Lack of *In Vivo/In Vitro* Correlations for 50 mg and 250 mg Primidone Tablets

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Purpose. To determine if large differences in the *in vitro* dissolution profiles for primidone tablets would result in significant bioavailability differences.

Methods. Two separate bioavailability studies were conducted. The first study used 18 healthy subjects and compared the bioavailability of an old 50 mg tablet formulation, a new 50 mg tablet formulation, and a suspension containing 50 mg/ml of primidone. The second study enrolled 24 subjects who were to receive a new 250 mg tablet formulation, two lots of an old 250 mg tablet formulation and a 250 mg tablet from a second manufacturer. *In vitro* dissolution was conducted over 90 minutes, using USP 23 Apparatus 2 at 50 rpm, with 900 ml of water.

Results. Dissolution at 90 minutes for the old and new 50 mg tablets was approximately 20% and 100%, respectively. The dissolution of the four 250 mg tablets ranged from approximately 30% to 100%. The 50 mg tablet that dissolved slower had a longer Tmax and a 14% lower Cmax than the more rapidly dissolving tablet, but the AUC(0–∞) values differed by only 3%. Only nine subjects completed the 250 mg study because of side effects. The differences in Cmax and AUC(0–∞) among the four 250 mg tablets were less than 7%.

Conclusions. Even though there were large differences in the *in vitro* dissolution of the 50 mg and the 250 mg primidone tablets, the two 50 mg tablets were shown to be bioequivalent, as were the four 250 mg tablets.

KEY WORDS: primidone; bioavailability; human; pharmacokinetics; *in vitro* dissolution.

INTRODUCTION

There is continuing interest in developing in *vivo/in vitro* correlations as a means to reduce the need for human bioequivalence testing. Various speakers at a recent workshop discussed some of the efforts to better understand and anticipate the feasibility of such correlations (1). However, in general we are still not able to determine *a priori* if the dissolution profile for a dosage form will be predictive of human bioavailability. The dissolution specification for primidone tablets in USP 22 required not less than 75% dissolution in 45 min in 900 ml of water, using Apparatus 1 at 100 rpm (2). USP 23 requires not less than 75% dissolution in 60 min in 900 ml of water, using Apparatus 2 (3). Because of this change in specification, the innovator firm (Wyeth-Ayerst) reformulated their 50 mg and 250 mg primidone tablets. When the original 50 mg and 250 mg tablet formulations were developed they were shown to be bioequivalent to the innovator suspension. When the new 50 mg and 250 mg tablet formulations were developed the suspension was again employed as the reference product in the bioequivalence studies. However the old and new tablet formulations, which exhibited different dissolution characteristics, had not been directly compared in a bioequivalence study. In addition, a generic 250 mg primidone tablet formulation utilized the old 250 mg innovator tablet formulation in the bioequivalence study required for FDA approval. The present study was undertaken to directly compare the bioavailability of the old and new innovator 50 mg tablet formulations to the suspension. In addition, a second study compared two lots of the old innovator 250 mg tablet formulation, one lot of the new innovator 250 mg tablet formulation, and a generic 250 mg tablet formulation. 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 participants provided written informed consent.

EXPERIMENTAL

Dosage Forms

The formulations used in the first study were: Product 1 Wyeth-Ayerst, Mysoline® 50 mg tablet, old formulation, lot #389089, Exp. 9/94; Product 2 Wyeth-Ayerst, Mysoline® 50 mg tablet, new formulation, lot #3910800, Exp. 6/94; Product 3 Wyeth-Ayerst, Mysoline® suspension, 50 mg/ml, lot #3910399, Exp. 4/96. The formulations used in the second study were: Product 4 Wyeth-Ayerst, Mysoline® 250 mg tablet, new formulation, lot #3910115, Exp. 11/93; Product 5 Wyeth-Ayerst, Mysoline® 250 mg tablet, old formulation, lot #3890863, Exp. 5/94; Product 6 Wyeth-Ayerst, Mysoline® 250 mg tablet, old formulation, lot #3890738, Exp. 8/94; Product 7 Danbury Pharmacal, 250 mg tablet, lot #06174C, Exp. 11/93. All products were obtained from the Food and Drug Administration. The dissolution testing and bioavailability studies were completed prior to the expiration dates for the tablets.

