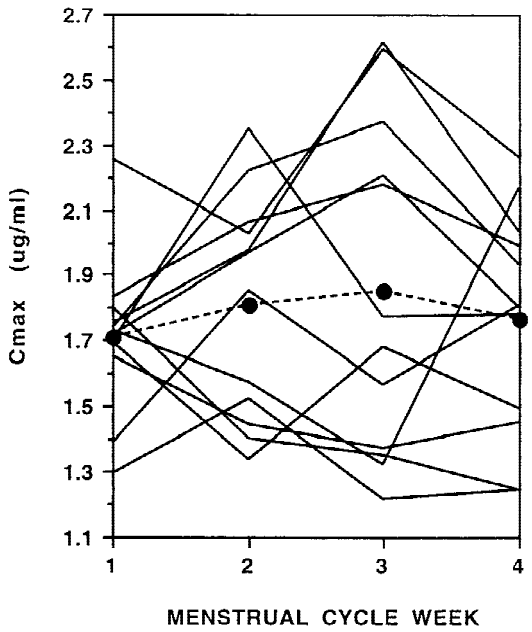

Fig. 2. Phenytoin Cmax in 12 females as a function of the week of the menstrual cycle. Mean values shown as (— · —).

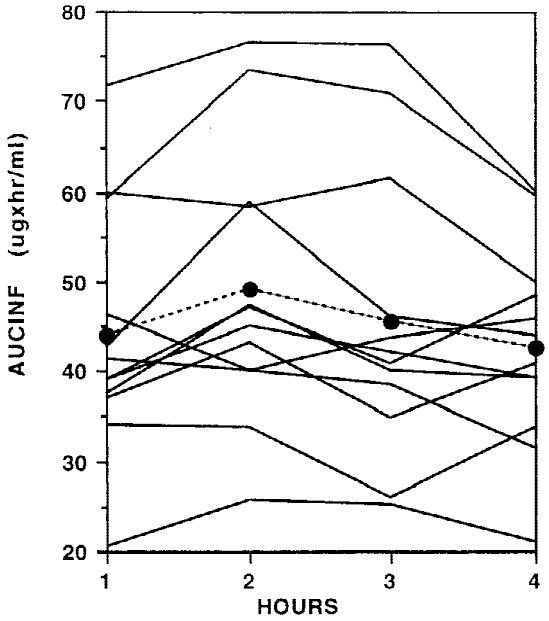

Fig. 3. Phenytoin AUC(0–∞) in 12 females as a function of the week of the menstrual cycle. Mean values shown as (— · —).

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