Simultaneous Investigation of Indinavir Nonlinear Pharmacokinetics and Bioavailability in Healthy Volunteers Using Stable Isotope Labeling Technique: Study Design and Model-Independent Data Analysis

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Abstract □ Indinavir follows nonlinear pharmacokinetics upon oral administration at clinical doses. A study employing the stable isotope administration technique in a three-treatment design was conducted to identify the source of the nonlinearity and to determine the dose-dependency of systemic bioavailability. In treatment A, 400 mg of unlabeled indinavir (D_0) was coadministered orally with 16 mg of a hexadeutero analogue of indinavir (D_6) intravenously. In treatment B, 800 mg of D_0 po was coadministered with 16 mg of D_6 intravenously. In treatment C, 16 mg of iv D_6 was infused concurrently with 16 mg iv of D_0. Plasma concentrations of D_0 and D_6 were determined by an LC/MS/MS assay method. Concentrations of indinavir in plasma increased greater than dose-proportionally over the 400- to 800-mg dose range. No meaningful kinetic isotope effects were found in treatment C. Plasma concentrations of D_6 were dependent on the coadministered D_0-indinavir dose and were lowest in treatment C, higher in treatment A, and highest in treatment B. The bioavailability of indinavir was high (60–65%) and comparable between the 400- and 800-mg doses. There was a significant contribution of nonlinear kinetics in the systemic circulation to the observed disproportional increase in plasma concentrations following oral dosing. The high bioavailability at clinically relevant doses suggests a high degree of saturation of first-pass metabolism. These results further demonstrate that the concomitant administration technique in combination with the LC/MS/MS method can provide a realistic and reliable means of elucidating important pharmacokinetic properties of drug candidates during product development.

Introduction

Characterization of biopharmaceutical and pharmacokinetic attributes of a drug candidate is an integral part of the drug product development. When properly studied and evaluated, such information provides the scientific basis for the optimal definition of dosage regimens. Among the many important properties are the pharmacokinetics of drug absorption and disposition and their dependence on dose or concentration. Procedures for bioavailability assessment differ, depending on whether linear kinetics prevail in the therapeutically relevant concentration range.

For a drug exhibiting linear pharmacokinetics, as demonstrated by proportional increases in plasma concentration with dose, plasma clearance can be assumed to be independent of the concentration. Clinical studies for determination of the systemic bioavailability are often conducted in a crossover fashion. The bioavailability of the oral treatment relative to the reference intravenous treatment is estimated by comparing the dose-normalized areas under the drug concentration curve (AUC). The crossover study design eliminates the intersubject variation and results in improved reliability of the estimates.

For a drug with nonlinear disposition kinetics, its clearance is concentration-dependent, and the above-cited crossover bioavailability study design may not be valid.[1−4] Under special circumstances, concomitant administration of the intravenous reference dose and the oral dose may provide a viable approach of obtaining reliable bioavailability estimates.[1−3,5] In this approach, the reference treatment is distinguished from the oral treatment by the use of a tracer dose of the isotopically labeled drug.[2,4,6] With recent advances in the LC/MS/MS assay detector technology,[7,8] simultaneous tracking and quantitation of both the unlabeled and labeled analytes can be reliably achieved.[9,10] Due to its potential of high specificity and sensitivity, the technique is most useful when the labeled dose, given intravenously, is small in mass relative to that of the unlabeled dose given orally. With the concurrent dosing study design, the intrasubject variation is eliminated. Further, stable isotope-labeled analogues may be repetitively administered in clinical studies. The concurrent dosing design may also provide a means of identifying the source(s) of pharmacokinetic nonlinearity.

Indinavir, a potent HIV protease inhibitor, exhibited greater than dose-proportional increases in plasma concentrations following oral administration in clinical studies.[11,12] Possible causes of the observed nonlinearity include the dose-dependent absorption or first-pass metabolism, nonlinear systemic disposition, or both. Based on the LC/MS/MS assay technology, a clinical study was conducted to examine the source(s) of the nonlinearity and to compensate for the observed nonlinearity and thereby enabling estimates of the systemic bioavailability in the therapeutic dose range. The purpose of this communication is to present the design and findings of the study.