Dissolution Testing

The dissolution of each tablet formulation was determined in FDA Laboratories, using the USP Apparatus 2 at 50 rpm, with 900 ml of water as the dissolution medium (3). Six tablets of each formulation were studied. Samples were withdrawn at 15, 30, 45, 60 and 90 minute intervals.

50 mg Tablet Bioavailability Study

This study compared the old and new innovator 50 mg tablet formulations and the innovator suspension (50 mg/ml), given as 50 mg doses. Eighteen healthy male subjects received each dose in a three-way crossover design. The subjects, who ranged in age from 21 to 34 years, were randomly assigned to one of six dosing sequences. All subjects were evaluated with a medical history, clinical chemistry (SMA 18/90), CBC, urinalysis and ECG prior to entering the study. The doses were administered at one week intervals. On each of the three dosing

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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 50 mg primidone dose with 180 ml of room temperature water. No food was permitted until a standard lunch was served four hours after dosing. Ten milliliter blood samples were obtained before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 15, 25, 30, 34 and 49 hours after dosing. Samples were collected by venipuncture or indwelling catheter into heparinized evacuated tubes. Plasma was removed by centrifugation at 4°C and the plasma was stored in glass vials at −20°C until analyzed.

250 mg Tablet Bioavailability Study

This study compared a new formulation of the innovator 250 mg tablet to two lots of the old innovator 250 mg tablet formulation and one lot of a generic 250 mg tablet formulation each given as single 250 mg doses. Twenty-four healthy male subjects were to receive each dose in a four-way crossover design. The subjects, who ranged in age from 20 to 33 years, were randomly assigned to one of four dosing sequences. The study protocol was identical to the 50 mg tablet protol described previously except: a) The subjects were given a light breakfast consisting of corn flakes, milk, orange juice, and two slices of white toast 45 min prior to the dose, in order to reduce the potential for nausea; b) Seven milliliter blood samples were obtained before dosing and at 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24, 32, 48, 56 and 72 hours after dosing.

Primidone Plasma Analysis

The primidone plasma concentrations were assayed by the completely validated HPLC method described below. For the 50 mg dose study a 1 ml plasma sample was combined with 1 ml of pH 6, 0.05 M phosphate buffer and 0.5 ml of internal standard solution (20 μg/ml of 4-methyl-primidone in 1% methanol). The mixture was extracted with 8 ml of 70:30 chloroform:isopropanol. After shaking and centrifuging, the aqueous phase was discarded and the organic layer was evaporated to dryness. The residue was dissolved in 3 ml of pH 6, 0.05 M phosphate buffer and extracted with 6 ml of hexane. The hexane layer was discarded and the remaining aqueous phase was extracted with 8 ml of 70:30 chloroform:isopropanol. After shaking and centrifuging, the organic phase was evaporated to dryness. The residue was then reconstituted with 100 μl of mobile phase. Plasma standards were prepared from drug-free human plasma fortified with 0.5 ml aliquots of primidone in water to contain 0.065, 0.13, 0.26, 0.52, 1.043 and 1.83 μg/ml of primidone. Quality control plasma samples were also prepared to contain 0.042, 0.70 and 1.40 μg/ml of primidone. 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 (1.0 ml/min); a WISP 710B autosampler (40 μl injection); a Model 490 variable wavelength detector (215 nm and 0.05 AUFS); and a Novapak C18 column (300 cm × 3.9 mm, 10 micron). The mobile phase contained 27:73 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 15 min. The primidone and 4-methylprimidone were from Sigma Chemical. The specificity of the method was determined by examining HPLC chromatograms from extracted drug-free pooled human plasma, as well as chromatograms prepared by injecting solutions containing primidone, 4-methylprimidone, phenobarbital and phenylethylmalonamide (PEMA, Alltech Associates). The latter two compounds are known metabolites of primidone.

