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A Population Pharmacokinetic Analysis of Nelfinavir Mesylate in Human Immunodeficiency Virus-Infected Patients Enrolled in a Phase III Clinical Trial

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A population pharmacokinetic analysis was conducted on nelfinavir in patients infected with human immunodeficiency virus (HIV) who were enrolled in a phase III clinical trial. The data consisted of 509 plasma concentrations from 174 patients who received nelfinavir at a dose of 500 or 750 mg three times a day. The analysis was performed using nonlinear mixed-effect modeling as implemented in NONMEM (version 4.0; double precision). A one-compartment model with first-order absorption best described the data. The timing and small number of early postdose blood levels did not allow accurate estimation of volume of distribution (*V*/*F*) and the absorption rate constant (k_a). As a result, two models were used to analyze the data: model 1, in which oral clearance (CL/*F*), *V*/*F*, and k_a were estimated, and model 2, in which *V*/*F* and k_a were fixed to known values and only CL/*F* was estimated. Estimates of CL/*F* ranged from 41.9 to 45.1 liters/h, values in close agreement with previous studies. Neither body weight, age, sex, race, dose level, baseline viral load, metabolite-to-parent drug plasma concentration ratio, history of liver disease, nor elevated results of liver function tests appeared to be significant covariates for clearance. The only significant covariate-parameter relationship was concomitant use of fluconazole on CL/*F*, which was associated with a modest reduction in interindividual variability of CL/*F*. Patients who received concomitant therapy with fluconazole had a statistically significant reduction in nelfinavir CL/*F* of 26 to 30%. Since serious dose-limiting toxicity and concentration-related toxicities are not apparent for nelfinavir, this effect of fluconazole is unlikely to be of clinical significance.

Nelfinavir mesylate (Viracept) is a protease inhibitor approved for use in the treatment of human immunodeficiency virus (HIV) infection by the Food and Drug Administration (FDA) in March 1997. The currently recommended adult dose is 750 mg three times a day (TID) taken with or after food (Agouron prescribing information, Agouron Pharmaceuticals, La Jolla, Calif., 1998), and an alternative 1,250-mg twice-daily dosage regimen has been recently approved by the FDA. Pharmacokinetic studies of nelfinavir have previously been conducted in phase I and phase II of clinical drug development (9, 11). When nelfinavir was administered at a dose of 500 or 750 mg TID, the peak plasma concentration (C_{max}) at steady state was in the range of 3 to 4 μg/ml and the time to peak concentration (T_{max}) was in the range of 2.5 to 3 h (9). The half-life of nelfinavir has been reported in these studies as 3 to 5 h (9, 11).

Nelfinavir, like the other currently available protease inhibitors, ritonavir, indinavir, saquinavir, and amprenavir, is metabolized by the cytochrome P450 system (6, 7, 9, 10, 13; C. A. Lee, B.-H. Liang, E. Y. Wu, H. M. Grettenberger, T. M. Sandoval, K. E. Zhang, and B. V. Shetty, Abstr. 4th Natl. Conf. Hum. Retroviruses Related Infect., 1997; J. L. Lillibridge, C. A. Lee, Y. K. Pithavala, T. M. Sandoval, E. Y. Wu, K. E. Zhang, E. L. Mazabel, M. Zhang, and B. M. Kerr, Abstr. 5th Int. ISSX Meet., abstr. 109, 1998; E. Y. Wu, T. M. Sandoval, K. E. Zhang, H. M. Grettenberger, B. R. Hee, C. A. Lee, S. Webber, and B. V. Shetty, Abstr. 5th Int. ISSX Meet., abstr. 110, 1998; M. H. Zhang, Y. K. Pithavala, C. A. Lee, J. H. Lillibridge, E. Y. Wu, T. M. Sandoval, R. G. Daniels, and B. M. Kerr, Abstr. 12th Int. Symp. Microsomes Drug Oxidations, abstr. 264, 1998). In vitro studies have shown that CYP3A4 and CYP2C19 are the predominant isoenzymes involved in the metabolism of nelfinavir (Lillibridge et al., 5th Int. ISSX Meet.; Wu et al., 5th Int. ISSX Meet.; Zhang et al., 12th Int. Symp. Microsomes Drug Oxidations). Other isoenzymes CYP2D6 and CYP2C9 are involved to a lesser extent (13; B. Kerr, G. Yuen, R. Daniels, B. Quart, and R. Anderson, Abstr. Natl. Conf. Hum. Retroviruses Related Infect., 1997; Lee et al., 4th Natl. Conf. Hum. Retroviruses Related Infect., Wu et al., 5th Int. ISSX Meet.). Nelfinavir has one major metabolite, M8 (also referred to as nelfinavir hydroxy-*t*-butylamide or AG1402), in human plasma. This metabolite has been shown to have an antiviral potency in vitro similar to that of nelfinavir (10; Lillibridge et al., 5th Int. ISSX Meet.; Zhang et al. 12th Int. Symp. Microsomes Drug Oxidations). The generation of this metabolite appears to be exclusively catalyzed by CYP2C19 (5; Lillibridge et al., 5th Int. ISSX Meet.; Wu et al., 5th Int. ISSX Meet.). Studies have demonstrated that CYP2C19 exhibits genetic polymorphism (8); 2 to 6% of Caucasians and 18 to 22% of Asians are poor metabolizers of CYP2C19.