Materials and Methods

Equation 1 forms the basis of computing the bioavailability (F) of a given dose (D) based on the observed drug concentration ($C(t)$):

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$$F_{po} = \frac{F_{iv} D_{iv} \int_0^\infty CL_{po}(t) C_{po}(t) dt}{D_{po} \int_0^\infty CL_{iv}(t) C_{iv}(t) dt} \quad (1)$$

where CL(t) is the clearance at time t, and subscripts iv and po denote the mode of administration. In general, the time profile of CL(t) is concentration-dependent and is unknown in a system where the disposition kinetics are nonlinear. The integrated quantities in eq 1 represent the respective amount of the drug which has been eliminated from the systemic circulation following the administration of test and reference treatments. In the present case, F_{iv} is assumed to be unity.

Upon simultaneous administration of a tracer intravenous dose and an oral dose, the systemically available drug from both doses is subject to the same time-course of clearance, CL(t):

$$F_{po} = \frac{D_{iv}}{D_{po}} \frac{\int_0^\infty CL(t) C_{po}(t) dt}{\int_0^\infty CL(t) C_{iv}(t) dt} \quad (2)$$

Under the situation where $C_{iv}(t)$ is an exact multiple of $C_{po}(t)$ at all times, the ratio of the two would remain constant:

$$z = \frac{C_{iv}(t)}{C_{po}(t)} \quad (3)$$

Substituting eq 3 into eq 2:

$$F_{po} = \frac{D_{iv}}{D_{po}} \frac{\int_0^\infty CL(t) C_{iv}(t) dt}{z \int_0^\infty CL(t) C_{iv}(t) dt} = \frac{D_{iv}}{z D_{po}} \quad (4)$$

Expressions similar to eqs 2 and 3 in bioavailability assessment under concurrent administration conditions have been discussed and reported previously.[5] In actual practice, due to differences in the absorption rate and infusion rate, eq 3 is virtually impossible to achieve since the ratio of the two concentrations will invariably fluctuate with time. As an approximation, an apparent z_{app} is defined as follows:

$$z_{app} = \frac{\int_0^\infty C_{iv}(t) dt}{\int_0^\infty C_{po}(t) dt} = \frac{AUC_{iv}}{AUC_{po}} \quad (5)$$

By analogy to eq 4, the bioavailability is estimated by the following:

$$F_{po} \cong \frac{D_{iv}}{z_{app} D_{po}} \quad (6)$$

In a nonlinear disposition system, the bioavailability computed with eq 6 would not be exact but would be an approximation. The degree of the imperfection would depend on the degree to which the concentration ratios, shown in eq 3, fluctuated with time. The magnitude of fluctuations in such a system might be minimized, but not eliminated, with properly designed clinical studies. To reduce the errors, other study designs, such as delaying the iv bolus or varying the infusion period to various fractions of plasma peak time (T_{max}), have been discussed previously.[5]

With the present approach, the estimation of bioavailability is based on the comparison of dose-normalized AUCs, and specific assumptions regarding the underlying mechanism(s) of the nonlinearity are not required. This represents a model-independent approach, with its computational procedures being identical to that of the conventional AUC method. It is worth noting that in a linear pharmacokinetic system, the accuracy of bioavailability estimation is independent of the fluctuations shown in eq 3.

Study Subjects and Dosing Regimens—The study was conducted as an open-label, three-period, single-dose study with 12 healthy subjects (6 males and 6 females, age range: 21 to 31 years; mean age, 25.3 years; weight range: 53 to 92 kg; mean weight, 70.9 kg). The protocol was approved by the Medeval Independent Ethics Committee, and informed consent was obtained from all subjects. The study subjects received doses of the unlabeled indinavir (D_0) as well as indinavir which was labeled with six deuterium atoms on the pyridomethyl side chain (D_6). The three treatments were as follows:

Treatment A: 400 mg D_0 orally as one 400-mg dry-filled capsule (DFC) plus 16-mg D_6 dose intravenously as an infusion.