The assay used for the 250 mg study was identical to the procedure used for the 50 mg study, except for the following: a) A 0.5 ml plasma sample was extracted; b) The primidone standard curve was prepared over a concentration range of 0.1 to 8.12 μg/ml; and c) The control samples were prepared to contain 0.42, 2.12 and 6.35 μg/ml of primidone. In addition phenobarbital and PEMA were added to the standard curve samples at concentrations ranging from 0.1 to 3.0 μg/ml, and control samples also contained phenobarbital and PEMA at concentrations of 0.15, 0.3 and 0.75 μg/ml.

Pharmacokinetic and Statistical Analysis

The maximum plasma concentration (C_{MAX}) and time to reach the maximum concentration (T_{MAX}) were determined by inspection of the data. The AUC to infinite time (AUC(0−∞)), was calculated using the linear trapezoidal rule to the last time point, with extrapolation to infinite time (4).

The statistical analysis was performed using the GLM procedure from the SAS statistical package on a VAX 8000 computer. The two, one-sided 90% confidence intervals for C_{MAX} and AUC(0−∞) were computed, using log-transformed data (5).

RESULTS AND DISCUSSION

Assay

The standard curves exhibited good linearity ($r^2 \geq 0.992$). The precision for the assay of the standards and controls was also good, with relative standard deviations of 3–17%, 4–17% and 3–13% for primidone, phenobarbital and PEMA, respectively. The retention times for PEMA, primidone, internal standard and phenobarbital were approximately 3, 4, 6 and 8 min, respectively.

In Vitro Dissolution

Figure 1 illustrates the dissolution results for the 50 mg and 250 mg tablets included in this study. These data clearly indicate that the new formulations (Products 2 and 4) are much more rapidly dissolved than the old formulations (Products 1, 5 and 6) or the generic product (Product 7).

50 mg Tablet Bioavailability Study

All 18 subjects successfully completed the study, although the majority of the participants reported feeling light headed, dizzy, nausea or fatigue. No significant clinical abnormalities were found in the post-study clinical evaluations. Mean plasma concentration-time profiles for the three 50 mg dosage forms are shown in Figure 2. The mean bioavailability parameters are summarized in Table I. The plasma concentration-time profiles for the new 50 mg tablet formulation (Product 2) and the suspension (Product 3) were essentially superimposable, and both formulations were more rapidly absorbed than the old 50

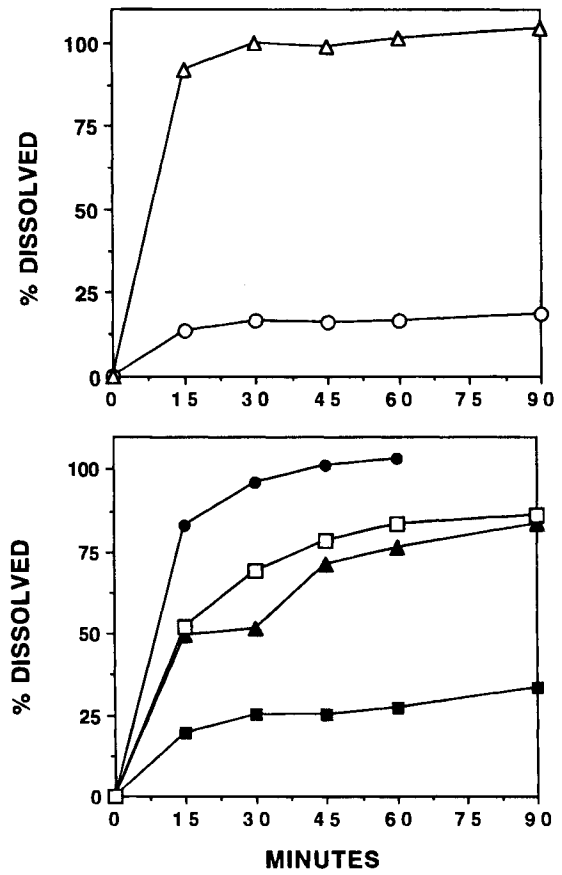

Fig. 1. *In vitro* dissolution results for two 50 mg primidone tablets: Products 1 (○) and 2 (△); and four 250 mg primidone tablet formulations, Products 4 (●), 5 (■), 6 (▲) and 7 (□).

