Pharmacokinetics of Nelfinavir and Efavirenz in Antiretroviral-Naïve, Human Immunodeficiency Virus-Infected Subjects when Administered Alone or in Combination with Nucleoside Analog Reverse Transcriptase Inhibitors

Patrick F. Smith,^{1,6*} Gregory K. Robbins,^{2,6} Robert W. Shafer,^{3,6} Hulin Wu,^{4,6} Song Yu,^{2,6} Martin S. Hirsch,^{2,6} Thomas C. Merigan,^{3,6} Jeong-Gun Park,^{2,6} Alan Forrest,^{1,6} Margaret A. Fischl,^{5,6} Gene D. Morse,^{1,6} and the ACTG 384-5006 Team⁶

University at Buffalo, School of Pharmacy and Pharmaceutical Sciences, Buffalo, New York¹; Harvard University, School of Medicine, Boston, Massachusetts²; Stanford University, School of Medicine, Palo Alto, California³; University of Rochester School of Medicine and Dentistry, Rochester, New York⁴; University of Miami, School of Medicine, Miami, Florida⁵; and Adult AIDS Clinical Trials Group, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland⁶

Received 2 December 2004/Returned for modification 16 January 2005/Accepted 5 May 2005

Pharmacokinetic studies were conducted with human immunodeficiency virus-infected patients receiving efavirenz, nelfinavir, or both agents at weeks 4 and 32. Reductions of 25% and 45% were observed in the mean nelfinavir area under the concentration-time curve and minimum concentration of the drug in serum, and there was a 31% more rapid half-life for patients receiving both drugs compared to patients receiving nelfinavir alone. There were no significant differences in efavirenz pharmacokinetics.

Therapy for antiviral-naïve human immunodeficiency virus (HIV)-infected individuals usually includes dual nucleoside analogue reverse transcriptase inhibitors with a nonnucleoside reverse transcriptase inhibitor and/or a protease inhibitor (10). Adult AIDS Clinical Trials Group (AACTG) protocol 384 was initiated to evaluate if efavirenz or a protease inhibitor would be more effective with a dual nucleoside analogue reverse transcriptase inhibitor backbone and whether two sequential three-drug regimens were superior to a single four-drug regimen. The primary study results reported that a regimen of zidovudine-lamivudine-efavirenz was as effective as zidovudine-lamivudine-nelfinavir (NFV)-efavirenz, but the four-drug regimen exhibited a longer time to treatment failure (7, 8).

A pharmacokinetic substudy was conducted to examine nelfinavir, M8, and efavirenz pharmacokinetics after 4 weeks and 32 weeks of therapy. A previous healthy-volunteer study similarly evaluated this interaction, utilizing a thrice-daily regimen of nelfinavir, and reported no effect of efavirenz on nelfinavir pharmacokinetics (W. D. Fiske, I. H. Benedek, S. J. White, K. A. Pepperess, J. L. Joseph, and D. M. Kornhauser, *Abstr. Conf. Retrovir. Oppor. Infect.*, abstr. 349, 1998).

Adult HIV-infected patients were randomized to receive zidovudine-lamivudine or didanosine-stavudine plus efavirenz (600 mg daily), nelfinavir (1,250 mg twice daily), or efavirenz-nelfinavir in a double-blind fashion with matching placebos.

Steady-state pharmacokinetics of efavirenz, nelfinavir, and its M8 metabolite were determined at weeks 4 and 32. Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h following oral dosing, and the exact times of the prior three doses were recorded. Efavirenz, nelfinavir, and M8 concentrations were measured by use of a validated assay method which has been previously described (6). Measurements performed on blinded samples demonstrated a variation for efavirenz ranging from 4.6 to 7.0% and 6 to 15% for M8 and nelfinavir. The limits of quantitation were 0.050 µg/ml for efavirenz and M8 and 0.100 µg/ml for nelfinavir. Pharmacokinetic parameters were determined by standard noncompartmental methods (WinNonlin Professional 4.1; Pharsight Corporation, Cary, NC). Statistical comparisons between treatment groups were by repeated-measures mixed-effects modeling.

For nelfinavir, 73 intensive pharmacokinetic studies were conducted: 36 studies were with patients receiving nelfinavir alone, and 37 were with patients receiving the combination of nelfinavir and efavirenz. Forty patients were studied at week 4, and 26 were studied at week 32. For M8, assay results were available for 34 subjects receiving nelfinavir alone and 27 subjects receiving nelfinavir and efavirenz concurrently. Pharmacokinetic parameters for nelfinavir and M8 are summarized in Table 1. For efavirenz, 77 pharmacokinetic studies were conducted with 46 patients. Totals of seven and eight patients were studied only at weeks 4 and 32, respectively.

