Evaluation of Bioequivalence of Highly Variable Drugs Using Clinical Trial Simulations. II: Comparison of Single and Multiple-Dose Trials Using AUC and Cmax[1]

Ahmed A. El-Tahtawy,[2,4] Thomas N. Tozer,[3] Ferrin Harrison,[2] Larry Lesko,[2] and Roger Williams[2]

Received August 6, 1997; accepted October 4, 1997

Purpose. Evaluating of the effects of high intrasubject variability in clearance (CL) and volume of distribution (V), on 90% confidence intervals (CIs) for AUC (Area Under the concentration Curve) in single and multiple-dose bioequivalence studies. The main methodology was Monte Carlo simulation, and we also used deterministic simulation, and examination of clinical trials. The results are compared with those previously observed for Cmax (maximum concentration.)

Methods. The time course of drug concentration in plasma was simulated using a one-compartment model with log-normal statistical distributions of intersubject and intrasubject variabilities in the pharmacokinetic parameters. Both immediate-release and prolonged-release products were simulated using several levels of intrasubject variability in single-dose and multiple-dose studies. Simulations of 2000 clinical bioequivalence trials per condition (138 conditions) with 30 subjects in each crossover trial were carried out. Simulated data were compared with data from actual bioequivalence trials.

Results. The current simulations for AUC show similar probabilities of failure for single-dose and multiple-dose bioequivalence studies, even with differences in the rate of absorption or fraction absorbed. AUC values from prolonged-release scenario studies are more sensitive to changes in the first order absorption rate constant ka, and to variability in CL and V than AUC from studies of immediate-release studies.

Conclusions. We showed that multiple-dose designs for highly variable drugs do not always reduce intrasubject variability in either AUC or Cmax, although the behavior of AUC differs from Cmax. Single dose AUC to the last quantifiable concentration was more reliable than either single dose AUC extrapolated to infinity, or multiple dose AUC during a steady-state interval. Multiple-dose designs may not be the best solution for assessing bioequivalence of highly variable drugs.

KEY WORDS: Bioequivalence; highly variable drugs; extent of absorption; rate of absorption; Monte Carlo simulations; single-dose; multiple-dose bioequivalence trial.

INTRODUCTION

The current U.S. FDA bioequivalence regulations require that AUC and Cmax means of ratios of test and reference formulations lie within specific bounds. Products are considered bioequivalent if the 90% confidence intervals of the means of the ratios lie between 0.8 and 1.25. For drugs with highly variable measures and customary numbers of subjects, bioequivalence may not be established even for identical products. Bioequivalence of highly variable drugs is a debatable and important issue that needs to be resolved (1–3). Highly variable drugs have been defined as those with intrasubject coefficients of variation (CV) greater than 30% in either the Cmax or AUC metric (1–3). High intrasubject variability can lead to an inability to declare identical drug products bioequivalent because the 90% confidence interval widens beyond the acceptance criteria. The increase in the number of subjects needed to reduce the 90% confidence interval sufficiently to pass the test may be excessive. To assess bioequivalence of highly variable drug products *in vivo* with a reasonable study size, several changes in study design have been proposed (1). One suggestion is to evaluate bioequivalence at steady-state. Another suggestion is to use replicate study designs to evaluate intrasubject variability. The steady-state suggestion is the subject of this report.

In a previous publication (4), simulated and clinical data were used to examine Cmax in single-dose versus multiple-dose bioequivalence testing of drugs, with intrasubject CVs in clearance less than 20%. That work was extended to highly variable drugs (5). The latter work showed that multiple-dose designs need not improve the performance of Cmax over single-dose designs, and often made performance worse. The current investigation examines the effect of intrasubject variability, study design, and release characteristics on the probability of failing a bioequivalence test using AUC for highly variable drugs and compares these results with the corresponding results for Cmax (5).

METHODS

Monte Carlo Simulations

Simulations were performed using a one-compartment model with first-order absorption and elimination (6) for immediate-release and prolonged-release products. Each subject received a single 1000-mg dose or multiple 1000-mg doses orally of the immediate-release product every 8 hours until steady-state was achieved. Single dose blood sampling times were 0, 0.5, 1, 2, 4, 8, 16, 24, and 36 hours. Multiple dose blood sampling times were 0, 0.5, 1, 2, 4, 6, and 8 hours, within a steady-state dosing interval, for immediate-release products. An additional sampling time of 48 hours was used for single dose studies of prolonged-release products. A 12-hour dosing interval was chosen for the multiple-dose prolonged-release studies with sampling times 0, 0.5, 1, 2, 4, 6, 8, and 12 hours. To account for lack of assay reproducibility and model misspecification, a random error for drug concentration at each sampling time was assumed to be log-normally distributed with a coefficient of variation (CV) of 15%.

Area under the curve to the last measurable time point after a single dose, AUC(0-lqc), was calculated using the trapezoidal rule to the time of the last quantifiable concentration. Let C(t) be the last quantifiable drug concentration. Let kel be the terminal elimination rate constant. Total area under the curve, AUC(0–∞), was estimated by adding to AUC(0–lqc)

[1] The views expressed in this paper are those of the authors and do not necessarily represent the policy of the Food and Drug Administration.

