Bioequivalence of Methylphenidate Immediate-Release Tablets Using a Replicated Study Design to Characterize Intrasubject Variability

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Purpose. To determine the relative bioavailability of two marketed, immediate-release methylphenidate tablets. The study used a replicated study design to characterize intrasubject variability, and determine bioequivalence using both average and individual bioequivalence criteria.

Methods. A replicated crossover design was employed using 20 subjects. Each subject received a single 20 mg dose of the reference tablet on two occasions and two doses of the test tablet on two occasions. Blood samples were obtained for 10 hr after dosing, and plasma was assayed for methylphenidate by GC/MS.

Results. The test product was more rapidly dissolved *in vitro* and more rapidly absorbed *in vivo* than the reference product. The mean Cmax and AUC($0 - \infty$) differed by 11% and 9%, respectively. Using an average bioequivalence criterion, the 90% confidence limits for the Ln-transformed Cmax and AUC($0 - \infty$), comparing the two replicates of the test to the reference product, fell within the acceptable range of 80–125%. Using an individual bioequivalence criterion the test product failed to demonstrate equivalence in Cmax to the reference product.

Conclusions. The test and reference tablets were bioequivalent using an average bioequivalence criterion. The intrasubject variability of the generic product was greater and the subject-by-formulation interaction variance was borderline high. For these reasons, the test tablets were not individually bioequivalent to the reference tablets.

KEY WORDS: methylphenidate; average bioequivalence; individual bioequivalence; human; pharmacokinetics; replicated design.

INTRODUCTION

When the first generic methylphenidate tablet was approved by the U.S. Food and Drug Administration (FDA), an *in vivo* bioequivalence study was not required because of the AA coding in the "Orange Book" (1), and a determination of bioequivalence was based on *in vitro* dissolution testing alone. The dissolution properties of the generic product were subsequently re-examined because of reports that the generic and innovator products were not therapeutically equivalent in patients. An *in vivo* bioequivalence study was also initiated to compare the generic and innovator tablets and to explore if differences in the *in vitro* dissolution of the two formulations were predictive of possible differences in the *in vivo* bioavailability of the two dosage forms.

EXPERIMENTAL

Dosage Forms

Product 1 was the reference 20 mg methylphenidate tablet (Ciba Pharmaceutical Co., Lot 1B106121). Product 2 was the test 20 mg methylphenidate tablet (MD Pharmaceuticals, Lot W532B01).

Dissolution Testing

The dissolution of both tablet formulations was determined using the USP basket method, at 100 rpm, with 900 ml of water as the dissolution media (2). Six tablets of each formulation were studied.

In Vivo Study Design

A four-way single dose, replicated crossover bioequivalence study was conducted in 20 healthy male volunteers between the age of 20 and 33 years. The research followed the 1964 Declaration of Helsinki and was approved by both the Institutional Review Board of the University of Tennessee and the Risk Involving Human Subject Committee of the FDA. All subjects were evaluated with a medical history and tests for clinical chemistry (SMA 18/90), CBC, urinalysis and ECG prior to entering the study. The 20 subjects were divided into four groups. Each group received the two products in a different sequence: Group 1—Products 1, 1, 2 and 2; Group 2—Products 1, 2, 2 and 1; Group 3—Products 2, 2, 1 and 1; and Group 4—Products 2, 1, 1 and 2. One week elapsed between doses. On each of the four dosing days, the subjects reported to the clinical laboratory in the morning after an overnight fast and received 180 ml of water to hydrate the subjects and facilitate catheter placement. One hour later each subject received a 20 mg methylphenidate tablet 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 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 8 and 10 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 −70°C until analysis.

Analysis of Methylphenidate in Plasma

A previously described (3–5) gas chromatographic/mass spectrometry method was used to determine the methylphenidate plasma concentrations. The method used deuterated methylphenidate as an internal standard. Standard curves were

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prepared each day that subject samples were assayed, using methylphenidate fortified human plasma at concentrations of 1, 2, 3.9, 7.8, 11.7 and 14.6 ng/ml. Quality control samples were also prepared at methylphenidate concentrations of 2.1, 7.0 and 12.3 ng/ml in human plasma. All samples from a given subject were assayed together, along with standards and controls.

