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Research Article

The Role of Metabolites in Bioequivalency Assessment. I. Linear Pharmacokinetics without First-Pass Effect

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The estimation of bioequivalency using metabolite data was investigated for immediate release formulations with drugs exhibiting linear pharmacokinetics and no first-pass effect. This was accomplished by generating parent drug and metabolite plasma level profiles assuming formation and excretion rate-limited pharmacokinetic models with absorption rate constants obtained from bivariate normal distributions and designated random errors. Simulation results indicated that bioequivalence determination using C_{max} of parent drug and metabolite was independent of the metabolite models as evaluated by confidence interval approach. However, a clear difference with respect to the outcome of bioequivalence evaluation arises depending upon the utilization of C_{max} values for the parent drug and metabolite. The major reason for this disparity was attributed to the minimal effect of the absorption process for the parent drug on the formation of the metabolite. This phenomenon results in an apparent lower intrasubject variability for C_{max} of the metabolite and, in turn, a tighter confidence interval for C_{max} of the metabolite in comparison with the parent drug. The simulated results have been found to be in agreement with the bioequivalency data for acetohexamide, allopurinol, procainamide, and sulindac. In all cases, the interval of the 90% confidence limit for C_{max} of the metabolite is always smaller than that of the parent drug, regardless of the drug pharmacokinetics and the level of error contained in the data.

KEY WORDS: parent drug; metabolite; bioequivalence; absorption rate; peak concentration; time to peak.

INTRODUCTION

The implementation of the Drug Price and Patent Term Restoration Act in 1984 has drawn renewed attention to the issue of bioequivalence (1,2). It has been established that evaluation of bioequivalence between formulations is made on the basis of comparisons of the rate and extent of drug absorption (3). To achieve this goal, a bioequivalence study is generally conducted in a crossover fashion on human subjects comparing the test with the reference formulation. Plasma profiles of the drug are constructed by measuring drug concentrations in the plasma after drug administration. The rate and extent of drug absorption are estimated through the pharmacokinetic parameters (area under the curve, C_{max}, and T_{max}) derived from the plasma profiles of the subjects.

In general, the target species for measurement in bioequivalence studies is the parent drug whenever the drug is either not metabolized or is the only reported therapeutically active moiety. Often, however, the parent drug is subject to complex metabolic pathways following drug administration, resulting in the formulation of considerable levels of metabolite(s). In some cases, metabolites may be inert substances which simply act as the degradation products of the parent moiety. In other cases, metabolites may exhibit various degrees of therapeutic activity compared with the parent drug. Alternatively, some metabolites may be responsible for the adverse reactions observed during drug therapy. Questions are thus raised as to which entity should be measured in bioequivalence studies in order to ensure an accurate estimation of bioequivalency between formulations.

While drug metabolism and the kinetics of drug metabolites have been widely studied (4–9), the role of metabolites in bioequivalence determinations has been neglected (10). Traditionally, it has been suggested that the metabolite level in the systemic circulation after the administration of the parent drug is of particular concern when the metabolite makes a significant contribution to the overall drug efficacy and/or side effect. In these circumstances, bioequivalence assessment is often made based upon the pharmacokinetic data of the parent drug and its metabolite, as long as both species are measurable at the sampling site. On the other hand, a need for the determination of metabolite levels arises when the parent drug has a relatively short half-life and/or its levels are too low to be measured. Analysis of the parent drug, however, would be limited or precluded in this case and bioequivalence evaluation would have to rely on the metabolite data.

The aims of this paper are (a) to investigate the effect of

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metabolite models on the bioavailability/bioequivalence determination, (b) to explore the influence of formulation variation and/or subject variability relevant to metabolite models on the assessment of bioequivalency, and (c) to determine the degree of correlation for bioequivalence estimates (i.e., AUC, C_{max}, and T_{max}) between parent drug and metabolite data obtained from simulation and bioequivalence studies. The scenarios presented are limited to immediate-release drug products.

METHODS

Pharmacokinetic Simulations

Simulations were done representing drug and metabolite plasma concentration time curves following a single oral administration of an immediate-release formulation using CONSAM (11). Metabolite models employed in the simulations (Fig. 1) described the formation of a single metabolite, with the elimination of the metabolite being rate-limited either by its formation in the body (Model I) or by its excretion from the systemic circulation (Model II). The parameters used for the simulations are presented in Table I (12–14). Assumptions have been made that the test product was absorbed faster than the reference product (14) and the absorption of drug from the gastrointestinal tract is complete for all formulations.