[2] Food and Drug Administration, Center for Drug Evaluation and Research, 5600 Fishers Lane, HFD-860, Rockville, Maryland 20857.

[3] School of Pharmacy, University of California San Francisco, San Francisco, California 94143.

[4] To whom correspondence should be addressed. (e-mail: Eltahtawya @cder.fda.gov)

0724-8741/98/0100-0098$15.00/0

the calculated remaining area, C(t)/kel. The rate constant was determined from the least squares slope of the ln concentration-time curve of the last three points (6). AUC(0–τ) during a steady-state dosing interval on multiple dosing, was calculated by the trapezoidal rule. Cmax was observed directly from the single-dose or multiple-dose data. For multiple-dose simulations, steady state was assumed to have been reached by the time of the tenth dose. Log-transformed measures for each trial were analyzed by SAS (®SAS Institute, Inc.) using a SUN Sparcstation 10. The 90% confidence intervals for the test/reference ratios were calculated as described previously (7). The 90% confidence interval was compared with the acceptable regulatory criteria, 0.8–1.25. If either limit fell outside the range, the product was declared to have failed the test.

The effect of high intrasubject variability in disposition on the probability of failing to declare bioequivalence for single-dose and multiple-dose trials using immediate-release and prolonged-release formulations was examined in five scenarios, as listed below and in Table 1. In the first four scenarios, the fraction absorbed (F) was held constant.

Table 1. Mean Parameter Values and Their Levels of Variability for the Five Scenarios Simulated

	Default values	
	Mean	Coefficient of variation
Dose (mg)	1000	0%
CL (L/hr)	1.72	50%
V (L)	20	25%
Dosing interval, hours	8 (12 for *Scenario IV*)	0%
Accumulation Index	2 (1.5 for *Scenario IV*)	0%
ka (hour)	Various	50%

Intrasubject variability, %CV			
Level	Correlation	Clearance	Volume of distribution
I	1.0	0%	0%
II	0.9	15%	8%
III	0.75	25%	12%
IV	0.5	35%	17%

Scenarios

Formulations with no differences: Scenario I

Formulations with Differences in ka

	Scenario II Immediate-release		*Scenario III* Intermediate-release		*Scenario IV* Prolonged-release	
	Test	Reference	Test	Reference	Test	Reference
ka1	1.65	1.5	0.165	0.15	0.081	0.09
ka2	1.8	1.5	0.18	0.15	0.072	0.09
ka3	1.95	1.5	0.195	0.15	0.067	0.09

Formulations with Differences in F and ka

Scenario V—Intermediate-release

ka(test) = 0.165 hr^{-1}, ka(reference) = 0.15 hr^{-1}

Ratio of bioavailabilities F (test/reference) = 1.0, 1.05, 1.1, 1.2 and 1.25.

Scenario I—Same (Equivalent) Formulations

Bioavailability (F) and first-order absorption rate constant (ka) were equal for both the test and the reference products, i.e., the same drug formulation was readministered to each subject. Three distinct measures [single dose, AUC(0–∞) and AUC(0-lqc); multiple doses, AUC(0-τ)] were used in analysis. Four different levels of intrasubject variability, and single and multiple dose designs were used for a total of 12 conditions.

Scenario II—Immediate-Release Formulations with Differences in ka

Extent of absorption (F) was equal for both the test and reference formulations. AUC(0-lqc) was analyzed only at the three highest levels of intrasubject variability in CL and V, combined with 3 levels of ka ratios [ka(test) = 1.65, 1.8, 1.95, ka(reference) = 1.5]. Three ka ratios, four levels of intrasubject variability in clearance (CL) and volume of distribution (V), and single and multiple dosing were tested, for a total of 48 conditions.

Scenario III—Intermediate-Release Formulations

The conditions tested were the same as those in *Scenario II* except the mean ka values were lower: [ka(test) = 0.165, 0.18, 0.195, ka(reference) = 0.15], for 24 conditions.

Scenario IV—Prolonged-Release Formulations

This scenario differs in two ways from *Scenarios II* and *III*. First, mean ka values of the test product were lower [ka(test) = 0.081, 0.072, 0.067, ka(reference) = 0.090] than the intermediate-release product to reflect prolonged-release conditions. Second, dosing interval (τ) was increased from 8 to 12 hours, with the accumulation index $1/(1 - e^{-k\tau})$ consequently decreased from 2 to 1.5, for 24 sets of conditions.

Scenario V—Formulations with Differences in F and ka

Fifteen situations arose from three levels of intrasubject variability, with five levels of the test/reference ratio for F, per Table 1. The ka test/reference ratio was 10% for single-dose and multiple-dose studies. This scenario produced 30 unique sets of conditions.

The five scenarios produced 138 different conditions, and the clinical trials were repeated 2000 times for each condition, with 30 patients per trial. Thus, pharmacokinetic analyses and statistical evaluations were done on 276,000 simulated clinical trials (8,280,000 subjects).