Pharmacokinetic and Statistical Analysis

The maximum plasma concentration (Cmax) and time to reach the maximum concentration (Tmax) were determined by inspection of the data. The area under the plasma concentration-time curve to 10 hours [AUC(0 − 10)] and the AUC to infinite time [AUC(0 − ∞)] were calculated using standard methods (6).

To determine average bioequivalence, the statistical analysis was performed using the GLM procedure from the SAS statistical package on a VAX 8000 computer. The statistical significance (p value) for differences between mean values for Cmax, Tmax and AUC(0 − ∞) were determined from the analysis of variance for both replicates. The two, one-sided tests (7) were carried out by computing 90% confidence intervals for Cmax and AUC(0 − ∞) using Ln-transformed data for the individual replicate sets, as well as the means of both sets of replicates.

To determine individual bioequivalence, the statistical analysis was carried out using the criterion described in the FDA's draft guidance (8). The variance terms, i.e., intrasubject variability and subject-by-formulation interaction, were estimated by a method of moments, using a saturated model for efficient estimates (9,10). The means were estimated by ordinary least squares estimates using equal weighting across sequences since there were an equal number of subjects in each sequence. For two-treatment designs, this estimator is the best linear unbiased estimator of the mean treatment effect (11). The 95% upper confidence bound was computed using a non-bootstrap procedure (12–13). 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 ≤0, the individual bioequivalence limit specified in the draft guidance.

RESULTS AND DISCUSSION

The test formulation was more rapidly dissolved than the reference formulation although both tablet formulations met