To simulate the crossover design of a bioequivalence study, bivariate normal distributions of k_a were generated. While k_a was varied to represent the test and reference products, the k_f and k_e values were assumed to be constant for each subject. The correlation coefficient (r = 0.26) for k_a within each individual between the test and the reference product was based upon the observed C_{max} values in a typical bioequivalence study for procainamide whose metabolite elimination has been reported to be excretion rate limited (12).

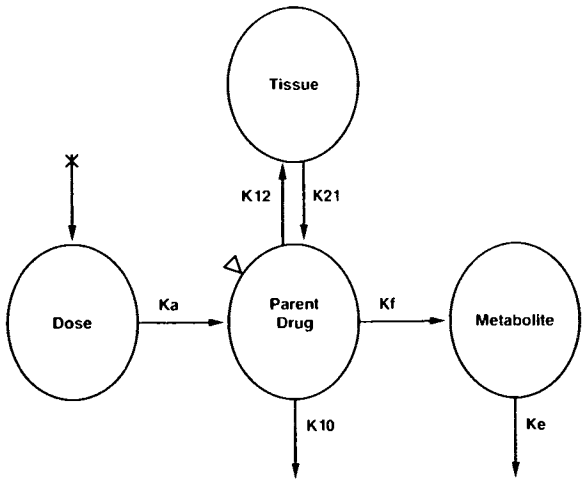

Fig. 1. Compartmental metabolite model used for the simulation where k_a = first-order absorption rate constant, k_{12} and k_{21} = intercompartment transfer constants, k_{10} = first-order elimination rate constant for parent drug from central compartment, k_f = formation rate constant of metabolite, and k_e = first-order elimination rate constant for metabolite.

Table I. Parameter Values Used in Simulations[a]

	Model I	Model II
Dose, mg	500	500
k_a, hr^{-1} (test/ref.)	0.75/0.43	0.75/0.43
k_{12}, hr^{-1}	0.527	0.527
k_{21}, hr^{-1}	0.174	0.174
k_{10}, hr^{-1}	0.3	0.3
k_f, hr^{-1}	0.09	0.2
k_e, hr^{-1}	0.2	0.09
$V_{d,p}$, L	100	100
$V_{d,m}$, L	100	100

[a] $V_{d,p}$: volume of distribution for parent drug. $V_{d,m}$: volume of distribution for metabolite.

Normally distributed error (high CV at 49% and low CV at 20%) was added to k_a to account for potential variability in assay, formulation, and/or subject differences (Table II). To investigate the effect of metabolite models on the pharmacokinetic behavior of parent drug and its metabolite, normally distributed error (CV, 20 vs 49%) was also added to the rate-limiting step of each model. The sampling time for the simulated data was at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hr for parent drug and at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 36 hr for metabolite.

Simulations were also performed in an attempt to study the influence of the magnitude of random error on the model specification as well as the performance of parent drug and its metabolite. In order to do so, two types of error addition were tested. First, equal sizes of random error (CV, 49%) were added to k_a and the rate-limiting step of each model. Second, in lieu of the rate-limiting step, variation (CV, 20%) was added to the non-rate-limiting step of the model. This resulted in four cases generated for each model (Table II).

All simulations were performed by varying two rate

Table II. Random Error (% CV) Used for Parameters in the Simulations

	Model I	Model II	Simulation characteristics
Case 1	k_a 49% k_f 20%	k_a 49% k_e 20%	High CV for k_a and low CV for k_f or k_e (rate-limiting step of Models I and II, respectively)
Case 2	k_a 20% k_f 49%	k_a 20% k_e 49%	Low CV for k_a and high CV for k_f or k_e (rate-limiting step of Models I and II, respectively)
Case 3	k_a 49% k_f 49%	k_a 49% k_e 49%	Equal CVs for k_a, k_f, and k_e (rate-limiting step of Models I and II, respectively)
Case 4	k_a 49% k_e 20%	k_a 49% k_f 20%	High CV for k_a and low CV for k_e or k_f (non-rate-limiting step of Models I and II, respectively)

constants at a time while keeping a third rate constant fixed so as to minimize the number of potential simulation outcomes. The procedure was repeated in order to generate 20 sets of data (i.e., 20 subjects) for each case. The bioavailability parameters, i.e., AUC(0–inf), C_{max}, and T_{max}, for the test and reference product were determined following simulations. The AUC(0–inf) values were derived from the exact equation, while the C_{max} and T_{max} were observed values from simulated data.

