The Role of Metabolites in Bioequivalency Assessment. III. Highly Variable Drugs with Linear Kinetics and First-Pass Effect

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Purpose. Simulated pharmacokinetic (PK) studies were done to determine the effect of intrinsic clearance (CL_{INT}) on the probability of meeting bioequivalence criteria for extent (AUC) and rate (Cmax) of drug absorption when the absorption rate and fraction absorbed (F) were formulated either to be equivalent or to differ by 25%.

Methods. Simulated PK studies were done using a linear first-pass model with CL_{INT} values ranging from 15 L/HR to 900 L/HR. Test/Reference absorption rate constants (Ka) and fraction absorbed (Fa) ratios of 1.0 or 1.25 were used for all simulations. The impact of the value of CL_{INT} and its intrasubject variation upon the probability of concluding bioequivalence at the two different Ka and F ratios was studied. Additionally, the effect of fraction metabolized i.v., (Fm) on the probabilities of concluding equivalence was studied at values of 0.25 and 0.75.

Results. When CL_{INT} values were raised above those for liver blood flow, the frequency of trials in which bioequivalence was correctly declared decreased when parent AUC was used as a bioequivalence criterion. Only when CL_{INT} exceeded liver blood flow did the metabolite become important in assessing extent of absorption.

Conclusions. The Cmax for the parent drug provided the most accurate assessment of bioequivalence. The Cmax for the metabolite was insensitive to changes related to rate of input, and when CL_{INT} exceeded liver blood flow, evaluation of the metabolite Cmax data may lead to a conclusion of bioequivalence for products that were not.

KEY WORDS: intrinsic clearance; metabolites; bioequivalence.

INTRODUCTION

The importance of metabolite evaluation to the approval of generic drug products has assumed pivotal proportions since the passage of the Drug Price Competition and Patent Term Restoration Act of 1984. However, there remains a great deal of concern related to their proper role in the determination of rate and extent for bioequivalence studies. Recent research has indicated that metabolites may not need to be considered when intrasubject variability is low, and that their importance increases as the level of intrasubject variability in their elimination and level of pharmacologic activity rises (1).

Other authors have argued that for drugs with a first-pass effect, the ratio of Test/Reference AUC's for the metabolite versus the parent drug will not be bioequivalent. These arguments were based upon the assumption that the metabolite data was independent of the F_H, the fraction of drug escaping first pass (2). It has been proposed that when a parent drug and metabolite are equipotent and present in about the same concentration, then bioequivalence should be declared even if only one of the moieties meets the confidence interval (3). A major unresolved issue concerns the relative sensitivities of the parent drug and metabolite to pharmacokinetic variability for different types of formulations (i.e., immediate vs. controlled-release) and for drugs exhibiting nonlinear kinetics. This ultimately affects their usefulness to accurately assess "true differences" between formulations, and thus raises the important question as to which species is best suited to measure bioequivalence. Of even greater importance to bioequivalence determination is how the parent and metabolite confidence intervals (CI) respond to true difference between the test and reference means for AUC and Cmax.

The objective of this study was to further investigate the response of parent and metabolite confidence intervals to equivalent and inequivalent immediate-release formulations. Of special interest was whether the result was influenced by the level of variability in the renal clearance of the metabolite (Clr), and of the magnitude of CL_{INT}. Simulations were designed with controlled levels of error to represent typical bioequivalence trials and to maximize metabolite impact on bioequivalence by having only one formed metabolite. Results from simulated studies were compared to experimental data for some highly variable drugs with high CL_{INT} to determine if the simulated results were supported by clinical data.

METHODS

Monte Carlo Simulations

The simulations were all done with random error (based upon a log-normal distribution) added to the model parameters for the following:

Renal clearance-7.2 L/HR; Hepatic clearance-45 L/HR; Systemic clearance-52.2 L/HR; Liver blood flow-90 L/HR; Volume of distribution parent-100 L; and Volume of distribution metabolite-100 L. Simulations were done at ratios of 1.0 and 1.25 for the absorption rate constant (Ka) and fraction available (F) using the following values:

	Test	Reference
Ka	0.75 hr-1	0.75 hr-1
Ka	0.94 hr-1	0.75 hr-1
F	0.99	0.99
F	0.79	0.99

These simulations were done using a linear first-pass model previously presented by Weiss (4) shown in Fig. 1. Sampling times were: 0, 0.25, 0.75, 1, 1.25, 1.5, 2.0, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 60, 84 and 108 hrs after dosing.