Stochastic variation in CL, and V was introduced by a random number generator, Rannor(0), in the SAS system, which creates a standard normal random deviate with a mean of zero and standard deviation of one. A bivariate log-normal distribution for all pharmacokinetic parameters was used with a correlation between treatment periods. The parameters were kept constant over each full treatment period. The correlation coefficient between treatment periods (ρ) was modeled at 1.0, 0.9, 0.75, 0.5. Intrasubject variability for CL and V corresponding to these levels of correlation is listed in Table 1. The intrasubject variability decreases with the correlation, reaching 0.0 at a value of one. This relationship is a result of intrasubject variability

depending on the difference between normally distributed treatment periods, divided by a mean. At correlation one, the random variables are equal, and their difference is zero.

The difference between formulations was simulated by creating bivariate random deviates for ka, for the test and reference formulations, using a moderate correlation ($\rho = 0.5$). Table 1 summarizes the parameters and the levels of variability used in the simulations.

Each *Scenario* has a symmetric factorial design of simulated clinical trials, including various combinations of ρ and CV and the pharmacokinetic parameters of interest, ka, F, CL, and V. Sequence and period were randomized to mimic the usual two-period crossover bioequivalence study.

RESULTS

The test and reference formulations have the same mean extent and rate of absorption in *Scenario I.* Figure 1(a,b) presents the distributions of the logarithms of test/reference ratio AUC values, for single and multiple-dose regimens in *Scenario I,* with low (Level II) and high (Level IV) intrasubject variability. The mean value for ln(AUC ratio) in *Scenario I* is near zero as expected, with very similar distributions for single and multiple-dose. For low intrasubject variability (Fig. 1a), the standard errors are SEM(SD) = 0.0010 and SEM(MD) = 0.0009. For high intrasubject variability (Fig. 1b), a similar distribution of the means is observed, since the standard errors are SEM(SD) = 0.002 and SEM(MD) = 0.002. Single and multiple-dose studies appear to have similar probabilities of failure for AUC, despite the level of intrasubject variability. In *Scenario I* for low intrasubject variability, intrasubject AUC

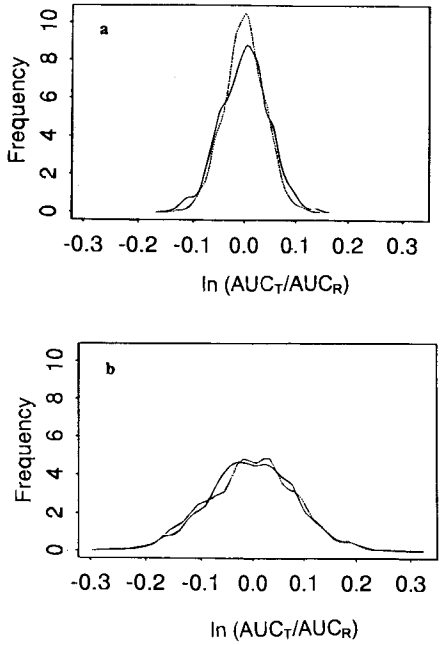

Fig. 1. Frequency distributions of mean ln AUC for 2000 bioequivalence trials simulated for single-dose (solid lines) and multiple dose (dashed lines) in *Scenario I* (no difference in ka or F between the test and reference). Fig. 1a represents the case of low intrasubject variability in CL and V (CV of 15% and 8%, respectively). In contrast, Fig. 1b shows the distribution for high intrasubject variability in CL and V (CV of 35% and 17%, respectively).

CV estimates were CV(SD) = 18% and CV(MD) = 16%. For high intrasubject variability, AUC CV estimates rose to CV(SD) = 32% and CV(MD) = 33%.

We examined Cmax performance in highly variable drugs in (5), now we examine AUC, and compare them in Table 2. The test and reference formulations are the same, so the ln ratios of Cmax and AUC are expected to be near zero, with a near zero probability of failure. Figure 2 shows AUC doing as expected when the intrasubject variability is low, as is seen at Levels I and II for which the probability of failure is 5% or less. When the intrasubject variability is high [CV(CL) = 35%, CV(V) = 17%, Level IV], AUC, like Cmax, incorrectly fails in both single (32%), and multiple-dose (36%) bioequivalence studies. This result shows the performance of ln(AUC) in single-dose and multiple-dose studies is greatly influenced by variability in disposition. Figure 2 also shows a consistently lower failure rate for AUC(0–lqc) over AUC(0–∞).

Table 2 summarizes the distribution (mean ± SEM, %ICV, mean CI) of AUC and Cmax log ratios with identical test and reference formulations. We test for the presence of bias to show the validity of the simulations. The largest of eight ratios of Z = |mean|/SEM, absolute mean over standard error of the mean, is 1.34, for a smallest p-value of 0.18 for bias. With all the p-values 0.18 or larger, we find no significant evidence of bias in the ratios of metrics, for these simulations of equivalent drugs, and the simulations appear valid.

