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Pharmacokinetic study of the interaction between rifabutin and delavirdine mesylate in HIV-1 infected patients

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Abstract

The oxidative metabolism of delavirdine, a non-nucleoside inhibitor of HIV-1 reverse transcriptase, is mediated in part by cytochrome P450 3A. The influence of rifabutin, an inducer of certain human cytochrome P450 isozymes, on the steady-state pharmacokinetics of delavirdine was investigated in 12 HIV-positive patients with CD4 counts ranging from 75 to 671/mm^3. Both the control group ($n = 5$) and the rifabutin group ($n = 7$) received 400 mg delavirdine mesylate every 8 h for 30 days; subjects in the rifabutin group took a 300 mg, once-daily dose of rifabutin on study days 16–30. Harvested plasma from serial blood samples collected after dosing on days 15, 16, and 30 was assayed for delavirdine and its *N*-desalkyl metabolite concentrations using a reversed-phase HPLC method. Blood samples obtained on days 16 and 30 were also assayed for rifabutin by HPLC. Delavirdine mesylate alone or in combination with rifabutin was well-tolerated. On day 30, statistically significant differences between groups were observed for all delavirdine pharmacokinetic parameters ($P < 0.046$). After coadministration of rifabutin and delavirdine mesylate for 2 weeks, oral clearance of delavirdine increased five-fold, resulting in lower steady-state plasma delavirdine concentrations. Rifabutin pharmacokinetic parameters were similar to those previously reported. Concomitant use of delavirdine and rifabutin at the recommended dose for each drug is discouraged. Maintaining therapeutic concentrations of delavirdine in patients on both medications may require dose modification. © 1997 Elsevier Science B.V.

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1. Introduction

Delavirdine mesylate, a non-nucleoside reverse transcriptase (RT) inhibitor, is under development as a potential therapeutic agent for the treatment of acquired immunodeficiency syndrome (AIDS). Delavirdine belongs to a class of compounds known as bisheteroarylpiperazines which bind to HIV-1 RT at a different site than the nucleoside analogs (Romero et al., 1991). The anti-HIV activity of delavirdine has been investigated in several in vitro assay systems; the IC50 of delavirdine is 0.26 μM (Dueweke et al., 1993). In 25 primary HIV-1 isolates, delavirdine blocked viral replication in peripheral blood lymphocytes with a mean 50% effective concentration of 0.066 μM (Dueweke et al., 1993). At a concentration of 3 μM, delavirdine totally prevented the spread of HIV_{IIIB} in MT-4 cell cultures (Dueweke et al., 1993). In other in vitro studies, delavirdine has exhibited synergy with zidovudine or zalcitabine over a wide range of concentrations (Chong et al., 1994).

Delavirdine exhibits nonlinear pharmacokinetics with oral clearance decreasing by about 50% and apparent half-life increasing by approximately 300% as the delavirdine mesylate daily dose increases from 400 to 1200 mg (Batts et al., 1993). In patients receiving 1200 mg/day, the median delavirdine steady-state trough concentration is about 8 μM (Freimuth et al., 1996a,b).

Rifabutin, a semisynthetic spiropiperidyl derivative of rifamycin S, is indicated for prevention of disseminated *Mycobacterium avium* complex (MAC) diseases in patients with advanced HIV infection. The minimum inhibitory concentration of rifabutin against *M. avium* intracellular isolates from patients with AIDS (83%) is about 0.25 μg/ml (Heifets et al., 1986; 1987). Rifabutin appears to be rapidly but incompletely absorbed from the gastrointestinal tract, reaching peak plasma concentrations in 2–3 h (Skinner et al., 1989). The terminal half-life of rifabutin is approximately 36 h (Skinner et al., 1989; Strolin-Benedetti et al., 1990). Linearity in the pharmacokinetics of rifabutin has been established for doses up to 900 mg in HIV-positive patients (Della Bruna et al., 1983). The bioavailability of rifabutin decreases after repeated administration, presumably due to induction of presystemic extrahepatic metabolism (Strolin-Benedetti et al., 1990). Rifabutin can induce hepatic microsomal enzymes in man, but to a lesser extent than rifampin (Perucca et al., 1988).

Delavirdine is believed to be metabolized by members of the cytochrome P450 3A subfamily (CYP3A) (Voorman et al., 1995). Because rifabutin may induce certain cytochrome P450 isozymes that mediate drug metabolism in humans, concomitant administration of rifabutin and delavirdine mesylate may result in increased delavirdine clearance. This study was conducted to determine if rifabutin, administered at the recommended dosage for MAC prophylaxis, affects the clinical pharmacokinetics of delavirdine. The pharmacokinetics of rifabutin were also assessed during treatment with delavirdine mesylate. The ratio of 6-β-hydroxycortisol to free cortisol, a putative endogenous marker of CYP3A activity (Ged et al., 1989), was monitored in this study to assess the effects of both delavirdine and rifabutin on CYP3A activity.

