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COMMUNICATION

Bioequivalence Parameters of Parent Drug and Its First-Pass Metabolite: Comparative Sensitivity to Sources of Pharmacokinetic Variability

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ABSTRACT

The relative susceptibilities of a drug and its first-pass metabolite to various forms of pharmacokinetic variability were studied. Plasma concentrations of both species were simulated under conditions of interindividual variability in intrinsic hepatic clearance, intraindividual variability in hepatic clearance, and/or a concentration-dependent model for assay error. In comparison to the metabolite, the plasma concentrations of the parent drug displayed a heightened sensitivity to all forms of error. The bioequivalence parameters, AUC, C_{max}, and T_{max} of the parent drug and the metabolite were determined from an abbreviated data set. Compared to the metabolite, the AUC and C_{max} of the parent drug were much more sensitive to the added variability. The T_{max} of both species displayed similar variability. For a given level of manufacturer risk, a much larger group of subjects would be necessary to demonstrate bioequivalence with respect to the drug than with respect to the metabolite.

INTRODUCTION

The approval process for generic products was simplified and expedited by the enactment of the Drug Price Competition and Patent Term Restoration Act in 1984, so that consumers could benefit from the economic advantages of these products in a more timely manner. Approval for a generic product is obtained if the manufacturer can demonstrate that the rate and extent of release of the drug from the dosage form are similar to those of the innovator product. In the United States, the 90% confidence intervals of the ratios of *AUC*, C_{max}, and sometimes T_{max} of the generic to the innovator product must fall within the limits of 80% and 125%.

Current bioequivalence requirements for a drug that forms an active metabolite stipulate that bioequivalency must be demonstrated for *both* parent drug and the

metabolite. Thus, the number of parameters associated with the test increases from two or three (AUC, C_{max}, and possibly T_{max}) to four or six. From a statistical perspective, the need to simultaneously satisfy all the requirements for both the drug and the metabolite, decreases consumer (type I) error and increases producer risk, or type II risk, for a given sample size (1).

Both the parent drug and the active metabolite are products of the dose, and as such their concentration–time profiles are a function of the bioavailability characteristics of the dosage form. However, each species has a different origin and has a different fate. The drug is provided directly from its release from the dosage form and subsequent absorption into the systemic circulation. The metabolite, on the other hand, is an indirect product of the dosage form, produced by the biotransformation of the parent drug. Consequently the degree of variability associated with each of the two species, and the impact of this variability on the concentration time curves and bioequivalence parameters, may be different. By extension, the rigor of the bioequivalency test may be different for the parent drug and the metabolite.

There are many sources of variability and error that can impact upon the pharmacokinetic response. These include inter- and intravariability of any of the pharmacokinetic parameters, and assay and timing error. In the case of a drug that is converted to an active metabolite, variability in hepatic clearance is of particular concern. Firstly, this process often displays large variability (2) and secondly, it is likely to impact the parent drug and the metabolite concentrations in a different manner. Additionally, owing to the potentially large disparity between the drug and metabolite concentrations, assay error, which is frequently concentration dependent, is also of particular concern in this situation.

This study was undertaken to study the relative sensitivity of a parent drug and its first-pass active metabolite to variability in hepatic clearance and to concentration-dependent assay error. The plasma concentration–time profiles and the bioequivalence parameters derived from them were studied after a series of simulations using a pharmacostatistical model in which several types of variability were added.

METHODS

The Pharmacokinetic Model

A one-compartment open model, with first-order absorption (k_a = 6 hr^{-1}) was created in STELLA (High Performance Systems, Inc., Hanover, NH). Complete absorption of the dose through the gastrointestinal membrane was assumed for all simulations and the rate of absorption was also assumed to remain constant throughout the study. The drug was assumed to undergo metabolism in the liver by a single, linear enzymatic process. Intrinsic hepatic clearance was set at 210 liter/hr and a value of hepatic blood flow of 90 liter/hr was used (3). It was assumed that the drug did not bind to the plasma proteins. Hepatic clearance was calculated using the well-stirred venous equilibrium model (4,5). The hepatic extraction ratio was calculated to be 0.7 and the fraction of the dose escaping hepatic extraction (maximum oral bioavailability), which in this case was equal to the overall bioavailability, was 0.3. The drug was also assumed to undergo minimal renal clearance (1.2 liter/hr). The volume of distribution was set at 181 liters. It was assumed that the metabolite was eliminated by renal excretion alone (renal clearance equaled 20 liter/hr). The metabolite's volume of distribution was set to 173 liters. The ratio of the half-lives of the drug (1.95 hr) and the metabolite (5.99 hr) was 1 to 3.

