High Variability in Drug Pharmacokinetics Complicates Determination of Bioequivalence: Experience with Verapamil

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Purpose. For the assessment of bioequivalence it is assumed that drug clearance in each subject on each of the study days is the same and any observed differences in AUC and/or Cmax between a brand and generic formulation are due to differences in bioavailability. We hypothesized that this assumption was invalid for highly variable drugs such as verapamil and tested it by comparing bioavailability for the brand vs itself.

Methods. To avoid any contribution from potential formulation differences, we evaluated bioavailability for Isoptin SR 240 mg tablets in 9 healthy volunteers on 2 occasions separated by 1 week as part of a larger study. A validated HPLC assay was used to measure serial blood samples over 36 hours.

Results. The AUC_{0-t} varied 3.8 fold among subjects and 5/9 subjects had >30% difference in AUC_{0-t} on the 2 days. After log transformation, the mean $AUC_{0-t} \pm$ %cv (ng·h/mL) on Occasion 1 (878 ± 38) was 23% greater ($p = 0.031$) than on Occasion 2 (713 ± 41). The 90% confidence interval of Occasion 1/Occasion 2 was 106–143%. The Cmax varied >9 fold (30–278 ng/mL) among subjects. The intra-subject difference between days ranged from −46% to +298%. The 90% confidence interval was 72–152% for Cmax. Since the same lot of Isoptin was used in the same subjects on 2 occasions, the observed differences must be due to biological variability in verapamil pharmacokinetics, not formulation differences.

Conclusions. The intra-subject biological variability complicates bioequivalence assessment and can lead to an erroneous assumption of bioinequivalence.

KEY WORDS: highly variable drugs; drug clearance; bioequivalence assessment; verapamil.

INTRODUCTION

Bioequivalence of two drug products is usually determined by conducting a randomized two-way crossover study (1). Bioavailability parameters such as AUC (reflecting extent of absorption) and Cmax (reflecting rate of absorption), calculated from the measured drug levels in blood, are then compared between the two drug products in each subject. In the US, the Food and Drug Administration (FDA) requires the 90% confidence limits for the percentage ratio (test/reference) of AUC and Cmax (based on their logarithms) to be between 80 and 125% for the conclusion of bioequivalence (2). For Cmax of uncomplicated drugs in Canada, the confidence limits are not required due to the inherently more variable nature of Cmax than AUC; only the mean ratio needs to be within 80 to 125% (3). An intermediate position has been taken in the European Community (EC), where a wider acceptance range for the confidence limits of Cmax (eg. 70–143%) has been suggested (4).

One of the assumptions for the assessment of bioequivalence in a crossover design is that drug clearance in each subject on the two study days remains the same and any observed differences in AUC and/or Cmax between the two drug products are due to differences in bioavailability. However, this assumption is not valid for highly variable drugs like verapamil (5) due to its high first pass metabolism (6). For these drugs even a small change in drug disposition, caused by physiological changes such as changes in blood flow to the gut from exercise, anxiety, food, etc., can have a significant impact on the bioavailability parameters.

The assumption of constant clearance for verapamil was tested as part of a larger study in which the bioavailability of the brand (Isoptin SR) and a generic formulation of verapamil sustained release (SR) tablets were compared. The results of the study are presented and the potential implication on the assessment of bioequivalence of highly variable drugs are discussed.

METHODS

Subjects

Twenty healthy, male volunteers, age 20–47 years (mean, 30 years) and weight 62–99 kg (mean, 77 kg), participated in the study. Each subject underwent a complete medical examination consisting of medical history, physical examination, blood biochemistry, hematology and urinalysis. The study was approved by the Apotex Research Independent Ethics Committee, and written informed consent was obtained from each subject before enrollment.

Study Design

A single-dose, two-formulation, three-period crossover design was used for the study. After a 10-hour overnight fast in the clinic, half of the subjects received a single oral dose of 240 mg of verapamil in the form of a generic verapamil SR 240 mg tablet in study period 1 and in the form of an Isoptin SR 240 mg tablet in study periods 2 and 3. One subject from this group withdrew from the study after period 2 due to personal reasons. His clinical samples were not analyzed. The other 10 subjects had Isoptin SR first followed by the generic verapamil SR on periods 2 and 3. The dosing sequence was randomly assigned to the subjects. Each study period was separated by 7 days. The Isoptin SR tablets were purchased from a retail pharmacy in the Toronto area and are, presumably, representative of the product on the Canadian market.