Treatment B: 800 mg D_0 orally as two 400-mg DFC's plus 16-mg D_6 dose intravenously as an infusion.

Treatment C: Simultaneous intravenous infusion of 16 mg D_0 and 16 mg D_6 over 30 min.

Subjects fasted from midnight before drug administration and remained fasted until 2 h after the dosing. In treatments A and B, each oral D_0-indinavir dose was administered at 0 h with 240 mL water, and each intravenous D_6 dose was administered as a 30-min infusion, starting at 15 min and ending at 45 min following the ingestion of the oral dose. The infusion regimen was designed to minimize the fluctuations in the ratio between the expected plasma concentrations of D_0 and D_6 over time. Previous clinical studies[11–13] indicated an average T_{max} of 0.8 h and often with a lag time <0.25 h in absorption following oral administration.

Treatment C was designed to examine (a) the disposition kinetics of indinavir at concentrations significantly lower than that generally achieved following oral dosing; and (b) the possible in vivo enzymatic isotope effect on the metabolism (and therefore the systemic disposition) of indinavir. Depyridomethylation is known to be one of the CYP3A-mediated oxidative biotransformations of indinavir in humans.[14,15]

Treatments A and B were administered according to a randomized two-period crossover design. Treatment C was administered in the third period. All iv solutions contained 0.05 mg/mL of D_6-indinavir. In treatment C, the iv solution also contained 0.05 mg/mL of D_0-indinavir. All indinavir doses were expressed as the milligram equivalent of anhydrous free base of unlabeled drug.

Plasma samples for assay of indinavir were obtained in treatments A and B at 0 (predosing), 20, 25, 35 and 45 min, and at 1, 1.5, 2, 4, 6, 8, 10, 12, 16, and 24 h following oral dosing; and in treatment C at 0, 5, 10, 20, 30, and 45 min, and at 1, 1.5, 2, 4, 6, 8, 10, 12, 16, and 24 h postdosing. All samples were stored at −20 °C until assayed.

Analytical Method—Concentrations of D_0-indinavir and D_6-indinavir in plasma were quantified by an LC/MS/MS method.[16] Briefly, the analytes and internal standard were isolated from plasma via liquid−liquid extraction with methyl *tert*-butyl ether. Multiple reaction monitoring of the parent → product ion combinations of m/z 614 → 465, 620 → 471, and 654 → 505 were used to quantify D_0-indinavir, D_6-indinavir, and internal standard, respectively, in the resulting extracts. The method was validated over the concentration range of 1 to 200 ng/mL for each analyte, using 1-mL aliquots of plasma. Precision of the assay, as measured by the coefficient of variation, ranged from 0.9 to 4.3% and 0.9 to 6.2% for D_0-indinavir and D_6-indinavir, respectively. Indinavir concentrations were converted to a molar basis using molecular weights of 613.81 for D_0-indinavir and 619.81 for D_6-indinavir. The limit of quantification was 1.63 and 1.61 nM for D_0 and D_6, respectively.

Pharmacokinetic Analysis—Peak plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) following oral administration were obtained by inspection. All AUC values were computed up to the last sampling time point at 24 h without extrapolation, and were obtained by the modified trapezoidal method using stable piecewise cubic polynomials.[17] For the intravenously administered plasma concentration data, AUC values were obtained as the sum of AUC up to the end of infusion and the AUC after the end of infusion. Plasma concentrations below the limit of quantification were treated as zero in all calculations. Apparent plasma clearance was computed as the quotient of D_6-dose/D_6-AUC.