Variability

Inter- and intrasubject variability in hepatic clearance and a concentration-dependent model for assay error were added to the pharmacokinetic model as described below. Each type of error was initially studied in isolation and then in combination with one and/or both of the other types of error.

Interindividual variability It was assumed that intrinsic clearance was normally distributed within the population, with a mean value of 210 liter/hr. The coefficient of variation of intrinsic hepatic clearance was set at 20%, 40%, and 50%, a range based upon published values of variability (6). Within a given simulation, intrinsic clearance remained constant; that is, no intraindividual variability was included.

Intraindividual variability in hepatic clearance A constant coefficient of variation model was used for intraindividual variability in hepatic clearance. Thus, intraindividual variability caused the hepatic clearance to deviate from the mean value in an individual, by an amount that was proportion to the mean value in that individual. Thus:

$$Clh_{i,j} = Clh_i \times (1 + \lambda)$$

where: $Clh_{i,j}$ represents hepatic clearance in individual i at time j; Clh_i represents the population predicted hepatic clearance in individual i; λ is a normally distributed random variable with an average value of 0. The

standard deviation of λ represents the coefficient of variation for intraindividual variability

The coefficient of variation for intraindividual variability was set at 10%, 20%, and 40%, which are within the ranges reported in the literature (6). In the case of intraindividual variability, hepatic clearance changed randomly according to the statistical model described above, at each iteration stage.

Assay error When a drug undergoes extensive first-pass metabolism, the concentration of parent drug is usually much lower than that of the metabolite. Assay error is often dependent on concentration and, while absolute error is greater at higher concentrations, the relative error usually increases as the concentration decreases. Thus, it is likely that a drug and its first-pass metabolite will be subject to different amounts of assay error. To incorporate this phenomenon in the simulations, the coefficient of variation of the assay error was considered as a linear function of the plasma concentration over a given range. Above and below the range it was assumed to be a minimum and a maximum constant, respectively. The maximum coefficient of variation was chosen such that it would apply to concentrations of the parent drug at around three half-lives after drug administration. Thus, for both drug and metabolite, the coefficient of variation was arbitrarily assumed to decrease from 0.2 at a low concentration of 20 μg/liter and less, down to a minimum of 0.05 at concentrations of 100 μg/liter and above.

Simulations Simulations were carried out using a fixed dose of 50 mg. Each of the three types of variability were initially studied in isolation. The combined effects of all three types of variability were then studied: for one data set the coefficient of variation of inter- and intravariability were set at 10% and 20%, respectively, and for a second set these parameters were set at 20% and 40%, respectively. The combined effects of intrasubject variability (10%, 20%, and 40% coefficient of variation) and assay error were studied. For each statistical model 20 sets of plasma concentration–time data were simulated simultaneously for the drug and the metabolite. Plasma concentrations were obtained from 0 to 24 hr after the dose, and were generated by numerical integration every 0.1 hr using Euler's method. Plots were prepared using the whole data set.

Bioequivalence Parameters The bioequivalence parameters were determined from an abbreviated data set, designed to mimic the timed samples of a bioequivalence study. Thus, assessments were based on the simulated concentrations at: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hr. The whole data set was used for the evaluation of the metabolite parameters. The parameters for the drug were based on the 0- to 6-hr plasma concentrations, since 6 hr represented about three elimination half-lives for the drug and at this time drug concentrations had reached or were approaching maximum assay error. The elimination rate constant was determined from the slope of the regression line that best fit the terminal (last three data points) portion of the log-linear concentration–time curve. The area under the curve (*AUC*) from zero to the last measured sample was determined using the trapezoidal rule. The *AUC* from the last measured sample to infinity was determined by dividing the last plasma concentration by the elimination rate constant. The highest plasma concentration of the abbreviated data set and the time at which it was measured were defined as C_{max} and T_{max}, respectively.

