The Clinical Pharmacokinetics of Rifampin and Ethambutol in HIV-Infected Persons with Tuberculosis

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Background. The pharmacokinetics of rifampin and ethambutol in HIV-infected patients with tuberculosis (TB) are incompletely characterized. We examined the pharmacokinetics of rifampin and ethambutol in a cohort of patients with HIV-related TB who were treated in the United States.

Methods. Serum drug concentrations were determined 2, 6, and 10 h after dosing in 36 HIV-infected patients with TB who were taking rifampin and in 49 who were taking ethambutol. Observed serum concentrations were compared with published normal ranges and published data.

Results. With daily dosing of rifampin (600 mg), 26 (77%) of 34 patients (95% confidence interval [CI], 59%–89%]) had a low maximum concentration of rifampin (<8 μg/mL), and 12 (35%; 95% CI, 20%–54%) had a very low maximum concentration (<4 μg/mL). With intermittent rifampin dosing (600 mg), 13 (68%) of 19 patients (95% CI, 44%–85%) had a low maximum concentration of rifampin, and 5 (26%; 95% CI, 11%–50%) had a very low maximum concentration. With daily ethambutol dosing (20 mg/kg), 33 (69%) of 48 patients (95% CI, 55%–81%) had a low maximum concentration of ethambutol (<2 μg/mL), and 18 (38%; 95% CI, 24%–53%) had a very low maximum concentration (<1 μg/mL). With intermittent ethambutol dosing (50 mg/kg twice weekly or 30 mg/kg thrice weekly), 13 (72%) of 18 patients (95% CI, 47%–88%) had a low maximum concentration of ethambutol (<4 μg/mL), and 5 (28%; 95% CI, 12%–54%]) had a very low maximum concentration (<2 μg/mL).

Conclusions. In HIV-infected patients with TB who are receiving rifampin and ethambutol, low maximum concentrations of rifampin and ethambutol were common. For patients with HIV-related TB, therapeutic monitoring of rifampin and ethambutol levels may help clinicians achieve target serum concentrations.

Tuberculosis (TB) treatment failures and relapses of TB have been associated with low serum drug concentrations [1–8]. Previously, we reported that serum pyrazinamide concentrations in HIV-infected patients with TB were generally in the normal range, even in patients with advanced immunosuppression [9].

Rifampin is the most important anti-TB drug, because it shortens the duration of treatment from 18 months to 9 months; when given with pyrazinamide, it shortens the duration to 6 months [10–13]. Single-drug resistance to rifampin is associated with HIV infection, although the precise mechanism is not known. Acquired rifampin resistance is associated with low isoniazid concentrations (for once-weekly isoniazid-rifapentine regimens) and with low isoniazid and rifabutin concentrations (for twice-weekly isoniazid-rifabutin regimens) [7, 8].

Ethambutol is "the fourth" anti-TB drug, and it is used to prevent further drug resistance while susceptibility results are pending [10, 14, 15]. It may replace isoniazid or rifampin in the treatment regimen when drug resistance is found, although it is not nearly as potent as these agents [10].

Clinically, serum concentrations of anti-TB drugs are collected 2 and 6 h after administration of the dose in an attempt to determine the rate and extent of absorption [9, 13, 16]. Although practical, this approach does not provide a robust measure of the area under the concen-

Received 16 April 2005; accepted 13 July 2005; electronically published 28 October 2005.

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Clinical Infectious Diseases 2005; 41:1638–47
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1058-4838/2005/4111-0015$15.00

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tration-time curve (AUC). We sought to characterize the pharmacokinetics of rifampin and ethambutol in HIV-infected patients with TB, to estimate the frequency and extent of low drug concentrations, and to determine whether a sparse but practical sampling strategy could provide some measure of the AUC. We also examined factors associated with low drug concentrations.

METHODS

Recruitment of subjects. The study was open to sites in the Adult AIDS Clinical Trials Group. HIV-infected individuals aged ⩾13 years with culture-confirmed TB were eligible for the study; subjects with unconfirmed TB could be enrolled presumptively, pending culture results [9]. Patients received at least 2 of the 4 first-line anti-TB drugs (i.e., isoniazid, rifampin, pyrazinamide, and ethambutol).

Individuals were excluded if they had an aspartate aminotransferase level of >10 times the upper limit of normal, a total bilirubin level of >2.5 times the upper limit of normal, a serum creatinine level of >3 times the upper limit of normal, and/or a creatinine clearance rate of <50 mL/min. Patients receiving antacids, sucralfate, didanosine, or imidazoles were eligible for the study if the administration of these agents could be separated from the administration of the anti-TB drugs by ⩾2 h.

Baseline evaluations included $CD4^+$ T lymphocyte counts within 60 days of study entry. Institutional review board–approved consent was obtained from all participants. Participating sites followed the US Department of Health and Human Services guidelines for human experimentation or stricter guidelines provided by the sites' institutional review boards.