Bioequivalence Studies

Normal, healthy male volunteers between 18 and 50 years of age participated in the studies. All studies were conducted in a crossover fashion with a washout interval of 1 to 2 weeks between the phases of the treatment. Participants in the study were randomly assigned to the sequence of treatment. Bioavailability parameters of AUC(0–inf), C_{max}, and T_{max} were obtained from the studies. The AUC(0–t), where t represents the last measurable time point, was calculated using the linear trapezoidal rule and the AUC(t–inf) was estimated by extrapolation as described previously (15). The AUC(0–inf) was thus the sum of AUC(0–t) and AUC(t–inf). The C_{max} and T_{max} were observed values following the administration of the drug.

Acetohexamide

Twenty-four subjects completed this two-treatment, two-period crossover clinical trial. The drug treatments were generic acetohexamide tablets and reference Dymelor tablets. After fasting overnight, each subject received a single 500-mg tablet with 180 ml of water. Blood samples were drawn at 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hr after drug administration.

Serum levels of acetohexamide and its metabolite, hydroxyhexamide, were determined by a reverse-phase high-performance liquid chromatography (HPLC) described previously (16). Standard curves were linear over the range of 0.2 to 50 μg/ml for both compounds. The interday assay precision (% CV) for acetohexamide was 2.3% at 50 μg/ml, 5.7% at 0.5 μg/ml, and 16% at 0.2 μg/ml, while that for hydroxyhexamide was 3.0% at 50 μg/ml, 3.9% at 0.5 μg/ml, and 10% at 0.2 μg/ml. The sensitivity of the assay was 0.2 μg/ml for both compounds.

Allopurinol

The study was conducted as a two-treatment, two-period crossover in 20 subjects comparing a generic product of allopurinol tablets with reference Zyloprim tablets. The subjects were fasted overnight (10 hr) prior to and 5 hr after the start of the study. Each subject was given a 300-mg dose of allopurinol with 240 ml of water. Blood samples were taken at 0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hr after dosing.

Plasma samples were assayed for allopurinol and its major metabolite, oxypurinol, using an HPLC method adapted from the literature (17). The assay was linear for both allopurinol and oxypurinol over the range of 0–6 μg/ml. The intraday precision (% CV) for allopurinol was 2% at 2 μg/ml and 4.3% at 0.1 μg/ml, while that for oxypurinol was 1.8% at 2 μg/ml and 5.6% at 0.1 μg/ml. The interday precision for allopurinol was 8.9% at 4 μg/ml and 13% at 0.5 μg/ml, while that for oxypurinol was 8.6% at 4 μg/ml and 13.1% at 0.5 μg/ml. The sensitivity of the assay was 0.1 μg/ml for both compounds.

Procainamide HCl

A four-treatment, four-period crossover design was carried out using 22 volunteers. The drug treatments were generic procainamide HCl capsules (250, 375, and 500 mg) versus the reference product, Pronestyl capsules, 500 mg. Following an overnight fast, each subject was given either two 250-mg tablets or a single tablet (375 or 500 mg) with 180 ml of water. Blood was withdrawn at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, and 24 hr.

Plasma concentrations of procainamide and its major metabolite, *N*-acetylprocainamide, were determined by an HPLC method described earlier (18). The assay was linear over the concentration range of 0.05–6 μg/ml for both compounds. The intraday precision (% CV) was 0.6–3.5% for procainamide and 0.4–1.2% for *N*-acetyl procainamide. The interday precision was 1.4–3.7% for procainamide and 0.5–1.2% for *N*-acetyl procainamide. The sensitivity of the assay was 0.5 μg/ml for both the parent drug (CV, 2.1%) and the metabolite (CV, 1.0%).

Sulindac

This study was conducted as a two-treatment, two-period crossover in 28 subjects comparing a generic formulation of sulindac tablets with reference Clinoril tablets. Subjects were fasted overnight for 10 hr, and a 200-mg tablet was administered orally to each subject with 250 ml of water. Blood samples were collected at 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 10, 13, 24, 36, and 48 hr after dosing.

Plasma levels of sulindac and its active metabolite, sulindac sulfide, were measured by an HPLC method (19). Standard curves were linear over the concentration range of 0.2–10 and 0.1–10 μg/ml for sulindac and sulindac sulfide, respectively. The assay reproducibility (% CV) for sulindac was 2.2% at 0.2 μg/ml and 5.4% at 3.0 μg/ml, and that for sulindac sulfide was 6.5% at 0.1 μg/ml and 4.7% at 3.0 μg/ml. The sensitivity of the assay was 0.2 μg/ml for sulindac and 0.1 μg/ml for sulindac sulfide.