RESULTS

The simulated concentration–time curves for the parent drug and the metabolite are shown in Figs. 1 through 4. Table 1 shows the 90% confidence intervals of the three bioequivalence parameters of the drug and its metabolite. The intervals are expressed as a percentage of the mean value. For all situations the parent drug displayed a greater sensitivity to variability than did the metabolite. The effect of variable hepatic clearance also had different qualitative effects on the two species. This is most readily apparent from the runs that incorporated interindividual variability alone, since these contained no noise within a given simulation. The *AUC* and C_{max} of the parent drug were highly sensitive to altered clearance. T_{max}, however, was not affected (Figs. 1–4). In contrast, T_{max} and C_{max} of the metabolite were sensitive to changes in hepatic clearance but the *AUC* was not (Figs. 1–4, Table 1).

The concentration-dependent assay error, as expected, had greater effects on the lower concentrations of the parent drug than the metabolite (data not shown). This compounded the difference in the ultimate variability experienced by the parent drug and metabolite. The combination of assay error with intra- and interindividual variability, particularly with the larger coefficients of variation, were associated with very large variability in the plasma concentration time profile of the parent drug (Figs.3 and 4).

The data in Table 1 illustrate that in all situations, the 90% confidence intervals (expressed as a percentage of the mean) of *AUC* and C_{max} of the parent drug, were several orders of magnitude larger than those of the

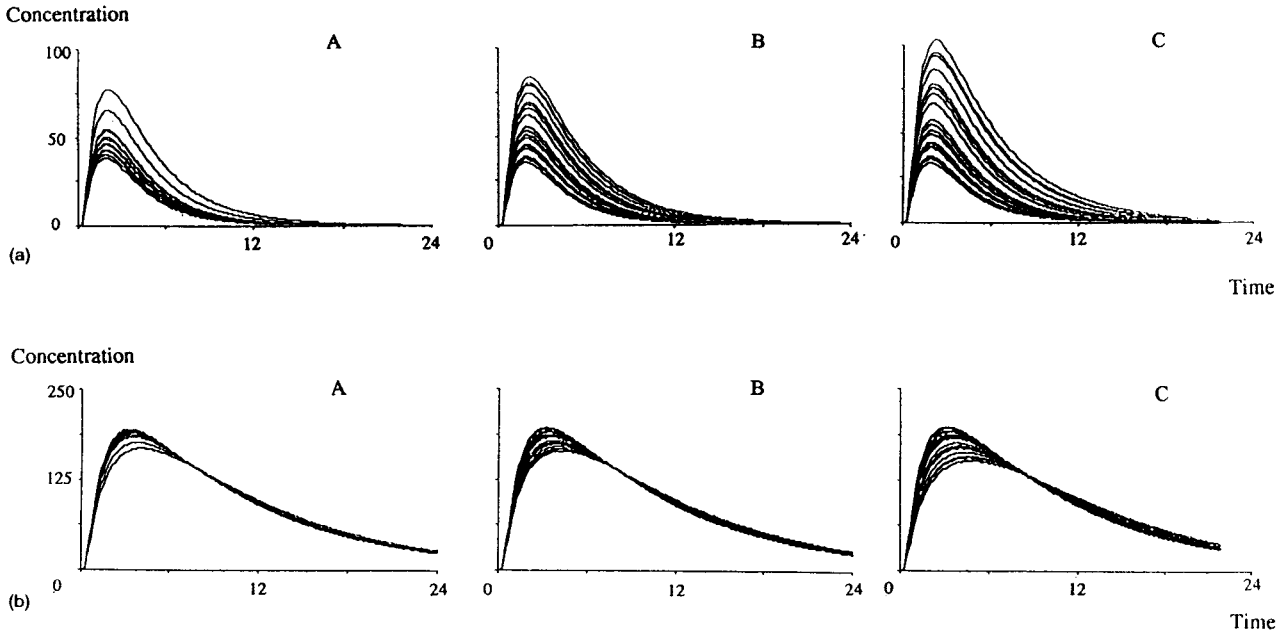

Figure 1. Plasma concentrations (μg/liter) of the parent drug (a) and the metabolite (b) as a function of time (hr) after a dose. Plasma concentrations were simulated as described under Methods using models that incorporated various degrees of interindividual variability. The coefficient of variation of interindividual variability was 20%, 40%, and 50% in panels A, B, and C, respectively.