2. Materials and methods

Seventeen HIV-positive volunteers, between 18 and 55 years of age and within 15% of ideal body weight, were enrolled in the study after giving written informed consent and a medical history. The study was approved by the Office of Research-Institutional Review Board, Evanston Hospital. All subjects were required to have written documentation of HIV-1 infection by serologic (ELISA), Western Blot, or HIV-1 culture, and a CD4 lymphocyte subset $\geq 100/mm^3$ and $\leq 500/mm^3$ performed at time of screening (within 30 days of study entry). Additionally, subjects were required to have a Karnofsky performance status > 60. Acceptable screening labs with adequate baseline organ function including the following laboratory values were also required: (1) hemoglobin ≥ 9.5 gm/dl; (2) absolute neutrophil count $\geq 1000/mm^3$; (3) platelets $\geq 100\,000/mm^3$; (4) creatinine ≤ 1.6 mg/dl, or estimated creatinine clearance > 50 ml/min; (5) AST, ALT, and alka-

line phosphatase ≤ 2.5 times the upper limit of normal; and (6) bilirubin ≤ 2.5 mg/dl. All subjects were required to have a negative urine drug screen for drugs of abuse. Concomitant noninvestigational medications were allowed except for lamivudine, stavudine, zalcitabine, and known enzyme inducing or enzyme inhibiting agents.

2.1. Design

This was an open-label, parallel-group, multiple-dose study in HIV-positive subjects who were randomized to either the control group or the rifabutin group. Patients received drug on an outpatient basis, except for trough blood draw days when they received the morning dose(s) at the clinic and for the pharmacokinetic evaluation periods when they remained in the clinic for dosing and sequential blood draws. Subjects in both groups received a 400 mg oral dose of delavirdine mesylate administered three times a day on study days 1–29; one delavirdine mesylate dose was taken on the morning of day 30. Subjects in the rifabutin group received a once-daily, 300 mg dose of rifabutin on days 16–30. Delavirdine mesylate was administered as four 100 mg tablets taken approximately 2 h after or 1 h before meals. The rifabutin treatment was taken as two 150 mg capsules in the morning with delavirdine mesylate. Each dose was taken with at least 180 ml (6 ounces) of water. On blood draw days, subjects were instructed to report fasted to the clinic and drug was administered by clinic personnel.

2.2. Safety evaluation

Reported medical events, safety laboratory evaluations, and vital signs at screening and during the course of the study comprised the primary safety variables of this study. Physical exams were conducted at screen and at the end of the study.

2.3. Sample collection

Venous blood samples for determination of delavirdine and its *N*-desalkyl metabolite were collected immediately prior to the morning dose on days -1, 3, 7, 11, 18, 22, and 26. On days 15, 16, and 30, samples were obtained before the morning dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 8 h after administration of delavirdine mesylate. Additional samples at 12, 16, 20, and 24 h after the last dose on day 30 were also collected. Venous blood samples for determination of rifabutin were collected immediately prior to the rifabutin dose on days 18, 22, and 26, and at the following times after the rifabutin dose on days 16 and 30: predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h. Blood samples were stored on ice, then centrifuged at 3000 rpm for 20 min at 4°C. Plasma was harvested, immediately frozen at -20°C, and stored frozen until assayed for delavirdine and its *N*-desalkyl metabolite or rifabutin.

All urine over a 4 h morning interval on study days -1, 2, 15, 16, and 30 was collected into 8 ml 50% acetic acid. Each subject emptied his/her bladder just prior to the start of the urine collection period. Specimens were refrigerated during the collection period. When the 4 h collection was complete, the urine was pooled, well-mixed, and the weight was recorded. One 25 ml aliquot was saved in a plastic storage specimen vial and frozen at -20°C until assayed for free cortisol and 6-β-hydroxycortisol.

2.4. Bioanalytical methods

Plasma samples were assayed for delavirdine and desalkyl delavirdine concentrations using validated, sensitive, and specific isocratic HPLC methods, one for the upper concentration range and one for the lower concentration range (Staton et al., 1995). In brief, delavirdine, desalkyl delavirdine, and the internal standard (U-88822) were extracted from plasma by protein precipitation with acetonitrile, the supernatant mixed with buffer and directly injected. Chromatographic separation was achieved using a cyano guard column (Brownlee CN) and a cyano analytical column (DuPont Zorbax SB CN) with a mobile phase of 10 mM KH_2PO_4 (pH 6.0)/acetonitrile (67:33 v/v). The analytes were detected by fluorescence at an excitation wavelength of 295 nm and an emissions filter at 418 nm. The retention times of the primary analytes were ~ 2.6 min (desalkyl

delavirdine), ~6.8 min (U-88822), and ~8.0 minutes (delavirdine).