Estimation of Procainamide Pharmacokinetic Parameters

The individual plasma data of procainamide and its metabolite, *N*-acetyl procainamide, for each subject ($N = 22$) were fitted simultaneously using CONSAM. Individual subject data were inversely weighted by the variance of the assay error.

The pharmacokinetic model and estimates of model parameters used for the procainamide data were obtained from the literature (12). During data fitting, the intercompartmental rate constants and elimination rate constant from the central compartment were kept constant, while the other model parameters were allowed to vary to give the best fit of individual subject experimental observations.

Statistical Analysis

Analyses of variance were performed for AUC and C_{max} using SAS General Linear Models (GLM) procedures (20). The statistical model was partitioned into sequence, subject within sequence, period, treatment and an error term. The root mean square of the error term divided by the reference mean was used as an estimate of intrasubject variability for the parameters. The two one-sided hypotheses at the $\alpha = 0.05$ level of significance were tested by constructing the 90% confidence intervals for the difference of the two means (test versus reference) (21).

RESULTS

Tables III and IV summarize the mean values for the major bioavailability parameters following simulation for the formation rate-limited (Model I) and excretion rate-limited (Model II) models with the four different error scenarios.

Rigorous analysis of AUC values from the data is limited due to the inability to characterize truly some parameters required for the assessment of AUC, namely, fraction absorbed (F) and volume of distribution (V_d) of the drug in individuals following oral administration. Therefore, the primary focus of the data in this paper is the analysis of the rate of drug absorption. However, as expected from the constraints imposed on metabolite kinetics, a general trend shows that the AUCs of metabolite for Model I are lower compared with those of Model II. Moreover, irrespective of the location or magnitude of random error added to the model, within each model (equivalent dose) identical mean values of AUCs have been observed for both test and reference formulation across all cases as a result of the assumption that F equals to 1 in all simulations.

On the other hand, the rate of drug absorption, as indicated by C_{max} and T_{max} of the parent drug, reflected the distinction in the pharmacokinetic models and error structure assigned to each case. The difference in the absorption rate, k_a, of the drug between the test and the reference formulation was expressed in the C_{max} values of the parent drug to a higher degree than those of the metabolite. The T_{max} values of parent drug and metabolite are shorter for the test product than for the reference product, which agrees with the assumption of the present simulation. Furthermore, between-model comparisons revealed that metabolites in Model I peak much earlier than in Model II.

The coefficients of variation associated with the mean values of bioavailability parameters, listed in Tables III and IV, are indicative of intersubject variability. An examination of the data for the formation rate-limited model (Table III) shows that the intersubject variability of AUC and C_{max} for the metabolite are significantly greater than found for the parent drug in Cases 2 and 3 but not in Cases 1 or 4. Therefore, it appears that when a high percentage error is added to the rate-limiting step of the model, i.e., k_f for Model I, the random error imposed upon the model is directly transferred to the metabolite data regardless of the error size for k_a.

A similar relationship has been found for Model II (Table IV) in terms of the magnitude of intersubject variability with respect to metabolite kinetics and the size of random error added to the model. This is exemplified by Case 2, where the addition of high variation (49% CV) to the rate-limiting step, k_e, results in a greater degree of intersubject variability for AUC and C_{max} of the metabolite in comparison to the parent drug. In contrast, as shown in Table IV, less intersubject variability is found for metabolite C_{max} when a low variation of 20% CV is added to the rate-limiting step, k_e (Case 1), or for metabolite AUC and C_{max} when random error is loaded to the non-rate-limiting step, k_f (Case 4). The only exception noted for Model II is Case 3, where similar intersubject variability has been obtained for the C_{max} of the parent drug and metabolite as a result of the addition of equal variation (49% CV) to k_a and k_e.

For most cases, no direct relationship has been observed between the structure of random error and the intersubject variability associated with T_{max}, except for Cases 2 and 3 in Model II (Table IV), with a larger variation in the parameter for metabolite relative to parent drug.

As mentioned previously, a limitation of the present simulations is in the prediction of the extent of drug absorption. Since the errors associated with V_d and F have not been estimated, the reported AUC values reflected only apparent changes in k_a and, therefore, yielded essentially identical mean AUCs for parent drug for the test and reference formulation within each model.