Table 2 shows statistically significant differences between single and multiple-dose in EACH case, including the smallest

Table 2. Mean (test/reference) ± SEM[a], Intrasubject Coefficient of Variability (%ICV[b]), and 90% Confidence Intervals (CI)1 for ln AUC and ln Cmax Ratios at Low (II) and High (IV) Levels of Intrasubject Variability in CL and V When the Formulations, on Average, are the Same (Scenario I) and are Administered in Single or Multiple-Doses

	Low Intrasubject Variability (Level II)		
ln(AUC)	Mean ± SEM	%ICV	90% CI
Single	(−1.01 ± 1.05)E-3	18.2%	92.5–108.2
Multiple	(−7.20 ± 8.98)E-4	15.9%	93.4–107.1
	High Intrasubject Variability (Level IV)		
	Mean ± SEM	%ICV	90%CI
Single	(−1.86 ± 1.83 E-3	32.3%	87.4–114.8
Multiple	(−1.60 ± 1.87)E-3	33.1%	87.1–115.3
	Low Intrasubject Variability (Level II)		
ln(Cmax)	Mean ± SEM	%ICV	90% CI
Single	(9.95 ± 10.98)E-4	19.3%	92.2–108.9
Multiple	(−1.33 ± 0.99)E-3	17.5%	92.7–107.9
	High Intrasubject Variability (Level IV)		
	Mean ± SEM	%ICV	90% CI
Single	(−8.08 ± 14.3)E-4	24.5%	90.1–111.2
Multiple	(−2.33 ± 1.88)E-3	33.4%	86.9–115.3

[a] Mean, SEM, and CI are the mean values for 2000 simulated bioequivalence trials. The mean is consistently within two standard errors of 0, of equality of test to reference. This is not a surprise since the formulations are the same (ln ratio tends toward 0).

[b] %ICV is sqrt[exp(sigma^2)−1], given by Don Schuirmann. This is computed as sqrt{exp[MS CI half width*#patients/(2t^2)]−1} from the 2000 ln CIs, where t is the appropriate value from Student's t distribution. The %ICV is approximately the standard relative error of an individual=s test or reference.

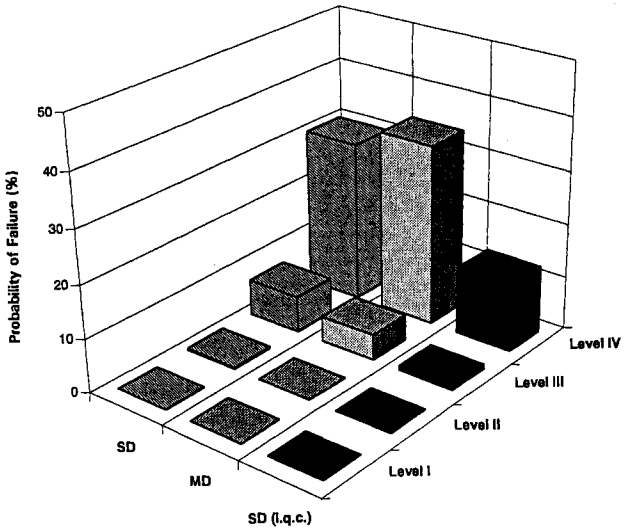

Fig. 2. Probability of failure of ln AUC for single and multiple dose studies for an immediate-release formulation with a ka ratio (test/reference) of one and 4 levels of intrasubject variability in clearance and volume (see Table 1, *Scenario I*). For single-dose studies, ln AUC(0–∞) and ln AUC(0–lqc) ratios are used while ln AUC(0–τ) ratio is used in multiple-dose studies.

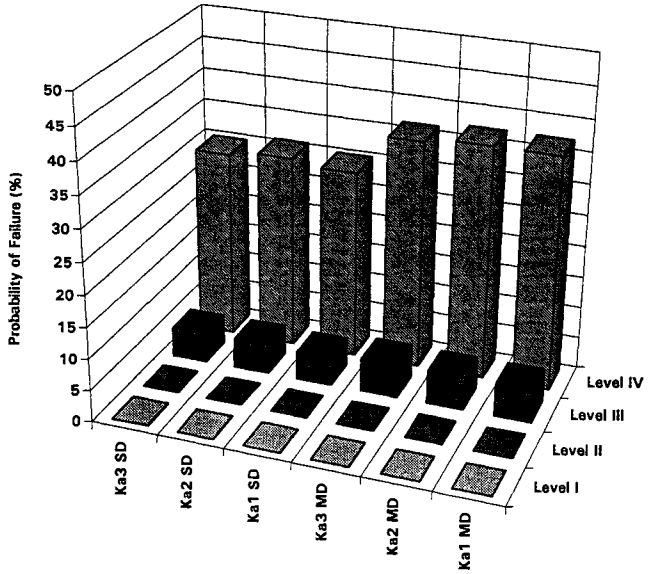

Fig. 3. Probability of failure of ln AUC(0–∞) as a function of intrasubject variability in clearance and volume for immediate-release formulations. Four levels of intrasubject variability and 3 different ratios of ka(test)/ka(reference) are tested (see Table 1, *Scenario II*).

difference of ICV(SD) = 33.1% versus ICV(MD) = 32.3%. With low intrasubject variability, multiple-dose gives lower %ICVs and narrower CIs than single-dose. With high intrasubject variability, multiple-dosing gives a higher %ICV and wider CIs than single-dose. The greatest difference between single and multiple-dose is with high intrasubject variability in Cmax, shown by increased variability (%ICV ratio or CI width). This increased variability means one needs 1.8 times as many multiple dose patients as single dose to show bioequivalence of identical formulations.