The following relationships were used to define model parameters:

$$CL_H = Q_H * CL_{INT}/Q_H + CL_{INT} \quad (1)$$

$$F_H = Q_H/Q_H + CL_{INT} \quad (2)$$

$$E_H = CL_{INT}/Q_H + CL_{INT} \quad (3)$$

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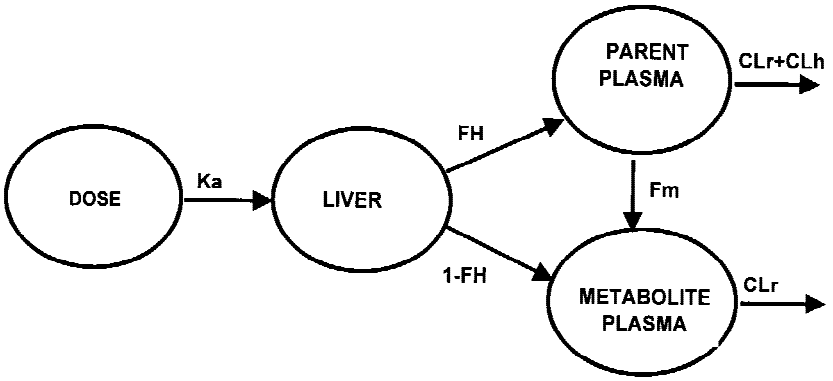

Fig. 1. First-pass pharmacokinetic model representing the liver used to simulate parent and metabolite plasma levels. Fm is the fraction of an intravenous drug converted to metabolite, F_H is the fraction of oral drug available, and CL_S is systemic clearance of parent. F_H and $(1 - F_H)$ are the the amount of parent drug absorbed and amount of metabolite formed via first-pass. CL_R is the renal clearance.

$$CL_s = CL_H + CL_R \quad (4)$$

Cl_s is the systemic clearance of the parent drug (hepatic and/or renal). Cl_p, which represents other clearances for the parent drug, was set equal to CL_R CL_{INT} is intrinsic clearance, Q_H is liver blood flow, F_H is the fraction escaping liver first-pass metabolism, Fm is the fraction of intravenous drug converted to metabolite, and E_H is the hepatic extraction ratio. The following intrinsic clearance values were studied: 900 L/HR, 90 L/HR, 48.6 L/HR, 30 L/HR, and 15 L/HR. The fraction metabolized, Fm based upon IV administration was set at 0.75 and reduced to 0.25 for selected simulations. The intrasubject variation was set at 40% for Ka, 40% for CL_{INT}, and 40% or 60% for Fm. Each simulation for 40% intrasubject variation on Fm was done at 20%, 40%, and 60%; intrasubject variability on CL_R, while simulations for which intrasubject variation was 60% on Fm were done at only 60% intrasubject variability on CL_R. Intrasubject variation in volume of distribution and liver blood flow was set at 20% (5,6,7). A summary of the simulations done for this study is presented in Table I. Bivariate log normal distributions were generated for Ka, CL_{INT}, ClR, and Fm using SAS (Statistical Analysis System, Cary, North Carolina) and plasma levels were calculated using SAAM (Simulation Analysis and Modeling, NIH, Bethesda, Maryland) (8). Random assay error (i.e., standard deviation) was added to each concentration as $\sigma_c = (0.2 * Cp+1)$ (9) where Cp is the generated concentration value. Fraction absorbed (F) was generated using a uniform distribution centered at 0.79 and 0.99 for test, and at 0.79 for reference. These generated plasma levels were statistically analyzed using SAS. The number of subjects for each simulation was 36, which was repeated 1,000 times for each case. The fraction of trials in which bioequivalence was declared was recorded. This value corresponds to the probability of declaring bioequivalence for parent drug and metabolite AUC and Cmax given a typical design of a clinical trial, human and analytical variability, data treatment procedures, and statistical analysis. All simulations were performed on a Compaq 5133 personal computer.