For delavirdine, between-day coefficients of variation (CV%) for back-calculated concentrations of calibration standards ranged from 1.4 to 6.2%, with mean accuracies within 7.0% of the nominal concentrations. Assay accuracy, expressed as the ratio (%) of the estimated to the theoretical quality control (QC) standard concentrations, for the low (0.052–11 μM) and high (0.22–55 μM) curves averaged between 90.0 and 98.5% for the QC standards. Assay precision for the high curve, expressed as the CV% of the estimated concentrations of QC standards, averaged between 2.2 and 4.9%. For desalkyl delavirdine, between-day CV% for back-calculated concentrations of calibration standards ranged from 1.3 to 6.0%, with mean accuracies within 8.4% of the nominal concentrations. Assay accuracy for the low (0.060–12 μM) and high (0.24–60 μM) curves averaged between 91.0 and 98.2% for the QC standards. Assay precision for the high curve averaged between 2.5 and 3.8%. Assay precision for the low curves could not be determined, since only one analysis group was conducted with the low-curve method.

Plasma samples were assayed for rifabutin concentrations using a validated, sensitive, and specific isocratic HPLC method (Lau et al., 1996). Rifabutin and the internal standard, sulindac, were acidified and extracted from plasma with a C8 solid-phase extraction cartridge (Varian BondElut) preconditioned with methanol, followed by water. The compounds were eluted with methanol, evaporated under nitrogen, and reconstituted in mobile phase (CH_3CN/0.5 M KH_2PO_4 and 0.5 M NaOAc; 47:53 v/v). Chromatographic separation was achieved using a C8 guard column (Brownlee Newguard RP8) and analytical column (DuPont Zorbax RX-C8). The analytes were detected by UV at 275 nm. The retention times of the primary analytes were ~7.0 min (sulindac) and ~11.0 min (rifabutin). The lower limit of quantitation for rifabutin was 5.0 ng/ml. Between-day CV% for back-calculated concentrations of calibration standards ranged from 1.9 to 8.0%, with mean accuracies within 6.6% of the nominal concentrations. Assay accuracy averaged between 93.5 and 101% for the QC standards. Assay precision averaged between 4.8 and 6.2%.

Urinary 6-β-hydroxycortisol concentrations were determined by a specific HPLC method. The urine sample containing 6-hydroxyprednisolone as the internal standard was applied to a BondElut C18 cartridge. After washing, the analytes were eluted with a mixture of ethyl acetate and cyclohexane and the solvent was evaporated. The residue was reconstituted in mobile phase (13% acetonitrile in 0.1 M KH_2PO_4) and injected onto a Beckman Ultrasphere C18 column. The analytes were monitored by UV detection at 238 nm. The assay was linear over the standard curve range of 50–25,000 ng/ml, with overall precision of 8% and recovery of 94 $\pm$ 3%.

Free cortisol levels in urine were determined using a similar method with two exceptions: cortisol and the internal standard, methylprednisolone, were extracted from urine using methylene chloride following an ethyl acetate/cyclohexane wash, and the mobile phase was 30% acetonitrile in 0.05 M KH_2PO_4. The assay was linear over the standard curve range of 10–5000 ng/ml, with overall precision of 9% and recovery of 88%.

2.5. Pharmacokinetic analysis

Delavirdine, desalkyl delavirdine, and rifabutin pharmacokinetic parameters were estimated using noncompartmental methods. Peak plasma concentration (C_{max}), minimum plasma concentration (C_{min}), and time to peak concentration (T_{max}) were determined by inspection of individual subject concentration-time curves. The fluctuation index (Fluc), a measure of the peak to trough variation, was calculated as the ratio of C_{max} to C_{min}. Elimination rate constants (λ_z) were estimated by least-squares regression of values within the terminal log-linear region of the plasma concentration-time curves. Time points for calculation of λ_z were selected by an iterative algorithm which minimized AIC (Akaike's Information Criterion) (Yamaoka et al., 1978). A minimum of three time points was used to calculate λ_z. In most cases, the number of time points selected for the regression line ranged from 8 to 12 for delavirdine, from 6 to 13 for desalkyl delavirdine,

and from 4 to 7 for rifabutin, with $R^2 > 0.90$. Half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. Area under the plasma concentration-time curve from time zero to the end time (τ) of the collection interval ($AUC_{0-\tau}$) was calculated using the trapezoidal rule. If the drug concentration was below the lower limit of quantitation at or before the end of the dosing interval, the AUC was extrapolated to time τ by estimating Ct_n (plasma concentration at time t_n) for $t_n \leq \tau$ using the least-squares regression equation for the terminal portion of the curve, and then applying the trapezoidal rule. For the first dose of rifabutin (day 16), the area under the curve was extrapolated to infinity ($AUC_{0-\infty}$) by adding C_T/λ_z to AUC_{0-T}, where C_T is the last quantifiable plasma concentration. Apparent oral clearance (Cl_{PO}) was calculated as Dose/AUC, where $AUC = AUC_{0-\tau}$ for steady-state data and $AUC = AUC_{0-\infty}$ for rifabutin on day 16. C_{ss}, the average steady-state plasma concentration, was determined as $AUC_{0-\tau}/\tau$. The ratio of metabolite formation clearance to metabolite elimination clearance for desalkyl delavirdine, Cl_f/Cl_m, was estimated as AUC_{met}/AUC_{par}, where met is the metabolite and par is the parent, delavirdine.