Each dose was administered with 240 mL of tap water. Light meals were served 4 and 10 hours after the administration of the dose. The same standardized meals were provided in all three periods. During each study day, no food other than the standardized meals served was permitted. Neither alcohol nor caffeine-containing beverages were allowed until 36 hours after dosing. Smoking was forbidden until 4 hours after the dose

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administration. Neither prescription nor over-the-counter drugs were permitted during participation in the study. Subjects were required to remain seated for the first 4 hours after drug administration and no strenuous physical activity was allowed until 36 hours after dosing.

Serial blood samples, using heparinized vacutainers, were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 18, 24, 30, and 36 hours after dosing. The blood samples were centrifuged and the plasma stored at −30°C until analyzed.

Analytical Assay

The concentration of verapamil in plasma was determined using a validated HPLC assay. The assay involved the addition of a 100 μL aliquot of an ethyl analogue of verapamil (internal standard) and 200 μL of saturated sodium phosphate solution to 0.5 mL of plasma, followed by extraction into 6 mL of extracting solvent, pentane:dichloromethane (2:1, v/v). The sample was centrifuged for 5 minutes and the organic phase was transferred into a clean polypropylene tube and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted in 200 μL of mobile phase and 50 μL was injected onto the HPLC column (Supelco LC-8-DB, 3 μm, 150 mm × 4.6 mm). The mobile phase was a mixture of 0.1 M potassium phosphate (pH 2.8):acetonitrile:triethylamine (680:320:0.15, v/v), and was delivered at a flow rate of 1.1 mL/min. Detection was accomplished by fluorescence (excitation at 203 nm and emission at 310 nm). The detector response was linear in the concentration range, 2.5 to 300 ng/mL, with a coefficient of determination (r^2) >0.997. The limit of quantitation was 2.5 ng/mL and the inter-assay coefficient of variation (cv) at this concentration was 3.1% (n = 7). The intra-assay and inter-assay precision of the quality control samples were <10% cv; the accuracy was within 15% of the nominal concentrations.

Data Analysis

From the measured plasma concentration data, the area under the curve from time zero to the last measurable time point (AUC_{0-t}) was calculated by the linear trapezoidal method. The peak plasma concentration (Cmax) and its time of occurrence (tmax) were obtained directly from the plasma concentration-time curve. The terminal elimination rate constant (kel) was calculated by least squares regression of the natural logs of the last four plasma concentrations. AUC from time zero to infinity ($AUC_{0-\infty}$) was determined by adding to the appropriate AUC_{0-t} the quotient of the estimated last plasma concentration and kel. The elimination half-life ($t_{1/2}$) was obtained from ln 2/kel.

For the comparison of bioavailability between Isoptin SR (reference) and the generic verapamil SR (test) tablets, analysis of variance (ANOVA), including sequence, subject nested within sequence, period and formulation effects, were performed on the natural logarithmic transformations of AUC and Cmax data for periods 1 and 2 (7). The intra-subject cv was estimated by taking the square root of the error mean square and multiplying by 100%. In addition to ANOVA results, the percentage ratio for test versus reference and its 90% confidence interval were calculated (7). For the comparison within the reference and the test products on two occasions, the data for periods 2 and 3 were used and the ANOVA model contained only subject and occasion effects.

The SAS System for PC, Release 6.08, was used for all the statistical analyses.

RESULTS

There were no significant adverse events or protocol violations observed during the study. The most frequently experienced adverse event after dosing was headache.