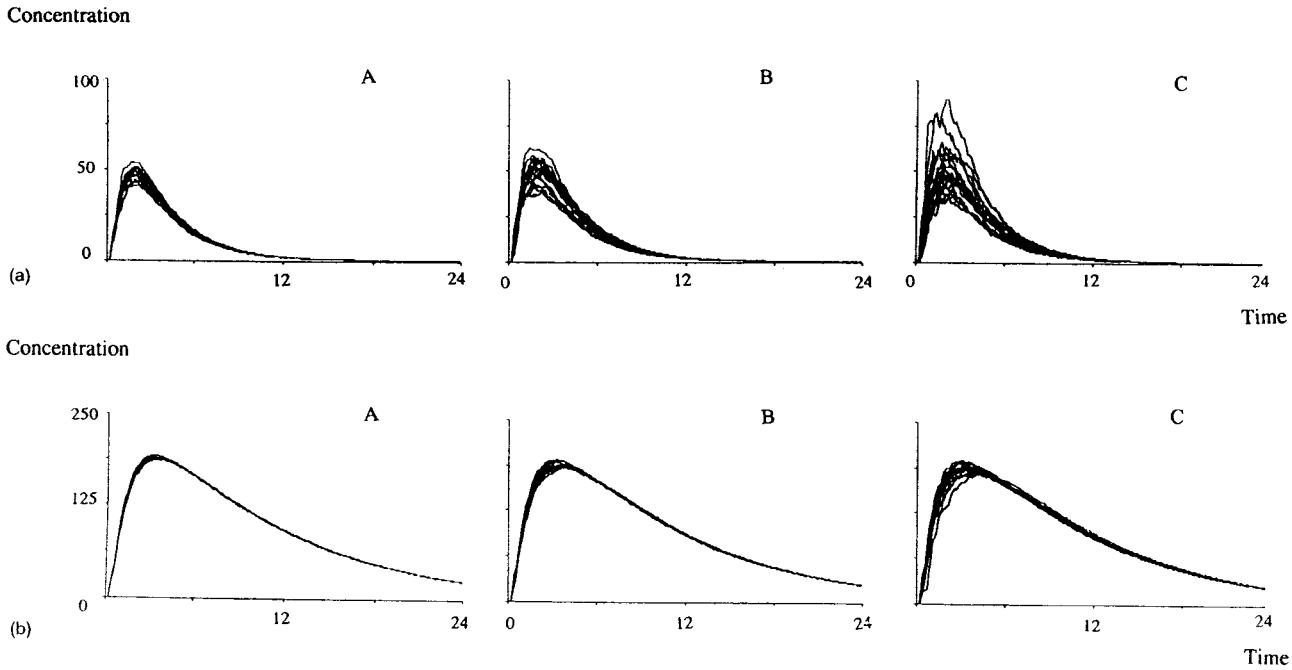

Figure 2. Plasma concentrations (μg/liter) of the parent drug (a) and the metabolite (b) as a function of time (hr) after a dose. Plasma concentrations were simulated as described under Methods using models that incorporated various degrees of intraindividual variability. The coefficient of variation of intraindividual variability was 10%, 20%, and 40% in panels A, B, and C, respectively.

