The Role of Metabolites in Bioequivalency Assessment. II. Drugs with Linear Pharmacokinetics and First-Pass Effect

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Simulations were conducted to address the question of whether metabolite data are required for bioequivalence evaluation of immediate release formulations with drugs exhibiting linear pharmacokinetics and first-pass effect. Plasma level-time profiles were generated for parent drug and metabolite using relevant rate constants obtained from a bivariate normal distribution and designated random error. Simulation results showed that the need for metabolite data (C_{max}) in the assessment of bioequivalence depends on the relative variability between the absorption process of the drug and first-pass route for metabolite(s). The importance of metabolite C_{max} data in the evaluation of rate of availability is clearly demonstrated for drugs with a high degree of intra-subject variation in the first-pass metabolism compared to the absorption process of the drug. Under such conditions, a wider confidence interval was found for the metabolite **rather** than parent drug. Opposite results were obtained when the intra-subject variance was high for drug absorption relative to first-pass effect. Discrepancies were observed for the scenarios in which the elimination pathway of the metabolite is more variable than the absorption process of the drug. The simulation results were in agreement with real bioequivalence data. It is thus recommended that, in the absence of the information on the relative variability of absorption and first-pass process, both parent drug and metabolite data be included for documentation of bioequivalence, should the metabolite(s) play an important role in the determination of efficacy and safety of the drug.

KEY WORDS: bioequivalence; rate of absorption; rate of availability; parent drug; metabolite; linear pharmacokinetics; first-pass.

INTRODUCTION

The role of metabolites in bioequivalence determination has gained increasing attention since the implementation of Drug Price Competition and Patent Term Restoration Act in 1984 (1-4). A consensus report (3) from the Bio-International '92 conference strongly favored use of parent drug data to assess bioequivalence and recommended consideration of metabolite data on a case-by-case basis. Yet, it was noted (3) that metabolites are indeed of prime importance in assessing bioequivalence under several situations as summarized below:

1. The parent drug cannot be measured due to insufficient sensitivity of the analytical method.
2. The parent drug undergoes a rapid and complete conversion to metabolite.
3. The parent drug levels give unreliable or unacceptable bioavailability parameters.
4. The parent drug and metabolite are equipotent, with the measured metabolite levels being higher than those for the parent compound.
5. The parent drug is inactive, and the metabolite is responsible for the efficacy and/or toxicity of the drug product.

One of the major concerns raised for metabolites, in the context of bioequivalence, has been focused on the relative variability between the measurement of metabolite(s) and parent drug (3). The question is which species should be employed to assess bioequivalency when the parent drug and metabolite do exhibit distinctly different degrees of variation. In this regard, theoretical consideration for equivalency in the extent of absorption using AUC of parent drug and metabolite has been discussed extensively (2,4,5). As for rate of absorption, a recent article (6) on conventional release formulations has concluded that bioequivalence decisions for linear drugs without first-pass effect should not be based solely on the metabolite C_{max} data. Nonetheless, the issue remains unresolved for drugs undergoing first-pass metabolism which is subject to differential inter- and intra-individual variabilities.

The aim of the present paper was to investigate the relationship between C_{max} values of the parent drug and metabolite from conventional release formulations for drugs exhibiting linear kinetics and first-pass metabolism. Simulations were conducted to examine whether variability residing in either parent drug absorption or metabolite formation would be equally reflected in C_{max} of both parent drug and metabolite. The simulation results were compared with experimental data obtained from several bioequivalence studies to determine if the simulated predictions support the clinical results.

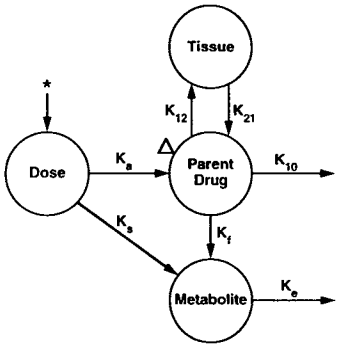

Fig. 1. Compartmental metabolite model used for simulation of drugs exhibiting linear kinetics with first-pass effect, where ka = first-order absorption rate constant, ks = first-order rate constant for the fraction metabolized via first-pass effect, kf = formation rate constant for metabolite from parent drug, k12 and k21 = intercompartment transfer constants, k10 = first-order elimination rate constant for parent drug from central compartment, and ke = first-order elimination rate constant for metabolite.