2.6. Statistical analysis

Due to the small sample size and high variability in parameter values, nonparametric methods were chosen to analyze the pharmacokinetic data. Pharmacokinetic parameters were compared between study days using the Wilcoxon signed-rank test. Delavirdine and desalkyl delavirdine parameters on day 15 were compared to the corresponding parameters on days 16 and 30, respectively. For rifabutin pharmacokinetic parameters, comparisons were made between days 16 and 30. Differences in parameters and 6-β-hydroxycortisol to free cortisol ratios between treatment groups on a given study day were assessed using the Wilcoxon rank sum test. Cortisol ratios on days 2, 15, 16, and 30 were compared to baseline (day -1 or day 15) using the Wilcoxon signed-rank test. Statistical significance was set at $P = 0.05$.

3. Results

Twelve subjects completed the study (2 females, one in each group, and 10 males). The mean (range) age, weight, and CD4 counts in the control group were 29 (25–30) years, 73 (62–100) kg, and 484 (290–671) cells/mm^3, respectively. In the rifabutin group, the mean (range) age, weight, and CD4 counts were 32 (22–45) years, 81 (53–96) kg, and 364 (75–514) cells/mm^3, respectively. At screening, all but two subjects had a CD4 count below 500 mm^3, which was one of the inclusion criteria for enrollment; however, for demographic purposes the CD4 count was calculated as the average of screening and predose determinations, resulting in values > 500/mm^3 in four subjects. The most common concomitant medication taken by the patients was zidovudine.

All 17 patients enrolled in the study reported at least one medical event. The most frequently reported events were headache, rash, pruritus, nausea, and elevated SGOT/SGPT which were equally reported in each treatment group. Twelve patients completed all aspects of the study. Ten patients experienced a rash that was probably associated with delavirdine mesylate use. However, two of these patients were also taking Bactrim, one having initiated Bactrim treatment less than 2 weeks prior to study start. In nine patients, onset of the rash varied between day 10 and day 13 of the study. Five patients were dosed through the rash, with resolution of rash occurring between day 12 and day 18 for these patients. One patient reported onset of rash on day 26, was dosed through the rash, and the rash resolved on day 37, 7 days after study completion. Four patients dropped from the study due to rash. One patient dropped from the study due to a sore throat. There were no clinically meaningful changes in vital signs in either treatment group. Safety laboratory data (hematology, clinical chemistries, urinalysis) were unremarkable with no clinically meaningful or drug-attributed changes occurring after drug administration.

Visual examination of morning trough delavirdine concentrations showed that steady state was reached for each subject by day 11 of dosing. Mean steady-state trough plasma

delavirdine concentrations averaged between 12.2 and 18.3 μM in the control group. In the rifabutin group, the average steady-state trough delavirdine concentration ranged from 19.8 to 26.6 μM through day 16; the mean trough level dropped to 9.3 μM after two doses of rifabutin (day 18) and reached a new steady state within 7 days after initiating rifabutin treatment. The steady-state morning trough level for delavirdine when taken with rifabutin averaged between 1.8 and 4.0 μM. Mean trough levels of desalkyl delavirdine were below 4.5 μM throughout the 30-day study period in both treatment groups. However, average metabolite concentrations were reduced by about 20% in the rifabutin group after delavirdine mesylate was administered with rifabutin.

Plasma delavirdine concentration-time profiles for morning dosing intervals on days 15, 16, and 30 are depicted in Figs. 1 and 2. In the control group, average drug concentrations were similar among days. Rifabutin had no effect on delavirdine concentrations on the first day the two drugs were coadministered (day 16). However, mean plasma delavirdine concentrations on day 30 were much lower than those observed on days 15 or 16. As was observed for delavirdine, average levels of desalkyl delavirdine on days 15, 16, and 30 were similar (Fig. 3). In the rifabutin group, there was no difference in desalkyl delavirdine concentrations between days 15 and 16 (Fig. 4). The metabolite concentration-time profile on day 30 was not as flat as that observed on days 15 and 16, with lower trough (0 and 8 h) concentrations on day 30. Metabolite levels between 0.75 and 4 h after dosing were similar among days in the rifabutin group.