The development of resistance to protease inhibitors, like that to other antiretroviral therapies, is of major concern. It has been observed that resistance is more likely to develop when plasma concentrations of protease inhibitors are sub-

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therapeutic (2). Therefore it is important to characterize the pharmacokinetics of nelfinavir in a representative patient population and to try and identify variables that may lead to lower plasma concentrations, which in turn may increase the risk of therapeutic failure. In particular, due to the extensive metabolism of nelfinavir by cytochrome P450 enzymes, there is the potential for drug interactions that affect nelfinavir concentrations to occur. This is especially of concern in patients with HIV disease, as these patients often take concomitant medications which are known to induce or inhibit cytochrome P450.

Thus, the objectives of this study were to determine the population pharmacokinetic parameters of nelfinavir and their variability in an HIV-infected patient population, to determine the influence of patient characteristics on the pharmacokinetic parameters of nelfinavir, and to investigate potential pharmacokinetic drug interactions.

MATERIALS AND METHODS

Concentration-time data were obtained from patients enrolled in a phase III clinical study, which was primarily designed to evaluate the safety and efficacy of nelfinavir in HIV-infected patients. A secondary objective of the study was to determine the population pharmacokinetic parameters of nelfinavir. Safety and efficacy results have been reported elsewhere (N. Clendennin, B. Quart, R. Anderson, M. Knowles, and Y. Chang, Abstr. 5th Conf. Retroviruses Opportunistic Infect., abstr. 372, 1998; M. Saag, M. Knowles, Y. Chang, S. Chapman, and N. J. Clendennin, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., 1997). This paper presents the results of the population pharmacokinetic analysis.

Study population. The study population included patients aged 13 years or older with HIV infection who had received either no prior antiretroviral therapy or less than 1 month of treatment with zidovudine (AZT). Patients were required to have a baseline plasma HIV RNA titer of ≥15,000 copies/ml. Participants in the trial were randomized to receive nelfinavir at 500 or 750 mg TID or placebo. All patients received concomitant therapy with AZT (200 mg TID) and lamivudine (150 mg twice a day). Randomization was stratified according to the patient's baseline CD4 count (less than 100 cells per μl, 100 to 300 cells per μl, or greater than 300 cells per μl) to ensure that the treatment groups were balanced. Concentration-time data were obtained from 174 participants who received one of the nelfinavir dosage regimens. These patients were enrolled at 27 investigative sites.

Exclusion criteria at baseline examination included prior antiretroviral therapy, therapy with immune modulators or vaccines within 1 month of baseline, patients of procreative potential who were not practicing double-barrier contraception, elevated liver function tests (LFTs) or hemoglobin or bilirubin levels, decreased neutrophil or platelet counts, renal insufficiency, acute pancreatitis or hepatitis, significant fever or diarrhea, malabsorption syndrome, severe intermittent medical conditions including opportunistic infections, active substance abuse, neoplastic disease requiring radiation or cytotoxic therapy, and females taking oral contraceptives. The appropriate institutional review boards approved the study, and written informed consent was obtained from all participants.

Plasma samples. The pharmacokinetic study was of an observational, population design. Patients had blood samples drawn for analysis of nelfinavir concentrations during clinical follow-up visits. In general, each individual provided two blood samples per visit: a predose trough sample and a postdose sample collected approximately 2 h after the dose taken during the visit. Doses of nelfinavir were given with food at these visits. The majority of individuals were sampled at weeks 2 and 8 of the study; hence all concentrations were considered to be at steady state. Ultimately, 509 samples from 174 patients were used in the pharmacokinetic analysis. This was an average of approximately three samples per patient (range of one to six).