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Table I. Parameter values used in the structure-model*

	Test	Reference
Dose, mg	100	100
F	0.6	0.6
k_a, hr^{-1}	1.35	0.75
k_s, hr^{-1}	0.45/0.9	0.25/0.5
k_f, hr^{-1}	0.308	0.293
k_e, hr^{-1}	1.152	1.094
k_{10}, hr^{-1}	0.086	0.082
k_{12}, hr^{-1}	0.094	0.094
k_{21}, hr^{-1}	0.119	0.119
$V_{d,p}$, L	213.9	213.9
$V_{d,m}$, L	10.04	10.04

* Scenario A & C: ka/ks = 3
Scenario B: ka/ks = 1.5
$V_{d,p}$: volume of distribution for parent drug
$V_{d,m}$: volume of distribution for metabolite

Table II. Random errors (% c.v.) used in the variance model

Parameter	Intersubject Variation	Intrasubject Variation
k_a		
Scenario A	60	80,40
Scenario B	60	80,40
Scenario C	20	25,10
k_s		
Scenario A	40	50,25
Scenario B	80	50,25
Scenario C	80	95,50
k_f	20	20,5
k_{10}	30	25,5
k_e	45	40,10

METHODS

Pharmacokinetic Simulations

Simulations were conducted using CONSAM (7) to generate drug and metabolite plasma concentration-time profiles following a single oral administration of a drug exhibiting linear kinetics with a first-pass effect. The pharmacokinetic model used in the simulations is shown in Figure 1 (8). The simulations apply to primary metabolites arising from metabolizing organs or first-pass metabolism and assume that metabolites formed by the first-pass route will not be subject to sequential elimination within the first-pass tissue. Moreover, luminal metabolism has been considered as a first-pass effect, and the metabolites formed in the gut lumen are available for absorption in the simulations.

Bivariate normal distributions (rho = 0.25 and 0.95) for the rate constants, ka, ks, kf, k10 and ke, were generated to simulate the two-treatment, two-period crossover design of a bioequivalence study (6). To account for potential variability in assay, formulation and/or subject differences, normally distributed errors were added to the rate constants. The sequence of the treatments was randomly assigned to each simulation.

Three scenarios (A, B and C) with different combinations of structural and variance models were employed in the simulations. The parameters used for the structural model are presented in Table I (8). The absorption of the drug was assumed to be faster for test formulation than for reference formulation (9). The structural model for Scenarios A and C represents the situation in which the absorption process (ka) for the parent drug is significantly faster than the first-pass process (ks) for the metabolite, while Scenario B represents the situation in which the input rates for two processes are comparable. Table II shows the magnitude of random errors assigned to the rate constants in the variance model. Both Scenarios A and B assume a higher or comparable intrasubject variance for ka than for ks, whereas Scenario C is defined by a smaller intrasubject variance for ka relative to ks.

Simulation was conducted for each scenario to generate data for 36 subjects in a crossover study. To minimize the number of potential simulation outcomes, all simulations were performed by varying three rate constants at one time while keeping the other parameters fixed. Three cases were thus generated for each scenario: 1) Case 1 with variance added to ka, ks and k10, 2) Case 2 with variance added to ka,

Table III. Key characteristics of bioequivalence studies

Drug	Subject No.	Dose	Reference Product	Sampling Time	Washout Interval	Analytical Method (ref.)
Triamterene/Hydrochlorothiazide	23	1 × 75/50 mg tablet	Maxide, Mylan	0-24 hrs	1 wk	HPLC (10)
Doxepin HCl	38	1 × 100 mg capsule	Sinequan, Roerig	0-120 hrs	3 wk	HPLC (11)
Isosorbide Dinitrate	20	1 × 20 mg tablet	Isordil, Ives	0-36 hrs	1 wk	GLC (12)
Metoprolol Tartrate	34	1 × 100 mg tablet	Lopressor, Ciba Geigy	0-36 hrs	1 wk	HPLC (13)
Amitriptyline HCl	24	2 × 25 mg tablet	Triavil, Merck	0-72 hrs	2 wk	GC (14)
Imipramine HCL	22	2 × 50 mg tablet	Tofranil, Geigy	0-72 hrs	2 wk	HPLC (15)
Nortriptyline HCl	26	1 × 75 mg capsule	Pamelor, Sandoz	0-216 hrs	4 wk	HPLC (16)