To further investigate the effect of the fluctuations in concentration ratio on the bioavailability estimates, a second analysis of the data with a modeling approach was taken. The model composed of three compartments with saturable elimination from the central compartment and saturable distribution in one of the peripheral compartments. Nonlinear regression analysis was employed to obtain the best-fit model parameters. The bioavailabilities of the oral doses were estimated using a mass balance approach. The computational procedures and the results based on this analysis have been reported.[18]

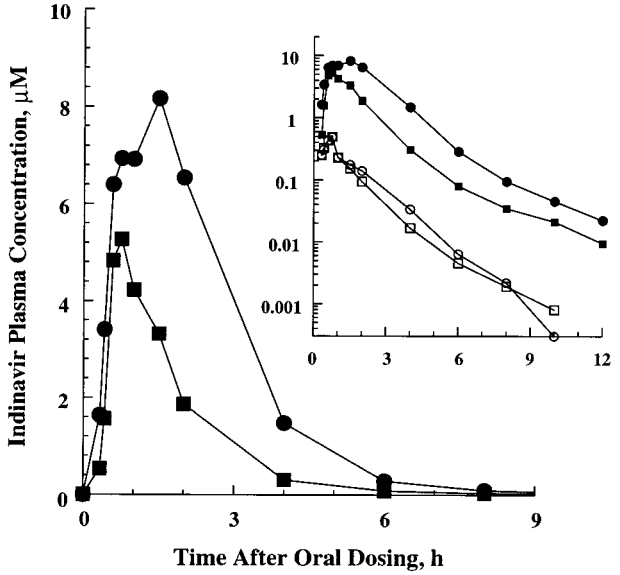

Figure 1—Mean plasma concentration profiles of D_0-indinavir following oral administration of D_0-indinavir in treatments A and B. Comparisons with the corresponding mean profiles of D_6-indinavir following intravenous administration of D_6-indinavir are shown in the inset. Key: ■ = 400 mg of D_0-indinavir po in treatment A; ● = 800 mg of D_0-indinavir po in treatment B; □ = 16 mg of D_6-indinavir iv in treatment A; ○ = 16 mg D_6-indinavir iv in treatment B.

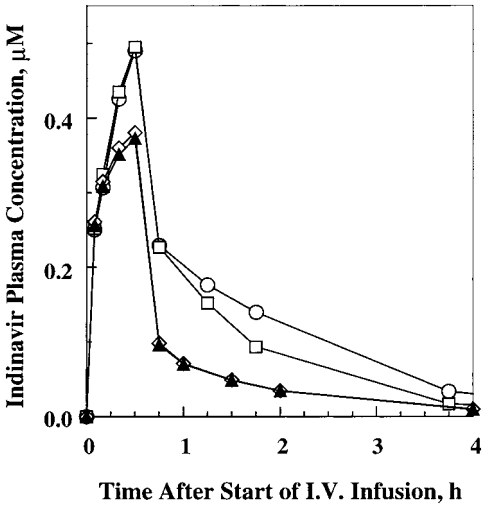

Figure 2—Comparisons of plasma concentration profiles of the intravenously administered indinavir from all three treatments. Key: □ = D_6 with 400 mg of oral indinavir in treatment A; ○ = D_6 with 800 mg of oral indinavir in treatment B; ◇ = D_6 in treatment C; ▲ = D_0 in treatment C.

Statistical Analysis—Statistical analysis was performed on the natural log-transformed individual bioavailability estimates based on dose-normalized D_6-AUC and D_0-AUC values. An ANOVA model for a two-period crossover design was used to test for the period effect. The systemic bioavailability for each treatment was estimated by the geometric mean (GM) ratio of the individual bioavailability estimates for that treatment. Ninety percent confidence intervals (CI) for the bioavailability estimates for each treatment were calculated on the log scale, and the limits were then exponentiated to yield the 90% CI for the GM ratio. To evaluate the kinetic isotope effect in treatment C, paired *t*-tests were performed to compare the AUC and the concentration at 30 min ($C_{30\ min}$) for D_0 and D_6. Dose-normalized geometric mean ratios and the corresponding 90% CI were similarly obtained.

Results

Concentration Profiles—Mean plasma concentration profiles of indinavir following the administration of oral and intravenous doses are shown in Figures 1 and 2. Summary values of the pharmacokinetic parameters are presented in Table 1.