To be consistent with the current evaluation procedures for bioequivalence studies, the confidence interval approach based upon the two one-sided t-test procedure has been used

Table III. Summary of Bioavailability Parameters for Model I (Formation Rate Limited)

	Case 1: k_a (49%), k_f (20%)		Case 2: k_a (20%), k_f (49%)		Case 3: k_a (49%), k_f (49%)		Case 4: k_a (49%), k_e (20%)	
	Test	Ref.	Test	Ref.	Test	Ref.	Test	Ref.
Parent drug								
AUC (μg · hr/ml)	12.87 (5.8)[a]	12.88 (5.8)	12.83 (13.3)	12.88 (13.8)	12.86 (12.8)	12.87 (12.8)	12.80 (0.00)	12.8 (0.1)
C_{max} (μg/ml)	1.83 (20.2)	1.25 (27.2)	1.66 (12.1)	1.18 (17.0)	1.82 (19.8)	1.24 (27.4)	1.83 (20.2)	1.25 (27.2)
T_{max} (hr)	1.27 (23.6)	1.85 (46.5)	1.37 (16.1)	1.77 (14.7)	1.27 (23.6)	1.93 (44.0)	1.25 (24.0)	1.85 (46.5)
Metabolite								
AUC (μg · hr/ml)	4.52 (20.1)	4.52 (20.1)	4.68 (42.7)	4.58 (45.0)	4.54 (43.6)	4.53 (43.7)	4.77 (34.2)	4.78 (34.1)
C_{max} (μg/ml)	0.25 (24.0)	0.22 (27.3)	0.26 (50.0)	0.22 (50.0)	0.26 (50.0)	0.23 (52.2)	0.25 (16.0)	0.22 (13.6)
T_{max} (hr)	4.60 (21.5)	6.50 (30.6)	4.80 (10.8)	6.40 (15.5)	4.55 (23.1)	6.30 (30.5)	4.55 (24.2)	6.95 (61.4)

[a] Coefficient of variation (%).

Table IV. Summary of Bioavailability Parameters for Model II (Excretion Rate Limited)

	Case 1: k_a (49%), k_e (20%)		Case 2: k_a (20%), k_e (49%)		Case 3: k_a (49%), k_e (49%)		Case 4: k_a (49%), k_f (20%)	
	Test	Ref.	Test	Ref.	Test	Ref.	Test	Ref.
Parent drug								
AUC (μg/hr/ml)	9.99 (0.00)[a]	9.99 (0.00)	9.99 (0.00)	9.99 (0.00)	9.99 (0.00)	9.99 (0.00)	9.93 (10.3)	9.93 (10.4)
C_{max} (μg/ml)	1.71 (20.5)	1.16 (28.5)	1.55 (11.6)	1.09 (15.6)	1.71 (20.5)	1.16 (28.5)	1.71 (21.6)	1.15 (27.8)
T_{max} (hr)	1.20 (25.0)	1.75 (38.9)	1.23 (24.4)	1.68 (14.3)	1.20 (25.0)	1.75 (38.9)	1.12 (28.6)	1.75 (38.9)
Metabolite								
AUC (μg · hr/ml)	20.38 (38.0)	20.39 (38.1)	21.40 (63.1)	21.41 (63.1)	21.51 (62.3)	21.52 (62.3)	17.91 (15.3)	17.92 (15.3)
C_{max} (μg/ml)	0.69 (11.6)	0.65 (13.9)	0.70 (25.7)	0.66 (30.3)	0.71 (23.9)	0.67 (28.4)	0.68 (19.1)	0.64 (21.9)
T_{max} (hr)	6.65 (29.3)	9.45 (42.9)	8.10 (54.8)	9.70 (41.3)	8.30 (69.9)	10.20 (52.4)	6.05 (19.0)	9.00 (43.0)

[a] Coefficient of variation (%).

for comparisons of C_{max} parameters in the simulations. The confidence intervals of T_{max}, however, were not computed in view of the fact that this parameter is used mainly as a qualitative check on the rate of absorption but rarely pivotal in a bioequivalence determination (1).

The resultant 90% confidence intervals for the difference between the test and the reference means of C_{max} are included in Table V. Unexpectedly, regardless of the pharmacokinetic model or error structure specified in the simulations, the 90% confidence intervals of the parent drug are consistently wider than those of the single metabolite formed. Furthermore, a remarkable decrease in the confidence limits of C_{max} has been found from parent drug to metabolite for each case in both models.