Table IV. Mean Cmax values (ng/ml) for Scenario A*

	Case 1		Case 2		Case 3	
	Test	Ref.	Test	Ref.	Test	Ref.
LLL						
P	148 (33)	123 (39)	145 (35)	122 (32)	151 (29)	107 (34)
M	1148 (24)	950 (18)	1183 (38)	1068 (44)	1144 (19)	1291 (15)
HHH						
P	141 (30)	107 (36)	142 (31)	104 (36)	150 (29)	112 (34)
M	1180 (21)	993 (17)	1350 (48)	969 (41)	1156 (17)	986 (19)
LLH						
P	148 (34)	120 (34)	143 (33)	121 (36)	161 (32)	129 (36)
M	1163 (20)	929 (20)	1300 (56)	970 (41)	1147 (22)	975 (21)
LHH						
P	146 (32)	122 (37)	146 (33)	117 (34)	154 (32)	129 (36)
M	1170 (22)	996 (13)	1303 (50)	999 (44)	1176 (23)	981 (19)
HLL						
P	143 (28)	110 (38)	156 (30)	114 (36)	152 (31)	119 (38)
M	1151 (20)	937 (15)	1230 (36)	1089 (56)	1109 (21)	932 (17)
HHL						
P	142 (33)	108 (39)	142 (30)	109 (36)	151 (32)	113 (35)
M	1154 (21)	994 (17)	1187 (35)	1069 (42)	1173 (19)	988 (19)
LHL						
P	145 (33)	119 (36)	144 (32)	122 (38)	150 (32)	129 (35)
M	1173 (19)	1001 (15)	1209 (40)	1103 (48)	1244 (22)	1055 (17)
HLH						
P	143 (28)	109 (36)	146 (31)	109 (39)	156 (29)	118 (36)
M	1164 (22)	939 (18)	1307 (50)	978 (44)	1238 (19)	1012 (18)

* P: parent drug, M: metabolite
( ): % coefficient of variation
Please see Appendix for other abbreviations

Table V. Mean Cmax values (ng/ml) for Scenario B*

	Case 1		Case 2		Case 3	
	Test	Ref.	Test	Ref.	Test	Ref.
LLL						
P	127 (36)	111 (40)	122 (35)	107 (38)	127 (36)	108 (38)
M	1609 (18)	1232 (14)	1578 (29)	1341 (38)	1963 (21)	1600 (15)
HHH						
P	121 (32)	98 (40)	123 (35)	96 (42)	122 (33)	96 (40)
M	1554 (18)	1307 (18)	1780 (50)	1304 (38)	1976 (18)	1730 (18)
LLH						
P	124 (37)	108 (37)	127 (36)	109 (39)	120 (34)	110 (38)
M	1583 (18)	1238 (16)	1587 (44)	1381 (38)	1938 (15)	1588 (13)
LHH						
P	123 (35)	108 (38)	123 (37)	107 (38)	123 (36)	109 (41)
M	1574 (19)	1237 (16)	1712 (41)	1286 (37)	1928 (18)	1613 (18)
HLL						
P	121 (33)	95 (38)	122 (32)	93 (38)	121 (32)	94 (37)
M	1581 (15)	1276 (14)	1633 (28)	1372 (35)	2004 (16)	1637 (15)
HHL						
P	123 (34)	96 (41)	120 (33)	94 (38)	123 (32)	97 (37)
M	1550 (17)	1306 (19)	1653 (37)	1476 (44)	1964 (18)	1655 (18)
LHL						
P	131 (39)	109 (37)	121 (33)	94 (42)	126 (38)	111 (38)
M	1554 (21)	1286 (17)	1653 (34)	1443 (35)	2022 (25)	1581 (20)
HLH						
P	122 (33)	92 (39)	125 (33)	96 (40)	119 (30)	96 (38)
M	1576 (15)	1278 (14)	1679 (31)	1296 (43)	1944 (16)	1654 (16)

* P: parent drug, M: metabolite
( ): % coefficient of variation
Please see Appendix for other abbreviations

ks and ke, and 3) Case 3 with variance added to ka, ks and kf. The pharmacokinetic parameter, C_{max}, for the test and reference formulation was determined following simulation.

Bioequivalence Studies

Table III outlines the key characteristics of bioequivalence studies (10-16). All studies were conducted in normal, healthy male volunteers between 18 and 50 years of age. The health status of the subjects was ascertained by their medical histories, physical examination, and clinical laboratory tests. The design for all studies was a single-dose, randomized, two-treatment, two-sequence, two-period crossover with an adequate washout interval between doses. In each study, plasma levels of parent drug and its metabolite(s) were measured following the administration of the drug. Peak concentration, C_{max}, was obtained directly from the plasma level-time profiles.