The orally administered indinavir data in the present study were generally consistent with those observed previously following oral administration,[11,12] with the exception of a trend of a slightly prolonged T_{max} following the 800-mg dose in treatment B relative to that of the 400-mg dose in treatment A. As expected, there was a disproportionate increase in plasma concentrations; an increase of 2-fold in the dose from 400 to 800 mg resulted in a 2.67-fold increase in AUC. However, as a result of the delayed T_{max} following the 800-mg dose, C_{max} did not increase disproportionally as is generally seen over a wider dose range.[11] Plasma concentrations of D_0-indinavir and D_6-indinavir in treatment C were virtually superimposed. Differences between the D_6-AUC and D_0-AUC, although statistically significant, were small, suggesting the absence of meaningful kinetic isotope effect.

Plasma concentrations of the intravenously administered D_6-indinavir varied considerably with treatment and were dependent on the magnitude of the coadministered D_0-indinavir concentrations. The D_6-AUC values were highest when coadministered with the 800-mg oral dose in treatment B, lower with the 400-mg oral dose in treatment A, and lowest with the 16-mg D_0-indinavir iv dose in treatment C. Plasma concentrations at the end of the 30-min infusion also demonstrated a similar trend. Additionally, in the presence of coadministered D_0-indinavir in the two oral treatments, plasma concentrations of D_6-indinavir declined more slowly after the termination of infusion and near the plasma T_{max} for D_0-indinavir. Adjusted for the increase in D_6-AUC from treatment A to treatment B (ratio = 1.22), the net increase in D_0-AUC was reduced to 2.19-fold. These observations are consistent with nonlinearity in the systemic disposition kinetics contributing significantly to the disproportionate increase in D_0-indinavir concentrations over the 400- and 800-mg dose range.

Plasma Clearance—It was reported previously[11] that following oral administration, the indinavir terminal plasma concentration did not reach the log-linear phase until concentrations had declined to below ~0.1 to 0.5 μM. In the present study, the range of the total concentrations in treatment C (sum of D_0- and D_6-indinavir) did not completely fall within this range. However, concentrations generally did not exceed 0.8 μM and the computed apparent plasma clearance (1.29 L/min) was approaching the hepatic blood flow of 1.45 L/min. The decreased plasma clearances in treatments A and B reflected the increased D_6-AUC values in the presence of high concentrations of D_0-indinavir in these treatments and were consistent with the concentration-dependency of clearance in a nonlinear disposition system.

Bioavailability—The systemic bioavailability of indinavir appeared to be high, averaging 0.60 following the 400-mg dose, and 0.65 following the 800-mg dose. The corresponding values based on the modeling approach were 0.64 for both doses.[18] The dose-dependence of bioavailability values over this dose range was not statistically significant.

Discussion

This study was motivated by the desire to understand the underlying source(s) of the observed nonlinear pharmacokinetics in previous clinical studies and to examine the systemic bioavailability of indinavir. Recognizing the potential of bioavailability overestimation based on conventional crossover study design in a nonlinear system,[19] the concurrent administration technique was adapted for the present study. In addition, treatment C was incorpo-

Table 1—Geometric Mean of Pharmacokinetic Parameter Values and the Corresponding Ratios (90% CI) of Intravenously and Orally Administered Indinavir

analyte	pharmacokinetic measures	treatment A[e]	treatment B[f]	treatment C[g]
D_0 (po)	AUC_{0-24h}, μM·h	7.41	19.78	–
	AUC ratio[a]	–	2.67 (2.10, 3.40)	–
	C_{max}, μM	5.89	10.55	–
	C_{max} ratio[a]	–	1.79 (1.23, 2.53)	–
	T_{max}, h[b]	0.87 ± 0.40	1.20 ± 0.53	–
	T_{max} difference[a]	–	0.33 (0.0, 0.65)	–
	bioavailability	0.60 (0.54, 0.66)	0.65 (0.58, 0.72)	–
	bioavailability ratio[a]	–	1.09 (0.92, 1.29)	–
D_6 (iv)	apparent CL, L/min	0.85 (0.74, 0.98)	0.70 (0.62, 0.79)	1.29 (1.20, 1.38)
	AUC_{0-24h}, μM·h	0.510	0.620	0.337
	AUC ratio[c]	1.50 (1.32, 1.69)	1.83 (1.60, 2.09)	1.016 (1.010, 1.021)[d]
	AUC ratio[a]	–	1.22 (1.07, 1.39)	–
	C_{30min}, μM	0.472	0.478	0.377
	C_{30min} ratio[a]	–	1.01 (0.88, 1.17)	–
	C_{30min} ratio[c]	1.25 (1.04, 1.51)	1.27 (1.10, 1.46)	1.023 (1.016, 1.031)[d]