A closer look at the data (Table V) reveals that the intrasubject variability, as indicated by the root mean square of the error term divided by the reference mean, for the parent drug is markedly greater than found for the metabolite across all cases. This results in a much narrower confidence interval calculated for the metabolite relative to the parent drug. On the other hand, little correlation can be found between confidence limits and intersubject variability for C_{max} (Table V).

As indicated in Table V, neither model specification nor error configuration has a profound bearing on the size of within-subject variability of C_{max} for parent drug and metabolite. Nevertheless, it is noted that a decrease in intrasubject variability is obtained for the parent drug relative to metabolite in Case 2 for both models where low error (CV, 20%) was added to k_a, as opposed to other cases with high error being imparted to this rate constant. In addition, a further decrease in the intrasubject variability is observed for the metabolite in each case when going from Model I to Model II.

Perusal of the data from bioequivalence studies on drugs exhibiting linear pharmacokinetics without first-pass metabolism has been found to substantiate the simulated results. Tables VI and VII give examples of drugs with statistical summaries of C_{max}, T_{max}, and AUC for the parent compound and metabolite. As shown in Table VI, it is clear that,

Table V. Inter- and Intrasubject Variability and Confidence Intervals (CI) of C_{max} in the Simulations[a]

	Model I					Model II				
	Intersubject variability (%)		Intrasubject variability (%)	90% CI	CI width	Intersubject variability (%)		Intrasubject variability (%)	90% CI	CI width
	Test	Ref.				Test	Ref.			
Case 1										
P	20.2	27.2	26.5	(132, 161)	29	20.5	28.5	27.3	(133, 162)	29
M	24.0	27.3	10.1	(108, 119)	11	11.6	13.9	6.4	(103, 110)	7
Case 2										
P	12.1	17.0	15.0	(133, 149)	16	11.6	15.6	15.0	(134, 150)	16
M	50.0	50.0	8.7	(113, 123)	10	25.7	30.3	3.6	(104, 108)	4
Case 3										
P	19.8	27.4	26.8	(132, 162)	30	20.5	28.5	28.2	(132, 163)	31
M	50.0	52.2	9.4	(84, 93)	9	23.9	28.4	5.0	(103, 109)	6
Case 4										
P	20.2	27.2	26.4	(132, 161)	29	21.6	27.8	28.2	(133, 164)	31
M	16.0	13.6	10.0	(108, 119)	11	19.1	21.9	5.2	(103, 109)	6

[a] Intersubject variability expressed by the coefficient of variation associated with the mean. Intrasubject variability expressed by the root mean square of the error term divided by the reference mean. Confidence interval (CI) expressed as the percentage of the reference mean. P, parent drug; M, metabolite.

Table VI. Summary Data of Bioequivalence Studies on C_{max} Parameter for Drugs Following Linear Kinetics and Without First-Pass Effect[a]

Parent drug/metabolite	Dose	Subject No.	Mean C_{max} (μg/ml)		Intrasubject variability (%)	90% CI	CI width
			Ref.	Test			
Acetohexamide	500 mg	24	36.66	34.19	23.7	(81, 105)	24
Hydroxyhexamide		24	24.41	24.93	13.1	(95, 108)	13
Allopurinol	300 mg	20	1.57	1.70	31.9	(91, 126)	35
Oxypurinol		20	5.90	5.80	6.8	(95, 102)	7
Procainamide	500 mg	22	2.84	2.97	13.3	(98, 111)	13
N-Acetylprocainamide		22	0.93	0.93	9.0	(96, 105)	9
Sulindac	200 mg	28	6.6	6.8	37.5	(86, 120)	34
Sulindac sulfide		28	4.1	4.0	27.1	(84, 108)	24

[a] Confidence interval (CI) is expressed as the percentage of the reference mean. Intrasubject variability is expressed by the root mean square of the error term divided by the reference mean.

across the board, the magnitude of within-subject variability for C_{max} of metabolites is considerably smaller when compared with those of their respective parent compounds. As a result, tighter confidence intervals have been secured for the metabolite(s) than for the parent drug in each case. In contrast, no clear trend exists for the AUC parameter of the parent drug in comparison to the metabolite (Table VII). It should be noted that the terminal half-lives of these parent drugs and their metabolites vary from 1.4 to 3.8 hr and from 4.6 to 23.1 hr, respectively. However, in all cases the AUC(0–t) values account for greater than 80% of the total AUCs calculated.