Statistical Analysis

Analysis of variance (ANOVA) was performed for log-transformed C_{max} of both parent drug and metabolite using SAS General Linear Model (GLM) procedures (17). The statistical model was partitioned into sequence, subject within sequence, period, treatment and an error term. The two one-sided hypotheses at the $\alpha = 0.05$ level were tested by constructing 90% confidence interval for the ratio of the test and reference average (18). The % coefficient of variation (CV) of C_{max} directly obtained from ANOVA was taken as a measure of intrasubject variance. It is to be noted that the CV so obtained only reflects a crude estimate of intrasubject variance since this is calculated from the residual error term which accounts for all sources of variation unexplained by the model.

RESULTS

Tables IV-VI summarize the mean C_{max} values for the three different scenarios employed in the simulation. As described in the Methods section, the present simulations were done by varying three rate constants at one time while keeping others fixed. For the purpose of illustration, each simulation was given a three-letter designation. The first letter was for the intrasubject variance of ka, the second letter for ks and the third letter for k10, ke or kf, depending on the case chosen. The high value was represented by H, and the low value by L.

As shown in Tables IV-VI, the mean C_{max} is higher for the test formulation than for the reference formulation in all cases for both parent drug and metabolite, which is consistent with the major premise that the test formulation is absorbed faster than the reference formulation. The coefficients of variation associated with the mean values of C_{max} are indicative of intersubject variability. The intersubject CV may be greater for the parent drug than for the metabolite in Scenarios A and B, but not in Scenario C. A closer examination of the data for Scenarios A and B (Tables IV and V) revealed that metabolite CV tended to exceed parent drug CV in Case 2, although the phenomenon was less pro-

Table VI. Mean Cmax values (ng/ml) for Scenario C*

	Case 1		Case 2		Case 3	
	Test	Ref.	Test	Ref.	Test	Ref.
LLL						
P	144 (15)	117 (16)	144 (14)	112 (16)	150 (18)	126 (18)
M	1272 (22)	1072 (26)	1326 (31)	1148 (41)	1635 (23)	1384 (24)
HHH						
P	139 (19)	114 (18)	141 (19)	113 (17)	151 (15)	124 (18)
M	1276 (26)	1027 (31)	1401 (47)	1043 (48)	1595 (25)	1373 (28)
LLH						
P	137 (16)	119 (18)	142 (19)	119 (18)	151 (19)	125 (19)
M	1298 (23)	1057 (23)	1413 (47)	1061 (36)	1691 (24)	1407 (22)
LHH						
P	143 (18)	118 (17)	146 (18)	119 (18)	148 (16)	129 (17)
M	1298 (26)	1076 (33)	1422 (54)	1031 (47)	1637 (25)	1370 (25)
HLL						
P	143 (18)	114 (16)	141 (20)	112 (18)	154 (18)	120 (19)
M	1626 (24)	1432 (22)	1299 (31)	1135 (42)	1636 (24)	1384 (23)
HHL						
P	144 (19)	115 (19)	142 (18)	115 (17)	154 (22)	120 (21)
M	1286 (27)	1015 (29)	1349 (36)	1173 (54)	1690 (22)	1368 (27)
LHL						
P	147 (19)	122 (19)	141 (17)	119 (17)	145 (18)	122 (17)
M	1269 (25)	1033 (28)	1316 (37)	1124 (54)	1636 (22)	1359 (25)
HLH						
P	142 (19)	112 (15)	140 (16)	111 (17)	144 (17)	121 (19)
M	1271 (24)	1041 (22)	1463 (47)	1065 (40)	1697 (23)	1359 (20)

* P: parent drug, M: metabolite
( ): % coefficient of variation
Please see Appendix for other abbreviations

nounced for Scenario B as for Scenario A. It appears that when ke is highly variable in Case 2, the mean C_{max} and the associated % CV are high for metabolite of the test product, indicating a rate-limiting step for the elimination of the metabolite under such conditions.

Figures 2-4 present the width of 90% confidence intervals for C_{max} values under various scenarios in the simulations. For Scenario A (Fig. 2) where ka is three times faster than ks and intrasubject variance is smaller for ks compared to ka, a narrower confidence interval was obtained, in most cases, for metabolite than for parent drug. Accompanied with this decrease in the confidence limits of C_{max} from parent drug to metabolite was a reduction of intrasubject CV estimated from the ANOVA (data not shown). An exception occurred when the intrasubject variance was low for ka and high for ke. Under such circumstances, comparable or even wider confidence limits were observed for the metabolite as opposed to parent drug.