Pharmacokinetic assessments. Two sessions at which pharmacokinetic data were determined (hereafter, "pharmacokinetic sessions") were scheduled: one was during the daily TB treatment period, and one was during the intermittent treatment (twice-weekly or thrice-weekly). Dosages were as follows: rifampin, 600 mg for both daily and intermittent regimens (450 mg if the patient's weight was <50 kg); isoniazid, 300 mg if given daily and 900 mg if given intermittently (600 mg if the patient's weight was <50 kg); pyridoxine, 50 mg daily; pyrazinamide, 2.0 g if given daily (1.5 g if the patient's weight was <50 kg) and 2.5 g if given intermittently (2.0 g if the patient's weight was <50 kg); and ethambutol, 20 mg/kg if given daily, 30 mg/kg if given thrice weekly, and 50 mg/kg if given twice weekly (rounded to the nearest 200 mg).

Patients received standardized drug lots at pharmacokinetic sessions, as follows: isoniazid, 300-mg tablets (Danbury Pharmacal); pyridoxine, 50-mg tablets (Tischon Laboratories); rifampin, 150- and 300-mg capsules (Marion Merrill Dow); pyrazinamide, 500-mg tablets (Lederle Laboratories); and ethambutol, 400-mg tablets (Lederle Laboratories). Patients received a ⩾10-day course of a given rifampin course before pharmacokinetic sessions. Blood samples were collected at 2, 6, and 10 h after observed dosing.

Laboratory methods. Whole blood samples were collected in vacuum tubes, allowed to clot, and centrifuged for 5 min at 1000–2000 *g*. Serum was transferred to prelabeled cryovials that contained ascorbic acid, inverted several times, wrapped in foil, and frozen at −70°C within 1 h after collection. Frozen serum was shipped on dry ice for batch testing.

Rifampin concentrations were measured using a validated high-performance liquid chromatography assay with UV detection. The concentration of standards ranged from 0.5 to 20 μg/mL. The concentrations of quality controls were 0.12, 2.5, and 15 μg/mL. Total assay variation for controls was 9.3%–12.8%, with precision ranging from +5% to +14%. Ethambutol concentrations were measured using a validated gas chromatography assay. Standards ranged from 0.5 to 10 μg/mL. Quality controls were 0.35, 3.0, and 6.0 μg/mL. The within-day precision (percentage coefficient of variation) of validation-quality control samples was 2.2%–4.1%, and the overall validation precision was 2.8%–3.3%.

Calculation of pharmacokinetic parameters. When all 3 time points were available, the C_{max} was the maximum observed concentration, and T_{max} was the corresponding time. Otherwise, C_{max} and T_{max} were excluded from analysis. Ranges for 2-, 6-, and 10-h concentrations also were examined. AUC was calculated as a 3–time point AUC (linear trapezoidal rule [AUC-L]) and a similarly constructed 2–time point AUC (AUC-2,6 h for rifampin and AUC-2,10 h for ethambutol) [9]. Time-zero concentrations were not measured and were assumed to be 0 μg/mL for purposes of calculating the AUC. When the 6-h concentration was greater than the 10-h concentration, the elimination rate constant, half-life, and AUC-L were estimated using standard noncompartmental techniques.

Statistical methods. Observed C_{max} and 2-h concentration for rifampin and ethambutol were compared with published reference ranges [11–16]. A low C_{max} was defined as <8 μg/mL for rifampin and as <2 μg/mL for ethambutol given daily or <4 μg/mL for ethambutol given intermittently. Very low C_{max} was defined as <4 μg/mL for rifampin and as <1 μg/mL or <2 μg/mL for daily and intermittent ethambutol, respectively. Delayed absorption was defined as a T_{max} of >3 h.

For comparison, pharmacokinetic data from intensively sampled, healthy volunteers (hereafter, "volunteers"), HIV-negative patients with TB (group NIH A), and sparsely sampled HIV-negative patients with TB (group NIH B) from the United States were included [11–17]. NIH B patients had samples collected only at 2 and 6 h after administration of the dose. Volunteers were studied after administration of single doses, whereas NIH A and NIH B patients were studied at steady state. Median values at each time point were calculated for each data set.

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Concentrations for each drug were standardized for a common dose (rifampin, 600 mg; ethambutol, 20 mg/kg).

The 2-sided Wilcoxon rank sum test (on SAS software, version 6.12; SAS Institute), Fisher's exact test, and Pearson correlation coefficients were used to evaluate differences in pharmacokinetic parameters, as appropriate [9, 18, 19].