In an attempt to explore possible explanations for the underlying phenomenon, curve fitting has been done using the pharmacokinetic data from the bioequivalence study on procainamide HCl capsules. Figures 2 and 3 illustrate the fitted plasma profiles of procainamide and its major metabolite, *N*-acetylprocainamide, in one subject following the administration of a single 500-mg dose of procainamide HCl. The three rate constants pertinent to metabolite Model II, k_a, k_f, and k_e, were estimated from fitted data for each formulation. Table VIII presents the summary statistics for these three rate constants. Interestingly, the results of analysis of variance indicate that the intrasubject variability for k_a is much higher than for k_f and k_e, while a similar magnitude of within-subject variability is obtained for k_f and k_e.

DISCUSSION

Individual inherent enzyme activity is one of the potential sources of intrasubject variability arising for all the kinetic processes after drug administration. However, individual variation of drug absorption can exceed that of metabolite formation and/or elimination, as observed in the procainamide case. Hence, the absorption process for the parent drug from a solid dosage form may be more complex than the subsequent events of formation and/or elimination of its metabolite(s).

Since a drug has to be in solution before it is absorbed, the absorption process of a drug from a solid dosage form encompasses several stages including disintegration, dissolution, and partitioning. In this regard, therefore, formulation characteristics such as coatings and excipients not only play an important role but also contribute profound variability to the absorption rate of the drug. Clearly, aside from the intrinsic enzyme variability within each individual, the variability in the absorption process among individuals receiving the formulations is the major factor for the high variations of k_a found in the fitted data. It is possible that the residual error in the statistical analysis for k_a is more likely related to product variability rather than "true" intrasubject variability since the latter is often assumed to be very small for crossover designs in bioequivalence studies.

Table VII. Summary Data of Bioequivalence Studies on T_{max} and AUC Parameter for Drugs Following Linear Kinetics and Without First-Pass Effect

Parent drug/metabolite	Dose	Subject No.	Mean T_{max} (hr)		Mean AUC (μg · hr/ml)		AUC, 90% CI	AUC, CI width
			Ref.	Test	Ref.	Test		
Acetohexamide	500 mg	24	1.81	1.74	113.0	109.9	(93, 102)	9
Hydroxyhexamide		24	3.58	3.50	205.8	213.8	(95, 113)	18
Allopurinol	300 mg	20	1.28	1.44	3.7	4.0	(98, 119)	21
Oxypurinol		20	4.20	4.20	176.9	177.1	(97, 104)	7
Procainamide	500 mg	22	1.30	1.02	14.2	13.9	(95, 101)	6
N-acetylprocainamide		22	2.73	2.14	12.5	12.4	(94, 105)	11
Sulindac	200 mg	28	1.42	1.51	12.8	12.4	(88, 105)	7
Sulindac sulfide		28	2.33	2.59	28.4	26.8	(79, 110)	31

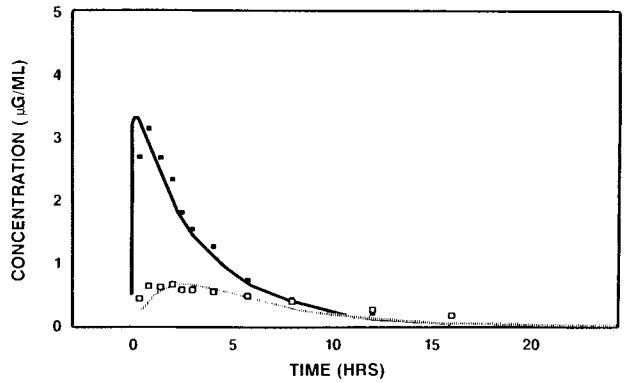

Fig. 2. Mean plasma concentrations of procainamide (■) and its metabolite, *N*-acetylprocainamide (□), in subject 16 following a single 500-mg Pronestyl capsule.

From the pharmacokinetic point of view, regardless of metabolite kinetics, peak concentrations of a drug and its metabolite(s) for one-compartment model can be described simply by the following two equations (15,6):

$$C_{max} = \frac{k_a F X_0}{V(k_a - k)} (e^{-k \cdot t_{max}} - e^{-k_a \cdot t_{max}}) \quad (1)$$

$$C_{max}(m) = A \cdot e^{-k \cdot t_p} + B \cdot e^{-k_e \cdot t_p} - C \cdot e^{-k_a \cdot t_p} \quad (2)$$

where C_{max} and $C_{max}(m)$ are the peak concentration of parent drug and metabolite, respectively; t_{max} and t_p are the corresponding peak time for parent drug and metabolite; F is the fraction of the administered dose X_O that is absorbed following oral administration; V is the volume of distribution of parent drug; k is the elimination rate constant of parent drug, which is a combination of k_f and k_{10} (see Fig. 1); and A, B, and C are coefficients which are functions of the various rate constants and fractions formed from the parent drug that are systemically available (6).