Plasma sample analysis. Plasma concentrations of nelfinavir and M8 were measured by a validated method using high-performance liquid chromatography and UV detection. The published version of this method (16) was modified slightly (the mobile phase consisted of 32.5% acetonitrile plus 7.5% methanol instead of 42% acetonitrile and the flow rate was adjusted to 1.5 ml/min) to accommodate the analysis of M8. The lower and upper limits of quantification were 0.05 and 10.0 mg/liter, respectively, for each analyte. For this study, assay correlation coefficients (16 analytical runs) exceeded 0.994 for nelfinavir and 0.997 for M8. Based on quality control samples, interday accuracy for the two analytes ranged from 97 to 101% of nominal concentration and interday precision expressed as a percent coefficient of variation was less than 10%.

Data preparation and pharmacokinetic analysis. Clinical, pharmacokinetic, and demographic data relevant to the population pharmacokinetic analysis were extracted from the raw data sets and merged and formatted using SAS (version 6.09) on an IBM ES-9000 computer. The pharmacokinetic analysis was performed using NONMEM (version 4.0, double precision) (S. L. Beal and L. B. Sheiner [ed.], NONMEM user's guide, NONMEM Project Group, University of California at San Francisco, San Francisco) on an IBM ES-9000 computer. Initially, the analysis was performed using the first-order (FO) method for parameter estimation, and the results were subsequently confirmed using the first-order conditional estimation (FOCE) method.

Pharmacokinetic model. Several pharmacokinetic models were used to fit the data. A one-compartment model and a two-compartment open model with first-order absorption and first-order elimination were tested. In addition, a zero-order input to a one-compartment model was tested using an input period of 3 h (the average value of T_{max} for nelfinavir [range of 2 to 4 h], as reported previously [Agouron prescribing information]). The first-order input to the one-compartment model was parameterized as the first order absorption rate constant (k_a), oral clearance (CL/F), and volume of distribution (V/F), and the zero-order input to the one-compartment model was parameterized as CL/F and V/F. The two-compartment model was parameterized as k_a, CL/F, volume of the central compartment (V_c/F), volume of distribution at steady state (V_{ss}/F), and intercompartmental clearance (Q). The model that best fit the data was selected for further analyses.

Statistical model. An exponential-error model and a proportional-error model were evaluated to describe interindividual variability. The models were $\theta j = \theta' \exp(\eta_{\theta_j})$ (exponential-error model) and $\theta_j = \theta'[1 + (\eta_{\theta_j})]$ (proportional-error model) where θ_j is the estimate for a pharmacokinetic parameter in the jth individual as predicted by the regression model, θ' is the population mean of the pharmacokinetic parameter, and η_{θ_j} represents the random variable with zero mean and variance ω^2 that distinguishes the jth individual pharmacokinetic parameter from the population mean value predicted by the regression model. Terms for interindividual variability were included for CL/F, V/F, and k_a.

Residual variability (including intraindividual variability) was modeled using either a proportional-error model or a combined proportional- and additive-error model. The equations used were $C_{ij} = C'_{ij}(1 + \varepsilon 1_{ij})$ and $C_{ij} = [C'_{ij}(1 + \varepsilon 1_{ij})] + \varepsilon 2_{ij}$ where C_{ij} is the observed serum concentration for the jth individual at time i, C'_{ij} is the model predicted serum concentration for the jth individual at time i, and $\varepsilon 1_{ij}$ and $\varepsilon 2_{ij}$ are the components of the proportional and additive errors (with zero mean and variance σ^2), respectively.

Data analysis strategy. The pharmacokinetic and statistical models were evaluated to determine the basic model that best fit the data. A statistically significant decrease ($P = 0.05$) in the minimum value of the objective function (as measured by the log likelihood difference) found when comparing reduced to full models, visual inspection of weighted residual plots, and an evaluation of the precision of pharmacokinetic parameter and variability estimates were used as criteria to determine the best basic model.

After the basic model was constructed, a model-building process was employed to examine the influence of patient covariates on the estimates of the pharmacokinetic parameters. The effects of the following patient covariates were evaluated: age, weight, sex, ethnic origin, dose, baseline HIV disease status, history of liver disease, elevated LFTs, metabolite-to-parent drug (M8-to-nelfinavir) plasma concentration ratio, and use of concomitant medications.