RESULTS

Patient Demographic Characteristics

Eight Adult AIDS Clinical Trials Group sites in the continental United States enrolled 59 patients. One patient tested HIV-1 negative, and 1 patient had negative TB culture results. Samples were lost for 5 patients. For medical reasons, not all patients received both rifampin and ethambutol. Of 52 patients, rifampin data were studied for 36; 34 patients received daily doses, and 21 received intermittent doses (19 received both, 15 received daily doses only, and 2 received intermittent doses only). For ethambutol, 49 subjects were studied: 48 received daily doses, and 20 received doses (19 received both, 29 received daily doses only, and 1 received intermittent doses only). The median age of subjects was 40 years, most patients were men, ⩾50% of patients were non-Hispanic black persons, and the median $CD4^+$ cell count was ⩽70 cells/mm^3 (table 1).

Drug Dosing and Pharmacokinetic Session Timing

Rifampin. All daily rifampin recipients had 2-, 6-, and 10-h concentrations determined, as did 19 (90%) of 21 intermittent rifampin recipients (2 did not have 10-h concentrations determined). The median time at which samples were obtained for determination of the 10-h concentration was 9:02 h after dosing (range, 7:55–10:10 h after dosing). The median daily and intermittent doses were 9.6 mg/kg (range, 6.6–14.7 mg/kg) and 8.7 mg/kg (range, 6.1–15.2 mg/kg), respectively. All daily rifampin recipients also received pyrazinamide and isoniazid; 97% also received ethambutol, and 26% also received antiretroviral therapy (with nucleosides only). For intermittent rifampin recipients, 67% also received pyrazinamide, 95% received isoniazid, 48% received ethambutol, and 19% received nucleoside reverse-transcriptase inhibitors. Patients had eaten ⩽2 h before taking the study medication for 91% of daily and 81% of intermittent dosing sessions. No patients had taken antacids within 2 h of taking rifampin.

Ethambutol. All daily ethambutol recipients had 2-, 6-, and 10-h concentrations determined, as did 18 (90%) of 20 intermittent ethambutol recipients (the 6-h concentration and the 10-h concentration were not determined for 1 patient each). The median time at which samples were obtained for determination of the 10-h concentration was 9:31 h (range, 7:10–10:10 h). The median daily dose was 20.2 mg/kg (range, 12.8–28.8 mg/kg), and the median intermittent dose was 42.2 mg/kg (range, 24.4–50.6 mg/kg). Ninety-four percent of daily ethambutol recipients received pyrazinamide, 92% received isoniazid, 75% received rifampin, and 44% received antiretroviral therapy (nucleoside reverse-transcriptase inhibitors, protease inhibitors, and/or nonnucleoside reverse-transcriptase inhibitors). Among the intermittent ethambutol recipients, 85% received pyrazinamide, 85% received isoniazid, 55% received rifampin, and 40% received antiretrovirals. Patients had eaten ⩽2 h before taking the study medication for 92% of daily and 75% of intermittent ethambutol dosing sessions. No patients had taken antacids within 2 h of taking ethambutol.

Pharmacokinetic Parameters

Rifampin. Median daily and intermittent 2-h concentrations and C_{max} values were considerably less than the expected C_{max} range (8–24 μg/mL) and were variable (table 2) [11–16]. The 2-h concentration was the C_{max} for 41% of daily and 58% of intermittent rifampin recipients, and the median T_{max} values were 5.97 h and 2.18 h, respectively. Thus, delayed rifampin absorption was common (tables 2 and 3).

Of 34 daily rifampin recipients, 26 (76%) had low C_{max} values, and 12 (35%) had very low C_{max} values (table 3) [11–16]. Of 19 intermittent rifampin recipients, 13 (68%) had low C_{max} values, and 5 (26%) had very low C_{max} values, consistent with incomplete rifampin absorption. The median AUCs were similar for daily and intermittent dosing groups; dosing frequency did not affect rifampin exposure. The coefficients of variation for AUC with daily and intermittent dosing were comparable and modest.

Median daily rifampin concentrations and comparison data are shown in figure 1. The median 2-h concentrations were dramatically different for the Adult AIDS Clinical Trials Group patients. The sparsely sampled HIV-negative patients with TB (NIH B) had median concentrations similar to those of the more intensively sampled patients (NIH A).

For HIV-infected patients with TB, the 2-h concentration and either the AUC-2,6h (for 34 daily rifampin recipients, $r = 0.93$; $P < .0001$) or the AUC-L (for 31 daily rifampin recipients, $r = 0.76$; $P < .0001$) were strongly correlated. Patients with higher (⩾100 cells/mm^3) or lower (<100 cells/mm^3) $CD4^+$ T lymphocyte counts had similar 2-h concentrations for both daily and intermittent dosing ($P = .298$ and .34, respectively) and similar AUC-L ($P = .49$ and .61, respectively). Daily rifampin concentrations increased slightly with food taken within 2 h of dosing (with food, the 2-h concentration was 3.9 μg/mL, and the AUC-L was 30.6 $\mu g \times h/mL$; without food, the 2-h concentration was 2.6 μg/mL, and the AUC-L was 21.7 $\mu g \times h/mL$; $P = .9$ for 2-h concentration, and $P = .3$ for AUC-L). Meanwhile, intermittent rifampin concentrations decreased slightly with food consumption (with food, the 2-h concentration was 5.1 μg/mL, and the AUC-L was $\mu g \times h/mL$; without

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Table 1. Demographic and clinical characteristics of study participants who received rifampin or ethambutol.

