Association between Acquired Rifamycin Resistance and the Pharmacokinetics of Rifabutin and Isoniazid among Patients with HIV and Tuberculosis

Marc Weiner,[1] Debra Benator,[2] William Burman,[3] Charles A. Peloquin,[4] Awal Khan,[5] Andrew Vernon,[5] Brenda Jones,[6] Claudia Silva-Trigo,[6] Zhen Zhao,[5] Thomas Hodge,[5] and the Tuberculosis Trials Consortium[a]

[1]University of Texas Health Science Center San Antonio and South Texas Veterans Health Care System, San Antonio, Texas; [2]Veterans' Affairs Medical Center and George Washington University Medical Center, Washington, D.C.; [3]Denver Public Health and Department of Medicine, University of Colorado Health Science Center, and [4]National Jewish Medical and Research Center and University of Colorado Schools of Pharmacy and Medicine, Denver, Colorado; [5]Division of TB Elimination, Centers for Disease Control and Prevention, Atlanta, Georgia; and [6]Los Angeles County/University of Southern California Medical Center, Los Angeles, California

Background. The occurrence of acquired rifamycin resistance despite use of directly observed therapy for tuberculosis is associated with advanced human immunodeficiency virus (HIV) disease and highly intermittent administration of antituberculosis drugs. Beyond these associations, the pathogenesis of acquired rifamycin resistance is unknown.

Methods. We performed a pharmacokinetic substudy of patients in a trial of treatment with twice-weekly rifabutin and isoniazid.

Results. A total of 102 (60%) of 169 patients in the treatment trial participated in the pharmacokinetic substudy, including 7 of 8 patients in whom tuberculosis treatment failure or relapse occurred in association with acquired rifamycin-resistant mycobacteria (hereafter, "ARR failure or relapse"). The median rifabutin area under the concentration-time curve ($AUC_{0–24}$) was lower for patients with than for patients without ARR failure or relapse (3.3 vs. 5.2 μg*h/mL; $P = .06$, by the Mann-Whitney exact test). In a multivariate analysis adjusted for $CD4^+$ T cell count, the mean rifabutin $AUC_{0–24}$ was significantly lower for patients with ARR failure or relapse than for other patients (3.0 μg*h/mL [95% confidence interval {CI}, 1.9–4.5] vs. 5.2 μg*h/mL [95% CI, 4.6–5.8]; $P = .02$, by analysis of covariance). The median isoniazid $AUC_{0–12}$ was not significantly associated with ARR failure or relapse (20.6 vs. 28.0 μg*h/mL; $P = .24$, by the Mann-Whitney exact test). However, in a multivariate logistic regression model that adjusted for the rifabutin $AUC_{0–24}$, a lower isoniazid $AUC_{0–12}$ was associated with ARR failure or relapse (OR, 10.5; 95% CI, 1.1–100; $P = .04$).

Conclusions. Lower plasma concentrations of rifabutin and, perhaps, isoniazid were associated with ARR failure or relapse in patients with tuberculosis and HIV infection treated with twice-weekly therapy.

Directly observed therapy prevents selective use of drugs and thereby generally prevents the acquisition of drug resistance during treatment of tuberculosis [1]. However, it has become evident that patients with HIV-related tuberculosis can acquire resistance to the rifamycin family of antibiotics (rifampin, rifabutin, and rifapentine) despite use of directly observed therapy [2–4]. This association was confirmed in the Tuberculosis Trials Consortium (TBTC)/US Public Health Service Study 22, in which 4 (13.3%) of 30 HIV-seropositive patients treated with once-weekly isoniazid and rifapentine developed failure of tuberculosis treatment or relapse of tuberculosis in association with acquired rifamycin-resistant mycobacteria (hereafter, "ARR failure or relapse"), whereas no cases of acquired rifamycin resistance were identified among 502 HIV-seronegative patients who were treated with the same regimen [5, 6]. Among patients with HIV-related tuberculosis, consistent risk factors for acquired rifamycin resistance were reported to be low $CD4^+$ T cell counts (or $CD4^+$ cell counts) and highly-intermittent (i.e., once- or

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[a] Members of the consortium are listed at the end of the text.

Reprints or correspondence: Dr. Marc Weiner, Div. of Infectious Diseases (111F), VAMC, 7400 Merton Minter Blvd., San Antonio, TX 78229-4404 (weiner@uthscsa.edu).

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twice-weekly) administration of tuberculosis therapy [4–8]. Apart from these associations, the pathogenesis of acquired rifamycin resistance remains speculative.

TBTC Study 23 involved HIV-infected patients with tuberculosis who were treated under direct supervision with a largely intermittent (i.e., twice-weekly) regimen containing rifabutin [7]. Although the overall rate of failure or relapse in the study (9 [5.3%] of 169 cases) was similar to that in previous studies, enrollment in the trial was stopped because 8 of the 9 patients with failure or relapse had acquired rifamycin resistance. To better understand the pathogenesis of acquired rifamycin resistance, we evaluated the pharmacokinetic parameters of isoniazid and rifabutin in patients enrolled in the TBTC Study 23.