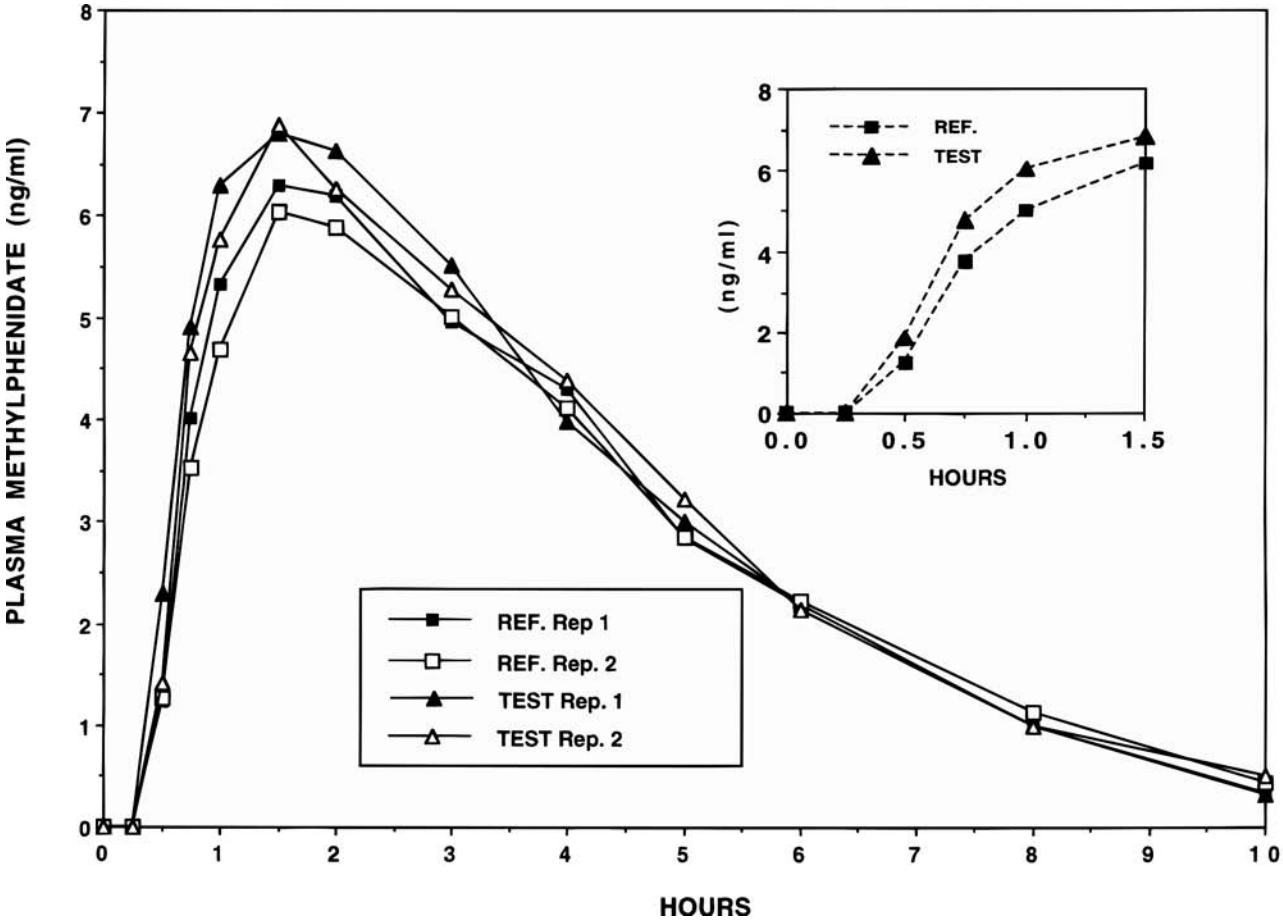

Fig. 1. Mean individual replicate methylphenidate plasma concentrations for two 20 mg tablet formulations in 20 subjects. Each subject received the test (▲, △) and reference (■, □) formulations twice (Rep., 1 and Rep. 2). Insert represents combined mean data for both replicates during the initial 1.5 hr after dosing.

Table I. Mean Pharmacokinetic Parameters[a]

Parameter	Mean value						% Test/ref combined
	Test product			Reference product			
	Rep. 1	Rep. 2	Combined	Rep. 1	Rep. 2	Combined	
Cmax (ng/ml)	7.71	7.45	7.59	7.01	6.70	6.85	111%
Tmax (hr)	1.50	1.61	1.55	1.86	2.08	1.97	80%
AUC(0 - 10) (ng × hr/ml)	30.65	30.34	30.50	28.81	28.39	28.60	107%
AUC(0 - ∞) (ng × hr/ml)	35.71	35.96	35.83	32.58	32.99	32.78	109%
K (hr-1)	0.292	0.293	0.293	0.309	0.301	0.305	96%
T1/2 (hr)	2.53	2.60	2.56	2.36	2.59	2.47	104%

[a] N = 20 for the first and second replicate; N = 40 for the combined replicates.

the USP requirement that no less than 75% should be dissolved in 45 min. The test formulation was 100% dissolved in 5 min, while the reference formulation was 43, 67, 82 and 100% dissolved at 5, 10, 15 and 25 min, respectively.

The mean of the back calculated concentrations for the standard curve samples were all within 1% of the nominal concentrations, and the coefficient of variation ranged from 8% for the highest concentrations to 16% for the lowest concentration (1 ng/ml), the latter value representing the limit of quantitation. The accuracy of the three levels of control samples ranged from 95% to 110% of the nominal concentrations.

All 20 subjects successfully completed the study. No side effects were reported and no significant clinical abnormalities were found in the post-study clinical evaluations. Mean plasma concentration-time profiles for the two products are shown in Fig. 1. Bioavailability parameters are summarized in Table 1. The test product was more rapidly absorbed than the reference product, based on the observation that the test product Tmax occurred approximately 0.4 hr before the reference Tmax ($p < 0.01$). In addition, the 0.5 and 0.75 hr plasma concentrations were 49% and 27% higher ($p < 0.05$) for the test product. Comparisons of the mean Cmax and AUC($0 - \infty$) were made using the combined data for both replicates. The mean Cmax differed by 11% ($p > 0.05$) and the mean AUC($0 - \infty$) by 9% ($p < 0.05$). The 90% confidence limits for Cmax and AUC($0 - \infty$) for the test formulation, based on Ln-transformed means for the combined replicate data, were 99–121% and 102–115%, respectively. Thus, the test and reference products were average bioequivalent using the Cmax means for the combined replicates. As shown in Table II, the means for replicates 1 and 2 were less than 5% different for both test and reference products. The same comparison for AUC($0 - \infty$) indicated only a 2% difference between each set of replicates. The slightly higher Cmax and a significantly shorter Tmax ($p < 0.01$) for the test product were consistent with the more rapid *in vitro* dissolution of the test product. For Cmax, the intraproduct comparison for the test product revealed that it was not bioequivalent to itself. This finding is probably attributable to the higher variability observed with the test product.