The results of comparison between the test and reference products based on the data for periods 1 and 2 revealed a significant difference ($p < 0.05$) in bioavailability between the two drug products as indicated by AUC_{0-t} and $AUC_{0-\infty}$. The 90% confidence intervals of the ratio of means were 56–92% and 63–92% for AUC_{0-t} and $AUC_{0-\infty}$, respectively. For Cmax, the difference in mean between the two products was not statistically significant but the 90% confidence interval was still well outside 80 to 125% (66–126%). Based on the error mean square, the intra-subject cv were 44, 33 and 58% for AUC_{0-t}, $AUC_{0-\infty}$ and Cmax, respectively. There were no significant differences ($p > 0.05$) in tmax, kel and $t_{1/2}$ between the two products.

Normally, these results would lead to a conclusion of bioinequivalence. However, these data showing "differences" may not be due to formulation differences, but to variability in drug clearance. For the purposes of examining the assumption of constant drug clearance and focussing on the highly variable nature of verapamil disposition, only the data for Isoptin SR from the 9 subjects studied with Isoptin SR on 2 separate occasions are presented in detail.

Plasma concentration profiles from two subjects displaying extensive variability in verapamil pharmacokinetics following administration of the brand on the two occasions are shown in Figure 1. The AUC_{0-t} and Cmax data are provided in Table I. The intra-subject comparison between the two occasions for AUC_{0-t} and Cmax are displayed in Figure 2.

There was a profound inter- and intra-subject difference in AUC_{0-t} and Cmax for Isoptin SR. The AUC_{0-t} varied 3.8 fold among subjects and 5 of 9 subjects had greater than 30% difference. Based on log transformation, the mean AUC_{0-t} on Occasion 1 was 23% greater ($p = 0.031$) than on Occasion 2. The intra-subject cv was 17% while the 90% confidence interval of Occasion 1/Occasion 2 was 106–143%. For Cmax, there was a greater than 9 fold variation among subjects and the intra-subject cv was 43%, reflecting a within subject Cmax difference ranging from −46% to +298%. The 90% confidence interval was 72–152% even though there was only a difference of 5% in means between the two occasions. There was no significant difference in tmax as the median tmax (5 hours) was identical between the two occasions.

Although the very wide confidence intervals for AUC_{0-t} and Cmax ratios between occasions are partly due to the small sample size (n = 9), the data reveal the potential difficulty of meeting the usual standards for bioequivalence for verapamil. Based on the results, the number of subjects required (80% power) to meet the FDA bioequivalence standards between the two occasions was estimated to be >1000 for AUC_{0-t} and 78 for Cmax, assuming the existence of the same differences in means and intra-subject cv (8).

With respect to the generic verapamil SR formulation, a similar degree of variability in bioavailability parameters can

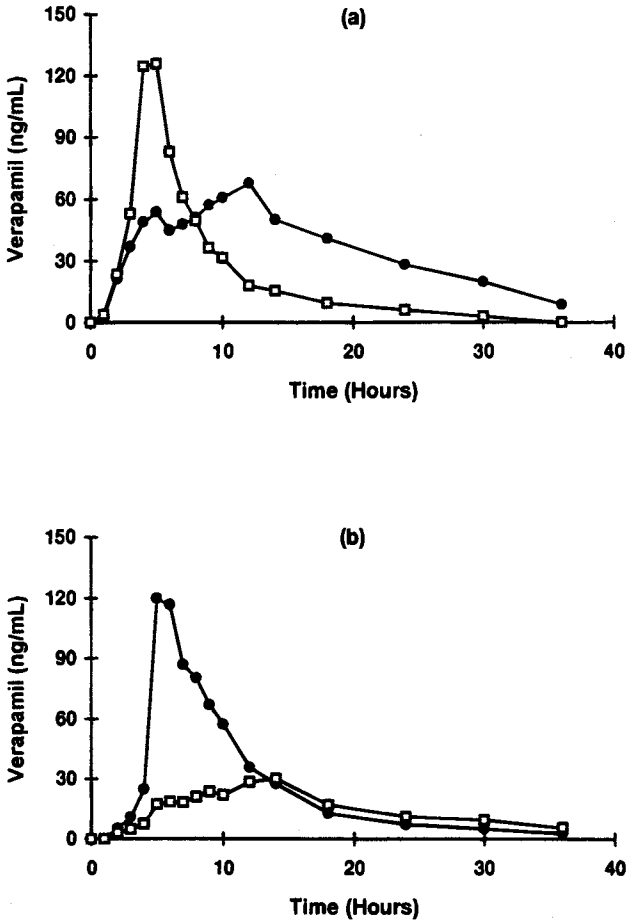

Fig. 1. Plasma concentrations of verapamil in two subjects after oral administration of an Isoptin SR 240 mg tablet on two occasions: ●, Occasion 1; □, Occasion 2. (a) Subject 4; (b) Subject 14.