Figure 3 shows increasing levels of intrasubject variation in CL and V, influencing the probability of failing the bioequivalence test for immediate-release formulations with differences in ka (see Table 1, *Scenario II*). AUC fails slightly more for single-dose than for multiple-dose studies with low intrasubject variability in CL and V. AUC fails comparably or less for single-dose with high variability. No effect was noted for the formulation differences in ka.

Figure 4 shows the probability of failure for AUC(0-lqc) to be almost half that for AUC(0–∞). This becomes apparent when the data in Fig. 3 are compared with those in Fig. 4. The extrapolated values for AUC(0–∞) usually produce biased estimates, and can lead to an increase in intrasubject variability. AUC(0–lqc) was also found the more reliable metric by (9). In *Scenario II,* single dose AUC(0–lqc) has a much lower probability of failure than steady state AUC(0–τ).

Prolonged-release formulations (*Scenario IV*) show a greater probability of failure for AUC as the level of intrasubject variation increases (Fig. 5). Unlike the result in immediate-release products, the single-dose design appears more sensitive to variability in rate of absorption than multiple-dose. The results (not shown) with intermediate-release products (*Scenario III*) are between those for immediate-release (Fig. 4) and prolonged-release (Fig. 5) products.

In *Scenario V,* the relationship between the fraction available (F) and the probability of failure of AUC was investigated at different levels of intrasubject variability for immediate-release formulations (Fig. 6). When the F(test)/F(reference) ratio was 1.25, the probability of failure of AUC exceeded 95% for both single and multiple-dose studies, for each level of intrasubject variability. When the F ratio was 1.2 or less, the failure rate increased as intrasubject variability increased. The probability curves show this result was virtually the same for both single and multiple-dose studies. Multiple-dose adminis-

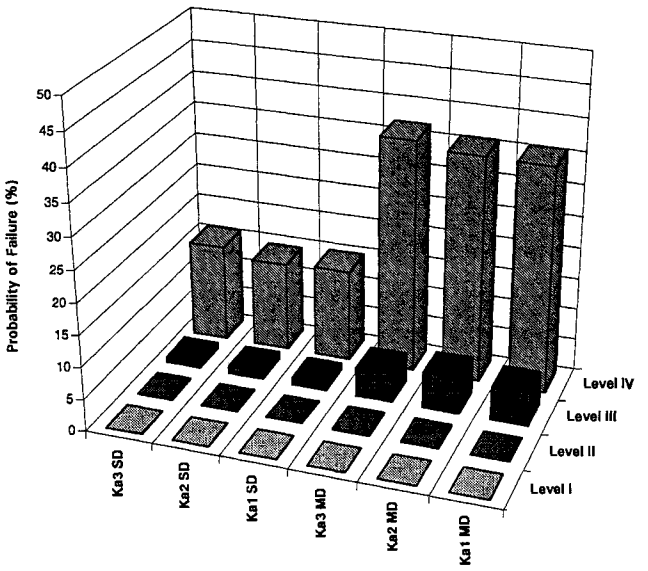

Fig. 4. Probability of failure of ln AUC(0–lqc) as a function of intrasubject variability in clearance and volume, under the same conditions as Fig. 3. (See Table 1, *Scenario II*).

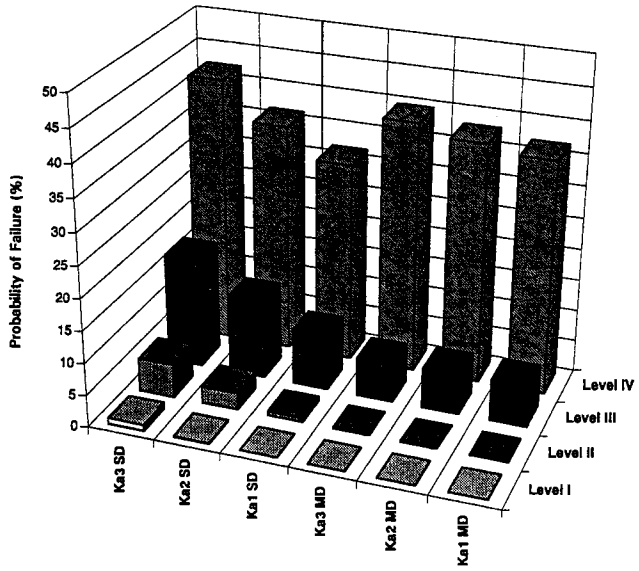

Fig. 5. Probability of failure of ln AUC(0–∞) for prolonged-release formulations as a function of intrasubject variability in clearance and volume and formulation differences in ka (see Table 1, *Scenario IV*). The ratios of ka(test)/ka(reference) are: $ka_1 = 0.081/0.09$; $ka_2 = 0.072/0.09$; $ka_3 = 0.067/0.09$.