METHODS

Experimental design. Patients were enrolled nonrandomly into the pharmacokinetic substudy. Of the 169 patients in treatment Study 23, 19 patients at 12 TBTC clinical sites were not enrolled because they had nearly completed or completed the course of study treatment before the start the pharmacokinetic substudy, 4 patients at 3 clinical sites were not enrolled because of participation in alternative TBTC pharmacokinetic studies, and 29 (88%) of 33 patients from 6 clinical sites were not enrolled because the TBTC clinical sites were unable to participate for logistical reasons. Of the remaining 113 patients at 14 TBTC clinical sites, 98 (87%) took part in the pharmacokinetic study. Patients were enrolled into the substudy in 2 phases (figure 1). In the prospective phase, 98 (58%) of the 169 HIV-infected patients with tuberculosis were enrolled during the 2-drug phase of treatment (generally, during months 3–6 after therapy initiation). The rifabutin dose was 300 mg, with adjustment for antiretroviral therapy when used with protease inhibitors or efavirenz [9]; the isoniazid dose was 15 mg/kg, with a maximum dose of 900 mg. Of these 98 patients, 1 experienced failure (i.e., had positive results of a sputum culture during the interval between month 4 and the end of treatment), and 2 experienced relapse after the course of study treatment was completed. Retrospective samples were obtained for pharmacokinetic analysis from 4 other patients (3 with relapse and 1 with failure) after they achieved a treatment end point: samples were obtained after completion of study therapy (for the 3 patients with relapse) or after failure. The pharmacokinetic substudy was approved and monitored by the institutional review boards at the Centers for Disease Control and Prevention (Atlanta, GA) and each study site. All patients gave written informed consent.

Sample collection and drug and genotype analyses. Blood samples were collected for analysis just before receipt of an observed dose and 1, 2, 3, 6, and 24 h afterward. Patients took medications under the same conditions (i.e., either a fasting or fed state) that they received TBTC Study 23 therapy. Standard techniques were used for validated high-performance liquid chromatography of plasma drug concentrations of isoniazid [10], rifabutin, and 25-desacetyl rifabutin and for N-acetyltransferase genotyping [11].

Statistical and pharmacokinetic analyses. Data for patients who underwent prospective sampling during study therapy and those who underwent retrospective sampling after completion of treatment were combined for the primary analyses, to compare all possible patients who achieved failure or relapse with all other patients enrolled in the study. However, 1 patient (patient G; table 1), who was sampled retrospectively after failure, was excluded from the isoniazid analysis, because, as part of a daily retreatment regimen, isoniazid 300 mg was administered for pharmacokinetic analysis. Another subject (patient E), who was studied retrospectively after relapse, was excluded from the rifabutin analysis because a blood sample was not obtained 24 h after drug administration. Finally, the pharmacokinetics of rifabutin (one 300-mg dose) in 2 subjects (patients D and F), both of whom were studied retrospectively after relapse, were adjusted for autoinduction by decreasing the estimated rifabutin area under the concentration-time curve (AUC_{0-24}) by 37.5% and by decreasing the maximal concentration by 11.6% [12]. In additional analyses, which did not adjust for rifabutin autoinduction, the association between outcome and the rifabutin AUC_{0-24} was examined only in patients who underwent prospective sampling while receiving study therapy. Because the metabolism of isoniazid does not undergo induction, isoniazid parameters were not adjusted [10, 13].

Analyses of pharmacokinetic parameters were performed using noncompartmental techniques (WinNonlin, version 4; Pharsight). Rifabutin is known to have an extended terminal elimination phase. Because of the sparse sampling scheme, it was not possible to accurately calculate the half-life of rifabutin. Therefore, rifabutin half-life was not included in this analysis.

Data analyses were performed using SAS software (SAS) and StatXact (Cytel). For dichotomous data, differences between groups were determined using Fisher's exact test or the χ^2 statistic. For nonnormally distributed data, the Mann-Whitney *U* test (table 2) or the Mann-Whitney exact test (tables 3 and 4) were used. Data were natural log–transformed if a normal distribution was rejected by the Shapiro-Wilk test or if variances of different groups were unequal and natural log transformation improved the validity of the analyses (tables 5 and 6 and the multivariate regressions). Transformed results were back-transformed to the original scale to obtain mean values and 95% CIs. We performed univariate analysis to compare epidemiologic, clinical, and treatment factors for patients with acquired rifamycin resistance with those for other patients in the study

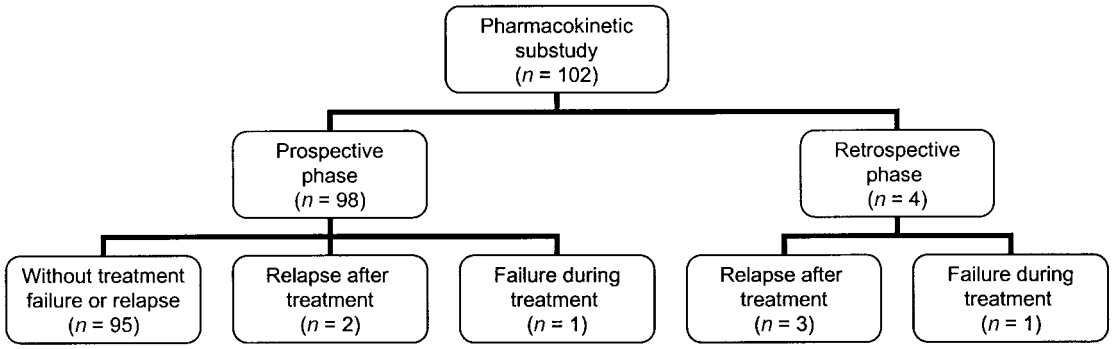

Figure 1. Flow of patients in a study of the pharmacokinetic properties of rifabutin and isoniazid in persons with HIV infection and tuberculosis

(table 5). In multivariate regression analysis, the natural log–transformed rifabutin AUC_{0-24} adjusted for autoinduction was examined as the dependent (continuous) variable, with the independent variables of ARR failure or relapse (present or absent; categorical) and $CD4^+$ cell count (in cells per milliliter; continuous) at the time of tuberculosis diagnosis. Also, multivariate logistic regression models were examined, with ARR failure or relapse (present or absent) as a dependent categorical variable and the independent variables (entered in a step-wise procedure) of rifabutin AUC_{0-24} adjusted for autoinduction (low [<4.5 μg*h/mL] or high [≥ 4.5 μg*h/mL]; categorical), the number of treatment doses (2, 3, or 5) per week during the induction phase (continuous), and $CD4^+$ cell count at the time of tuberculosis diagnosis (continuous) (table 4). For $CD4^+$ cell count, the OR denotes the odds for a unit decrease of 1 cell/mL.