Characteristic	Rifampin recipients (n = 36)	Ethambutol recipients (n = 49)
Age, years[a]		
24–29	6 (17)	7 (14)
30–39	11 (31)	16 (33)
40–49	14 (39)	20 (41)
⩾50	5 (14)	6 (12)
Sex		
Male	28 (78)	40 (82)
Female	8 (22)	9 (18)
Race/ethnicity		
White, non-Hispanic	3 (8)	3 (6)
Black, non-Hispanic	18 (50)	28 (57)
Hispanic, regardless of race	14 (39)	17 (35)
Other/unknown	1 (3)	1 (2)
Injection drug use at baseline		
Never	24 (67)	33 (67)
Currently	3 (8)	3 (6)
Previously	9 (25)	13 (27)
Homosexual activity		
Yes	16 (44)	22 (45)
No	19 (53)	26 (53)
Unknown	1 (3)	1 (2)
$CD4^+$ T cell counts, cells/mm^3[b]		
<100	20 (56)	28 (57)
100–199	8 (22)	11 (22)
200–299	4 (11)	5 (10)
300–399	2 (6)	3 (6)
400–499	2 (6)	2 (4)
Ingested food ⩽2 h before receipt of pharmacokinetic dose, *n/N* (%)		
Daily therapy recipients	31/34 (91)	44/48 (92)
Intermittent therapy recipients	17/21 (81)	15/20 (75)
Diarrhea and/or vomiting in past week, *n/N* (%)		
Daily therapy recipients	10/34 (29)	13/48 (27)
Intermittent therapy recipients	1/21 (5)	2/20 (10)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Percentages may not total to 100 because of rounding.

[a] Median age for both rifampin recipients and ethambutol recipients, 40 years.

[b] Median for rifampin recipients, 70 cells/mm^3; median for ethambutol recipients, 61 cells/mm^3.

food, the 2-h concentration was 7.0 μg/mL, and the AUC-L was 42.7 $\mu g \times h/mL$; $P = .7$ for 2-h concentration, and $P = .5$ for AUC-L). The study was not powered to detect such differences. The 2-h concentration and AUC-L values for daily and intermittent therapy were similar regardless of use of antiretroviral therapy ($P \geqslant .2$ for all).

Ethambutol. Median daily and intermittent 2-h concentration and C_{max} values were considerably less than the expected C_{max} ranges of 2–6 μg/mL and 4–12 μg/mL, respectively (table 4) [11–16]. The variability of 2-h concentration determinations was lower than that for rifampin. The 2-h concentration was the C_{max} for 90% of daily ethambutol and 78% of intermittent ethambutol recipients, and median T_{max} values were 2.02 h and 2.05 h, respectively. Thus, delayed ethambutol absorption was less common than with rifampin (tables 3 and 4).

Of 48 daily ethambutol recipients, 33 (69%) had low C_{max} values, and 18 (38%) had very low C_{max} values [11–16]. Of 18 intermittent ethambutol recipients, 13 (72%) had low C_{max} values, and 5 (28%)

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Table 2. Descriptive statistics for rifampin therapy.

Dosing frequency, measurement	Median	Minimum	Maximum	CV, %	No. of patients with value available
Daily					
C_{max}, μg/mL	5.49	1.06	11.21	48.7	34
T_{max}, h	5.97	1.96	10.04	51.3	34
2-h concentration, μg/mL	3.28	0.11	11.21	93.6	34
6-h concentration, μg/mL	3.65	0.41	9.08	53.6	34
10-h concentration, μg/mL	1.66	0.12	7.70	87.8	34
AUC, μg × h/mL	30.06	5.81	62.01	48.1	31
AUC-2,6h, μg × h/mL	17.01	3.68	47.67	60.6	34
Elimination rate constant	0.30	0.07	0.62	46.2	31
Half-life, h	2.30	1.12	10.45	69.3	31
AUC-L, μg × h/mL	29.21	7.13	69.58	52.6	31
Intermittent					
C_{max}, μg/mL	5.44	2.24	13.94	51.3	19
T_{max}, h	2.18	1.98	7.96	56.2	19
2-h concentration, μg/mL	4.69	0.07	13.94	75.8	21
6-h concentration, μg/mL	4.12	2.21	6.61	35.1	21
10-h concentration, μg/mL	2.30	0.39	6.97	68.6	19
AUC, μg × h/mL	30.51	9.84	66.69	47.5	18
AUC-2,6h, μg × h/mL	22.33	4.64	49.07	54.7	21
Elimination rate constant	0.19	0.12	0.45	38.3	18
Half-life, h	3.58	1.53	6.00	32.7	18
AUC-L, μg × h/mL	29.46	9.36	66.38	46.0	18