Table 3. Comparative Ratios of the Mean Values for AUC and Cmax Ratios, and the 90% Confidence Intervals (CI) for Different Single-Dose and Multiple-Dose Bioequivalence Studies

		AUC		Cmax	
Study Drug	N[a]	Ratio	90% CI P/F[c]	Ratio	90% CI P/F[c]
Single-Dose Studies					
Ca blocker 1[b]	24	0.95	84–104 P	0.96	91–115 P
antibiotic 1[b]	35	0.96	84–119 P	0.88	66–103 F
Ca blocker 2	14	1.15	98–135 F	1.11	98–128 F
antihistamine 1	42	0.79	65–92 F	0.75	63–86 F
antihistamine 2	24	0.97	69–118 F	0.84	67–98 F
Antiinflammatory 1	30	0.99	95–106 P	0.86	80–99 P
Multiple-Dose Studies					
Ca blocker 1[b]	24	0.87	79–92 F	0.96	93–109 P
antibiotic 1[b]	35	0.97	79–103 F	0.94	74–100 F
Ca blocker 2	24	0.91	78–93 F	0.94	85–102 P
antihistamine 1	13	1.32	119–146 F	0.99	78–121 F
Ca blocker 3	50	1.19	111–126 F	1.11	100–120 P
Antiinflammatory 1	32	0.99	93–106 P	0.84	70–94 F

[a] N = number of subjects in study.
[b] Single-dose and multiple-dose studies were conducted on the same subjects.
[c] P/F-pass/fail by CI in the 80–125 range.

tration under the conditions of low intrasubject variability (Level II, III) has a slightly lower probability of failure for AUC.

Clinical data submitted to the FDA in Table 3 includes examples of highly variable drugs failing the current bioequivalence criteria. Most of the multiple-dose studies in Table 3 fail. Single and multiple-dose studies were conducted on the same subjects for the first two drugs, with AUC passing both single dose studies, and failing both multiple dose studies, as we might suspect from the simulations in Table 2. These two drugs show no difference in Cmax failure between single and multiple dose, one passing and one failing. The results on these two drugs support the simulation results.

The pairs of independent trials in Table 3 show multiple doses studies can fail. Calcium (Ca) blocker 2 has results AUC-Fail, Cmax-Fail on 14 single-dose patients, and AUC-Fail, Cmax-Pass on 24 multiple-dose patients. With 71% more

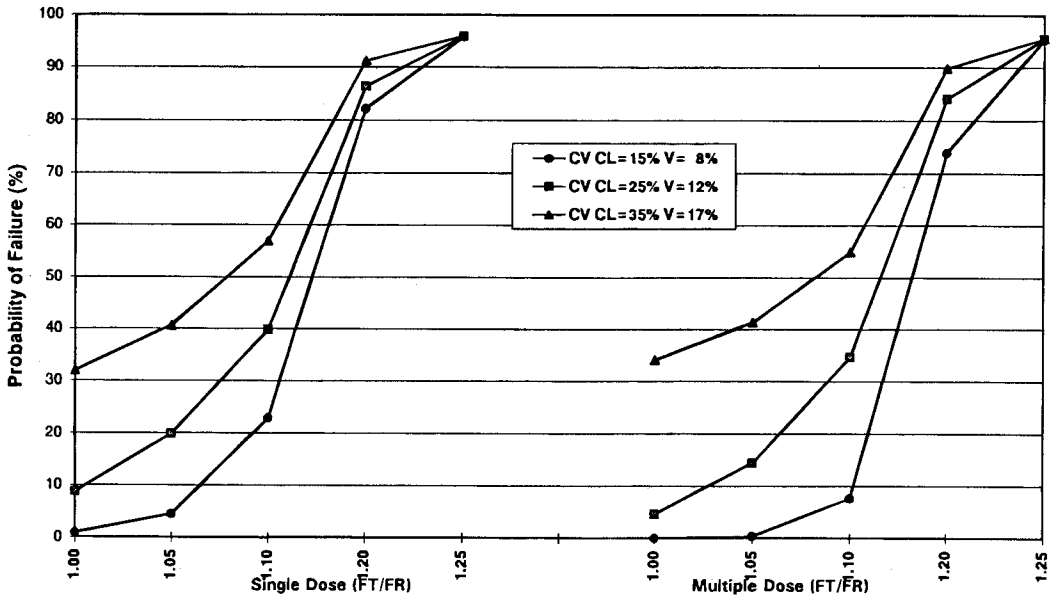

Fig. 6. Probability of failure of ln AUC(0–lqc) for intermediate-release ($ka_T = 0.165$, $ka_R = 0.15$) formulations of various values of F(test)/F(reference) (1, 1.05, 1.1, 1.2, 1.25 and intrasubject variabilities (level II to IV, see Table I, *Scenario V*).

patients, the partial success in the multiple-dose study is not much of an accomplishment. An antiinflammatory drug barely passes on Cmax in the 30 patient single-dose study at a ratio of 80, but fails in the 32 patient multiple dose study, supporting the thesis that single dose is better in highly variable drugs.