As with Scenario A, similar results have been obtained for Scenario B (Fig. 3), indicating a lack of influence on the relative size of intrasubject variability in C_{max} from changes in the fraction of total body clearance that furnishes the metabolite via first-pass route to the general circulation.

In an attempt to explore alternative situations by which a higher intrasubject variability may be observed for the metabolite than parent drug, additional simulations were performed assuming a lower intrasubject variance for ka than for ks (10-25% vs. 50-95%). As depicted in Fig. 4 (Scenario

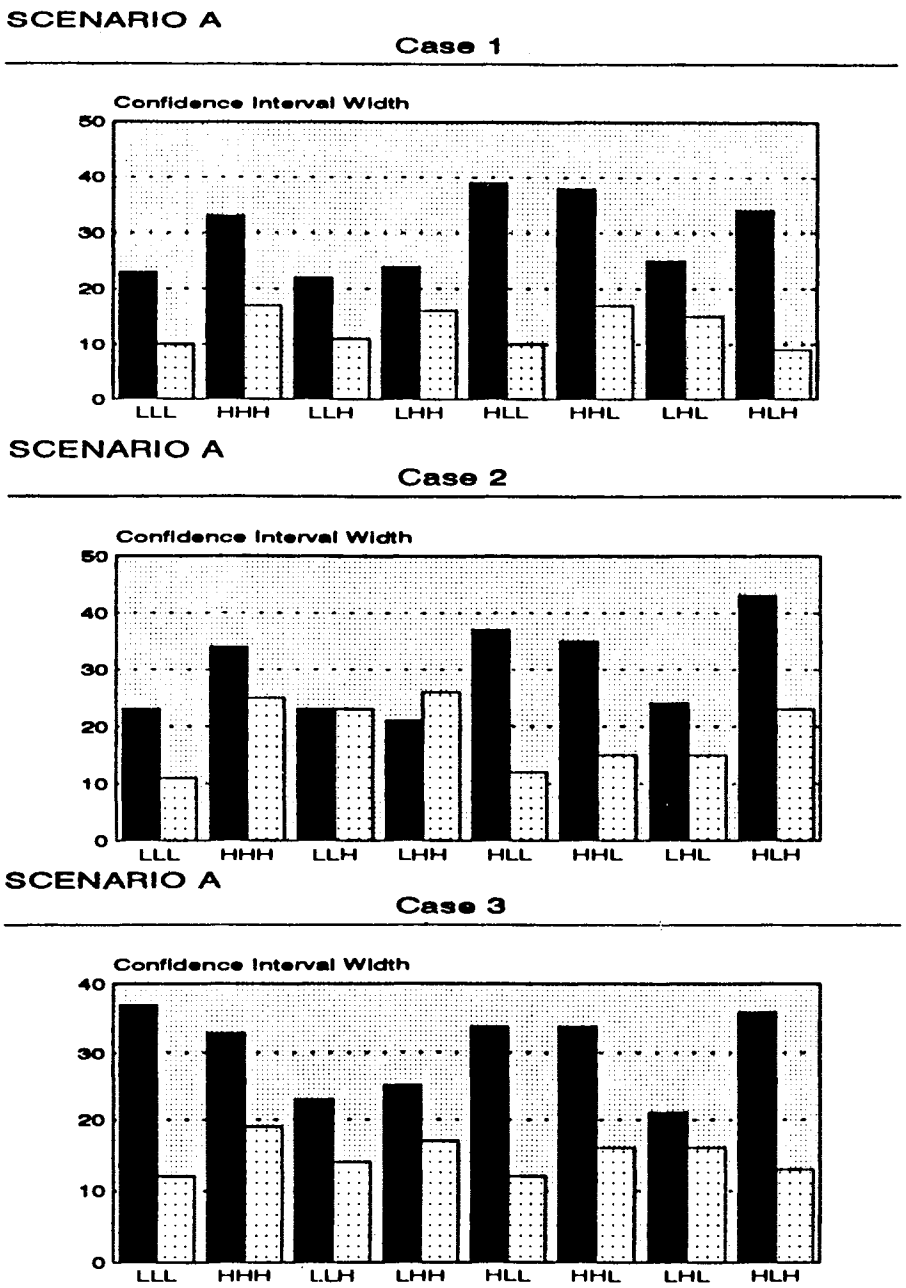

Fig. 2. 90% Confidence interval width of C_{max} for parent drug (■) and metabolite (□) under Scenario A in the simulations.