Age and weight were examined as continuous variables. Sex, dose, ethnic origin, and history of liver disease were examined as categorical variables. The effect of an increase in LFTs was examined as a dichotomous variable, a change of either grade two and higher or less than grade two. The HIV disease status of the patient at entry into the study was characterized by baseline CD4 count and baseline viral load. The CD4 count was categorized into three groups as follows: less than 100 cells per μl, 100 to 300 cells per μl, and greater than 300 cells per μl. Viral load measurements were also split into three categories: greater than 100,000 copies/ml, 50,000 to 100,000 copies/ml, and less than 50,000 copies/ml. The baseline level of circulating HIV RNA in plasma was estimated using an experimental branched-DNA signal amplification assay (second-generation Chiron branched-DNA assay). The M8-to-nelfinavir plasma ratio (a reflection of CYP2C19 enzyme activity) was investigated as a potential covariate both as a continuous variable and as a categorical variable. This was determined, where possible, from the M8-to-nelfinavir concentration ratio in the 2-h postdose plasma samples. Ratio values were empirically categorized into three groups: a ratio less than 0.1 was considered low, a ratio between 0.1 and 0.3 was considered intermediate, and a ratio greater than 0.3 was considered high (Lillibridge et al., 5th Int. ISSX Meet.). It should be noted that metabolite data were only available for 110 of the 174 patients in this study. As CYP3A4 and CYP2C19 play important roles in the elimination of nelfinavir (Kerr et al., Natl. Conf. Hum. Retroviruses Related Infect.; Lee et al., 4th Natl. Conf. Hum. Retroviruses Related Infect.; Zhang et al. 12th Int. Symp. Microsomes Drug Oxidations), concomitant medications known to inhibit or induce these isoenzymes were examined as potential covariates. The effects of macrolide antibiotics and quinolone antibiotics were examined as a class rather than on an individual basis to increase the number of patients in each covariate group. In this study, patients taking an azole antifungal consisted mainly of patients taking fluconazole; thus, the effect of fluconazole alone was investigated as was the effect of concomitant therapy with rifabutin.

A decrease in the minimum value of the objective function of 3.8 or greater following introduction of a single covariate into the model was considered statistically significant ($P = 0.05$) using the χ^2 distribution if the 95% confidence intervals (CI) for the estimate did not include the null value. If the change in the objective function was 3.8 or greater but the 95% CI for the estimate included the null value, the effect of the variable was considered to be of borderline

TABLE 1. Characteristics of 174 patients evaluated in the population pharmacokinetic analysis of nelfinavir

Characteristic	No. of patients	% Total study population
Sex (men/women)	155/19	89/11
Mean age (range)	37 (21–63)[b]	
Mean total body weight (range)	77.7 (42–140)[c]	
Baseline CD4 count (cells per μl)		
<100	35	20
<300 and >100	59	34
<300	80	46
Baseline viral RNA (copies/ml)		
>100,000	68	39
>50,000 and <100,000	43	25
>50,000	63	36
Race		
Caucasian	136	78
Black	21	12
Asian	3	2
Hispanic	7	4
Latin American	4	2
Native American	3	2
Concomitant medications		
Azole (fluconazole)	25 (23)	15 (13)
Macrolide	18	10
Quinolone	12	8
Rifabutin	5	3
History of liver disease	60	34
LFTs grade 2 or higher	51	29
Plasma M8-to-nelfinavir ratio[a]		
Low ratio (<0.1)	6	3
Intermediate ratio (>0.1 and <0.3)	67	38
High ratio (>0.3)	37	21

[a] M8 concentrations were available for only 110 of 174 patients in this study.
[b] In years.
[c] In kilograms.

significance and that covariate was not included in the full model. All significant variables were included in the full model. It was assumed that no significant interactions between covariate factors existed. If there was an interaction present for an effect that was not significant alone at the $P = 0.05$ level, then this effect would be minor and would not be likely to be of clinical significance. Thus, covariate effects were introduced individually and no covariate-covariate interactions were modeled.

A backward elimination process was then employed to eliminate covariates from the full model in order to develop the final model. An increase in the objective function of 3.8 or greater ($P = 0.05$) on removal of a covariate from the full model signified that the variable was important, and that covariate was retained in the final model.

Minimal pharmacokinetic model. It was difficult to obtain accurate estimates of V/F and k_a during this analysis. To assess the impact of possible misestimation of V/F and k_a on the ability to estimate CL/F, the parameter of most interest, V/F and k_a, were fixed to more-reliable values and only CL/F was estimated in the modeling process. Data from a traditional pharmacokinetic study consisting of intensive sampling per individual in a given dosing interval were analyzed using NONMEM to obtain values for V/F and k_a. These values were subsequently used to fix V/F and k_a in the analysis of the population data. Thus, analysis of the population data was performed using two models: model 1 in which all three pharmacokinetic parameters were estimated and model 2 in which fixed estimates of V/F and k_a were used and only CL/F was estimated.