Table I. Between Day Variation of AUC_{0-t} and Cmax with Isoptin SR 240 mg Tablets

	AUC_{0-t}(ng·h/mL)			Cmax (ng/mL)		
Subject	Occasion 1	Occasion 2	% Diff[a]	Occasion 1	Occasion 2	% Diff[a]
1	640	662	−3	77	81	−5
3[e]	1069	787	36	72	83	−13
4[e]	1257	783	60	68	126	−46
5[e]	762	540	41	36	39	−9
7	845	641	32	81	49	67
8	590	518	14	99	86	15
10	576	633	−9	67	79	−15
14	898	521	73	120	30	298
18[e]	1832	1984	−8	160	278	−42
Mean[b]	878	713[d]	23[c]	80	77	5[c]
%cv	38	41	—	42	66	—
Min	576	518	−9	36	30	−46
Max	1832	1984	73	160	278	298

[a] % Diff = (Occasion 1 − Occasion 2)/Occasion 2 × 100.
[b] Geometric mean.
[c] Calculated from the means of the parameter.
[d] Significantly different from Occasion 1 ($p = 0.031$).
[e] Smokers.

be observed. The mean AUC_{0-t} (±%cv) on Occasion 1 (1203 ng·hr/mL ± 41%) was 29% greater ($p = 0.0010$) than on Occasion 2 (930 ng·hr/mL ± 51%). The intra-subject cv was 18% while the 90% confidence interval of Occasion 1/Occasion 2 was 112–150%. The difference in Cmax between Occasion 1 (144 ng/mL ± 50%) and Occasion 2 (89 ng/mL ± 77%) was 63% with a 90% confidence interval of 122–217%. The intra-subject cv was 35%.

DISCUSSION

Verapamil, a calcium channel blocker, is commonly regarded as a drug with highly variable pharmacokinetics (5) primarily due to its high first pass metabolism (6). Drug products containing verapamil can be expected to exhibit variable bioavailability as shown in this study. The presented data clearly indicate that the bioavailability of Isoptin SR is highly variable, not only among, but also within subjects. Since the same lot of Isoptin SR was used in the same subjects on the two occasions, the observed differences must be due to biological variability in verapamil disposition or even absorption, not formulation differences. The plasma concentration profiles displayed in Figure 1 substantiate there are considerable changes in drug pharmacokinetics on different days with the same product. A similar degree of variation in drug levels between study days was also demonstrated by the generic verapamil SR tablet.

Although the high variability in verapamil pharmacokinetics might explain the observed differences in "bioavailability" between Isoptin SR and the generic verapamil SR tablets, the test product was reformulated. However, as shown in this study, the highly variable nature of the drug caused a substantial difference in mean for AUC_{0-t} and a large intra-subject cv for Cmax between the two occasions, thus resulting in the wide confidence intervals for both parameters. For a typical comparative bioavailability study with 24 subjects, this means that a conclusion of bioinequivalence between the brand and a generic product could result due to a high intra-subject variability of verapamil kinetics even if there were no formulation differences.

The results of our study, and its interpretation appear to have been corroborated by data presented in the discussion period of a session on Bioequivalence at an International Workshop on Statistical and Regulatory Issues on the Assessment of Bioequivalence held October 1995 in Dusseldorf. In a crossover study of Isoptin SR marketed in Germany (Knoll), the investigators found that the brand failed to pass against itself in a study of 24 subjects.

The findings in our study do not necessarily preclude the demonstration of bioequivalence in all studies of two sustained release verapamil formulations using the 90% confidence interval criterion. Because the effect of this between day variation is random, any given study has a chance of passing. On the other hand, our results do predict that, using current bioequivalence criteria, many studies evaluating truly bioequivalent formulations would needlessly fail unless an unreasonably large number of subjects was used. We view the use of large numbers of subjects (e.g. >24) as unacceptable for reasons of ethics and cost, although this issue will not be further developed in this presentation.