Experimental Data

Bioequivalence data from fasting single dose clinical trials for isosorbide dinitrate, terfenadine, and clomipramine (obtained from Abbreviated New Drug Application drug studies submitted to the Office of Generic Drugs) were evaluated. All subjects were males between the ages of 18–45 years and within 15% of ideal body weight. Drug washout periods

Table I. Simulation Scenarios for Fm = 0.75 with 40% and 60% Intrasubject Variation[1]

Intrasubject variation Fm Simulation	40% 0.75 Ratios	60% 0.75 Ratios	60% 0.25 Ratios
Number 1	Ft/Fr = 1.00	Ft/Fr = 1.00	—
	Kat/Kar = 1.00	Kat/Kar = 1.00	—
Number 2	Ft/Fr = 1.00	Ft/Fr = 1.00	Ft/Fr = 1.00
	Kat/Kar = 1.25	Kat/Kar = 1.25	Kat/Kar = 1.25
Number 3	Ft/Fr = 1.25	—	—
	Kat/Kar = 1.00	—	—

[1] Each scenario was performed at intrinsic clearance values of 15 L/HR, 30 L/HR, 48.6 L/HR, 90 L/HR, and 900 L/HR. Intrasubject variation on renal clearance (CL_R) was set at 60%, 40%, and 20% for each simulation when intrasubject variation on Fm = 40%. When intrasubject variation on Fm was 60% only 60% intrasubject variation on (CL_R) was investigated. The mean intrasubject variation on intrinsic clearance was 40%.

were one week for isosorbide dinitrate and terfenadine and three weeks for clomipramine. Study and analytical details are presented in Table II.

RESULTS

Monte Carlo Simulations

The effects of CL_{INT} and ClR on the probability of concluding bioequivalence using AUC parent and metabolite values is presented in Fig. 2. Fig. 2A indicates that for bioequivalent products, the parent drug adequately indicates bioequivalence until CL_{INT} exceeds liver blood flow. Then, up to the CL_{INT} of 90 L/HR, the parent drug is the better predictor of bioequivalence irrespective of the level of ClR error. At the CL_{INT} above liver blood flow the metabolite is the superior predictor of bioequivalence except at the 60% level of intrasubject variation in ClR, where the probability of concluding bioequivalence is comparable to the parent. In Fig. 2B, when the ratio for KaT/KaR = 1.25 (the rate constant for absorption test product/rate constant for absorption reference product) and FaT/FaR = 1.0 (fraction of drug available for test product/fraction of drug available for reference product), the parent drug again maintains a high probability of concluding bioequivalence for AUC until CL_{INT} exceeds liver blood flow. In Fig. 2C when KaT/KaR = 1.0 and FaT/FaR = 1.25, the probability of concluding bioequivalence is at the nominal level of 5% for parent and metabolite, consistent with the 25% difference in fraction absorbed between the formulations. Fig. 3A shows that the probability of concluding bioequivalence for Cmax even when KaT/KaR = 1.25 is always at or near 100% for metabolite, which indicates that there is not a direct relationship between Cmax and the underlying rate constant Ka. The probability of concluding bioequivalence of Cmax for the parent drug is similar to the metabolite until CL_{INT} approaches liver blood flow. At that time, the parent drug responds (i.e., probability of concluding bioequivalence decreases) to the 25% differences in Ka between test and reference products. Primary metabolite Cmax values were insensitive to changes in CL_{INT} although at 60% intrasubject variability in ClR, there was a small decrease in power. Fig. 3B indicates that with equivalent PK parameters (that is, KaT/KaR = 1.0 and FaT/FaR = 1.0) the parent drug Cmax CI shows sensitivity as CL_{INT} exceeds liver blood flow, again demonstrating that observed changes in the Cmax CI for the parent in high clearance drugs can be a result of changes in CL_{INT} and not only in Ka. The probability of concluding bioequivalence for Cmax using metabolite data was not greatly affected by changes in CL_{INT} for products with equivalent Ka's. Fig. 4A and 4B represent the effects of change in Fm (with 60% intrasubject variation on CL_R and Fm) on the probability of concluding bioequivalence for AUC and Cmax when KaT/KaR = 1.25 and FaT/FaR = 1 as a function of CL_{INT}. The data show that the parent drug and metabolite have comparable power to predict bioequivalence until CL_{INT} exceeds liver blood flow. As CL_{INT} increases, however, the metabolite shows the greater probability of indicating bioequivalence for AUC. There appeared to be no major impact of Fm on power. In Fig. 4B Cmax for parent was an insensitive predictor of bioequivalence until CL_{INT} exceeded liver blood flow at Fm = 0.25 and at Fm = 0.75. Again, the probability of concluding bioequivalence using metabolite Cmax was not affected by changes in CL_{INT} and Fm.