Mean values of delavirdine pharmacokinetic parameters for the control and rifabutin groups are provided in Tables 1 and 2. No day 16 pharmacokinetic parameters were calculated for one of the subjects in the rifabutin group due to

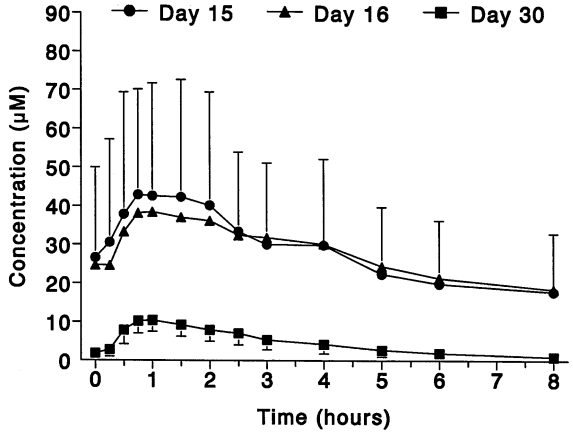

Fig. 2. Mean (S.D.) steady-state plasma delavirdine concentrations in rifabutin group ($n = 7$) on delavirdine mesylate 400 mg three times a day (days 1–30) and rifabutin 300 mg once daily (days 16–30).

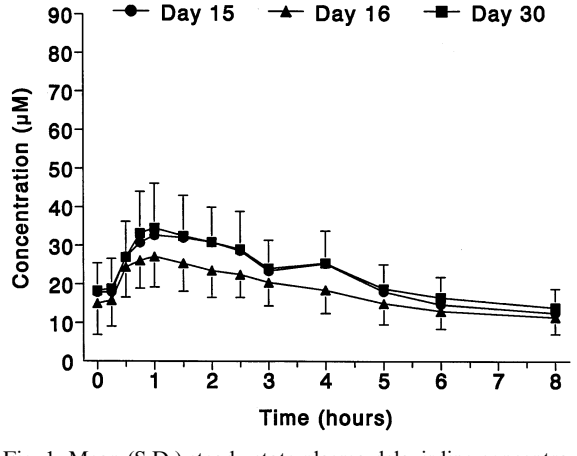

Fig. 1. Mean (S.D.) steady-state plasma delavirdine concentrations in control group ($n = 5$) on delavirdine mesylate 400 mg three times a day for 30 days.

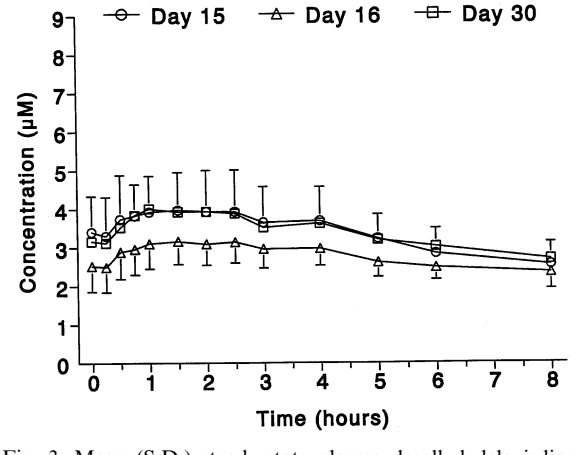

Fig. 3. Mean (S.D.) steady-state plasma desalkyl delavirdine concentrations in control group ($n = 5$) on delavirdine mesylate 400 mg three times a day for 30 days.

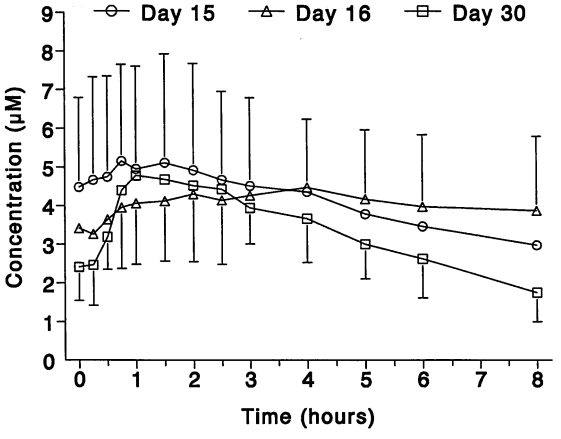

Fig. 4. Mean (S.D.) steady-state plasma desalkyl delavirdine concentrations in rifabutin group ($n = 7$) on delavirdine mesylate 400 mg three times a day (days 1–30) and rifabutin 300 mg once daily (days 16–30).

missed sample collections between 0.25 and 3 h after dosing. There were no significant differences in pharmacokinetic parameters between treatment groups on day 15 or day 16 ($P > 0.33$). On day 30, statistically significant differences between groups were observed for all parameters ($P < 0.046$). Cl_{PO} was higher, C_{ss}, C_{min}, and C_{max} were correspondingly lower, T_{max} occurred earlier, the fluctuation index was higher, apparent half-life was shorter, and Cl_f/Cl_m was greater for subjects in the rifabutin group relative to the control group. Within-group comparisons showed no significant differences in parameters between study days 15 and 16 or between study days 15 and 30 for the control group. After coadministration of rifabutin and delavirdine mesylate for 2 weeks, significant differences in all pharmacokinetic parameters except T_{max} were observed relative to administration of delavirdine mesylate alone in the rifabutin group. Delavirdine Cl_{PO} increased by approximately five-fold, resulting in lower steady-state plasma delavirdine concentrations. Cl_f/Cl_m was significantly higher, the apparent half-life was significantly shorter, and the fluctuation index was significantly higher after concurrent administration of delavirdine mesylate and rifabutin (day 30) than after delavirdine mesylate treatment (day 15).