Only AUC_{0-t} data were presented in detail in this report, but the same conclusion can be obtained with $AUC_{0-\infty}$ as AUC_{0-t} contributed more than 90% of $AUC_{0-\infty}$. AUC_{0-t} was

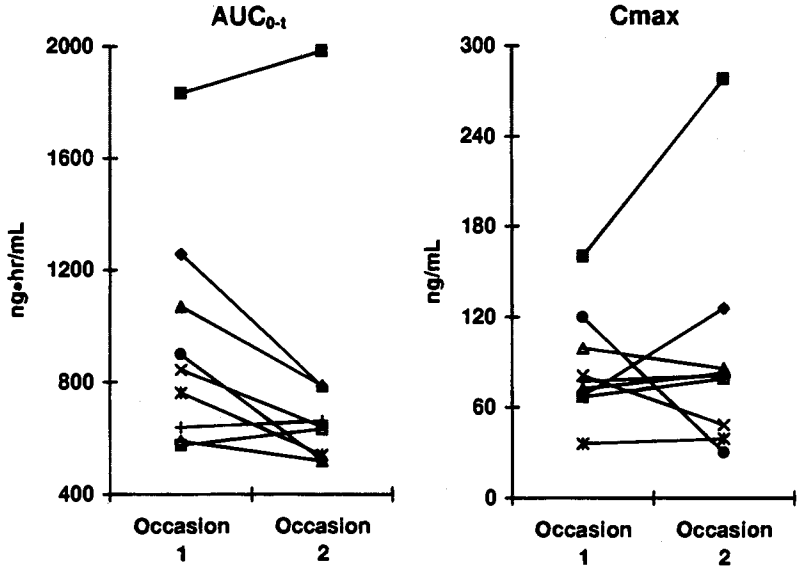

Fig. 2. Inter- and intra-subject variations of bioavailability with Isoptin SR 240 mg tablets. Each set of symbol represents data from one subject.

chosen because, unlike $AUC_{0-\infty}$, its determination is not complicated by the estimation of the terminal elimination rate constant which could be somewhat inaccurate for slow release formulations due to the possibility of an incomplete absorption phase. For the same reason, the terminal elimination half-life was not used, nor recommended for "correcting" AUC's due to apparent changes in drug clearance.

In light of the above data, the appropriateness of the current bioequivalence criteria needs to be reconsidered. One possibility is to widen the bioequivalence limits ("goalpost") for highly variable drugs from 80–125% to 70–143% as recommended in a recent report of a joint American Association of Pharmaceutical Scientists/FDA workshop (1995) on evaluation of orally administered highly variable drugs and drug formulations (9). Another possibility is to define the acceptance limits based on a multiple of the reference intra-subject cv as proposed by Boddy *et al* (10) recently. Applying their approach would also result in a widening of the acceptance limits for highly variable drugs.

The concept of studying both the test and reference products on two different occasions (4 way crossover) could also be considered under certain situations. For example, the proposal by L. Endrenyi in Bio-International 92 (11) has merit. Endrenyi suggested that two formulations could be judged bioequivalent if there were a similar degree of variation between and within products, after repeated administration of the products to an individual, when evaluating highly variable drugs like verapamil. However, increasing the numbers of studies required for demonstrating bioequivalence introduces other problems which would need addressing.

In conclusion, the assumption of constant drug pharmacokinetics between days may not be valid for highly variable drugs such as sustained release verapamil. The high intra-subject variability causes the "bioavailability" of the drug product to vary from day to day. This complicates the assessment of bioequivalence and can lead to an erroneous conclusion of bioinequivalence between the brand and a generic product under the currently accepted bioequivalence criteria. The most reasonable approach, at this point in time, appears to be that recommended at the AAPS/FDA workshop in 1995, which focussed on changing the bioequivalence limits to 70–143% for highly variable drugs, but keeping the 90% confidence interval criterion, to maintain assurance (9).

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