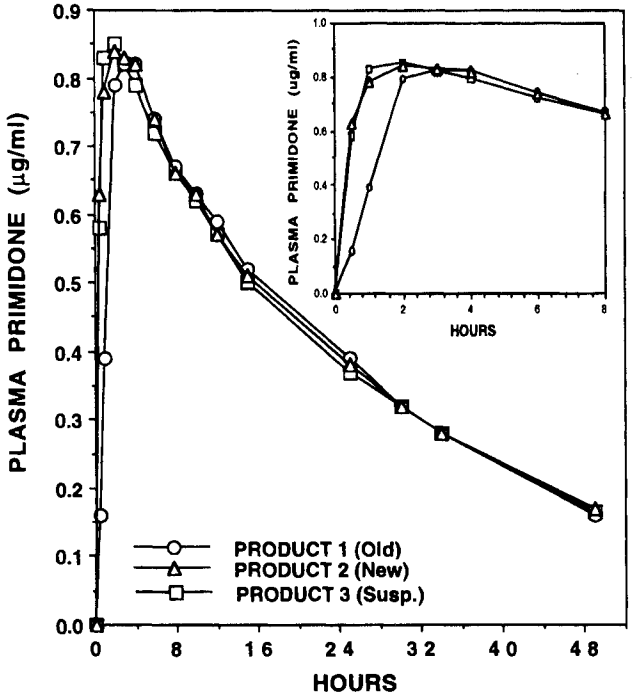

Fig. 2. Mean primidone plasma concentrations in 18 subjects after dosing with two different 50 mg primidone tablet formulations (Products 1 and 2) and a suspension (Product 3). Insert represents mean data during the initial 8 hr after dosing.

mg tablet formulation (Product 1). The slower absorption of Product 1 is seen in the differences in the mean plasma concentrations during the first hour after dosing, and the longer T_{MAX}. There was also a 14% difference between the mean C_{MAX} values for Products 1 and 2 ($p < 0.05$). The longer T_{MAX} and lower C_{MAX} for Product 1 were consistent with the slower dissolution of this product. There were no significant differences ($p > 0.05$) among the three products for mean AUC(0−∞) or half life. The two, one-sided 90% confidence limits, using log-transformed data for C_{MAX} and AUC(0−∞), comparing each product to the other two, were all within the limits of 80–125%. Thus, in spite of the differences seen in the dissolution profiles for the old and new 50 mg tablet formulations, the two tablet formulations and the suspension were found to be bioequivalent. It is also of interest to consider the bioequivalence of the formulations in the individual subjects. Figure 3 illustrates the ratios for C_{MAX} and AUC(0−∞) for the new and old 50 mg tablet formulations. In five of the eighteen subjects the new formulation resulted in a C_{MAX} which was more than 20% greater than for the old formulation. The C_{MAX} ratios for the other thirteen subjects were within ±20%, and all AUC(0−∞) ratios were within ±20%. While this trend toward higher C_{MAX} values in certain individuals is of some concern, currently there are no statistical criteria that are routinely used to determine individual subject bioequivalence.