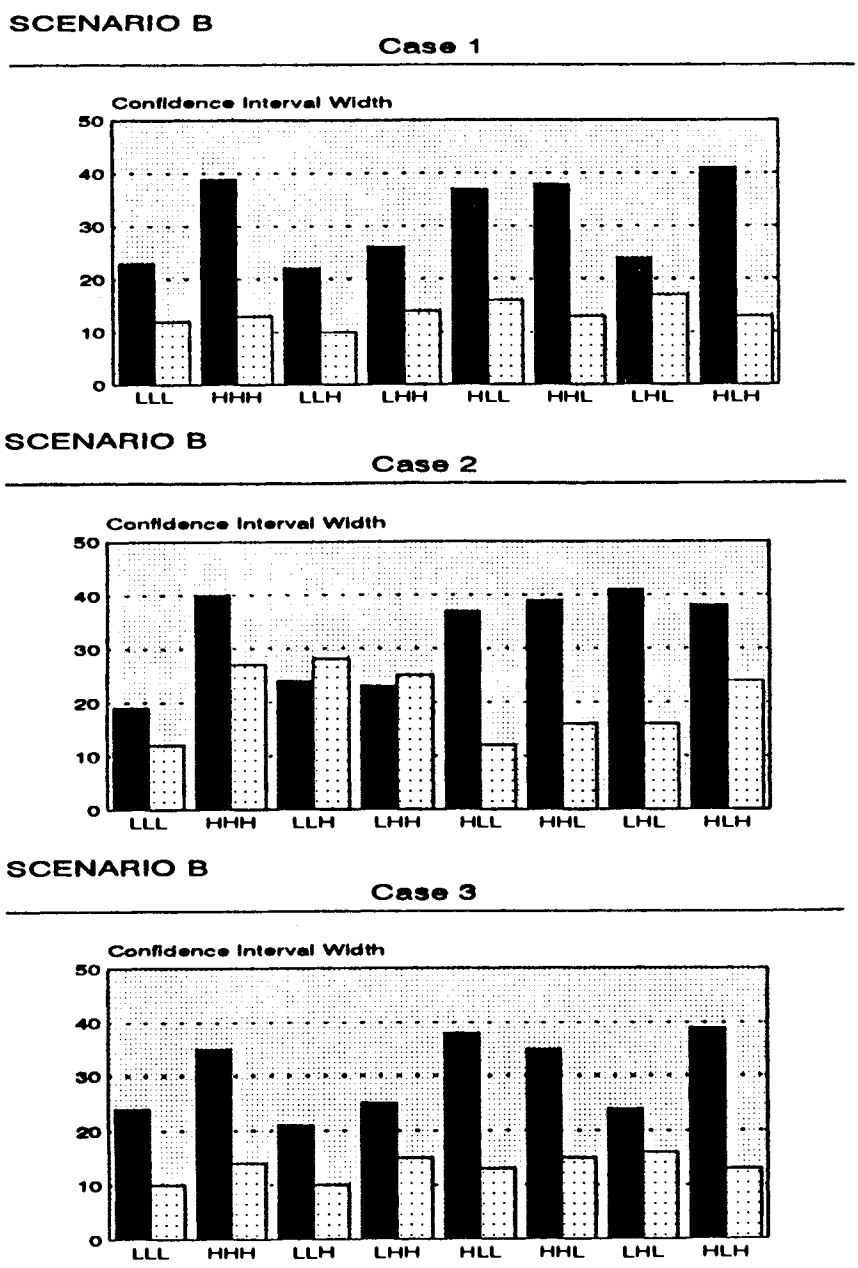

Fig. 3. 90% Confidence interval width of C_{max} for parent drug (■) and metabolite (□) under Scenario B in the simulations.

C), the simulation results in an increase in the intrasubject CV of C_{max} for the metabolite, and in turn, a tighter confidence interval for the parent drug rather than the metabolite. It appears that the width of the confidence interval for the parent drug and metabolite directly reflects the intrasubject variability of ka and ks, respectively.

Table VII presents summary statistics of C_{max} and T_{max} parameter for *in vivo* bioequivalence studies on drugs following linear kinetics with a first-pass effect. The clinical data have been found to agree with the simulation results. While wider confidence intervals were obtained in many cases for the parent drug than for metabolite, opposite results were observed for imipramine and nortriptyline. The latter may exemplify either the situations in Scenario C where the intrasubject variation is high for the first-pass route (ks) relative to the absorption process (ka) of the drug, or those cases in Scenarios A or B where a higher intrasubject variability lies in ka rather than ks, but the intrasubject variability of ke (or more generally, clearance) is even greater compared with ka.