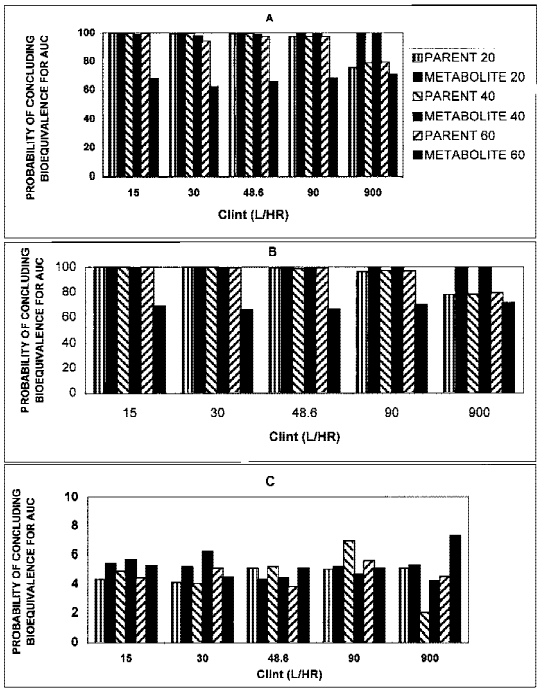

Fig. 2. The effects of CL_{INT} (intrinsic clearance) on the probability of concluding bioequivalence for parent drug and metabolite AUC as a function of metabolite intrasubject variability of 20%, 40%, and 60% on renal clearance (Clr) when the ratios for KaT/KaR and FaT/FaR are: A. KaT/KaR = 1.0, FaT/FaR = 1.0; B. KaT/KaR = 1.25 and FaT/FaR = 1.0; C. KaT/KaR = 1.0 and FaT/FaR = 1.25.

Table II. Study Characteristics for Isosorbide Dinitrate, Terfenadine, and Clomipramine

	Subjects	Dose	Assay CV[1] at LOQ[2]	Accuracy	Linear range
Isosorbide Dinitrate	28	40 mg	2.0%	107% (4 ng/ml)	1–60 ng/ml
2-ISMN			2.7%	112% (4 ng/ml)	1–60 ng/ml
5-ISMN			2.3%	101% (20 ng/ml)	5–300 ng/ml
Terfenadine	25	120 mg	3.1%	90% (0.3 ng/ml)	0.1–5 ng/ml
Metabolite M1			8.6%	99.6% (25 ng/ml)	10–201 ng/ml
Clomipramine	36	50 mg	3.3%	96.5% (1 ng/ml)	0.5–99 ng/ml
Desmethylclomipramine			4.2%	98.6% (1 ng/ml)	0.5–20 ng/ml

[1] Coefficient of Variation.
[2] Limit of Quantitation.