250 mg Tablet Bioavailability Study

Only nine of the initial twenty-four subjects completed all four study phases. During the first dosing phase ten subjects dropped from the study because of significant nausea, vomiting or dizziness. Several subjects required antiemetic medication by

Table I. Mean (CV%) Primidone 50 mg Tablet and Suspension Bioavailability Parameters (N = 18)

Parameter	Old tablet Product 1	New tablet Product 2	Suspension Product 3
C_{MAX} (µg/ml)	0.88 (14)	1.02 (19)	0.93 (12)
T_{MAX} (hr)	2.45 (35)	1.56 (71)	1.76 (71)
AUC (0–49 hr) (µg × hr/ml)	20.70 (13)	21.15 (13)	20.83 (13)
AUC (0–∞) (µg × hr/ml)	26.04 (13)	26.72 (13)	26.39 (14)
Half Life (hr)	21.2 (8)	22.8 (8)	21.9 (11)

Two, one-side 90% confidence limits for log-transformed data

Comparison	Confidence limit
Ln Cmax:	
Product 2 vs 1	88–101%
Product 3 vs 1	102–117%
Product 3 vs 2	108–124%
AUC(0−∞):	
Product 2 vs 1	96–102%
Product 3 vs 1	99–102%
Product 3 vs 2	100–106%

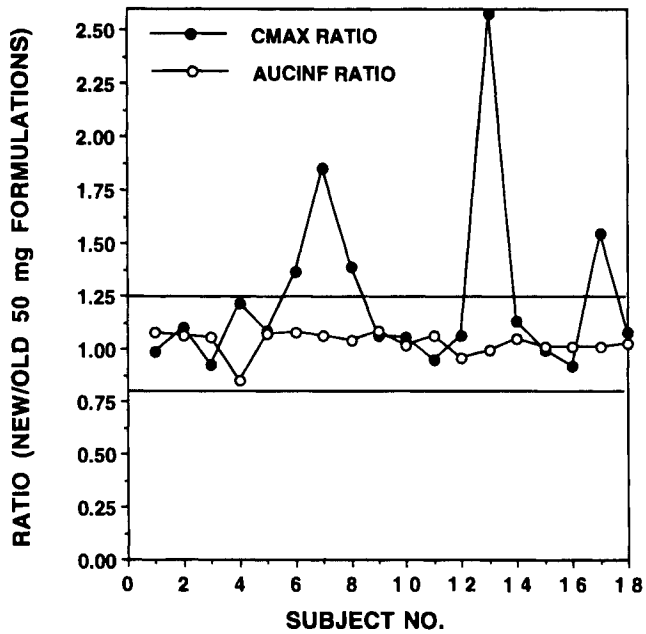

Fig. 3. C_{MAX} and AUC(0 − ∞) ratios (New Formulation/Old Formulation) in each subject for the 50 mg primidone tablet study.

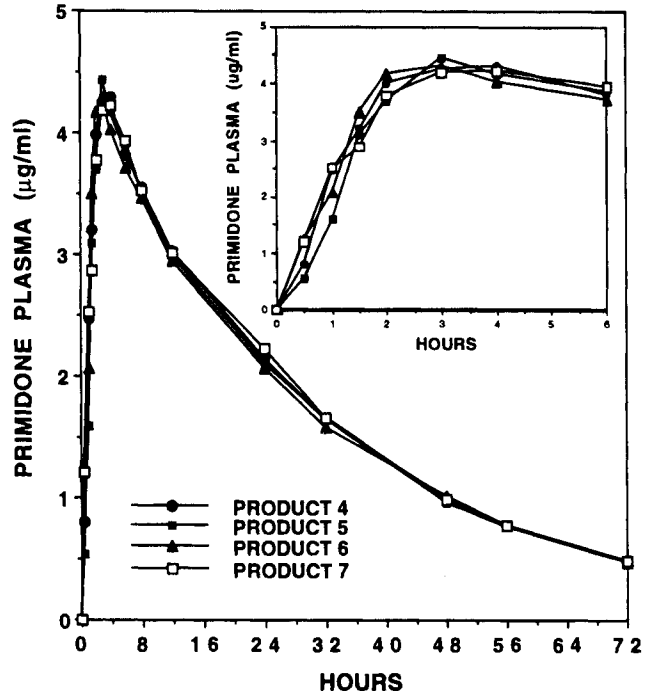

Fig. 4. Mean primidone plasma concentrations in 9 subjects after dosing with two different lots of an old 250 mg primidone tablet formulation (Products 4 and 6), a new 250 mg tablet formulation (Product 5), and a 250 mg tablet formulation from a second manufacturer (Product 7). Insert represents mean data during the initial 6 hr after dosing.