Average values of desalkyl delavirdine pharmacokinetic parameters for the control and rifabutin groups are shown in Tables 3 and 4. No significant differences in pharmacokinetic parameters between treatment groups were observed on day

Table 1
Mean (S.D.) delavirdine pharmacokinetic parameters after oral administration of delavirdine mesylate 400 mg three times a day for 30 days, control group ($n = 5$)

Parameter	Day 15	Day 16	Day 30
Cl_{PO} (l/h)	4.8 (2.1)	5.4 (1.8)	4.3 (1.5)
C_{ss} (μM)	22 (8.3)	18 (5.7)	23 (7.4)
C_{min} (μM)	13 (5.0)	11 (5.2)	14 (4.8)
C_{max} (μM)	34 (13)	27 (7.8)	35 (12)
T_{max} (h)	1.2 (0.3)	1.0 (0.3)	1.2 (0.3)
Fluc	2.7 (0.4)	2.7 (0.7)	2.5 (0.3)
$t_{1/2}$ (h)	4.7 (0.87)	5.1 (1.3)	4.6 (0.78)
Cl_f/Cl_m	0.16 (0.04)	0.16 (0.03)	0.16 (0.03)

Table 2
Mean (S.D.) delavirdine pharmacokinetic parameters after oral administration of delavirdine mesylate 400 mg three times a day (days 1–30) and rifabutin 300 mg once daily (days 16–30), rifabutin group ($n = 7$)

Parameter	Day 15	Day 16[a]	Day 30
Cl_{PO} (l/h)	4.4 (2.1)	4.4 (2.2)	24 (13)[b]
C_{ss} (μM)	28 (20)	28 (18)	4.6 (2.0)[b]
C_{min} (μM)	18 (15)	18 (14)	0.96 (0.70)[b]
C_{max} (μM)	44 (30)	40 (24)	11 (3.2)[b]
T_{max} (h)	0.96 (0.3)	1.0 (0.3)	0.83 (0.2)[b]
Fluc	2.8 (0.7)	2.6 (1.0)	14 (4.1)[b]
$t_{1/2}$ (h)	4.8 (0.86)	6.6 (2.9)	2.1 (0.64)[b]
Cl_f/Cl_m	0.17 (0.05)	0.17 (0.06)	0.83 (0.29)[b]

[a] $n = 6$.
[b] $P = 0.022$ compared with day 15.

Table 3
Mean (S.D.) desalkyl delavirdine pharmacokinetic parameters after oral administration of delavirdine mesylate 400 mg three times a day for 30 days, control group ($n = 5$)

Parameter	Day 15	Day 16	Day 30
C_{ss} (μM)	3.4 (0.82)	2.8 (0.44)	3.4 (0.59)
C_{min} (μM)	2.5 (0.56)	2.2 (0.50)	2.7 (0.44)
C_{max} (μM)	4.1 (1.1)	3.2 (0.56)	4.1 (0.83)
T_{max} (h)	1.6 (0.5)	1.6 (0.5)	1.7 (0.6)
Fluc	1.6 (0.2)	1.5 (0.2)	1.5 (0.1)
$t_{1/2}$ (h)	8.8 (2.3)	14 (7.2)	9.7 (1.6)

Table 4
Mean (S.D.) desalkyl delavirdine pharmacokinetic parameters after oral administration of delavirdine mesylate 400 mg three times a day (days 1–30) and rifabutin 300 mg once daily (days 16–30), rifabutin group ($n = 7$)

Parameter	Day 15	Day 16[a]	Day 30
C_{ss} (μM)	4.1 (2.1)	4.1 (1.7)	3.4 (0.95)
C_{min} (μM)	3.0 (1.6)	3.2 (1.2)	1.8 (0.75)[b]
C_{max} (μM)	5.2 (2.8)	4.5 (1.7)[b]	4.8 (1.0)
T_{max} (h)	1.2 (0.7)	2.9 (1.4)	1.2 (0.4)
Fluc	1.7 (0.1)	1.4 (0.05)[b]	3.0 (0.8)[b]
$t_{1/2}$ (h)	8.1 (1.3)	21 (12)[b]	4.2 (0.50)[b]

[a] $n = 6$.
[b] $P \leq 0.036$ compared with day 15.