[a] Relative to treatment A. [b] Arithmetic mean ± SD. [c] Relative to the intravenous D_6 dose in treatment C. [d] Relative to the intravenous D_0 dose in treatment C. [e] Treatment A: 400 mg of D_0 orally plus 16 mg of D_6 intravenously. [f] Treatment B: 800 mg of D_0 orally plus 16 mg of D_6 intravenously. [g] Treatment C: Simultaneous 16 mg of D_0 and 16 mg of D_6 intravenously.

rated to serve as a reference for identifying the source of nonlinearity.

While there are a number of factors that could result in the nonlinear disposition kinetics, plasma protein binding did not appear to be a contributing factor in the present case. Binding of indinavir to plasma protein is not extensive (39% unbound) and is not dependent on concentration up to 80 μM, which is significantly higher than the C_{max} of 10.6 μM achieved following the 800-mg dose. Other potential causes include saturable elimination and tissue binding. A more detailed study on the mechanism(s) of the systemic nonlinearity can be found elsewhere (Stone et al., unpublished results).

Increasing the mass as a result of isotope labeling generally increases the bond stability in the drug molecule.[20,21] In the present case, the deuterium isotope was chosen to be incorporated on the pyridomethyl moiety of the indinavir because of its relative ease of chemical syntheses. Since the bond cleavage leading to depyridomethylation is one of the metabolic pathways for indinavir,[15] the possibility that the metabolic rate and therefore the overall disposition kinetics of indinavir might be perturbed could not be ruled out. The most desirable deuterated analogue of indinavir would have contained the labeling placed at the metabolically stable site of the molecule. However, such an ideal analogue of indinavir was not available at the time of the study. Any significant difference in the disposition kinetics of indinavir between the two analogues of the drug would have made the D_6 a poor surrogate for D_0 in data analysis.

Prior to initiation of the present human study, the potential for the isotope effect was examined. In vitro studies with D_6 using rat liver and human microsome preparations indicated modest decreases (~23%) in the formation of the dealkylated product.[13] Intravenous administration of a 1:1 mixture containing the D_0 and D_6 in rats at a total dose of 10 mg/kg indicated no discernible difference in plasma concentration profiles.[13] In vitro metabolism studies indicated depyridomethylation was only one of the multiple CYP3A4-mediated pathways in the oxidative metabolism of indinavir in humans.[15] These experiments suggested that the potential kinetic isotope effects in human would be negligible, which was confirmed by the data from treatment C of the present study.

The mass of the intravenous D_6-indinavir dose administered in treatments A and B was considerably lower than that of the D_0 oral doses. The D_0-AUC data observed in the present study did not appear inconsistent with the historical data,[11] suggesting the D_6 dose did not have significant effect on the absorption or disposition processes of the D_0 oral doses.

The first-pass metabolism of indinavir has been characterized to be mediated by the CYP3A isoform of the cytochrome P450 enzyme system, primarily in the liver but also to some extent in the intestine.[22,23] This same isozyme is also known to be involved in the metabolism of other protease inhibitors ritonavir, saquinavir, and nelfinavir. It has been recently reported[24] that protease inhibitors as a class are also substrates for p-glycoprotein (Pgp) which functions as a transmembrane efflux pump.[25–27] Thus, in addition to the first-pass metabolism, the apical expression of Pgp in the epithelial cells of the GI tract and on the bile canalicular surface of hepatocytes would function as biological barriers and further limit the absorption for this class of drugs. The high bioavailability results of the present study would suggest a reduced impact of the Pgp-mediated transporter system at clinically relevant doses of indinavir.[28,29]