intramuscular injection or suppository. Two additional subjects withdrew after the second phase, and three more after the third phase. Adverse events were reported for all four products, with no tendency for a greater incidence with any given product. The adverse reactions resolved overnight, and no significant clinical abnormalities were found in the post-study clinical evaluations.

The data were analyzed using only the nine subjects that completed all four phases of the study. Mean plasma concentration-time profiles for the four 250 mg dosage forms are shown in Figure 3. The mean bioavailability parameters are summarized in Table II. The plasma concentration-time profiles for the four 250 mg tablet formulations were essentially superimposable. In spite of the differences seen in the *in vitro* dissolution profiles, there were no significant differences ($p > 0.05$) for C_{MAX} or AUC(0−∞) among the four products. The mean C_{MAX} and AUC(0−∞) values differed by less than 7% and 2%, respectively. In addition, the mean T_{MAX} values differed by 0.3 hr or less. The two, one-sided 90% confidence limits for log-transformed C_{MAX} and AUC(0−∞) for all comparisons among the four products were within the limits of 80–125%. Thus, in spite of the small number of subjects that completed the study, it was still possible to demonstrate bioequivalence based on the confidence limits, because of the relatively low variability seen in the study. The C_{MAX} ratios for the new 250 mg tablet formulation and the generic formulation were also calculated, as described above for the 50 mg tablet study. The ratios for nine subjects ranged from 0.87 to 1.10.

No attempt was made to rigorously analyze the phenobarbital and PEMA results because the concentrations of these metabolites were below the limit of sensitivity (0.1 µg/ml) for many of the sampling times. Compared to the mean C_{MAX} for primidone (range 2.6–6.4 µg/ml) the highest concentrations of phenobarbital and PEMA did not exceed 0.4 µg/ml after the single 250 mg dose. In addition, the blood sampling protocol was designed to focus on the primidone kinetics, and did not permit a good estimation of phenobarbital, since this metabolite has a half life of approximately 100 hr (6).

The results of these two studies provide an example of an *in vitro* dissolution test for immediate-release dosage forms that was not predictive of *in vivo* bioavailability. There were clear

Table II. Mean (CV%) Primidone 250 mg Tablet Bioavailability Parameters (N = 9)

Parameter	Product 4	Product 5	Product 6	Product 7
C_{MAX} (µg/ml)	4.45 (11)	4.56 (11)	4.76 (17)	4.53 (12)
T_{MAX} (hr)	2.72 (38)	2.78 (34)	2.51 (40)	2.83 (52)
AUC (0–72 hr) (µg × hr/ml)	125.3 (16)	123.8 (16)	123.2 (13)	126.4 (16)
AUC (0–∞) (µg × hr/ml)	140.5 (18)	139.8 (19)	139.7 (15)	142.1 (17)
Half Life (hr)	21.9 (13)	22.1 (13)	23.0 (12)	21.9 (9)

Two, one-side 90% confidence limits for log-transformed data

Comparison	Confidence limit
Ln Cmax:	
Product 2 vs 1	93–109%
Product 3 vs 1	98–114%
Product 3 vs 2	92–108%
AUC(0 − ∞):	
Product 2 vs 1	93–106%
Product 3 vs 1	94–106%
Product 3 vs 2	95–108%

differences in the dissolution profiles for the tablets included in this study. The new formulations were rapidly and completely dissolved and the old formulations were slowly and incompletely dissolved. However all formulations were found to be bioequivalent. Thus the current dissolution test for primidone (3), which has a low water solubility of 0.6 mg/ml (7), was too discriminating.

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