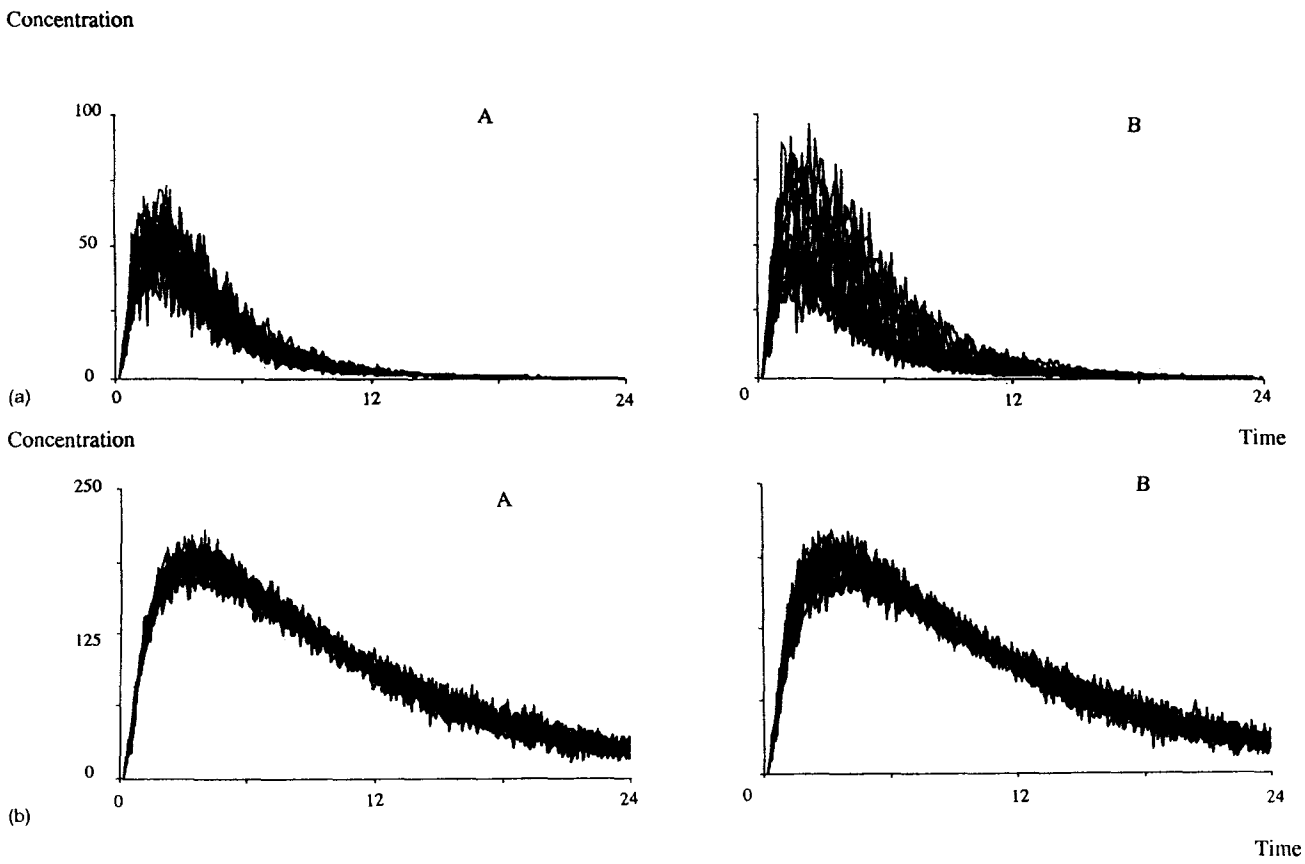

Figure 3. Plasma concentrations (μg/liter) of the parent drug (a) and the metabolite (b) as a function of time (hr) after a dose. Plasma concentrations were simulated as described under Methods using models that incorporated a concentration-dependent model for assay error, interindividual variability, and intraindividual variability. The coefficients of variation of interindividual variability and intraindividual variability were 10% and 20%, respectively, in panel A, and 20% and 40%, respectively, in panel B. The model for assay error, which is described in the Methods section, was the same for all simulations.

metabolite. The 90% confidence intervals of T_{max} for the drug and metabolite were within the same range. This is probably because, as illustrated in Fig. 1, T_{max} for the metabolite was more sensitive to variability in hepatic clearance than that of the parent drug. With large values (40–50%) of the coefficient of variation for inter- and/or intraindividual error, the 90% confidence intervals for the drug became very wide.

DISCUSSION

The study demonstrated how pharmacostatistical models can be used to investigate the manner in which pharmacokinetic variability is translated to different responses. The pharmacokinetic model used here was based on the relatively common situation of a drug that undergoes extensive first-pass metabolism (extraction ratio of 0.7) to produce an active metabolite. As a consequence of the extensive first-pass metabolism, the concentration of the metabolite was much higher than that of the parent drug. Statistical models were incorporated for hepatic clearance using a constant coefficient of variation model. Assay error was also incorporated into the model. It was assumed that the assay error was proportional to the model-predicted concentration and that the coefficient of variation decreased with increasing concentrations, a phenomenon frequently observed. Values of the pharmacokinetic and statistical model parameters were all chosen to be within the range of the values published in the literature for therapeutic drugs.