Finally, we analyzed factors associated with the rifabutin AUC_{0-24}. In analysis of covariance (ANCOVA), the natural log–transformed rifabutin AUC_{0-24} adjusted for drug dose was examined as the dependent (continuous) variable, with the independent (categorical) variables of demographic, clinical, and laboratory factors (table 6). The group means of the natural log of the rifabutin AUC_{0-24} and the 95% CIs of these means were then transformed back into their original units.

Table 1. Characteristics of patients who participated in the pharmacokinetic (PK) substudy associated with Tuberculosis Trials Consortium Study 23 and in whom tuberculosis treatment failure or relapse occurred in association with acquired rifamycin-resistant mycobacteria.

Patient	Time of PK sampling	Outcome	Dosing schedule	AUC (dose in mg)		Comments
				Rifabutin[a]	Isoniazid	
A	Prospective	Failure	Twice weekly	1.38 (300)	21.71 (900)	Following the initiation of efavirenz-based antiretroviral therapy after PK sampling, the estimated rifabutin AUC would have been lower, because the rifabutin dose was not increased.
B	Prospective	Relapse	Twice weekly	3.81 (300)	23.58 (900)	…
C	Prospective	Relapse	Twice weekly	1.95 (300)	6.46 (900)	…
D	Retrospective	Relapse	Single dose	4.28 (300)	19.47 (900)	…
E	Retrospective	Relapse	Single dose	ND (300)	61.39 (900)	Plasma rifabutin concentrations were very low, but the patient was not included in the main rifabutin analysis because a specimen was not obtained 24 h after sampling and very late rifabutin absorption could not be ruled out.
F	Retrospective	Relapse	Single dose	2.75 (300)	11.43 (900)	…
G	Retrospective	Failure	Several doses[b]	9.67 (300)	ND (300)	PK was performed during receipt of concurrent nelfinavir and nevirapine antiretroviral therapy. The rifabutin AUC before protease inhibitor treatment was likely lower than that at the time of PK sampling. Also, isoniazid concentrations were almost undetectable, but isoniazid AUC was not included in the isoniazid analysis because PK was performed after administration of a nonstandard isoniazid study dose.

NOTE. AUC, area under the concentration-time curve; ND, not included in the analysis; PK, pharmacokinetic.

[a] Rifabutin AUC_{0-24} with 2 cases with single dose studies adjusted for autoinduction.

[b] One dose was administered on the day before PK sampling, another dose was administered 2 days before PK sampling, and a third dose was administered 5 days before PK sampling.

Table 2. Demographic and clinical characteristics of patients enrolled in Tuberculosis (TB) Trials Consortium (TBTC) Study 23 who did or did not participate in the pharmacokinetic (PK) substudy.

Characteristic	Patients in PK study (n = 102)	Patients not in PK study (n = 67)	P[a]
Age, years	41 (35–46)	39 (33–45)	.25
Male sex	79 (78)	53 (79)	1.00
Race/ethnicity			
Black	57 (56)	29 (43)	.12
White, Hispanic	35 (35)	19 (28)	.41
White, non-Hispanic	7 (7)	12 (18)	.04
Born in Mexico	22 (22)	10 (15)	.32
Homeless within past year	30 (30)	15 (22)	.29
Injection drug use within the past year	10 (10)	7 (10)	.46
Alcohol abuse within the past year	38 (38)	19 (28)	.23
Body mass index at the time of TB diagnosis	21.5 (18.5–24.1)	20.9 (18.6–23.1)	.55
Site of involvement			
Pulmonary	56 (55)	37 (55)	.97
Extrapulmonary and pulmonary	37 (36)	17 (25)	.14
Presence of cavitation on chest radiograph	18 (18)	3 (5)	.01
Bilateral lung involvement on chest radiograph	20 (20)	3 (5)	.02
$CD4^+$ cell count at the time of TB diagnosis	101 (51–245)	55 (29–135)	.01
Log_{10} HIV RNA load at the time of TB diagnosis	5.3 (4.7–5.7)	5.3 (4.9–5.3)	.89
Use of twice weekly treatment during first 2 months of TB therapy	48 (48)	27 (42)	.52

NOTE. Data are no. (%) of patients or median value (interquartile range).

[a] By the Mann-Whitney U test (for continuous variables) and the Fisher's exact test or χ^2 statistic (for dichotomous variables).

RESULTS

Patient characteristics. The demographic and clinical characteristics of the 102 patients in the pharmacokinetic substudy were similar in most respects to the characteristics of the other HIV-seropositive patients in Study 23 who did not participate (table 2). However the $CD4^+$ cell counts were higher and cavitation was more common among patients who underwent pharmacokinetic sampling, suggesting that this population had somewhat less advanced HIV disease. Fewer non-Hispanic white persons participated in the present study.