For an immediate release dosage form, k_a is often expected to be substantially larger than k_f and k_e. Under such conditions, the term $C \cdot e^{-k_a \cdot t_p}$ in Eq. (2) will be relatively small compared with $A \cdot e^{-k \cdot t_p}$ or $B \cdot e^{-k_e \cdot t_p}$. Accordingly, while intrasubject variability exists for both C_{max} values of parent drug and metabolite, there is a clear distinction as to the origin of the variability for each species. For the parent drug, intrasubject variance of C_{max} originates from k_a and k (where $k = k_f + k_{10}$), whereas that of the metabolite derives mainly from k and k_e. It is apparent that a significantly higher variability will result for C_{max} of parent drug than the metabolite because of the dominance of k_a in its estimation.

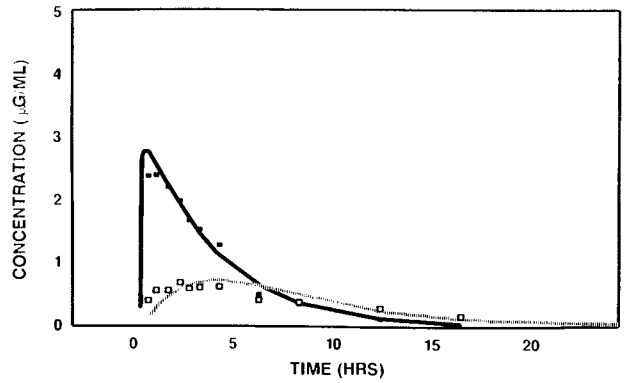

Fig. 3. Mean plasma concentrations of procainamide (■) and its metabolite, *N*-acetylprocainamide (□), in subject 16 following a single 500-mg generic procainamide HCl capsule.

Table VIII. Summary of Rate Constants for Fitted Data from a Bioequivalence Study Following Administration of a Single 500-mg Dose of Procainamide[a]

Rate constant	Mean (% CV) Test	Mean (% CV) Ref.	Mean diff. (%)	Intrasubject variability (%)
k_a, hr^{-1}	2.17 (110.3)	1.36 (142.9)	0.81 (59.6)	120.9
k_f, hr^{-1}	0.71 (57.1)	0.63 (48.0)	0.08 (12.7)	30.7
k_e, hr^{-1}	0.26 (35.0)	0.31 (35.6)	0.05 (16.1)	29.9

[a] Intrasubject variability is expressed by the root mean square of the error term divided by the reference mean.

The importance of pharmacokinetics and pharmacodynamics of drug metabolites has been readdressed in a recent workshop sponsored by Pharmaceutical Manufacturers Association (PMA) (10). It is widely accepted that the knowledge of metabolite pharmacokinetics can aid in the evaluation of bioequivalence among different formulations, when metabolites make a significant contribution to the overall therapeutic effect. Nonetheless, the role of metabolites in bioequivalence is challenged by the fact that metabolite formation is a sequence secondary to the absorption of the parent drug, and thus the appearance of metabolite(s) in the blood may be too remote, from the absorption process of the drug, to differentiate adequately formulation differences in the absorption rate of the parent drug. The above speculation, in fact, is strongly supported by the data presented in this manuscript.

The results of the simulation are admittedly striking in that, regardless of the metabolite model and random errors specified, the intrasubject variability of C_{max} is greatly diminished from parent drug to metabolite, which invariably leads to a significant reduction in the width of the confidence interval for the metabolite compared with the parent drug. As a consequence, using identical statistical criteria or decisional criteria for bioequivalence, while the test product is often found to be bioequivalent to the reference product based on metabolite data, bioinequivalency may be claimed between the two formulations based on parent drug data. This applies to immediate-release drug products where the drug follows linear kinetics and exhibits no complications of first-pass effect. Conceivably, if parent drug data are unavailable, differences in the rate of drug absorption between test and reference products may be indiscernible using metabolite data alone.

At the present time, it is unknown whether the same conclusions can be drawn for drugs following nonlinear kinetics or for drugs with a first-pass effect. However, in light of the significant differences in pharmacokinetics between controlled-release and immediate-release dosage form, it is speculated that results of simulations for controlled-release drug products may deviate from the present findings for immediate-release formulations.

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