Briefly, the data from the traditional study consisted of 190 observations from 19 HIV-infected patients who received nelfinavir monotherapy at doses of 500 or 750 mg TID. Data were obtained from a phase II clinical trial, the results of which have previously been reported (9). Each patient provided 10 plasma samples at the following times: predose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 h postdose during a steady-state dosing interval on day 28 of treatment. No patient was taking drugs that were known inhibitors or inducers of CYP3A4.

RESULTS

The demographic characteristics of the 174 patients in the population analysis are summarized in Table 1. The mean age was 37 years (range, 21 to 63 years), and mean total body weight was 78 kg (range, 42 to 140 kg). The patient population was predominantly male (89%) and Caucasian (78%).

A one-compartment model and a two-compartment model with first-order absorption and first-order elimination were used to fit the data. It was not possible to obtain model convergence when the two-compartment model was fit to the data; thus a one-compartment model was used. The first-order absorption model provided a significantly better fit to the data than the zero-order absorption model. Thus, the best basic model consisted of the one-compartment model with first-order absorption and first-order elimination. Interindividual variability was best described using an exponential-error model, and intraindividual variability was best described by a combined proportional- and additive-error model.

Convergence was achieved using a one-compartment model employing model 1 (all three pharmacokinetic parameters were estimated) and model 2 (V/F and k_a were fixed to estimates obtained from analysis of the traditional pharmacokinetic data). The mean parameter estimates (95% CI are in parentheses) from analysis of the traditional data were as follows: CL/F, 39.1 liters/h (30.1 to 48.1 liters/h); V/F, 229 liters (161 to 297 liters); k_a, 0.845 h^{-1} (0.60 to 1.13 h^{-1}). Thus, V/F and k_a were fixed to 229 liters and 0.845 h^{-1}, respectively, in model 2.

The basic population pharmacokinetic parameter estimates (95% CI are in parentheses) from the fit of model 1 to the data were as follows: CL/F, 40.7 liters/h (37.7 to 43.7 liters/h); V/F, 731 liters (531 to 931 liters); k_a, 1.22 h^{-1} (0.70 to 1.74 h^{-1}). The variability in the estimate of CL/F, expressed as approximate percent coefficient of variation, was 36%. When a term for interindividual variability was included in V/F, the value tended towards zero. Thus it was not possible to model interindividual variability in V/F. A large degree of interindividual variability, 214%, was associated with k_a. Model 2 provided estimates of CL/F and its variability of 39.4 liters/h (36.3 to 42.5 liters/h) and 39%, respectively. These estimates agreed well with the estimates obtained using model 1. In turn, estimates of CL/F using both models agreed favorably with the estimate that was obtained from analysis of the traditional data.

The patient covariates that significantly influenced CL/F using both models 1 and 2 are shown in Table 2. A baseline CD4 count less than 100 cells per μl and concomitant use of either a macrolide antibiotic or fluconazole resulted in a significant decrease in the estimate of CL/F. The magnitudes of reduction in CL/F using either model 1 or model 2 were similar and were 17 to 19, 23 to 24, and 26 to 27% for CD4 count and macrolide and fluconazole use, respectively. A significant increase in CL/F of 55% was observed in patients taking rifabutin using model 1. In contrast, a non-statistically significant increase of 37% in CL/F was observed using model 2. Due to the lack of a consistently statistically significant effect of rifabutin and the small number of patients in the study taking rifabutin, this covariate was excluded from the model-building procedure. Neither age, weight, dose level, sex, ethnic origin, baseline viral load, M8-to-nelfinavir plasma ratio, history of liver disease, nor LFTs grade two or higher appeared to influence CL/F in this group of patients.

The full model consisted of a low baseline CD4 count and concomitant use of either a macrolide antibiotic or fluconazole. A backward elimination process was then employed to eliminate nonsignificant covariates from the full model to develop the final model. The final model parameters using both models are shown in Table 3. The equations for CL/F were as follows. For model 1 (all parameters estimated), $CL/F = 42.7(1 - 0.256 \cdot \text{flu})$ liters/h where flu = 1 if the patient was taking concomitant fluconazole therapy. For model 2 (V/F and

TABLE 2. Summary of analyses of significant effects of patient covariates tested alone in CL