Association between rifabutin $AUC_{0–24}$ and treatment outcome. The median rifabutin $AUC_{0–24}$ was lower for patients with ARR failure or relapse than for patients without ARR failure or relapse (3.3 vs. 5.2 $\mu g^*h/mL$; $P = .06$, by the Mann-Whitney exact test) (figure 2*A* and table 3). This association was also seen in an analysis restricted to the 96 patients who underwent prospective sampling during the treatment trial (2.0 vs. 5.2 $\mu g^*h/mL$; $P = .01$, by the Mann-Whitney exact test). ANCOVA adjusted for $CD4^+$ cell count revealed that the mean rifabutin $AUC_{0–24}$ was significantly less for patients with ARR failure or relapse than for other patients (3.0 $\mu g^*h/mL$ [95% CI, 1.9–4.5] vs. 5.2 $\mu g^*h/mL$ [95% CI, 4.6–5.8]; $P = .02$). Similarly, multivariate logistic regression analysis adjusting for $CD4^+$ cell count revealed that a low rifabutin $AUC_{0–24}$ (<4.5 $\mu g^*h/mL$) was associated with failure or relapse (table 4). There was no association between the $AUC_{0–24}$ of the desacetyl rifabutin metabolite and acquired rifamycin resistance (table 3).

In an analysis of patients who underwent prospective sampling, the median peak concentration of rifabutin was significantly lower for patients with acquired rifamycin resistance, compared with patients without failure or relapse (0.15 vs. 0.45 $\mu g/mL$; $P = .03$, by the Mann-Whitney exact test). However, a statistically significant difference between median concentrations was not detected in an analysis that included all patients (0.30 vs. 0.45 $\mu g/mL$; $P = .14$, by the Mann-Whitney exact test; table 3).

Isoniazid $AUC_{0–12}$ and treatment outcome. Patients with ARR failure or relapse had a lower median isoniazid $AUC_{0–12}$ than did patients without failure or relapse (20.6 vs. 28.0 $\mu g^*h/mL$; $P = .24$, by the Mann-Whitney exact test) (table 3 and figure 2*B*). The power of this comparison was limited by the small number of patients ($n = 6$) that could be included in the analysis. Furthermore, it is notable that patient G, who was not included in the analysis because of the 300-mg isoniazid dose he received, had isoniazid concentrations that were barely detectable. Univariate analysis of data for 74 patients revealed

Table 3. Pharmacokinetic parameters for patients in whom tuberculosis treatment failure or relapse occurred in association with acquired rifamycin-resistant mycobacteria (ARR) and patients without ARR-associated failure or relapse.

	Patients with ARR failure or relapse		Patients without ARR failure or relapse		
Pharmacokinetic parameter	No.	Median (interquartile range)	No.	Median (interquartile range)	P[a]
Isoniazid[b]					
AUC_{0-12}	6	20.6 (11.4–23.6)	89	28.0 (16.6–45.5)	.24
Maximal concentration	6	7.8 (2.5–8.6)	91	7.5 (5.0–10.1)	.59
Rifabutin[c]					
AUC_{0-24}	6	3.28 (1.95–4.28)	94	5.22 (3.97–7.39)	.06
Maximal concentration	6	0.30 (0.15–0.46)	95	0.45 (0.29–0.58)	.14
Rifabutin[d]					
AUC_{0-24}	6	4.11 (1.95–6.85)	94	5.22 (3.97–7.39)	.31
Maximal concentration	6	0.32 (0.15–0.53)	95	0.45 (0.29–0.58)	.21
Rifabutin[e]					
AUC_{0-24}	3	1.95 (1.52–3.35)	94	5.22 (3.97–7.39)	.01
Maximal concentration	3	0.15 (0.09–0.31)	95	0.45 (0.29–0.58)	.03
Desacetyl rifabutin[d]					
AUC_{0-24}	6	0.77 (0.47–1.13)	85	0.72 (0.55–1.60)	.69
Maximal concentration	6	0.05 (0.03–0.09)	94	0.06 (0.04–0.10)	.64
Desacetyl rifabutin[e]					
AUC_{0-24}	3	0.45 (0.17–0.52)	94	0.71 (0.47–1.26)	.08
Maximal concentration	3	0.03 (0.01–0.04)	94	0.06 (0.04–0.10)	.10

NOTE. AUC, area under the concentration-time curve.

[a] By the Mann-Whitney exact test.

[b] Data are for patients sampled prospectively ($n = 94$) or retrospectively ($n = 3$) for pharmacokinetics relative to Study 23 continuation-phase therapy.

[c] Data are for patients sampled prospectively or retrospectively for pharmacokinetics relative to Study 23 continuation-phase therapy. Rifabutin pharmacokinetic parameters were adjusted for autoinduction [12] in 2 patients with acquired rifamycin resistance sampled retrospectively after Study 23 continuation-phase therapy and sampled after administration of a single dose of rifabutin.

[d] Data are for patients who underwent prospective or retrospective sampling and are not adjusted for induction.

[e] Data are for patients who underwent prospective sampling.

that presence of the isoniazid acetylator type, determined by detection of the N-acetyl-transferase type 2 genotype, was not associated with ARR failure or relapse ($P = .14$, by the χ^2 statistic). However, a logistic regression model of 5 events that adjusted for rifabutin AUC_{0-24} revealed that a low isoniazid AUC_{0-12} (natural log transformed) was significantly associated with ARR failure or relapse (OR, 10.5; 95% CI, 1.1–100; $P = .04$). As seen in figure 3, all patients with acquired rifamycin resistance had low $AUCs_{0-24}$ for rifabutin, isoniazid, or both.