NOTE. AUC-L, area under the concentration-time curve calculated using the linear trapezoidal rule; AUC-2,6h, area under the concentration-time curve calculated using the 2- and 6-h concentrations; AUC-2,10h, area under the concentration-time curve calculated using the 2- and 10-h concentrations; C_{max}, maximum concentration; CV, coefficient of variation; T_{max}, time of maximum concentration.

had very low C_{max} values, which is consistent with incomplete ethambutol absorption. The median AUC was higher for intermittent dosing than for daily dosing, which is to be expected, given the higher doses given in the intermittent regimen.

Median daily ethambutol concentrations and comparison data are shown in figure 2. The median 2-h concentrations were substantially lower for the Adult AIDS Clinical Trials Group patients. The sparsely sampled NIH B patients had median concentrations resembling those for the NIH A patients.

For HIV-infected patients with TB, 2-h concentration and either AUC-2,10h (for 48 daily ethambutol recipients, $r = 0.98$; $P < .0001$) or AUC-L (for 44 daily ethambutol recipients, $r = 0.93$; $P < .0001$) were strongly correlated. Patients with higher (≥100 cells/mm^3) or lower (<100 cells/mm^3) $CD4^+$ T lymphocyte counts showed similar 2-h concentration and AUC-L values for both daily and intermittent dosing ($P \geq .4$ for all). Similarly, consumption of food ≤2 h before administration of the dose did not have a large effect (with food, the daily 2-h concentration was 1.6 μg/mL, and the AUC-L was 8.2 μg × h/mL; without food, the 2-h concentration was 1.8 μg/mL, and the AUC-L was 12.7 μg × h/mL; $P = .9$ for 2-h concentration, and $P = .2$ for AUC-L). For intermittent dosing with food, the 2-h concentration was 2.7 μg/mL, and the AUC-L was 14.0 μg × h/mL; without food, the 2-h concentration was 4.0 μg/mL, and the AUC-L was 15.6 μg × h/mL ($P = .3$ for 2-h concentration, and $P = .9$ for AUC-L). The 2-h concentration and AUC-L values for daily and intermittent therapy were similar regardless of use of antiretroviral therapy ($P \geq .2$ for all).

DISCUSSION

In this study of HIV-infected patients with TB, the median rifampin 2-h concentration and C_{max} frequently were less than the reported range of 8–24 μg/mL [11–16]. Rifampin induces its own clearance during the first week of treatment, independent of rifampin's well-known induction of cytochrome P450 enzymes [10, 16, 20]. Autoinduction produces shorter rifampin half-lives and lower AUCs, compared with single doses, whereas the reduction in C_{max} appears to be small [7, 8, 20, 21]. This is demonstrated in figure 1 and 2: volunteers were studied after administration of single doses, but the comparator groups were studied at steady state. Furthermore, the published C_{max} ranges reflect steady state data from patients with TB that were sub-

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Table 3. Median serum concentrations of ethambutol and rifampin in HIV-infected patients with tuberculosis.

Variable	Ethambutol recipients	Rifampin recipients
Daily treatment recipients		
No. of patients	48	34
C_{max}		
Median μg/mL (range)	1.54 (0.17–4.73)	5.49 (1.06–11.21)
Low, % of patients (95% CI)	69 (55–81)	77 (59–89)
Very low, % of patients (95% CI)	38 (24–53)	35 (20–54)
T_{max}		
Median h (range)	2.02 (1.96–8.79)	5.97 (1.96–10.04)
Delayed, no. (%) of patients	5 (10)	20 (59)
Intermittent treatment recipients		
No. of patients	18	19
C_{max}		
Median μg/mL (range)	3.08 (0.62–6.63)	5.44 (2.24–13.94)
Low, % of patients (95% CI)	72 (47–88)	68 (44–85)
Very low, % of patients (95% CI)	28 (12–54)	26 (11–50)
T_{max}		
Median h (range)	2.05 (1.95–10.01)	2.18 (1.98–7.96)
Delayed, no. (%) of patients	4 (22)	8 (42)

NOTE. C_{max}, maximum concentration; T_{max}, time of maximum concentration.

sequently validated using data for healthy volunteers [21]. Therefore, our AUC estimates were expected to be lower than those from studies of single doses, but our C_{max} values were expected to be within the published ranges.