Hypothesis	Parameterization (CL/*F*)	Null value	θ4 (95% CI)	Change in objective function	*P*
Model 1 (do the following affect CL/*F*?)					
CD4 < 100 cells per μl	$\theta1(1 - \theta4 \cdot cd4)$	0	0.19 (0.02, 0.36)	10.014	<0.01
Concomitant medications					
Fluconazole	$\theta1(1 - \theta4 \cdot flu)$	0	0.26 (0.11, 0.40)	17.225	<0.01
Macrolide	$\theta1(1 - \theta4 \cdot mac)$[a]	0	0.24 (0.02, 0.46)	10.784	<0.01
Ribabutin	$\theta1(1 + \theta4 \cdot rif)$[b]	0	0.55 (0.32, 0.77)	6.035	<0.05
Model 2 (do the following affect CL/*F*?)					
CD4 < 100 cells per μl	$\theta1(1 - \theta4 \cdot cd4)$	0	0.17 (0.01, 0.33)	6.978	<0.01
Concomitant medications					
Fluconazole	$\theta1(1 - \theta4 \cdot flu)$	0	0.27 (0.15, 0.40)	18.531	<0.01
Macrolide	$\theta1(1 - \theta4 \cdot mac)$	0	0.23 (0.06, 0.41)	10.132	<0.01
Rifabutin	$\theta1(1 + \theta4 \cdot rif)$	0	0.37 (0.17, 0.57)	3.012	Not significant

[a] mac, value of 1 for concomitant macrolide therapy and 0 otherwise.
[b] rif, value of 1 for concomitant rifabutin therapy and 0 otherwise.

k_a fixed), CL/*F* = $41.9(1 - 0.273 \cdot flu)$ liters/h where flu was as defined above.

The estimates of CL/*F* for patients not taking fluconazole derived from the two models were comparable, 42.7 and 41.9 liters/h for models 1 and 2, respectively. Additionally, the effect of fluconazole was similar in both models, resulting in a reduction in clearance of 26 to 27%. Interindividual variability in CL/*F* was reduced marginally from 36 to 34% and from 39 to 36% using models 1 and 2, respectively. After controlling for use of fluconazole, use of a macrolide antibiotic or a low baseline CD4 count did not significantly affect CL/*F* in either model. Thus it was not necessary to include either of these covariates in the final models for CL/*F*.

This data set was also analyzed using the FOCE method in NONMEM. The only covariate found to be of statistical significance was concomitant use of fluconazole. In contrast to the FO method, a low CD4 count, concomitant use of a macrolide antibiotic, and concomitant use of rifabutin were either of borderline significance or not significant at all when tested alone in CL/*F* and thus were not included in the full model. Thus, the final model included only the effect of concomitant fluconazole. Parameter estimates for the final models are shown in Table 3. The estimates of CL/*F* using FOCE were 44.9 and 45.1 liters/h for models 1 and 2, respectively. These results compare favorably with the results obtained from the FO method, 42.7 and 41.9 liters/h, respectively. Additionally, the reductions in CL/*F* observed in patients taking concomitant fluconazole of 26 and 30% for models 1 and 2, respectively, are in good agreement with the reductions of 26 and 27% obtained using the FO method.

DISCUSSION

The pharmacokinetic parameters of nelfinavir were best described by a one-compartment open model with first-order absorption and first-order elimination. It proved difficult to

TABLE 3. Final population pharmacokinetic parameters for model 1 and model 2[a]

Parameter (units)[e]	Model 1		Model 2	
	Parameter estimate (95% CI)	Interpatient variability[b] (95% CI)	Parameter estimate (95% CI)	Interpatient variability[b] (95% CI)
FO method				
θ_1[c] (liters/h)	42.7 (39.3–46.1)	34 (27–39)	41.9 (38.7–45.1)	36 (28–43)
θ_2 (liters)	736 (501–971)	NI[f]	229 (fixed)	81 (65–94)
θ_3 (h^{-1})	1.19 (0.200–2.18)	142	0.845 (fixed)	NI
θ_4[c]	0.256 (0.111–0.401)	NA[g]	0.273 (0.150–0.396)	NA
σ_1^2	0.106 (0.028–0.184)	NA	0.025 (−0.015–0.064)[d]	NA
σ_2^2 (mg/liter)^2	0.265 (0.014–0.516)	NA	0.537 (0.300–0.778)	NA
FOCE method				
θ_1[c] (liters/h)	44.9 (40.9–48.9)	34 (28–40)	45.1 (41.6–48.6)	34 (27–39)
θ_2 (liters)	769 (363–1,175)	53	229 (fixed)	99 (76–117)
θ_3 (h^{-1})	1.34 (−1.76–4.44)	58	0.845 (fixed)	NI
θ_4[c]	0.26 (0.13–0.40)		0.305 (0.181–0.429)	
σ_1^2	0.09 (0–0.18)	NA	0.04 (−0.005–0.08)[d]	NA
σ_2^2 (mg/liter)^2	0.28 (0–0.55)	NA	0.47 (0.28–0.66)	NA