Factors associated with rifabutin AUC. ANCOVA revealed that, for demographic, clinical, and treatment factors adjusted for dose, the mean rifabutin AUC_{0-24} was significantly higher for patients who were younger (<40 years old) or receiving protease inhibitor–based antiretroviral therapy (table 6). Multivariate ANCOVA revealed that dose, age, and receipt of protease inhibitor–based antiretroviral therapy were significantly associated with the rifabutin AUC_{0-24}.

DISCUSSION

This study suggests that the pharmacokinetics of rifabutin and isoniazid are important in the pathogenesis of acquired rifamycin resistance in HIV-infected patients with tuberculosis. A clear association was found between decreased rifabutin AUC_{0-24} and acquired rifamycin resistance, despite the small number of patients with acquired rifamycin resistance available for analysis. This association was retained in an analysis limited to prospectively sampled patients and in multivariate analysis adjusting for $CD4^+$ cell count, a previously identified risk factor for acquired rifamycin resistance. It is notable that our analysis probably overestimated rifabutin AUC for patients with acquired rifamycin resistance (table 1).

Table 4. Results of multivariate logistic regression analysis, with adjustment for CD4⁺ cell count, of the association between low rifabutin $AUC_{0–24}$ and the outcome of tuberculosis treatment failure or relapse in association with acquired rifamycin-resistant mycobacteria.

Variable	OR (95% CI)	*P*, by the Wald test	*P*, by logistic likelihood ratio test
Low rifabutin $AUC_{0–24}$[a]	23 (2–279)	.01	.003
CD4⁺ cell count	1.04 (1.00–1.08)[b]	.07	.0001

NOTE. $R^2 = 0.46$ for the logistic regression model.

[a] <4.5 μg*h/mL.

[b] Denotes the OR for a decrease in CD4⁺ cell count of 1 cell/mL.

In this pharmacokinetic study, the median isoniazid $AUC_{0–12}$ was lower among patients with ARR failure or relapse than among other patients (20.6 vs. 28.0 μg*h/mL; $P = .24$), but the median difference was not statistically significant in univariate analysis. However, in a multivariate logistic regression model adjusting for rifabutin concentrations, lower isoniazid $AUC_{0–12}$ was significantly associated with ARR failure or relapse. Furthermore, results of additional comparisons suggested that low isoniazid AUC may be important in the pathogenesis of acquired rifamycin resistance. The median isoniazid $AUC_{0–12}$ for all patients enrolled prospectively in the present study was significantly lower than that for HIV-negative patients sampled prospectively in other TBTC studies (28 μg*h/mL [interquartile range, 17–45 μg*h/mL] vs. 49 μg*h/mL [32–67 μg*h/mL]; $P < .0001$, by the Mann-Whitney *U* test) [14]. The isoniazid pharmacokinetic data are also consistent with

Table 5. Univariate analysis of demographic, clinical, and treatment factors for patients with and patients without acquired rifamycin resistance.

Characteristic	Patients with acquired rifamycin resistance ($n = 7$)	Patients without acquired rifamycin resistance ($n = 95$)	*P*[a]
Demographic			
Age, years	39 (37–42)	41 (34–47)	.65
Male sex	6 (86)	74 (78)	1.00
Race/ethnicity vs. all others			
White, Hispanic	5 (71)	32 (34)	.10
White, non-Hispanic	0 (0)	7 (100)	1.00
Black	2 (29)	54 (57)	.24
Born in Mexico	4 (57)	19 (20)	.04
Body mass index at the time of TB diagnosis	19.9 (17.8–26.0)	21.5 (18.6–24.1)	.77
Clinical			
Site of TB involvement			
Pulmonary only	2 (29)	54 (57)	.24
Both pulmonary and extrapulmonary	3 (43)	34 (36)	.70
Presence of cavitation on chest radiograph	1 (14)	19 (20)	1.00
Bilateral lung involvement on chest radiograph	1 (14)	17 (18)	1.00
CD4⁺ cell count at the time of TB diagnosis	28 (21–43)	112 (55–259)	.001
Log_{10} HIV RNA load at the time of TB diagnosis	5.4 (5.2–5.6)	5.3 (4.7–5.8)	.69
TB treatment			
Twice-weekly therapy during induction phase	5 (71)	44 (46)	.26
Pharmacokinetics, μg*h/mL			
Isoniazid $AUC_{0–12}$	20.6 (11.4–23.6)	28.0 (16.6–45.5)	.24
Rifabutin $AUC_{0–24}$	3.28 (1.95–4.28)	5.22 (3.97–7.39)	.06

NOTE. Data are no. (%) of patients or median value (interquartile range). TB, tuberculosis

[a] By the Mann-Whitney exact test (for continuous covariates) and by χ^2 analysis or Fisher's exact test (for dichotomous factors).

Table 6. Effects of demographic, clinical, and treatment factors on the rifabutin area under the concentration-time curve (AUC) for 100 patients from Tuberculosis Trials Consortium (TBTC) Study 23 who were enrolled in a substudy of pharmacokinetics.