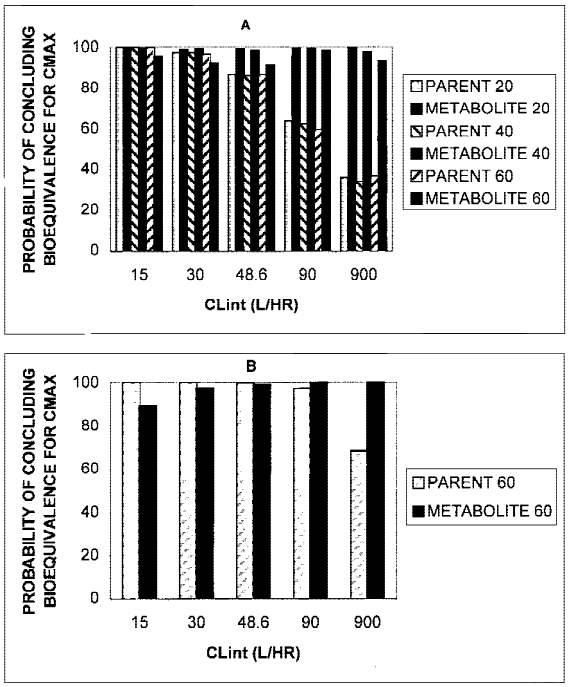

Fig. 3. The probability of concluding bioequivalence for Cmax using the simulation conditions in Fig. 1. The ratios for KaT/KaR and FaT/FaR are: A. KaT/KaR = 1.25 and FaT/FaR = 1.0; B. KaT/KaR = 1.0 and FaT/FaR = 1.0 (60% Clr only).

The experimental data in Table III show that for terfenadine and isosorbide dinitrate, drugs with high CL_{INT} (10,11) the metabolite CI has a smaller range for AUC and Cmax compared with that of the parent compound because the intrasubject variation for the metabolite is smaller. For the clomipramine metabolite the intrasubject variability for only AUC was greater than the parent drug. For all three drugs, the intrasubject variation for Cmax was less for the metabolite than the parent, resulting in much smaller CI centered near 1.0. This decreased variability means one needs fewer subjects and has greater statistical power to declare bioequivalence with respect to Cmax for the metabolite than for the parent drug.

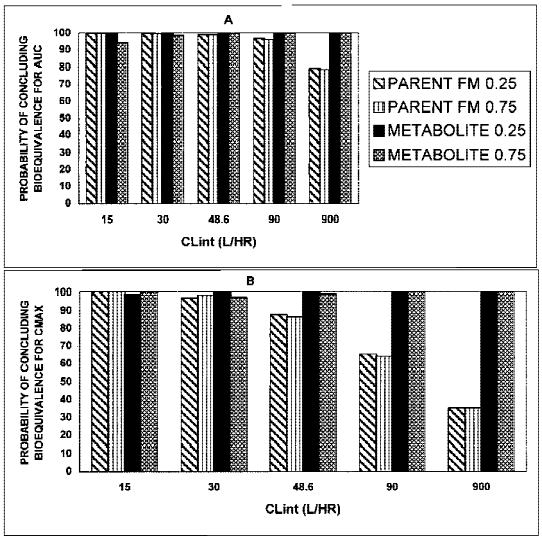

Fig. 4. Effect of Fm (fraction of drug metabolized intravenously) on the probability of concluding bioequivalence for AUC, (A) and Cmax, (B) a function of CL_{INT}. The ratios for KaT/KaR and FaT/FaR are KaT/KaR = 1.25 and FaT/FaR = 1.0, respectively.