Rifampin is a concentration-dependent killer of microorganisms, including *Mycobacterium tuberculosis* [16, 22, 23]. Similar to aminoglycosides and fluoroquinolones, rifampin's most important pharmacodynamic parameters are the ratio of C_{max} to MIC and the ratio of AUC to MIC [16, 22]. For *M. tuberculosis* in vivo, this would apply to actively multiplying extracellular organisms. On the basis of the anaerobic Wayne model of *M. tuberculosis,* this also would apply to the "persis-

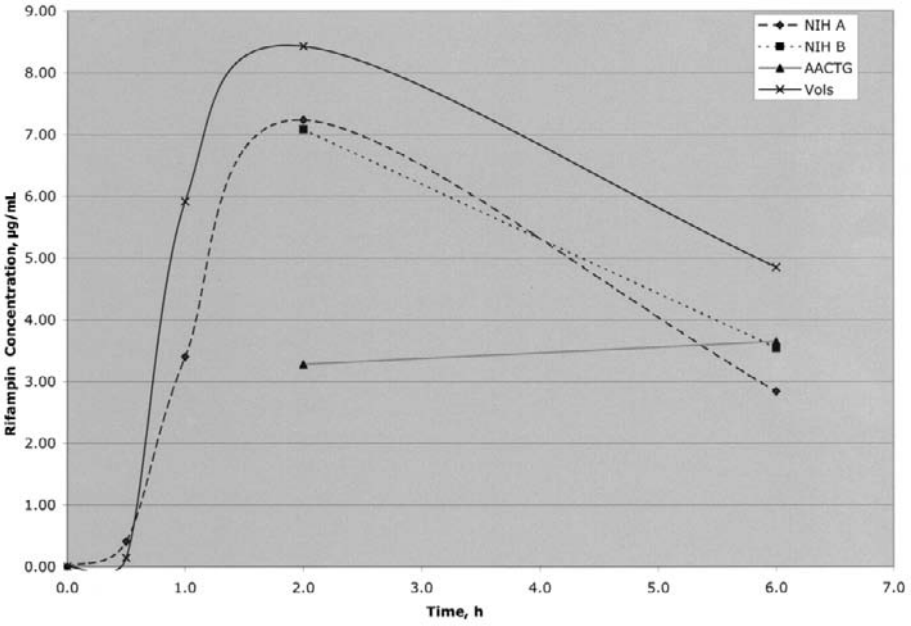

Figure 1. Concentration-versus-time profiles for patients taking rifampin daily, compared with profiles from separate studies of healthy volunteers who underwent intensive sampling (Vols) and HIV-negative patients with tuberculosis who underwent intensive sampling (NIH A) or sparse sampling (NIH B). AACTG, Adult AIDS Clinical Trials Group.

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Table 4. Descriptive statistics for ethambutol therapy.

Dosing frequency, measurement	Median	Minimum	Maximum	CV, %	No. of patients with value available
Daily					
C_{max}, μg/mL	1.54	0.17	4.73	66.5	48
T_{max}, h	2.02	1.96	8.79	57.7	48
2-h concentration, μg/mL	1.54	0.12	4.73	68.0	48
6-h concentration, μg/mL	0.64	0.04	2.12	63.4	48
10-h concentration, μg/mL	0.42	0.04	1.10	59.9	48
AUC, μg × h/mL	8.09	1.52	19.39	54.4	44
AUC-2,6h, μg × h/mL	8.77	0.99	26.31	63.5	48
Elimination rate constant	0.17	0.04	0.33	35.7	44
Half-life, h	4.09	2.01	17.25	52.2	44
AUC-L, μg × h/mL	7.68	1.51	19.06	54.6	44
Intermittent					
C_{max}, μg/mL	3.08	0.62	6.63	55.8	18
T_{max}, h	2.05	1.95	10.01	71.6	18
2-h concentration, μg/mL	3.08	0.49	7.48	66.1	20
6-h concentration, μg/mL	1.48	0.20	2.68	51.4	19
10-h concentration, μg/mL	0.81	0.09	6.63	123.1	19
AUC, μg × h/mL	13.05	2.98	23.61	48.9	17
AUC-2,6h, μg × h/mL	17.52	2.74	45.04	65.8	19
Elimination rate constant	0.24	0.07	0.30	31.8	17
Half-life, h	2.93	2.34	10.74	58.5	17
AUC-L, μg × h/mL	13.14	2.97	23.27	48.6	17

NOTE. AUC-L, area under the concentration-time curve calculated using the linear trapezoidal rule; AUC-2,6h, area under the concentration-time curve calculated using the 2- and 6-h concentrations; AUC-2,10h, area under the concentration-time curve calculated using the 2- and 10-h concentrations; C_{max}, maximum concentration; CV, coefficient of variation; T_{max}, time of maximum concentration.