Characteristic	Rifabutin AUC_{0-24}		Rifabutin AUC_{0-24} adjusted for rifabutin dose	
	Mean (95% CI)	*P*	Mean (95% CI)	*P*[a]
Rifabutin dose ⩽300 mg				
Yes (*n* = 80)	4.70 (4.21–5.25)	.008	…	
No (*n* = 20)	6.69 (5.05–8.88)		…	
Median age <40 years				
Yes (*n* = 50)	5.68 (4.99–6.47)	.03	5.74 (4.98–6.61)	.01
No (*n* = 50)	4.48 (3.79–5.30)		4.44 (3.85–5.11)	
Male sex				
Yes (*n* = 79)	5.00 (4.45–5.62)	.77	4.98 (4.43–5.59)	.62
No (*n* = 21)	5.21 (3.96–6.84)		5.31 (4.24–6.65)	
White, non-Hispanic				
Yes (*n* = 7)	5.56 (3.81–8.11)	.63	5.62 (3.80–8.30)	.58
No (*n* = 93)	5.01 (4.48–5.61)		5.00 (4.50–5.57)	
White, Hispanic				
Yes (*n* = 35)	5.56 (4.73–6.54)	.19	5.60 (4.76–6.59)	.15
No (*n* = 65)	4.79 (4.16–5.51)		4.77 (4.20–5.42)	
Black				
Yes (*n* = 56)	4.70 (4.01–5.50)	.13	4.66 (4.06–5.34)	.09
No (*n* = 44)	5.53 (4.83–6.33)		5.59 (4.79–6.51)	
Median body weight <65 kg				
Yes (*n* = 50)	5.20 (4.47–6.06)	.47	5.19 (4.49–6.00)	.49
No (*n* = 50)	4.81 (4.12–5.61)		4.82 (4.17–5.58)	
Median body mass index <22				
Yes (*n* = 50)	5.32 (4.60–6.14)	.24	5.39 (4.65–6.24)	.15
No (*n* = 50)	4.68 (3.98–5.50)		4.63 (4.01–5.34)	
Presence of other AIDS-defining condition(s)				
Yes (*n* = 9)	5.87 (4.15–8.32)	.38	5.70 (4.04–8.04)	.47
No (*n* = 91)	4.97 (4.44–5.57)		4.99 (4.47–5.55)	
Median HIV RNA viral load <32,000 copies[b]				
Yes (*n* = 46)	5.33 (4.58–6.20)	.34	5.18 (4.50–5.97)	.65
No (*n* = 46)	4.81 (4.14–5.59)		4.94 (4.29–5.70)	
$CD4^+$ cell count ⩽75 cells/mm^3[c]				
Yes (*n* = 32)	5.18 (4.20–6.39)	.63	5.22 (4.35–6.28)	.54
No (*n* = 61)	4.88 (4.27–5.59)		4.86 (4.26–5.55)	
Use of protease inhibitor–based antiretroviral therapy				
Yes (*n* = 22)	6.52 (5.37–7.92)	.004	6.79 (5.47–8.42)	.0009
No or NRTI only (*n* = 66)	4.42 (3.86–5.07)		4.36 (3.85–4.94)	
Use of efavirenz-based antiretroviral therapy				
Yes (*n* = 7)	6.81 (4.82–9.62)	.05	4.96 (3.36–7.31)	.76
No or NRTI only (*n* = 66)	4.42 (3.86–5.07)		4.58 (3.99–5.26)	

NOTE. Characteristics were recorded at the time samples were obtained for pharmacokinetic analysis, unless otherwise indicated. NRTI, nucleoside reverse-transcriptase inhibitor.

[a] By analysis of covariance.

[b] Measured at the time of (or if unavailable, before) enrollment into TBTC Study 23 at a median of 66 days (interquartile range [IQR], 31–100 days) before samples were obtained for pharmacokinetic analysis.

[c] Measured at enrollment into TBTC Study 23 at a median of 49 days (IQR, 27–89 days) before samples were obtained for pharmacokinetic analysis.

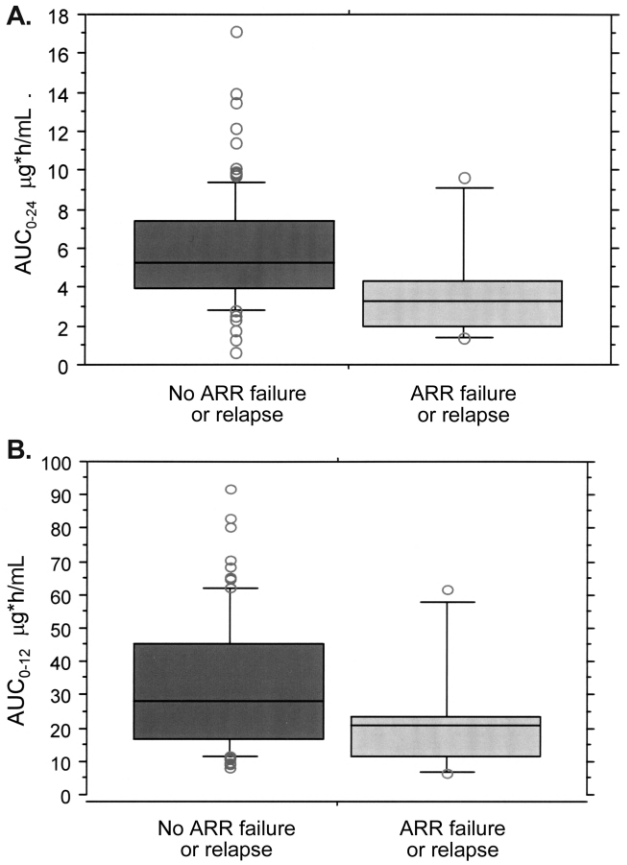

Figure 2. *A,* Rifabutin area under the concentration-time curve ($AUC_{0–24}$) for patients in whom tuberculosis treatment failure or relapse occurred in association with acquired rifamycin-resistant mycobacteria (hereafter, "ARR failure or relapse") ($n = 6$), compared with other patients ($n = 94$). *B,* Isoniazid $AUC_{0–12}$ for patients with ARR failure or relapse ($n = 6$), compared with other patients ($n = 89$). The rifabutin $AUC_{0–24}$ was adjusted for 2 patients who underwent pharmacokinetic sampling after receipt of a single dose of rifabutin [12]. *Boxes (lines),* 25th and 75th percentiles (median values); *brackets,* 10th and 90th percentiles; *circles,* outliers.

findings in a murine model of tuberculosis, in which bactericidal activity was highly correlated with the ratio of the isoniazid AUC to the isoniazid MIC [15].