[a] Model 1, all three parameters are estimated; model 2, *V* and k_a are fixed to 229 liters and 0.845 h^{-1}, respectively.
[b] Approximate coefficient of variation.
[c] CL/*F* = $\theta1(1 - \theta_4 \cdot flu)$ liters/h where flu = 1 if the patient was taking concomitant fluconazole therapy and 0 otherwise.
[d] This estimate was close to zero and the 95% CI included the null value, indicating that this term was not required in the residual error model when using model 2.
[e] θ_2, *V*/*F*; θ_3, k_a.
[f] NI, not identifiable.
[g] NA, not applicable.

obtain accurate estimates of V/F and k_a in the analysis of this data set. In previous phase I studies, V/F has been estimated as 2 to 7 liters/kg (140 to 490 liters for a 70-kg man) (Agouron prescribing information). The estimate from this analysis was much larger (734 liters) and was associated with a large CI. The estimate of k_a was also associated with a large CI and a very large degree of interindividual variability. The ability to obtain accurate estimates of pharmacokinetic parameters depends on the timing and number of plasma samples. In this study, only one early level per dosing interval was taken in each individual. This level was taken at essentially the same time in every individual, 2 h after the dose. This is close to the reported T_{max} of nelfinavir (2.5 to 3 h) (9), a period of the concentration-time profile associated with inherent variability as plasma concentrations change markedly. It is likely that this inherent variability in observed plasma concentrations within an individual, the lack of variability in the timing of the early postdose levels between individuals, and the small number of early samples per individual made it difficult to obtain accurate estimates of V/F and k_a. Additionally, the difficulty in estimating these parameters may be compounded by the lack of information in the data set on factors that can influence the absorption and/or bioavailability of nelfinavir. Studies have demonstrated that the C_{max} and area under the concentration-time curve (AUC) of nelfinavir are two to three times higher in fed than in fasted subjects (Agouron prescribing information; B. D. Quart, S. K. Chapman, J. Peterkin, et al., Abstr. 2nd Natl. Conf. Hum. Retroviruses Related Infect., abstr. 167, 1995). In this study the quantity and type of food were not strictly controlled, which may have contributed to the variability in the rate of absorption (14). Due to the problems encountered in estimating V/F and k_a in this particular data set, the estimates that were obtained should be interpreted with caution.

In order to assess any influence of misestimation of V/F and k_a on the estimation of CL/F, two models were used in the analysis. In model 1, all three parameters (CL/F, V/F, and k_a) were estimated. In model 2, the values of V/F and k_a were fixed to estimates obtained from analysis of traditional pharmacokinetic data. The estimates obtained for CL/F and the influence of fluconazole on CL/F were in good agreement between the models. Thus, the estimation of CL/F and the evaluation of the effect of covariates on CL/F in this study were not affected by poor estimates for V/F and k_a. Previously, Wade et al. investigated the effect of misspecification of k_a on the ability to estimate CL using sparsely sampled simulated data (15). They found that misspecification of k_a did not markedly affect the ability to estimate CL. The estimates obtained for CL/F were 42.7 and 42.0 liters/h using models 1 and 2, respectively. These values compare favorably with previous estimates of the steady-state CL/F of nelfinavir of 37.4 (17) and 46.0 liters/h (calculated as dose/AUC from 0 to 8 h [AUC_{0-8}]) (9).

Azole antifungal agents are commonly prescribed for patients with HIV disease for the treatment and prophylaxis of fungal infections (4). In this study, 25 of 174 patients (15%) were receiving concomitant azole therapy. Since 23 of these 25 patients were taking fluconazole, it was only possible to assess the effect of this azole antifungal on the CL/F of nelfinavir. Fluconazole is a known inhibitor of CYP3A4 and CYP2C19, the primary isoenzymes involved in the metabolism of nelfinavir (Wu et al., 5th Int. ISSX Meet.; Zhang et al., 12th Int. Symp. Microsomes Drug Oxidations). This study found that patients receiving concomitant therapy with fluconazole experienced a statistically significant reduction in CL/F of 26 to 27% compared to patients not taking fluconazole. This effect on the CL/F of nelfinavir is probably attributable to inhibition of CYP2C19 since the concentrations of the principal metabolite, M8 (which is generated only by CYP2C19 in vitro), in plasma are markedly decreased in the presence of fluconazole (Zhang et al., 12th Int. Symp. Microsomes Drug Oxidations). However, it is unlikely that an effect of this magnitude would be clinically significant and would warrant dosage adjustment since serious dose-limiting toxicities and concentration-related toxicities are not apparent with nelfinavir. A controlled, prospective study with intensive pharmacokinetic sampling would allow this modest effect of fluconazole to be quantified with improved confidence.