The use of a constant-rate infusion rather than a bolus intravenous administration in the study was designed to reduce the magnitude of fluctuations in the D_6/D_0 concentration ratios and to improve the reliability of bioavailability estimates.[5] Ideally, the infusion rates may be controlled to match closely the rate of drug delivery to the systemic circulation. In actual practice, the superposition can only be approximated since the drug input after an oral dose is not known a priori. Figure 3 shows the (D_6/D_0) plasma concentration ratios following the two oral treatments. These data indicated relatively constant ratios after about 1.5 h post oral dosing. However, substantial fluctuations were noted during the earlier time points. Prior to the start of the infusion at 15 min, D_0-concentrations were unmatched with ratios effectively zero for a brief period. The ratios then increased rapidly and reached the maximum within 5 min once the D_6 infusion was initiated. It should be noted that a perfect alignment of T_{max} with the end of infusion would only reduce, but not eliminate, the fluctuations, due to the differences in the input rate between the two administration modes. The magnitude of errors associated with such imperfection has been reviewed using model simulations.[5]

Cross comparisons of the bioavailability estimates based on the present method and those based on the modeling approach[18] are presented in Figure 4. Although the two computational procedures differed from each other, the results were numerically comparable, confirming the im-

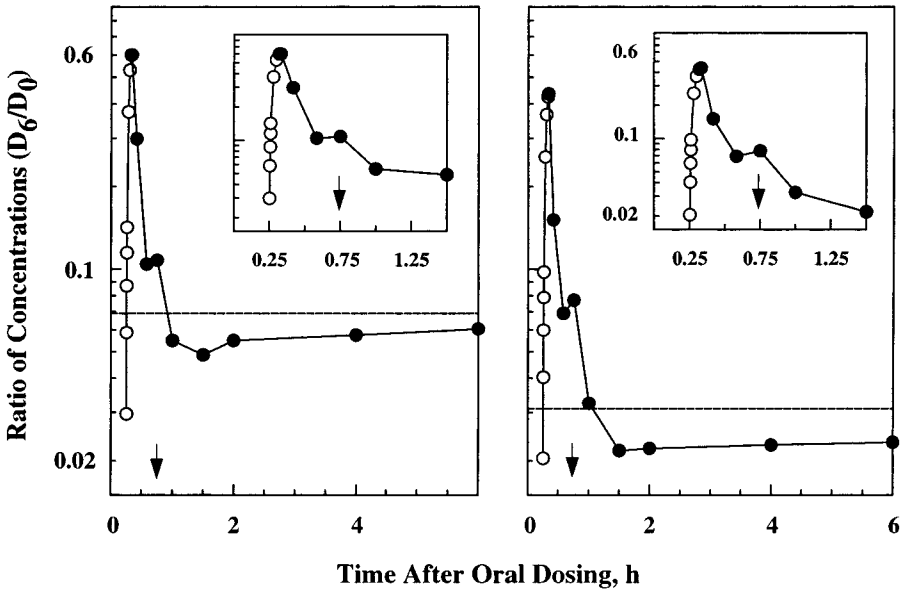

Figure 3—Ratios of plasma concentrations (D_6-indinavir/D_0-indinavir) following the administration of 16 mg of D_6-indinavir iv with 400 mg of D_0-indinavir po in treatment A (left panel) or with 800 mg of D_0-indinavir po in treatment B (right panel). Solid circles represent median ratios and open circles represent medians of simulated ratios during the first 5 min immediately after the initiation of the iv infusion. Simulated ratios were obtained using interpolated concentration values between 0 and 5 min for the po and iv curves, which were obtained by the piecewise cubic polynomial functions.[17] The arrows indicate the end of infusion at 0.75 h. The horizontal lines represent the apparent Z_{app} based on eq 5. The expanded initial ratios are shown in the insets.