ters" responsible for treatment failures and for relapses [16, 24, 25]. Given the frequently low rifampin concentrations in our study and in other studies of HIV-infected patients with TB, the 600-mg standard dose may be suboptimal for this population [22, 26]. Published data support weight-based dosing (10 mg/kg) over a standard 600-mg dose [11, 12]. When feasible, therapeutic drug monitoring may be a reasonable option for similar patient populations. When rifampin is given daily or thrice weekly, nearly all toxicities have been found to be idiosyncratic rather than dose related. Therefore, higher daily (or perhaps thrice-weekly) doses of rifampin (20 or 30 mg/kg) should be studied as a possible approach to maximize the effect of treatment [22]. In contrast, high rifampin doses given more intermittently may produce a flulike syndrome, and in HIV-infected persons, more highly intermittent regimens are associated with the emergence of rifamycin resistance [8, 10, 28–30]. Another consideration that may merit additional study is whether continuation of treatment with other anti-TB drugs beyond the induction period prevents the poor outcomes associated with subtherapeutic drug concentrations. For settings in which therapeutic drug monitoring may not be an option, additional studies to characterize whether early surrogate markers of response (such as 2-month sputum culture conversion rates) may be predictive of suboptimal pharmacokinetic parameters would be valuable.

Ethambutol is primarily bacteriostatic against *M. tuberculosis* at achievable serum concentrations [14–16, 21]. Low ethambutol doses—and, by extension, low serum concentrations—fared no better than placebo in patients with TB [15, 27]. Given the frequently low ethambutol concentrations in our patients when they were given doses of 20 mg/kg daily, this dosage may not be adequate to suppress drug-resistant subpopulations present in patients with TB. Therefore, ethambutol doses of 25 mg/kg may be preferable initially, and therapeutic drug monitoring may be used to verify the adequacy.

Optimal sampling time studies suggest that 2- and 6-h postdosing samples are reasonable for rifampin and acceptable for ethambutol [11, 12, 14]. Comparisons of the sparsely sampled HIV-negative patients with TB (NIH B) with more intensively sampled HIV-negative patients (NIH A) and healthy volunteers showed similar median concentration-versus-time curves (figures 1 and 2). Our data suggest that a 2-point AUC-2,6h (rifampin) and AUC-2,10h (ethambutol) are comparable to a 3-point AUC-L. Further, 2-h concentration showed a good

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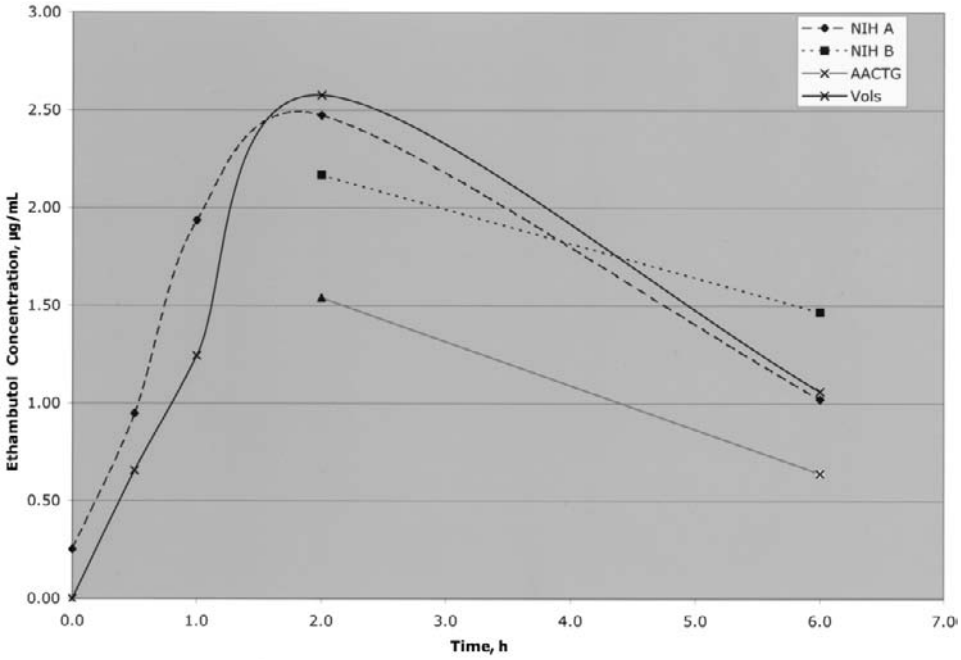

Figure 2. Concentration-versus-time profiles for patients taking ethambutol daily, compared with profiles from separate studies of healthy volunteers who underwent intensive sampling (Vols) and HIV-negative patients with tuberculosis who underwent intensive sampling (NIH A) or sparse sampling (NIH B). AACTG, Adult AIDS Clinical Trials Group

correlation with both AUC-2,6h (rifampin) and AUC-2,10h (ethambutol) and AUC-L. From this, we conclude that the 2- and 6-h sampling strategy is a reasonable approach clinically. In situations in which only 1 sample can be obtained, the 2-h sample may be acceptable; however, use of a single 2-h sample will not distinguish between delayed absorption and malabsorption.