An effect of similar magnitude was observed in patients who were taking ritonavir concomitant with therapy with fluconazole (1, 5), whereas patients treated with indinavir concomitant with fluconazole therapy experienced a nonsignificant increase in CL/F (3). The inconsistency of fluconazole's inhibitory effect towards the various protease inhibitors in vivo may be accounted for by different contributions of cytochrome P450 isoenzymes (CYP3A4 versus non-CYP3A4) to the metabolism of these drugs and differing sensitivities of the various P450 isoenzymes to fluconazole.

Concomitant use of a macrolide antibiotic and a baseline CD4 count less than 100 cells per μl were statistically significant covariates for CL/F when tested alone (Table 2). However, the influence of concomitant fluconazole therapy was greater, and once this effect had been incorporated into the final model for CL/F, the effects of concomitant use of a macrolide antibiotic and a low baseline CD4 count were no longer statistically significant. It should be noted that 16 of the 35 patients with a low CD4 count were taking fluconazole and 8 of the 18 patients receiving concomitant macrolide therapy were also taking fluconazole. Thus, the observed influence of a low CD4 count and concomitant macrolide therapy may be explained by the high percentage of patients with these characteristics who were also taking fluconazole.

In this study, the CYP2C19-metabolizing status of the patient was evaluated indirectly as a covariate in CL/F by means of the M8-to-nelfinavir plasma ratio. The M8 metabolite is predominantly formed by the cytochrome P450 isoenzyme, CYP2C19 (Lillibridge et al., 5th Int. ISSX Meet.; Wu et al., 5th Int. ISSX Meet.). Previous studies (Lillibridge et al., 5th Int. ISSX Meet.; Zhang et al., 12th Int. Symp. Microsomes Drug Oxidations) indicate that the plasma M8-to-nelfinavir ratio is substantially reduced and plasma nelfinavir concentrations are modestly increased when CYP2C19 enzyme activity is impaired (genotypic poor metabolizers and/or concomitant drugs that inhibit CYP2C19). In this study, the plasma M8-to-nelfinavir ratio was not found to be a significant covariate for CL/F of nelfinavir. However, five of the six patients in this study with a low plasma M8-to-nelfinavir ratio (ratio, <0.1) were also receiving fluconazole. Thus, the lack of significance for the plasma M8-to-nelfinavir ratio as a covariate may be explained in part by a correlation with concomitant use of fluconazole (itself a significant covariate).

The effect of rifabutin on the CL/F of nelfinavir was inconsistent using the models in this analysis. It is probable that this study did not have sufficient power to assess the influence of rifabutin on the CL/F of nelfinavir, since only 5 of the 174 patients in the study were taking rifabutin. In both models, there was a trend for concomitant rifabutin to increase the CL/F of nelfinavir (from 37 to 55%). However, only when all three pharmacokinetic parameters (k_a, V/F, and CL/F) were estimated did the increase achieve statistical significance. There is evidence from other clinical studies that rifabutin induces the metabolism of nelfinavir when given TID (Agouron prescribing information). It is likely that a larger number of patients taking concomitant therapy with rifabutin in this

study population would have confirmed the existence of a significant effect on CL/*F*.

The FO method in NONMEM involves linear assumptions, whereas the FOCE method does not. These assumptions increase the risk of obtaining biased parameter estimates. However, the FOCE method is computationally more complex than the FO method, resulting in a substantially longer time to run an analysis. A limited comparison of some linear approximation methods (e.g., the FO method) with computationally more intensive approximation methods (e.g., the FOCE method) has shown that the linear approximation methods perform adequately (12). In this study, the population pharmacokinetic parameters were obtained initially by implementing the FO method in NONMEM and the results were confirmed using the FOCE method. The final models using both methods did compare favorably, showing that, in this instance, the FO method provided adequate estimates that were similar to the estimates obtained with the FOCE method.

In conclusion, the population pharmacokinetic parameters of nelfinavir were best described using a one-compartment model with first-order absorption. Estimation of V/F and k_a was difficult using this data, and the values obtained should be interpreted with caution. The estimate of CL/*F* was approximately 42.7 liters/h. There appeared to be no effect of body weight, age, sex, or ethnicity on the CL/*F* of nelfinavir in this study. Patients receiving concomitant therapy with fluconazole had a modest reduction in CL/*F* of 26 to 27%. This finding appears to be of little clinical significance given the safety of nelfinavir, and no dosage adjustment is indicated.

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