DISCUSSION

The utility of metabolites in determining bioequivalence depends upon the metabolite data's ability to reflect true formulation differences, not merely changes in distribution. Primary metabolites should be looked upon as a potential source of information additional to that of the parent drug, or, in certain situations, as a surrogate for the parent. Their true importance relies upon the quantifiability of the parent compound. The simulations show that metabolites have a role in determining the extent of bioequivalency whenever the CL_{INT} approaches liver blood flow. The parent drug data in Fig. 2A–C allowed accurate prediction of extent of bioequivalence when in fact the fractions absorbed were equivalent, or when the test rate and/or extent of absorption was increased by 25%. However, the metabolite data seemed to predict bioequivalence better than did the parent drug data whenever CL_{INT} approached and or exceeded liver blood flow. The experimental data for clomipramine are similar to the simulations represented in Fig. 2A and 2B in that clomipramine has a reported first-pass of 50% (12). The parent drug met the CI requirements while the metabolite did not, since the parent has less intrasubject variability and greater power. When the CL_{INT} exceeded liver blood flow in the simulations, the metabolite was the better predictor of extent of absorption when the products were bioequivalent. This was similar to terfenadine and isosorbide dinitrate compounds whose first-pass is reported to be approximately 99% (10,11). When the extent of absorption is equivalent and one looks at the effect of changing the fraction metabolized intravenously, Figure 4A indicates that the parent is still the best predictor until CL_{INT} values exceed liver blood flow, after which the metabolite begins to better predict bioequivalence, as seen in Figure 4A. The data also show that the probability of concluding bioequivalence for the metabolite depends little on the fraction metabolized intravenously, Fm. As Fm was decreased from 0.75 to 0.25, the probability of concluding bioequivalence for the metabolite was unchanged. The simulated and experimental data support those from previous studies (1) which showed that the Cmax for metabolite is insensitive to changes in Ka. It was previously shown that a 25% change in Ka only results in a 4–7% change in Cmax for the parent drug under several different conditions of simulation (13). The results from this study support those findings since in Figure 3A, the Cmax for the parent drug is unresponsive to the 25% difference in Ka until CL_{INT} exceeded liver blood flow. Conversely, use of the metabolite led to a conclusion of bioequivalence for Cmax 100% of the time, even with a difference of 25% in Ka. A dilemma occurs when the formulations are bioequivalent, but use of the parent (which responds to changes in CL_{INT}) leads to a conclusion of bioequivalence for Cmax only 70% of the time with bioequivalent products at a CL_{INT} = 900 L/HR. In this case, one obtains a more accurate prediction from the metabolite only because it is insensitive to formulation changes. Perhaps in cases such as these, use of a different metric for rate of absorption would be more

Table III. Relationship of Parent and Metabolite Mean AUC Infinity (AUCI) and Cmax Values, Intrasubject Variability, Test to Reference Ratio, and 90% Confidence Intervals[1]

Parent drug/metabolite	Intrasubject variability	*Test* AUCI reference	90% CI	Intrasubject variability	*Test* Cmax reference	90% CI
Terfenadine	48.3%	1.18	83–144	39.5%	1.08	86–122
Metabolite[2]	14.7%	1.03	101–119	16.6%	0.99	91–113
Isosorbide Dinitrate[2]	18.2%	1.01	93–110	21.0%	0.61	57–69
Isosorbide 2-Mononitrate	6.1%	1.06	103–109	11.2%	0.91	87–96
Isosorbide 5-Mononitrate	5.7%	1.06	104–109	11.0%	0.98	93–103
Clomipramine	27.7%	0.95	84–107	27.7%	0.92	82–104
Desmethyl clomipramine[2]	46.0%	0.93	69–102	22.3%	0.95	87–105

[1] All estimates are on the log scale.
[2] AUC value is to time t since a log linear phase could not be clearly defined.

appropriate (7,13,14) if, indeed, rate remains an issue for drugs that are highly cleared and attain only low plasma levels. For these cases, onset of action should also be considered. Table III illustrates the relative insensitivity of metabolite Cmax to absorption rates. For isosorbide dinitrate, the Cmax CI for the metabolite was within the acceptable range when the parent's was not.

In conclusion, pivotal decisions related to bioequivalence should be based upon parent drug data to determine bioequivalence in extent of absorption (AUC) for immediate release formulations, except when the drug has a CL_{INT} value equalling and/or exceeding liver blood flow and the drug exhibits high intrasubject variability. In those cases, the metabolite should be used instead. Other occasions when the metabolite may be of importance in assessing extent of absorption include nonquantifiable and low parent levels and safety issues related to metabolite levels.

The parent compound should always be used to determine equivalence in rates of absorption (i.e., Cmax), since the metabolite is not a satisfactory metric since it is more influenced by metabolism and distribution than formulation changes. When CL_{INT} exceeds liver blood flow, the use of metabolites to determine equivalent rate of absorption (Cmax) could increase consumer risk by falsely declaring products bioequivalent when they are not, due to the lack of correlation of Cmax for metabolites with changes in absorption.

The information in this article has addressed only immediate-release formulations. More information is required to determine if the conclusions related to immediate-release formulations also apply to sustained-release products and drugs that exhibit nonlinear kinetics.

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