As illustrated in Fig. 1, nelfinavir pharmacokinetic parameters differed significantly for subjects receiving both nelfinavir and efavirenz and subjects receiving nelfinavir alone, with a 25% reduction in the mean (standard deviation [SD]) nelfinavir area under the concentration-time curve from 0 to 12 h (AUC_{0-12}) (22.8 [11.2] versus 30.5 [13.6] µg · h/ml, $P = 0.01$),

* Corresponding author. Mailing address: Adult ACTG Pharmacology Support Laboratory, Laboratory for Antiviral Research, Department of Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, 219 Cooke Hall, University at Buffalo, Buffalo, NY 14260. Phone: (716) 645-2828, ext. 242. Fax: (716) 645-2886. E-mail: pfsmith@buffalo.edu.

TABLE 1. Nelfinavir and M8 pharmacokinetic parameters (combined weeks 4 and 32)

Drug(s) or parameter	Least-squares mean (SD) pharmacokinetic parameter							
	Nelfinavir				M8			
	$C_{\max}$ ($\mu\text{g/ml}$)	$C_{\min}$ ($\mu\text{g/ml}$)	AUC_{0-12} ($\mu\text{g} \cdot \text{h/ml}$)	$t_{1/2}$ (h)	$C_{\max}$ ($\mu\text{g/ml}$)	$C_{\min}$ ($\mu\text{g/ml}$)	AUC_{0-12} ($\mu\text{g} \cdot \text{h/ml}$)	$t_{1/2}$ (h)
NFV	4.3 (1.2)	1.1 (0.9)	30.5 (13.6)	4.2 (2.2)	1.2 (0.7)	0.24 (0.19)	7.9 (4.8)	2.9 (1.2)
NFV/EFV ^a	3.7 (1.7)	0.6 (0.5)	22.8 (11.2)	2.9 (1.5)	1.0 (0.5)	0.17 (0.11)	5.7 (3.6)	2.6 (0.9)
P^b	0.12	<0.01	0.01	<0.01	0.2	0.1	0.1	0.2

^a EFV, efavirenz.^b Repeated measures.

45% reduction in the mean (SD) minimum concentration of drug in serum ($C_{\min}$) (1.1 [0.9] versus 0.6 [0.5] $\mu\text{g/ml}$, $P < 0.01$), and a 31% more rapid half-life (4.2 [2.2] versus 2.9 [1.5] h, $P < 0.01$). Although the M8 metabolite AUC tended to be smaller, this difference was not statistically significant. The AUC ratios of M8 to nelfinavir with and without efavirenz were similar (0.26 versus 0.25). Pharmacokinetic parameters are summarized in Table 1.

The mean efavirenz plasma concentration-time profiles are shown in Fig. 2. The overall efavirenz mean (SD) AUC_{0-24} , maximum concentration of drug in serum ($C_{\max}$), and $C_{\min}$ were 48.8 (37.0) $\mu\text{g} \cdot \text{h/ml}$, 3.7 (1.9) $\mu\text{g/ml}$, and 1.8 (1.4) $\mu\text{g/ml}$, respectively. By repeated-measures analysis of variance, there were no statistically significant differences in efavirenz pharmacokinetic parameters with regard to either concomitant nelfinavir or duration of therapy. At week 4, the least-squares mean (SD) AUC_{0-24} was 45.1 (16.6), and that at week 32 was 48.2 (19.6) ($P > 0.05$). When efavirenz was combined with nelfinavir, the least-squares efavirenz mean (SD) AUC_{0-24} was 43.8 (17.1), compared to 52.6 (18.9) for efavirenz without nelfinavir ($P > 0.05$).

Efavirenz and nelfinavir both display complex pharmacokinetic characteristics during multiple dosing. Efavirenz is highly bound to plasma proteins, displays a prolonged plasma half-

life, is metabolized via cytochrome P450 2B6 and 3A4, and induces CYP450 activity during chronic administration (9). Numerous drug interactions have been reported between efavirenz and commonly prescribed medications (3). Nelfinavir is also highly bound to plasma proteins, is metabolized via 3A4 and 2C19 to an active metabolite (M8), which competitively inhibits 3A4 activity, and induces cytochrome P450 enzymes with chronic dosing. The M8 metabolite undergoes subsequent metabolism via 3A4 (2, 4, 5).

The pharmacokinetic results of efavirenz, both with and without a protease inhibitor, are consistent with previously published reports (9, 11). Prior studies have reported the half-life of efavirenz after repeated dosing to be approximately 40 to 50 h with no major influence noted when a protease inhibitor is combined (1, 5, 9). These data also provide new information, in that efavirenz pharmacokinetics were stable over 32 weeks.