Throughout all the simulations performed in this study, the bioavailability characteristics of the dosage

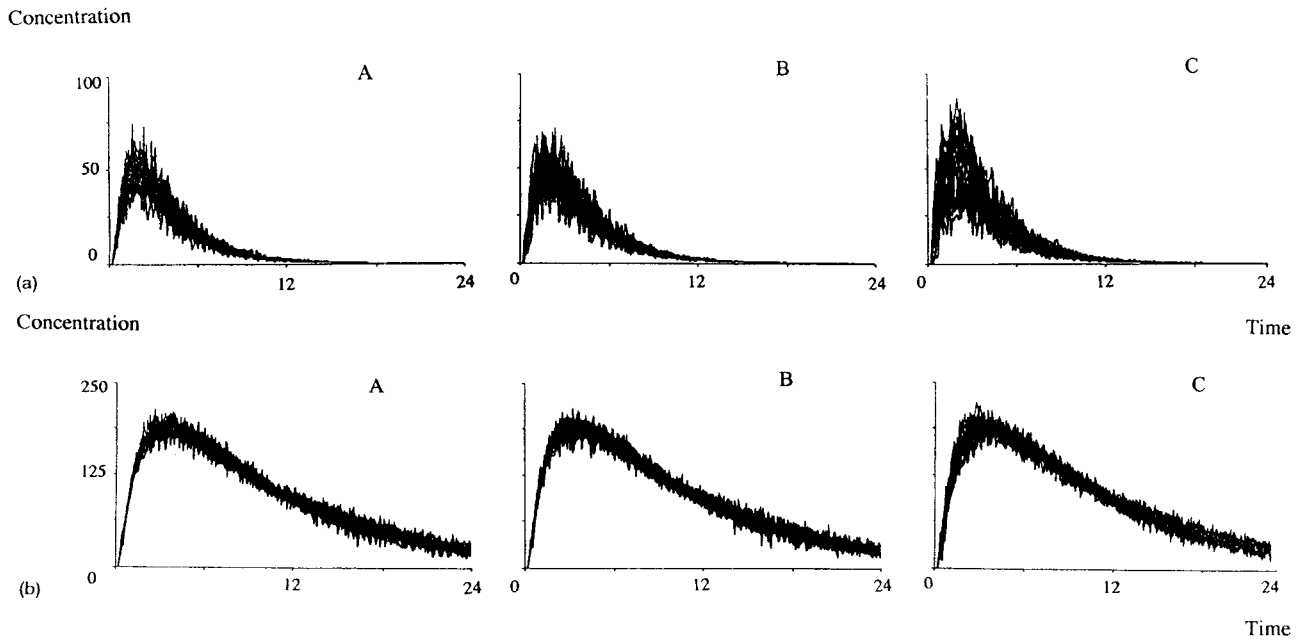

Figure 4. Plasma concentrations (μg/liter) of the parent drug (a) and the metabolite (b) as a function of time (hr) after a dose. Plasma concentrations were simulated as described under Methods using models that incorporated various degrees of intraindividual variability and a concentration-dependent model for assay error. The coefficient of variation of intraindividual variability was 10%, 20%, and 40% in panels A, B, and C, respectively. The model for assay error, which is described in the Methods section, was the same for all simulations.

form remained constant. Thus, variability in the bioequivalence parameters arose only from variability in a disposition parameter, hepatic clearance, and/or assay error. Maximum oral bioavailability (the fraction of the dose escaping extraction by the liver) would be affected by changes in hepatic clearance.

The simulations performed in the presence of interindividual variability alone clearly illustrated, by eliminating noise within a given simulation run, the different impact of altered hepatic clearance on the plasma concentration profiles for the drug and the metabolite. As would be predicted, based on fundamental pharmacokinetic principles, changes in hepatic clearance affected C_{max} and the *AUC* of the parent drug. For this hypothetical drug, the absorption rate constant was so much greater than the elimination rate constant that T_{max} was essentially insensitive to small changes in the elimination rate constant. The peak plasma concentration (C_{max}) and T_{max} for the metabolite were both affected by the changes in hepatic clearance of the parent drug. This is predictable since changes in hepatic clearance will directly affect the rate of formation of the metabolite. The *AUC* of the metabolite displayed little sensitivity to variations in hepatic clearance.