Findings from the simulation studies are reflected in the results of actual clinical bioequivalence trials (Table 3). Antihistamine 1 and antihistamine 2 are examples of immediate-release products, with high intrasubject variability (CV > 30%) in AUC and Cmax, that failed the bioequivalence test. Ca blocker 1 is an example of a drug that passes single-dose but not multiple dose trials. Ca blocker 2 is a drug product of moderate (20%) intrasubject variability and, on going from single-dose to multiple-dose trials, the confidence interval of Cmax met the bioequivalence criteria while that of AUC did not. The shrinkage and shift of the confidence interval on multiple dosing, like that of drugs of low intrasubject variability, is noteworthy. Ca blocker 3 has saturable first-pass metabolism, and AUC (but not Cmax) failed the bioequivalence test on multiple dosing in spite of the large number of subjects used. This result may be explained by AUC being influenced not only by clearance but also by extent of absorption (F).

With respect to the hypothesis that more subjects will yield a passing trial, consider the single dose study of 42 antihistamine 1 patients, failing in both metrics. A more dramatic example is the multiple dose study of 50 calcium blocker 3 patients, passing on Cmax but failing on AUC. These customary increases in numbers of patients do not necessarily give passing trials.

DISCUSSION

Simulating variability encountered in human bioequivalence trials helps reveal the ability of a given measure to find a true difference between two products, covering a more comprehensive variety of cases than are available from clinical trials. Simulating variability helps explain, predict, and add rigor to the debate on the use of various measures and bioequivalence designs, relative to examining only clinical trials and deterministic simulations.

A current recommendation (1–3,8) for highly variable drugs is to conduct the bioequivalence trial at steady state whenever a multiple-dose design is ethically acceptable. This report and (5) show that multiple dosing may not reduce the 90% confidence interval for AUC or Cmax ratios so that they fall within the currently specified limits (0.8–1.25). This is especially the case for Cmax (5). In addition to not helping, multiple-dose designs may make the situation worse by raising intrasubject variability, contrary to (8). This is especially true with prolonged-release formulations. As apparent from both the simulated data and clinical data, the multiple-dose study design may be more sensitive to clearance for the prolonged-release dosage formulations, even when true differences in ka are small.

Cmax is a measure of both input and disposition. Perhaps a measure of input alone would not show an increased probability of failure in multiple dose trials.

An explanation for the more frequent failure of Cmax after multiple dosing than after a single dose is shown in Fig. 7A. Variability in clearance produces a much greater variability in Cmax at steady state than after a single dose. This is a result of Cmax primarily reflecting clearance at steady state. The greater the extent of drug accumulation, the greater is this tendency. Variability in volume of distribution, on the other hand, produces much greater variability in Cmax after a single dose (Fig. 7B). The more rapid the absorption compared with disposition for immediate-release products, the greater the tendency for Cmax to reflect variability in volume of distribution after a single dose. Simulations in this study primarily show the results of variability in clearance, because clearance was more variable.

Usually, one expects intraindividual variability in volume of distribution to be small. In contrast, clearance has circadian and other periodic changes, due to changes in renal function, plasma protein concentration, hepatic function and blood flow. So clearance is expected to show greater intraindividual variability. For purposes of the simulations, both parameters were held constant over the time of the single dose and steady-state observations.

Bioavailability is another source of variability. A change in absorption (F) should change Cmax equally in single and multiple-dose studies, since Cmax primarily reflects V/F after a single dose and CL/F at steady state. If F varies between doses, one would expect more Cmax variability in single than multiple dose studies. This expectation is based on averaging F before the last dose. Changes in the rate-time profile of absorption, produced by variability in gastrointestinal physiology, especially by food, are expected to affect Cmax more after a single dose than at steady state. At steady state, the observation is the sum of the last dose and what remains from previous doses, with variability in Cmax damped by the remains from previous doses.

Observations on chronic dosing may reveal time-dependent changes in clearance and extent of absorption. However, determination of bioequivalence at steady state masks real differences in ka, regardless of whether the drugs has high or low variability. Comparing two drug products for extent of bioavailability at steady state does not provide a real advantage for drugs of low intrasubject variability. For highly variable drugs, the precision and bias of AUC(0–τ) were comparable to AUC(0–∞) in single dose studies. AUC(0–lqc) outperformed both AUC(0–∞) and AUC(0–τ).

This work emphasizes that multiple-dose study designs do not necessarily provide an advantage in bioequivalence testing of highly variable drugs. Perhaps bioequivalence criteria for highly variable drugs should be tailored to each drug, using the drug intrasubject variability and its relevant pharmacokinetic parameters. Stochastic simulations can help define the appropriate measure and confidence limits. To guard against any increase in consumer risk, perhaps intrasubject variability should be estimated using replicate designs. The therapeutic index and the steepness of the dose (concentration)-response relationship should be considered before widening the acceptance limits.