DISCUSSION

Our previous studies (6) have shown that C_{max} for the metabolite cannot be used to predict equivalency in the rate of absorption of immediate release formulations for drugs exhibiting linear pharmacokinetics without first-pass effect. Both simulation and real bioequivalence trials (6) revealed that the confidence intervals of C_{max} were always tighter for metabolite than parent drug, regardless of the metabolite

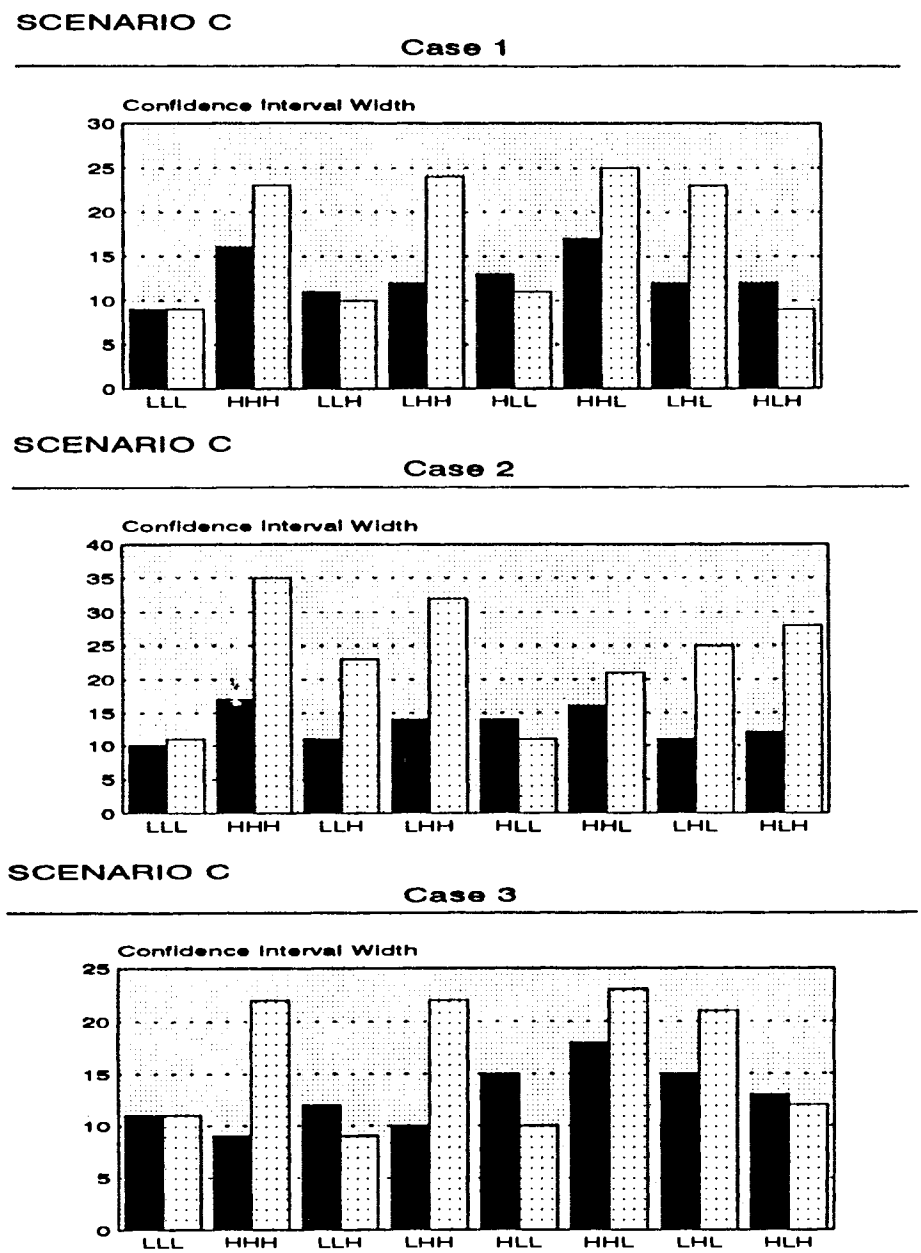

Fig. 4. 90% Confidence interval width of C_{max} for parent drug (■) and metabolite (□) under Scenario C in the simulations.

model and error structure contained in the data. A plausible explanation for this finding is that metabolite formation, in this case, appears to be a sequence secondary to the absorption of the drug, and thus reflects little information with respect to the complexity of the absorption process of the parent compound.