The association between acquired rifamycin resistance and low isoniazid concentrations was expected. Resistance is thought to emerge during periods of functional monotherapy, when strains resistant to the drug that is still present (rifabutin, in this case) would be selected. That this occurs almost exclusively among patients with severe immunodeficiency ($CD4^+$ cell count <75 cells/mm^3 in almost all cases) suggests that ineffective cellular immune function and marginal drug concentrations permit greater mycobacterial growth between doses of tuberculosis chemotherapy, as well as increased opportunity for development of rifamycin tolerance or selection of genomic resistance.

However, the important finding in this study was the association between low rifabutin concentrations and acquired rifamycin resistance. Mitchison [16] has suggested that rifamycins have unique sterilizing activity against slowly or intermittently metabolizing bacilli that remain after the initial phase of multidrug therapy and that low concentrations of rifamycins lead to inadequate mycobacterial suppression. Lower concentrations of rifabutin and isoniazid that were identified in our study are consistent with and extend the hypothesis. Notably, the rifabutin pharmacokinetic-pharmacodynamic parameters associated with treatment outcome in the present study (table 3) are similar to rifampin pharmacokinetic-pharmacodynamic parameters reported in a murine model of tuberculosis, in which the rifampin AUC/MIC was better correlated with bactericidal activity than was the rifampin peak concentration [17].

Another factor postulated to be important in the pathogenesis of acquired rifamycin resistance is the disparity in the half-lives of isoniazid and the long-acting rifamycins, rifabutin and rifapentine [5, 18]. The pharmacokinetic mismatch between rifapentine and isoniazid was thought to be one of the reasons for the high rate of acquired rifamycin resistance among patients with advanced HIV infection who were treated with once-weekly rifapentine plus isoniazid [5]. There is a similar pharmacokinetic difference between rifabutin and isoniazid (with plasma half-lives of 35 h and 2–5 h, respectively), so this mismatch might be thought to be important in the occurrence of acquired rifamycin resistance in patients receiving this twice-weekly regimen. However, 2 observational cohort studies have shown similar rates of acquired rifamycin resistance among

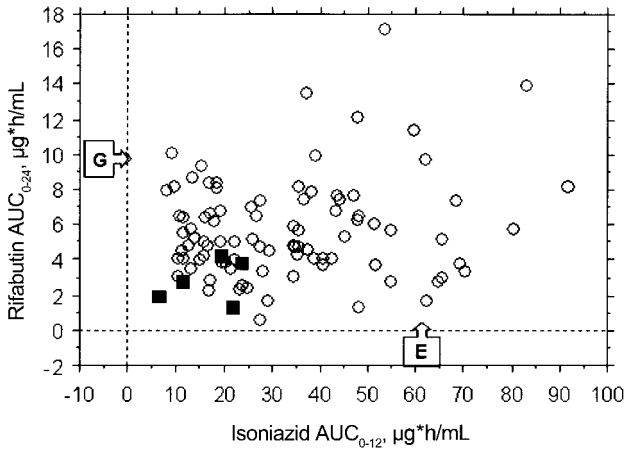

Figure 3. A comparison of rifabutin versus isoniazid area under the concentration-time curves (AUCs) for patients in whom tuberculosis treatment failure or relapse occurred in association with acquired rifamycin-resistant mycobacteria (hereafter, "ARR failure or relapse") ($n = 5$; *squares*) and patients without ARR failure or relapse ($n = 90$; *circles*). Patients E and G (*arrows;* table 1), who had ARR failure or relapse, had low rifabutin and isoniazid AUCs, respectively.

HIV-infected patients who received intermittent rifampin and isoniazid therapy, compared with patients who received rifabutin and isoniazid therapy [8, 19]. Because the half-lives of rifampin and isoniazid are similar, these studies suggest that mismatched pharmacokinetic properties are not a requirement for the development of acquired rifamycin resistance. However, malabsorption of isoniazid in the presence of characteristic rifampin pharmacokinetics can result in ARR-associated relapse [13].

In the present study, multivariate ANCOVA revealed that rifabutin AUC_{0-24} was significantly associated with age, use of protease inhibitor–based antiretroviral therapy, and rifabutin dose. The differences in the mean rifabutin AUC_{0-24} we observed for patients who did and patients who did not receive antiretroviral therapy (table 6) were similar to differences in the mean rifabutin AUC_{0-24} observed in other studies with longitudinal, cross-over designs and repeated pharmacokinetic sampling before and after the start of antiretroviral therapy. For example, the mean rifabutin AUC_{0-24} observed during baseline pharmacokinetic sampling of 7 patients with HIV-related tuberculosis who were receiving rifabutin (300 mg twice weekly) and no antiretroviral therapy increased by 33% after the patients initiated rifabutin therapy (300 mg twice weekly) and daily nelfinavir-based antiretroviral therapy (4.5 vs. 6.0 μg*h/mL; $P = .15$, by the paired t test) [20]. In addition, similar to the differences in the mean rifabutin AUC_{0-24} observed between groups in our study (table 6), the mean rifabutin AUC_{0-24} for 15 patients who initiated rifabutin therapy (600 mg twice weekly) and daily efavirenz-based antiretroviral therapy was higher than the baseline rifabutin AUC_{0-24} measured after receipt of rifabutin (300 mg twice weekly) and no antiretroviral therapy [21].