The model demonstrated that the plasma concentrations of the parent drug displayed a much greater sensitivity to the added variability than did the concentrations of the metabolite. The extensive hepatic extraction resulted in high concentrations in the metabolite, which were relatively insensitive to small changes in its extent of formation. Conversely, the low residual concentrations of parent drug were highly sensitive to these same changes. This phenomenon was also reflected in the differing susceptibilities of the *AUC*s of the two species. The *AUC* of the metabolite was much more resilient to the changes compared to that of the parent drug. Also as a consequence of its relatively low concentration, the parent drug was subject to a greater degree of assay error. Thus, the fairly typical concentration-dependent model for assay error used for these simulations further exacerbated the disparity in the variability of parent drug and metabolite. In summary, the *AUC* and C_{max} of the

Table 1

90% Confidence Range for the Bioequivalence Parameters of Parent Drug and Its Metabolite[a]

	90% Confidence Interval Range (% of the mean)					
	AUC		C_{max}		T_{max}	
	Drug	Metabolite	Drug	Metabolite	Drug	Metabolite
Interindividual error						
CV 20%	19.09	0.064	14.28	2.53	8.71	7.29
CV 40%	27.57	0.103	21.05	4.28	7.95	10.88
CV 50%	38.13	0.119	27.78	6.24	9.35	12.87
Intraindividual error						
CV 10%	4.79	0.034	5.36	0.80	8.71	0.00
CV 20%	8.43	0.063	9.10	1.42	10.74	10.88
CV 40%	16.46	0.139	19.68	2.18	12.76	14.29
Assay error	13.69	3.45	7.52	2.58	14.87	19.88
Inter- and intraindividual and assay error						
Inter Intra						
CV 20% 10%	14.83	3.22	9.98	2.82	19.99	16.18
CV 40% 20%	39.28	3.26	21.00	4.46	19.88	16.40
Assay and intraindividual error						
CV 10%	9.97	3.56	7.89	2.74	17.87	16.54
CV 20%	12.33	2.90	8.15	2.65	21.51	16.81
CV 40%	30.82	2.54	20.66	3.30	22.09	17.75

[a]The drug was absorbed by first-order absorption and underwent extensive first-pass metabolism. Details of the pharmacokinetic model may found under Methods. Inter- and intraindividual error in hepatic clearance were incorporated with different coefficients of variation (CV). Concentration-dependent assay error was incorporated. Details are given in methods section.

parent drug were much more variable than the corresponding parameters for the metabolite. Clearly, in real situations other factors will undoubtedly modify the overall variability in both species. These factors include variability in the dosage form, and inter- and intravariability of competing elimination pathways. Theoretically, these could either heighten or lessen the disparity in the variability of the two sets of parameters.

Under conditions of large variability in hepatic clearance and/or assay error, the variability associated with the bioequivalence parameters of the parent drug were so large that demonstration of bioequivalence would require an exceedingly large sample size to achieve the desired power. Yet, under the same conditions, bioequivalency could be demonstrated for the metabolite alone using a much smaller cohort of subjects. In the case of a drug that forms an active metabolite, the need to demonstrate bioequivalency for both drug and metabolite is in itself associated with a decrease in consumer risk and an increase in manufacturer risk. This increased rigor is compounded if, in addition, various sources of error lead to large variability in parent drug concentrations. In these conditions, it may be more equitable and economical to try to identify alternative approaches for analyzing bioequivalency data. A logical approach may be to normalize the two sets of concentrations according to potency and then combine them. In this way bioequivalency determination could be performed on a single (summed) species. However, this approach is complicated by the fact that the plasma concentration profiles for the drug and metabolite are not parallel. The peaks occur at different times, and T_{max} of each species displays a different sensitivity to changes in hepatic clearance. Alternatively, it may be possible for regulators to devise an appropriate and equitable system for weighting the two sets of parameters. Thus, the bioequivalency parameters of the entity displaying the greatest variability would be considered but would be given less weight than the other, more robust set of parameters.

Further studies are in progress to more fully investigate the relationship between different sources of variability and response.

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