For one extended-release Antiinflammatory study, intrasubject variability was above 25%. As shown in Table 3, the Cmax failure rate was lower in single versus multiple-dose designs when intrasubject variation was above 25%. Prolonged-release and immediate-release dosage forms may be far less sensitive to intrasubject differences in clearance and volume in

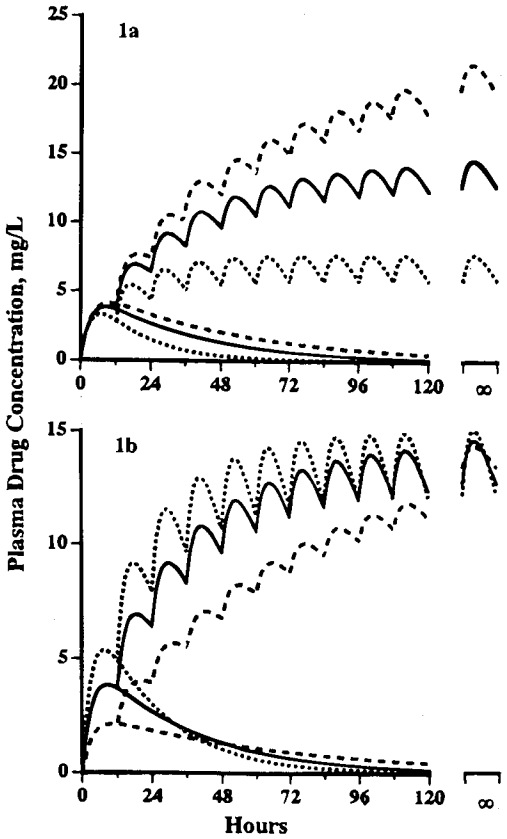

Fig. 7. The concentration-time curve and Cmax are greatly different due to variations in clearance (top graph) and volume of distribution (bottom graph) between single and multiple doses. When clearance (1.5(——), 3.0(- - - -), 1.0 (----) liters/hour), Cmax is only slightly altered after a single dose, but highly altered during a steady-state interval (insert on right of top graph). Conversely, when volume of distribution varies (50(——), 33.3 (- - - -), 100 (----) liters), Cmax after a single dose is highly altered, but little change occurs under steady-state conditions (insert on right of bottom graph). The simulations are based on a one-compartment model with first-order absorption. Default characteristics: bioavailability = 1.0; first-order absorption rate constant = 0.3 $hour^{-1}$, volume of distribution = 50 liters; clearance = 1.5 liter/hour. The single dose is 250 mg. The multiple-dose regimen is 250 mg every 12 hours.

single dose designs than in multiple dose. This is seen by the superimposability in Figures 4 and 5 of the multiple-dose probabilities of failure, for all ka values. At any level of intrasubject variability and ka ratio, prolonged-release formulations appear to have a higher probability of failure.

Clearly, the probability of failure of AUC is sensitive to intrasubject variability in disposition. Failure of AUC, and Cmax, can increase in multiple-dose compared with single-dose studies when intrasubject variability in disposition is high. AUC is more sensitive to changes in ka, and increased variability in CL and V for extended-release than in the immediate-release products. Increasing the acceptance criteria (from 0.8–1.25 to 0.75–1.33) may produce a large decrease in the probability of failure for highly variable drugs (5). This would increase the probability of the Cmax value meeting the acceptance criterion for formulations that are truly bioequivalent. The range should probably be widened only for drugs of suitably large therapeutic indices and high intrasubject variability. For drugs of unknown intrasubject variability, replicate designs may be needed to justify the assumption of high intrasubject variability. Other alternatives should be considered. These include selecting the best measure of absorption rate based on simulation of the particular situation in bioequivalence trials (10), indexing criteria to reference product variability and therapeutic index (11), and replicate designs for individual bioequivalence (12).

REFERENCES

1. K. K. Midha and H. H. Blume. Bio-International, Medpharm, Stuttgart, Germany, (1993).
2. BioInternational-94, Munich Germany, June 15–17, (1994).
3. V. Shah, A. Yacobi, W. H. Barr, L. Z. Benet, D. Breimer, M. R. Dobrinska, L. Endrenyi, W. Fairwheather, W. Gillespie, M. A. Gonzalez, J. Hooper, A. Jackson, L. J. Lesko, K. K. Midha, P. K. Noonan, R. Patnaik, and R. L. Williams. *Pharm. Res.* **13**:1590–1594, (1996).
4. A. A. El-Tahtawy, A. J. Jackson, and T. M. Ludden. *Pharm. Res.* **11**:1330, (1994).
5. A. A. El-Tahtawy, A. J. Jackson, and T. M. Ludden. *Pharm. Res.* **12**:1634, (1995).
6. M. Gibaldi and D. Perrier, Pharmacokinetics, Marcel Dekker, New York, (1982).
7. D. Schuirmann. *J. of Pharmacokin. and Biopharm.* **15**:657, (1987).
8. H. Blume, B. Scheidel, and M. Siewert. In: K. K. Midha and H. H. Blume (eds.), Bio-International, Medpharm, Stuttgart, Germany, 37–52, (1993).
9. F. Bois, T. N. Tozer, W. W. Hauck, M. L. Chen, R. Patnaik, and R. L. Williams. *Pharm. Res.* **11**:715, (1994).
10. F. Bois, T. N. Tozer, W. W. Hauck, M. L. Chen, R. Patnaik, and R. L. Williams. *Pharm. Res.* **11**:966, (1994).
11. L. Sheiner. *Statistics in Medicine.* **11**:1777, (1992).
12. R. Schall and R. L. Williams. *J. Pharmacokin. Biopharm.*, **24**:133–149, (1996).