Using the currently proposed individual bioequivalence criterion (8), the data summarized in Table III indicate that based on either constant- or reference-scaled method, the 95% upper confidence bound of AUC($0 - \infty$) fell within the acceptable bioequivalence limit. The upper bound of Cmax exceeded the bioequivalence limit because of both higher intrasubject variability of the test product compared to reference product and the presence of a borderline subject-by-formulation interaction.

Several reports to FDA's Therapeutic Inequivalency Action Coordinating Committee in the late 1980s and early 1990s suggested that the generic product studied in this report was not bioequivalent to the reference listed (pioneer) product [medicine did not last long enough (2 cases), was not effective (1 case), caused emesis (1 case), was associated with irritability (1 case), and caused a feeling of not being 'all there' (1 case)]. Other reports also seemed to suggest a more rapid onset and shortened duration of action for the generic product. Based on this study and these reports, FDA concluded that the generic and pioneer product were bioequivalent, but changed the Orange Book coding from "A" to "B" to indicate that an *in vivo* bioequivalence study should be performed for generic methyphenidate drug products. While the test and reference products in this study were determined to be bioequivalent using an average bioequivalence criterion, the test product was more variable and the study indicated a borderline subject-by-formulation interaction. The analysis using an individual bioequivalence criterion for Cmax suggested that the test and reference products were not as interchangeable as was indicated by the average criterion given in Table II. The additional analysis based on the individual bioequivalence criterion supports a conclusion that some of the clinical events described may have been present in certain individuals who manifested a more rapid absorption, more rapid onset of action, and a lessened duration of action.

Table II. Methylphenidate Cmax Statistics

Comparison	Mean (ng × hr/ml)	Percent	Confidence limits Ln cmax
REF. 1, REF. 2	7.01, 6.70	96–105%[a]	84–119%
TEST 1, TEST 2	7.72, 7.45	97–104%[b]	76–131%
TEST 1, REF. 1	7.72, 7.01	110%[c]	103–123%
TEST 1, REF. 2	7.72, 6.70	115%[c]	84–117%
TEST 2, REF. 1	7.45, 7.01	106%[c]	106–138%
TEST 2, REF. 2	7.45, 6.70	111%[c]	93–123%
Mean TEST, REF.	7.58, 6.85	111%[c]	99–121%

[a] (Ref. 1/Ref. 2) and (Ref. 2/Ref. 1).
[b] (Test 1/Test 2) and (Test 2/Test 1).
[c] (TEST/REF.).

Table III. Statistics Using an Individual Bioequivalence Approach

Parameter	Intrasubject std. dev.[a]			Subject-by-formulation interaction	95% upper confidence bound	
	Test	Reference	T/R ratio		Constant scaled	Reference scaled
Cmax	0.259	0.175	1.480 (0.969–2.262)[b]	0.143 (0–0.303)	0.0502 (fail)[c]	0.0852 (fail)[c]
AUC(0 − 10)	0.224	0.191	1.171 (0.766–1.789)	0 (0–0.164)	−0.408 (pass)	−0.0118 (pass)
AUC(0 − ∞ hr)	0.228	0.184	1.234 (0.808–1.886)	0 (0–0.112)	−0.0478 (pass)	−0.0108 (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] Range in parenthesis represents 90% confidence interval of the value indicated.
[c] Compared with the bioequivalence limit of ≤ 0, as specified in the FDA draft guidance.

These findings could arise both from the subject-by-formulation interaction and the increased variability observed for the generic product.

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