15 ($P > 0.21$). On day 16, significant between-group differences were observed for C_{ss} ($P = 0.036$) which was lower in the control group. C_{min} and the apparent half-life were significantly lower and the fluctuation index was significantly higher in the rifabutin group than in the control group on day 30 ($P < 0.043$). Metabolite parameters did not differ significantly between study days 15 and 16 or between days 15 and 30 for the control group. In the rifabutin group, significant differences in C_{max}, the fluctuation index, and apparent half-life were observed after 1 day of coadministration of rifabutin and delavirdine mesylate (day 16) relative to delavirdine mesylate alone (day 15). Except for half-life, these differences were small ($< 18\%$). On day 30, 2 weeks after initiating concurrent administration of the two drugs, desalkyl delavirdine C_{min} was significantly lower, the fluctuation index was significantly higher, and apparent half-life was significantly shorter than on day 15 (delavirdine mesylate alone).

Visual examination of individual subject trough rifabutin concentrations indicated that steady state was attained for each subject within 10 days of dosing (day 26), with an average steady-state trough rifabutin level of about 240 ng/ml. The mean trough rifabutin concentration dropped to 141 ng/ml after rifabutin was taken with only the morning dose of delavirdine mesylate on day 30. Rifabutin pharmacokinetic parameters are shown in Table 5. C_{max} was about 20% higher and Cl_{PO} was about 20% lower on day 30 than on day 16. However, these differences were not statistically significant.

The mean cortisol ratio (6-β-hydroxycortisol to free cortisol) on day 30 in the rifabutin group (9.6 ± 4.0) was significantly higher than that in the control group (4.6 ± 2.2; $P = 0.035$). However, within-group comparisons of cortisol ratios for subjects receiving rifabutin and delavirdine mesylate showed no significant difference between ratios on day 15 (pre-rifabutin; 9.1 ± 4.9) and day 16 (first dose of rifabutin; 8.4 ± 6.8) or day 30 (2 weeks of rifabutin; 9.6 ± 4.0). In both subject groups the cortisol ratio was highly variable. The ratio of 6-β-hydroxycortisol to free cortisol was significantly reduced from baseline (6.1 ± 2.5) after treatment with delavirdine mesylate for 24 h (3.3 ± 2.4; $P = 0.0038$). By day 15, the cortisol ratio (8.7 ± 7.3) was not significantly different from the baseline value ($P = 0.33$).

4. Discussion

Findings from studies with both rat and human liver microsomes have suggested that the oxidative metabolism of delavirdine is mediated, at least in part, by CYP3A, and that delavirdine inhibits its own metabolism both acutely and chronically (Voorman et al., 1995). Results of multiple-dose clinical studies have furthermore suggested that delavirdine has an acute inhibitory effect on CYP3A, as shown by reductions in the

Table 5
Mean (S.D.) rifabutin pharmacokinetic parameters after 300 mg rifabutin once daily on study days 16–30[a]

Parameter	Day 16	Day 30
Cl_{PO} (l/h)	49 (21)	41 (12)
AUC (ng h/ml)[b]	7372 (3894)	7973 (2446)
C_{ss} (ng/ml)	—	332 (102)
C_{min} (ng/ml)	—	141 (43)
C_{max} (ng/ml)	511 (113)	616 (207)
T_{max} (h)	3.4 (0.5)	2.9 (1.1)
$t_{1/2}$ (h)	10 (3.3)	11 (1.8)

[a] Rifabutin taken with delavirdine mesylate 400 mg three times a day through morning of day 30.
[b] $AUC_{0-\infty}$ on day 16; AUC_{0-24} on day 30.

urinary ratio of 6-β-hydroxycortisol to free cortisol (Batts et al., 1993; Borin et al., 1997). This acute effect was also observed in the present study, with day 2 cortisol ratios significantly reduced relative to baseline values; by day 15 (steady state), the ratios had returned to baseline values. In another multiple-dose clinical study in which the erythromycin breath test was used as an in vivo marker of CYP3A activity, delavirdine use was associated with both acute and chronic inhibition of CYP3A (Cheng et al., 1997). Chronic inhibition of CYP3A by delavirdine is also supported by results of drug interaction studies with saquinavir and with indinavir (Cox et al., 1997). Thus, the cortisol ratio test may be better suited for monitoring CYP3A induction rather than inhibition, since extra-hepatic production of 6-β-hydroxycortisol has been reported (Ged et al., 1989).