The nelfinavir and M8 pharmacokinetic data are consistent with previous reports (2, 5), including the extent of M8 metabolism (M8-to-NFV ratio, ~20 to 30%). It is difficult to draw conclusions about the influence of efavirenz induction on M8 formation from our study, due to the noncrossover study design. However, these results strongly suggest that chronic administration may be associated with a smaller amount of total nelfinavir plus M8. This is consistent with efavirenz induction of both 2C9 and 3A4. The influence of enzyme inducers on nelfinavir pharmacokinetics has been previously described during phenytoin administration with a lowering of the nelfinavir AUC (M. J. Shelton, D. Cloen, M. Becker, P. H. Hsyu, J. H. Wilton, and R. G. Hewitt, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 426, 2000). Induction effects of efavirenz on other HIV-1 protease inhibitors have also been described (1, 5). In addition, a stable AUC ratio of metabolite to parent was observed in both arms; thus, a possible reduction in oral bioavailability could also contribute to the observed results. Possible influences of patients with low nelfinavir concentrations dropping out of the nelfinavir-alone arms due to virological failure also cannot be ruled out.

Patients in this substudy were not evaluated for potential genetic polymorphisms in the primary CYP450 enzymes responsible for efavirenz (2B6 and 3A4) and nelfinavir (2C19 and 3A4) metabolism. Efavirenz exposure has been shown to be correlated with a single-nucleotide polymorphism of CYP2B6 (516G→T) (D. W. Haas, H. Ribaud, G. R. Wilkinson, R. Gulick, D. Clifford, T. Hulgan, et al., Abstr. 11th Conf. Retrovir. Oppor. Infect., abstr. 133, 2004). Genetic polymorphisms that result in a premature stop codon or altered splice

FIG. 1. Mean nelfinavir and M8 plasma concentrations ($\pm$ standard errors of the means) when the drug was administered with or without efavirenz (EFV).

FIG. 2. Mean $\log_{10}$ efavirenz plasma concentrations in HIV-infected subjects. The graphic inlay shows the efavirenz AUC_{0-24} for efavirenz administered alone (-NFV) or in combination with nelfinavir (+NFV).

site in CYP2C19 confer a poor metabolizer phenotype, resulting in reduced nelfinavir metabolism. The potential contributions of these polymorphisms to the observed results are unknown and should be evaluated in future studies. Knowledge of patient-specific genotypes would be expected to account for a portion of the observed pharmacokinetic variability and may improve our understanding of drug-drug interactions whereby the presence and/or magnitude of the interaction may depend upon the specific genotype and phenotype of a given patient.

A majority of pharmacokinetic studies of antiretrovirals consider acute therapy to be within one to two weeks and long-term therapy to be over the first month. In ACTG 384-5006, the design we employed allowed for the investigation of within-subject patterns over 32 weeks. The observation that nelfinavir exposure levels were similar between the three-drug nelfinavir arms but were lower in the four-drug arms suggests that efavirenz may have lowered nelfinavir and M8 concentrations through sustained induction.

With regard to interactions between nelfinavir and efavirenz, the only prior pharmacokinetic data that examined the combined administration were obtained from a seven-day study with healthy volunteers which utilized thrice-daily nelfinavir dosing. This study reported that nelfinavir plasma concentrations were increased after seven days by ~20%. These data are markedly different from those obtained in ACTG 384-5006. Possible reasons for these different findings may be related to studying healthy volunteers compared to HIV-infected individuals. The duration of drug exposure was much longer in the present study, reflecting chronic drug administration. In addition, potential contributions resulting from the use of twice-daily nelfinavir dosing in the current study, reflecting current clinical practices, cannot be ruled out.

This study was funded by the following grants from the following organizations: National Institute of Allergy and Infectious Diseases,