This pharmacokinetic substudy has several important limitations. Pharmacokinetic data were obtained for only 7 patients with ARR failure or relapse, resulting in decreased power and reliability of study observations. However, this pharmacokinetic substudy involving 102 patients, including 7 of 8 patients with acquired rifamycin resistance enrolled in the parent trial, is the largest study to date of drug pharmacokinetics in HIV-infected patients with tuberculosis and the first study in which the association between drug pharmacokinetics and acquired rifamycin resistance has been analyzed. Although patients were enrolled nonrandomly into the substudy from the treatment trial, most patients for whom pharmacokinetic data could be potentially studied participated in the substudy. In addition, demographic and clinical characteristics of the 102 patients in the pharmacokinetic study were similar to characteristics of the other patients in Study 23. Four patients underwent pharmacokinetic analysis after completing their initial course of treatment, and it is possible that the pharmacokinetic parameters for these patients would have been different if sampling had been performed prospectively during receipt of study treatment. However, after adjustment for the auto-inductive effects of rifabutin, pharmacokinetic parameters for patients who underwent sampling retrospectively were similar to those of patients who underwent sampling prospectively in this study. Furthermore, patients who were only prospectively evaluated during receipt of study treatment also demonstrated an association between decreased rifabutin AUC_{0-24} and acquired rifamycin resistance. Thus, it is unlikely that our use of retrospective sampling resulted in an analytic bias. Our sampling scheme (6 time points per patient) was not sufficient to determine some pharmacokinetic parameters (such as half-life) with accuracy. Also, pharmacokinetic studies were not performed during the initial, multidrug phase of treatment. However, the robustness of our findings suggests that this sampling scheme and study design were sufficient to detect relevant differences in pharmacokinetic parameters. In addition, $CD4^+$ cell counts and HIV loads were obtained before pharmacokinetic sampling was performed, so that correlations could not be evaluated between HIV surrogate markers at the time of sampling and drug pharmacokinetic properties. Residual confounding by measured or unmeasured variables is possible, but the association between acquired rifamycin resistance and low rifabutin AUC_{0-24} remained strong in analyses adjusting for risk factors for failure or relapse identified in the parent study.

In summary, our study offers strong evidence that an important factor in the pathogenesis of acquired rifamycin resistance is the abnormal pharmacokinetics of the drugs used to treat tuberculosis, and the results illustrate the usefulness of incorporating pharmacokinetic measurements into studies evaluating new tuberculosis treatment regimens. The relationship between drug pharmacokinetics and acquired rifamycin resistance was strongest for low rifabutin AUC_{0-24}, but data also suggested a relationship with low isoniazid AUC_{0-12}. Additional clinical trials are required before these results can be translated into clinical practice. One possible trial might involve therapeutic drug monitoring of rifabutin and isoniazid in patients with tuberculosis and advanced HIV disease [22]. However, the expense of obtaining drug concentration data at multiple time points is considerable, and our results do not lend themselves to simple criteria for interpretation of and action based on the results of the such data. Intensification of treatment by means of increased dosage frequencies [23] and dose sizes may ameliorate the risk of abnormally low plasma concentrations of antituberculosis drugs, but it requires additional study to determine optimally effective regimens. Current recommendations propose use of daily therapy, at least for the first 2 months of treatment, for patients with tuberculosis and advanced HIV disease [9].

MEMBERS OF THE CONSORTIUM

The participating clinical sites (principal investigators and study coordinators; numbers of patients enrolled) were as follows: Los Angeles County/University of Southern California Medical Center (Brenda Jones, Claudia Silva, and Maria Brown; 15), University of Texas Health Science Center in San Antonio/South Texas Veterans Health Care System (Marc Weiner, Melissa Engle, and Victoria Rodriguez; 12), Johns Hopkins University School of Medicine (Richard Chaisson, Tim Sterling, Kristina Moore, and Judith Hackman; 11), Nashville VA Medical Center (Douglas Kernodle, Anthony Chapdelaine, Guat-Siew McKee, and Linda Reeves-Hammock; 11), University of North Texas Health Science Center (Stephen Weis, Barbara King, and Norma Shaffer; 9), Houston VA Medical Center (Christopher Lahart, Ruby Nickson, Terry Scott, and Robert Awe; 9), New Jersey Medical School National TB Center–University of Medicine & Dentistry of New Jersey (Bonita T. Mangura, Marilyn Owens, and Cora Leus; 8), Bellevue Hospital Center, New York University (Neil Schluger, William Rom, Rany Condos, and Laurie Sandman; 6), Harlem Hospital Center (Wafaa El-Sadr, Frantz Medard, and Mary Klein; 4), Carolinas Medical Center (James Horton, Ann Boye, and Beth Quinn; 4), Denver Health and Hospitals (Randall Reves, William Burman, and Jan Tapy; 3), San Francisco VA Medical Center (Charles Daley and Llewellyn Stanton; 3), University of Manitoba (Earl Hershfield and Gerry Izon; 2), Duke University Medical Center and Durham VA Medical Center (Carol Dukes Hamilton and Ann Mosher; 2), Veterans Affairs Medical Center–Washington, D.C. (Fred Gordin, Debra Benator, Donna Sepulveda Conwell, Thomas Walsh, Margaret Lankford, and Charlotte Quinlan-Mauzy; 1), University of British Columbia Health Center (Mark Fitzgerald, Eduardo Hernandez, and Banafsheh Peyvandi; 1), and Hines VA Medical Center and Suburban Cook County TB District (Constance Pachuki, Mary Samuel, and James Gallai; 1).

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Potential conflicts of interest. All authors: no conflicts.

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