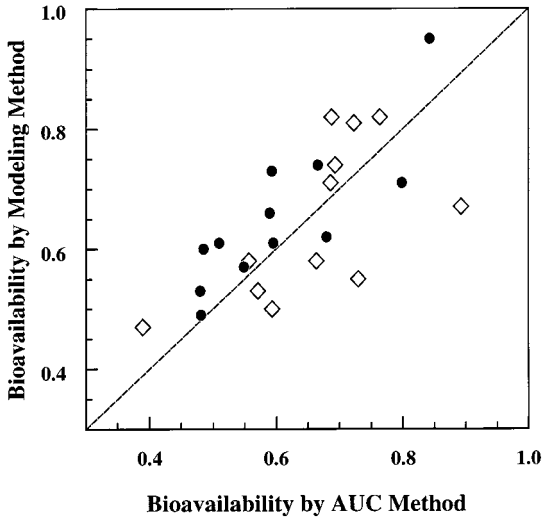

Figure 4—Comparison of the bioavailability estimates based on the present model-independent AUC method and that based on the kinetic modeling method.[18] The diagonal line represents the projected perfect agreement between the two estimation methods. Key: ● = 400-mg dose; ◇ = 800-mg dose.

portance of the delayed iv infusion regimen used in this study in reducing the imperfection of fluctuations in the (D_6/D_0) plasma concentration ratios. Lengthening the infusion period with a reduced input rate would likely have reduced the fluctuations at early time points (Figure 3) and improve the concordance between the two estimates for those data sets with greatly delayed T_{max}, although it also would have adversely affected the bioavailability estimates from the data sets with shorter T_{max}. On the other hand, errors in bioavailability estimates based on the modeling method would most likely arise from model miss-specification and thus would be model-dependent but would be less likely to be correlated with the D_0 T_{max}.

While the ratio of indinavir concentrations in blood to that in plasma was not available for humans, preclinical studies indicated the ratio to be approximately equal to unity for monkeys.[22] Thus, the plasma clearance and the blood clearance for indinavir could be assumed to be comparable for humans. It has been reported that the urinary excretion of intact indinavir constituted 5−12% of the dose over the 200−1000 mg dose range, indicating a significant contribution of hepatic metabolism to the overall elimination of the drug.[11,15] At low indinavir plasma concentrations, the extremely high clearance (1.29 L/min) relative to the hepatic blood flow of ∼1.45 L/min in man would suggest that at low doses, indinavir could be classified as a high hepatic extraction drug with low systemic bioavailability following oral administration,[30] and that dose-dependence of bioavailability would contribute significantly to the observed pharmacokinetic nonlinearity. In addition, the first-pass metabolism would gradually become more saturated with increasing doses, and as the doses approached the clinical range, the bioavailability would be high and would be only marginally dose-dependent.

In summary, the present study indicates that indinavir bioavailability was high (60−65%) and comparable over the 400−800 mg dose range and that there was a significant contribution of the nonlinearity in the systemic disposition to the observed nonlinear pharmacokinetics following oral administration. These results demonstrate that the concurrent administration approach can be used to identify the source(s) of pharmacokinetic nonlinearity and obtain bioavailability estimates in a nonlinear pharmacokinetic system. Characterizing important pharmacokinetic attributes of drug candidates during product development based on such an approach has become increasingly feasible as a result of recent advances in LC/MS/MS instrumentation.[31] The application of the model-independent method described in the present study requires that there is no kinetic isotope effect. The computed bioavailability is not exact and its reliability depends on the closeness of similarity between the plasma concentration profile following the test dose and that following the labeled tracer dose. However, the elaboration needed in the design and execution of such studies is compensated for by the simplicity in data analysis. With the conventional crossover study design, bioavailability in a nonlinear disposition system could only at best be estimated with a modeling

approach.[32] Additionally, due to the concentration-dependence in clearance, systemic bioavailability could be potentially overestimated unless the concentration ranges achieved in the intravenous treatment were sufficiently high and comparable to that achieved in the test treatment. For drugs with low aqueous solubility, it may not be feasible, if not impossible, to achieve the required plasma concentrations with clinically acceptable preparations.

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