Rifabutin, an inducer of hepatic microsomal enzymes in humans, affected the pharmacokinetics of delavirdine when the two compounds were coadministered in this multiple-dose study. Steady-state plasma delavirdine concentrations and pharmacokinetic parameters in the control group (delavirdine mesylate alone) were unchanged during the study. In the rifabutin group, delavirdine oral clearance increased by about fivefold after 2 weeks of concurrent dosing of delavirdine mesylate and rifabutin, resulting in an average steady-state trough drug concentration of only 1 μM compared to 18 μM when these patients were taking delavirdine mesylate alone. C_{min} in two subjects in the rifabutin group was below 0.50 μM on day 30. Additionally, the ratio of metabolite formation to elimination clearance for desalkyl delavirdine was significantly higher and delavirdine elimination half-life was significantly shorter when rifabutin and delavirdine mesylate were coadministered. These findings are consistent with induction of delavirdine metabolism by rifabutin. Although the relationship between plasma delavirdine concentrations and clinical efficacy has not yet been established, the reduction in delavirdine concentrations observed in this steady-state drug interaction study may likely be of clinical significance.

There appeared to be a trend for desalkyl delavirdine levels to also decrease after coadministration of delavirdine mesylate and rifabutin; however, only C_{min} was significantly lower (about 40%) on day 30 relative to day 15 (pre-rifabutin). The apparent elimination half-life of the metabolite on day 30 was also significantly shorter (about 50%) than that on day 15. These results suggest an inductive effect by rifabutin on desalkyl delavirdine. The approximately 2.5-fold increase in desalkyl delavirdine elimination half-life after 1 day of concomitant administration of delavirdine mesylate and rifabutin was somewhat surprising. Inspection of individual subject values and regressions confirmed an increase in apparent half-life on day 16 relative to day 15 in each subject. However, the apparent half-life of the metabolite was also higher ($\sim$63%) on day 16 than on day 15 in the control group, which suggests that the corresponding increase observed in the rifabutin group was not associated with rifabutin treatment.

The effect of rifabutin on the pharmacokinetics of delavirdine is consistent with reports on the interaction between rifabutin and clarithromycin, which is known to inhibit CYP3A (Gillum et al., 1993). In one study, concurrent administration of rifabutin (600 mg/day) and clarithromycin (1000 mg/day) resulted in a decrease in clarithromycin serum levels and no measurable effect on levels of the 14-hydroxy metabolite (Wallace et al., 1995). When the same dose of clarithromycin was taken with a 300 mg/day dose of rifabutin in another study, the area under the curve for clarithromycin decreased by about 50% while the area under the curve for the 14-hydroxy metabolite increased by about 40%, which is consistent with increased metabolism of clarithromycin in the presence of an enzyme inducer (Hafner et al., 1997). Trapnell et al. reported no significant effect of rifabutin (300 mg/day) on the pharmacokinetics of fluconazole (200 mg/day), which is excreted largely unchanged but is a weak inhibitor of cytochrome P450, in HIV-positive patients (Trapnell et al., 1993).

Although this study was not designed to look at the potential effect of delavirdine mesylate on rifabutin pharmacokinetics, it is possible to make some general observations in relation to published pharmacokinetic data for rifabutin. First of all,

except for the terminal half-life which was underestimated, rifabutin pharmacokinetic parameters in this study were similar to those previously reported for healthy volunteers and HIV-positive patients (Skinner et al., 1989; Strolin-Benedetti et al., 1990; Brogden and Fitton, 1994). The reason for the shorter half-life in the present study was that sampling was not continued for a sufficient time after dosing to accurately calculate the half-life of rifabutin, which is reported to range from 32 to 67 h for sampling times beginning no earlier than 24 h post dosing (Brogden and Fitton, 1994). Autoinduction of rifabutin metabolism has been previously reported after 28 days of multiple dosing, which results in a lower steady-state area under the curve (Skinner et al., 1989; Strolin-Benedetti et al., 1990). In the present study, the area under the curve did not differ significantly between the first dose (day 16) and the last dose (day 30). This is likely due to underestimation of the $AUC_{0\text{-}\infty}$ for the first dose of rifabutin, since the elimination rate constant was inaccurately determined. However, the cortisol ratio remained unchanged after 2 weeks of concurrent treatment with rifabutin and delavirdine, which suggests that CYP3A was not induced. It is therefore possible that delavirdine may have inhibited rifabutin metabolism, thus counteracting the autoinduction. The observed decrease (about 40%) in the mean trough rifabutin concentration after dosing of delavirdine mesylate was discontinued on day 30 is consistent with this hypothesis.

Additional work is needed to determine whether increasing the delavirdine mesylate dose will result in maintenance of adequate plasma delavirdine concentrations when delavirdine mesylate and rifabutin are coadministered. A more definitive study on the effect of delavirdine on the pharmacokinetics of rifabutin is also necessary to ensure that safe and therapeutic levels of rifabutin are achieved for the prevention of MAC disease in patients receiving delavirdine mesylate.

In summary, no drug-limiting adverse effects occurred during 2 weeks of concurrent administration of delavirdine mesylate and rifabutin in HIV-positive patients. However, rifabutin had a significant effect on the steady-state pharmacokinetics of delavirdine. Oral clearance of delavirdine was increased about five-fold, resulting in lower steady-state plasma delavirdine concentrations. Therefore, concurrent administration of delavirdine mesylate and rifabutin is discouraged.

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