On the other hand, for drugs with a first-pass effect, one may theorize that the presence of first-pass metabolism which occurs almost simultaneously with drug absorption may necessitate the use of metabolite data, in addition to the parent compound, for determination of bioequivalence among formulations. Indeed, the results of the present studies demonstrated that the above hypothesis is true for drugs with a high degree of within-subject variance in the first-pass route as opposed to the absorption process of the parent compound. It has been found that the C_{max} of metabolite consistently exhibits a wider confidence interval than the parent compound. In this regard, use of parent drug data alone for bioequivalence assessment may fail to reflect a true difference between formulations, should the metabolite be a major source for therapeutic effect and/or responsible for the toxicity of the drug product. Evidently, inclusion of metabolite data becomes imperative for decision-making related to the bioequivalence of these drugs.

It is noteworthy that our studies also revealed that if the intrasubject variability is high for the absorption process relative to the first-pass effect, metabolite data may not be critical for bioequivalence assessment. Under such conditions, the simulations indicated that only when the elimination pathway of the metabolite appears more variable than

Table VII. Summary data of bioequivalence studies on Cmax and Tmax for drugs following linear kinetics with first-pass effect

Parent Drug/ Metabolite	Mean Tmax (hr)		Mean Cmax (ng/ml)		Intrasubject Variability*	90% C.I.	C.I. Width
	Ref.	Test	Ref.	Test			
Triamterene	0.95	0.90	143.0	161.6	27.9%	(99, 127)	28
Hydroxy-triamterene sulfate	1.15	1.00	1928.0	1902.2	17.1%	(90, 107)	17
Doxepin	1.92	2.11	55.9	50.4	28.4%	(79, 102)	23
Desmethyldoxepin	4.53	5.01	11.9	11.0	16.2%	(86, 99)	13
Isosorbide Dinitrate	0.45	0.42	27.0	30.9	64.8%	(79, 150)	71
Isosorbide-2-mononitrate	0.85	0.86	38.4	43.9	21.0%	(103, 126)	23
Isosorbide-5-mononitrate	1.19	1.23	181.9	199.1	19.3%	(99, 120)	21
Metoprolol	1.8	1.8	140.3	150.0	17.4%	(100, 114)	14
Hydroxymetoprolol	1.9	1.9	144.7	154.1	9.3%	(103, 110)	7
Amitriptyline	4.21	3.92	24.8	25.6	18.5%	(94, 112)	18
Nortriptyline	12.33	15.42	9.8	9.8	12.0%	(94, 106)	12
Imipramine	3.2	3.0	57.1	58.7	14.3%	(95, 110)	15
Desipramine	8.3	9.9	21.5	21.4	19.2%	(89, 110)	21
Nortriptyline	7.85	8.00	31.0	29.9	11.7%	(91, 102)	11
Hydroxynortriptyline	6.35	7.57	26.7	26.2	19.3%	(89, 107)	18

* The % CV obtained from ANOVA was taken as an estimate of intrasubject variability

the absorption process of the parent compound, would the metabolite (if active) be needed for bioequivalence determination.

In summary, contrary to the general belief that measurement of the parent drug is the method of choice for bioequivalence decision, the present studies illustrated the importance of metabolite in the evaluation of rate of availability for linear drugs with a first-pass effect. The simulation results demonstrated that the need for metabolite data in the assessment of bioequivalence may rely on the relative variability between the absorption process for parent drug and first-pass route for metabolite(s). In light of the fact that information on the relative variability between these two processes is oftentimes unavailable from the actual bioequivalence studies, it would always be prudent to include both C_{max}'s of parent drug and metabolite for documentation of bioequivalence whenever the metabolite data are pertinent to the evaluation of clinical efficacy and/or safety of the drug product.

APPENDIX. GLOSSARY

Scenario

A: ka/ks = 3, intrasubject CV of ka $\geq$ ks.

B: ka/ks = 1.5, intrasubject CV of ka $\geq$ ks.

C: ka/ks = 3, intrasubject CV of ka $\leq$ ks.

Case

1: Variance added to ka, ks and k10.

2: Variance added to ka, ks and ke.

3: Variance added to ka, ks and kf.

HLH

Each simulation was given a three-letter designation. For example, in Case 1, the first letter was for the intrasubject variance of ka, the second for ks, and the third for k10. The high value was represented by H, and the low value by L.

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