We found that low and very low 2-h concentrations of rifampin and ethambutol were common in the HIV-infected patients with TB in our study. Drug absorption was heterogeneous and unpredictable, in contrast to our results for pyrazinamide [9]. Rifampin absorption was delayed in many patients, and in most cases of delayed absorption, the C_{max} was still low, suggesting that the predominant pattern was one in which absorption was both late and incomplete. Patient characteristics, such as $CD4^+$ T lymphocyte count and concomitant receipt of other drugs, did not predict poor drug absorption. Similarly, in a study from Kenya, diarrhea and $CD4^+$ T lymphocyte count did not predict drug malabsorption [5]. Of note, in the Kenyan study, all patients showed poor absorption, regardless of whether they had HIV infection. In contrast, our HIV-infected patients with TB had lower serum concentrations than did separately studied US patients with TB without HIV infection [15, 17]. This difference in drug absorption between African and North American patients with TB also was seen in a study performed in Botswana [26].

Preventing acquired drug resistance is the primary reason for using multiple anti-TB drugs. We did not collect treatment outcome data as part of this pharmacokinetic study. However, a recent study of once-weekly isoniazid and rifapentine therapy in HIV-uninfected patients showed that low isoniazid concentrations were associated with treatment failures, TB relapses, and the development of acquired rifamycin-resistant TB [7]. A second study of HIV-infected patients with TB showed that low isoniazid and rifabutin concentrations were associated with similar problems [8]. The development of rifamycin-resistant TB is associated with HIV infection and with highly intermittent treatment [7, 28–30]. The studies that have revealed that low serum concentrations are associated with poor outcomes, combined with our results and those of Tappero et al. [26], demonstrating that low serum concentrations are common, suggest that careful dosing and follow-up are required for HIV-infected patients with TB.

We did not detect a significant effect for recent food ingestion on drug absorption, but 81%–91% of patients ate within 2 h of taking the study medication, limiting power to detect differences. Previously, consumption of high-fat food was shown to reduce rifampin's C_{max} by 36%, with smaller reductions seen in the AUC [12]. It seems prudent to limit food intake near the time of anti-TB drug ingestion, to avoid any potential deleterious effects. Similarly, in this study, patients could receive certain agents (antacids, sucralfate, didanosine, and imidazoles) that may affect absorption. Because coadministration of such agents was separated by at least 2 h, only if there were delayed

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gastric emptying would there be the potential for these interfering substances to "catch" rifampin or ethambutol before those interfering substances left the stomach for the intestines. Our analyses of pyrazinamide therapy in the same cohort revealed that delayed absorption was rare—thus, the delayed rifampin and ethambutol absorption occurs within the intestines—and interfering substances should have played a minimal (if any) role in this study [9]. Certificates of analysis were not available for the study of anti-TB medications, nor was the bioavailability of these agents independently verified. However, by using medications from US sources used as part of routine clinical practice in the United States, the data should closely resemble results expected in routine clinical practice.

In summary, HIV-infected patients with TB treated with rifampin and ethambutol frequently had low serum concentrations of these agents, and in these patients, the pattern of absorption was unpredictable. Therapeutic drug monitoring for rifampin and ethambutol may assist in achieving target serum concentrations in such patients. Additional studies are needed to examine the role of higher initial doses of rifampin and ethambutol.

Acknowledgments

We are indebted to the patients for their participation in this study and to the other members of the AIDS Clinical Trials Group 309 protocol team (Gerald Friedland, Keith Chirgwin, Ana Martinez, Wafaa El Sadr, Marie Davidian, and Donald Smith), to the investigators at participating sites (Brenda Jones and Patricia Lopez, University of Southern California, Los Angeles; C. Robert Horsburgh and Raine Alexander, Emory University, Atlanta, GA; Beth Israel Medical Center, New York, NY; Mt. Sinai Medical Center, New York; SUNY Health Sciences Center at Brooklyn, NY; Rob Roy Macgregor and Doris Shank, University of Pennsylvania, Philadelphia; Memorial Sloan Kettering/St. Vincents Medical Center, New York; Case Western/Metro Health Medical Center (Cleveland, OH; Juan J. L. Lertora and David Mushatt, Tulane University, New Orleans, LA; University of California, Los Angeles/Harbour General Medical Center, Los Angeles; University of Alabama Medical Center); and to the staff of the many tuberculosis directly observed therapy programs that supported the study. Isoniazid was supplied by Danbury Pharmacal, vitamin B6 was supplied by Tischon Laboratories, rifampin was supplied by Merrel Dow, and rifampin and ethambutol were supplied by Lederle Laboratories.

Financial support. Adult AIDS Clinical Trials Group, funded by the National Institute of Allergy and Infectious Diseases (1U01AI46370, UO1A127673, A12015802, A6201, A1802, A5802, A2205, AO603, A5901, A1701, A2503, IUO1A13844, PHS NCRR MO1 RR05096, and AI 38855).

Potential conflicts of interest. All authors: no conflicts.

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