U01-AI38858; University at Buffalo ACTG Pharmacology Support Laboratory Harvard University, ACTU AI-27659; Harvard (Massachusetts General Hospital) (A0101), AACTG grant no. AI27659; NYU/Bellevue (A0401), AACTG grant no. AI27665, GCRC grant no. M01-R00096; Mount Sinai Medical Center (N.Y.) (A0404), AACTG grant no. U01-AI-27667 and GCRC grant no. M01-RR-00071; Stanford University (A0501), AACTG grant no. AI27666 and GCRC grant no. M01-RR00070; University of California, San Diego (A0701), AACTG grant no. AI27670 and GCRC grant; University of Rochester Medical Center and SUNY—Buffalo (Rochester) (A1101 and A1102), AACTG grant no. AI27658 and GCRC grant no. RR00044; University of Southern California (A1201), AACTG grant no. AI27673 and GCRC grant; University of Washington (A1401), AACTG grant no. AI27664 and GCRC grant no. M01-RR-00037; University of Minnesota (A1501), AACTG grant no. AI27661 and GCRC grant no. M01RR00400; University of Cincinnati (A2401), AACTG grant no. AI25897 and GCRC grant no. M01RR0884; Indiana University Hospital (A2601), AACTG grant no. AI25859 and GCRC grant no. M01RR00750; University of North Carolina (A3201), AACTG grant no. AI25868, GCRC grant no. RR00046, and CFAR no. AI50410; University of Puerto Rico (A5401), AACTG grant no. AI34832 and GCRC grant no. 1P20RR11126; Tulane University (A9426), AACTG grant no. AI35162; Harbor-UCLA (A0601), AACTG grant no. AI27660 and GCRC grant no. M01-RR00425; University of Pittsburgh (A1001), AACTG grant no. 5U01-AI46383 and GCRC grant no. M01-RR00056; and The Cornell Clinical Trials Unit and Chelsea Clinic (A7803 and A7804), AACTG grant no. AI46386 and GCRC grant no. M01RR00047.

REFERENCES

1. Aarnoutse, R. E., K. J. Grintjes, D. S. Telgt, M. Stek, Jr., P. W. Hugen, P. Reiss, P. P. Koopmans, Y. A. Hekster, and D. M. Burger. 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose zidovudine in healthy volunteers. *Clin. Pharmacol. Ther.* 71:57-67.
2. Baede-van Dijk, P. A., P. W. Hugen, C. P. Verweij-van Wissen, P. P. Koopmans, D. M. Burger, and Y. A. Hekster. 2001. Analysis of variation in plasma concentrations of nelfinavir and its active metabolite M8 in HIV-positive patients. *AIDS* 15:991-998.
3. Barry, M., F. Mulcahy, C. Merry, S. Gibbons, and D. Back. 1999. Pharmacokinetics and potential interactions amongst antiretroviral agents used to treat patients with HIV infection. *Clin. Pharmacokinet.* 36:289-304.
4. Capparelli, E. V., J. L. Sullivan, L. Mofenson, E. Smith, B. Graham, P. Britto, M. I. Becker, D. Holland, J. D. Connor, and K. Luzuriaga. 2001.

- Pharmacokinetics of nelfinavir in human immunodeficiency virus-infected infants. *Pediatr. Infect. Dis. J.* **20**:746–751.
5. **DiCenzo, R., A. Forrest, M. A. Fischl, A. Collier, J. Feinberg, H. Ribaud, R. DiFrancesco, and G. D. Morse.** 2004. Pharmacokinetics of indinavir and nelfinavir in treatment-naïve, human immunodeficiency virus-infected subjects. *Antimicrob. Agents Chemother.* **48**:918–923.
 6. **Frerichs, V. A., R. DiFrancesco, and G. D. Morse.** 2003. Determination of protease inhibitors using liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* **787**:393–403.
 7. **Robbins, G. K., V. De Gruttola, R. W. Shafer, L. M. Smeaton, S. W. Snyder, C. Pettinelli, M. P. Dube, M. A. Fischl, R. B. Pollard, R. Delapenha, L. Gedeon, C. van der Horst, R. L. Murphy, M. I. Becker, R. T. D'Aquila, S. Vella, T. C. Merigan, and M. S. Hirsch.** 2003. Comparison of sequential three-drug regimens as initial therapy for HIV-1 infection. *N. Engl. J. Med.* **349**:2293–2303.
 8. **Shafer, R. W., L. M. Smeaton, G. K. Robbins, V. De Gruttola, S. W. Snyder, R. T. D'Aquila, V. A. Johnson, G. D. Morse, M. A. Nokta, A. I. Martinez, B. M. Gripshover, P. Kaul, R. Haubrich, M. Swingle, S. D. McCarty, S. Vella, M. S. Hirsch, and T. C. Merigan.** 2003. Comparison of four-drug regimens and pairs of sequential three-drug regimens as initial therapy for HIV-1 infection. *N. Engl. J. Med.* **349**:2304–2315.
 9. **Smith, P. F., R. DiCenzo, and G. D. Morse.** 2001. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. *Clin. Pharmacokinet.* **40**: 893–905.
 10. **U.S. Department of Health and Human Services.** 2004. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. [Online.] <http://aidsinfo.nih.gov/guidelines>.
 11. **Villani, P., M. B. Regazzi, F. Castelli, P. Viale, C. Torti, E. Seminari, and R. Maserati.** 1999. Pharmacokinetics of efavirenz (EFV) alone and in combination therapy with nelfinavir (NFV) in HIV-1 infected patients. *Br. J. Clin. Pharmacol.* **48**:712–715.