

# Low Isoniazid Concentrations and Outcome of Tuberculosis Treatment with Once-Weekly Isoniazid and Rifapentine

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To understand why once-weekly isoniazid/rifapentine therapy for tuberculosis was less effective than twice-weekly isoniazid/rifampin, we studied human immunodeficiency virus-seronegative patients with either failure ( $n = 4$ ), relapse ( $n = 35$ ), or cure ( $n = 94$ ), recruited from a comparative treatment trial. In multivariate analyses that were adjusted for severity of disease, low plasma concentrations of isoniazid were associated with failure/relapse with once-weekly isoniazid/rifapentine (median isoniazid area under the concentration-time curve for 12 hours after the dose [AUC<sub>0-12</sub>] was  $36 \mu\text{g} \cdot \text{hour/ml}$  in failure/relapse versus  $56 \mu\text{g} \cdot \text{hour/ml}$  in control cases  $p = 0.005$ ), but not with twice-weekly isoniazid/rifampin. Furthermore, two patients who relapsed with *Mycobacterium tuberculosis* mono-resistant to rifampin had very low concentrations of isoniazid. Finally, isoniazid acetylase status determined by *N*-acetyltransferase type 2 genotype was associated with outcome with once-weekly isoniazid/rifapentine ( $p = 0.03$ ) but not twice-weekly isoniazid/rifampin. No rifampin pharmacokinetic parameter was consistently and significantly associated with outcome ( $p > 0.10$ ). Because low isoniazid concentrations were associated with failure/relapse, a drug with consistently greater area under the concentration-time curve than isoniazid may be needed to achieve highly active once-weekly therapy with rifapentine.

**Keywords:** tuberculosis; isoniazid; rifapentine; treatment; pharmacokinetics

Directly observed therapy is one of the key elements of efforts to improve tuberculosis control around the world. Intermittent dosing facilitates directly observed therapy by decreasing the number of required encounters between patient and the treatment provider. The development of rifapentine, a rifampin antibiotic with a much longer half-life

than rifampin (14–15 hours versus 2–5 hours, respectively) was undertaken with the hope that it would allow highly active once-weekly therapy. However, in three large randomized trials (1–3), once-weekly isoniazid/rifapentine was less effective than twice- or thrice-weekly isoniazid/rifampin in the last 4 months of treatment of active tuberculosis.

Two problems were identified in the randomized trials of once-weekly isoniazid/rifapentine: a higher rate of drug-susceptible relapse among human immunodeficiency virus (HIV)-negative patients and a substantial incidence of acquired rifampin-mono-resistance among the small number of HIV-positive patients treated with this regimen (4). Two theories have been proposed to explain these findings. Mitichison suggested that the dose of rifapentine used in all three trials (600–750 mg) may have been inadequate, analogous to the decreased activity of the 450-mg dose of rifampin, compared with the 600-mg dose, in early clinical trials of that rifampin (5). However, the occurrence of acquired rifampin-mono-resistance suggests that the activity of the companion drug, in this case isoniazid, was inadequate to prevent the selection of rifampin-resistant *Mycobacterium tuberculosis*. These two theories lead to substantially different interventions to improve the activity of once-weekly therapy: increasing the dose of rifapentine by the former suggestion versus improving the activity of the companion drug in the latter. To evaluate the reasons for decreased activity of once-weekly isoniazid/rifapentine, we evaluated pharmacokinetic parameters of isoniazid, rifapentine, and rifampin among patients enrolled in the Tuberculosis Trials Consortium (TBTC)/United States Public Health Service randomized trial involving this regimen (3). Some of the results of the pharmacokinetic substudy have been previously reported in the form of abstracts (6, 7).

## METHODS

### Experimental Design

TBTC/United States Public Health Service Study 22 was a randomized trial of once-weekly isoniazid/rifapentine versus twice-weekly isoniazid/rifampin in the continuation phase of treatment in 1,004 HIV-seronegative (3) and 71 HIV-seropositive (4) patients with drug-susceptible pulmonary tuberculosis. The crude rates of failure/relapse were 46/502 (9.2%) in the once-weekly arm and 28/502 (5.6%) in the twice-weekly arm (relative risk 1.64, 95% confidence interval 1.04–2.58;  $p = 0.04$ ). Patients in Study 22 were enrolled into the pharmacokinetic substudy in two phases. In the retrospective phase, 83 HIV-seronegative patients were enrolled after treatment. Four had failed (had a positive culture between 4 months and the end of therapy) and 33 had relapsed after treatment. Forty-six HIV-seronegative control cases (no evidence of relapse for 2 years after completing therapy) were matched by study site and sex. Retrospective cases underwent sampling after receiving one

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**TABLE 1. COMPARISON OF THE DEMOGRAPHIC, CLINICAL, AND RADIOGRAPHIC CHARACTERISTICS OF HUMAN IMMUNODEFICIENCY VIRUS–SERONEGATIVE CONTROL CASES AND TREATMENT FAILURE/RELAPSE CASES IN THE PHARMACOKINETIC SUBSTUDY WITH THE OTHER HUMAN IMMUNODEFICIENCY VIRUS–SERONEGATIVE CASES IN THE PARENT STUDY (TUBERCULOSIS TRIALS CONSORTIUM STUDY 22)**

Characteristic	All with Treatment Failure or Relapse		p Value	All Patients with Cure		p Value
	Patients in the PK Substudy, n = 40 n (IQR, %)	Patients Not in the PK Substudy, n = 34 n (IQR, %)		Patients in the PK Substudy, n = 93 n (IQR, %)	Patients Not in the PK Substudy, n = 837 n (IQR, %)	
Age, median yr	39.6 (31.0–50.4)	41.6 (32.5–51.1)	0.46	41.1 (34.6–51.6)	43.8 (33.2–55.0)	0.17
Sex, percent male	35/40 (88)	27/34 (79)	0.37	72/93 (77)	618/837 (74)	0.45
Race/ethnicity						
White	17/40 (43)	7/34 (21)	0.05	19/93 (20)	134/837 (16)	0.28
African American	12/40 (30)	17/34 (50)	0.08	40/93 (43)	330/837 (39)	0.50
Hispanic	6/40 (15)	7/34 (21)	0.53	21/93 (23)	218/837 (26)	0.47
Asian	3/40 (8)	3/34 (9)	0.84	8/93 (9)	125/837 (15)	0.10
Alcohol (> 1 drink/d)	20/40 (50)	20/34 (59)	0.45	44/93 (47)	362/836 (43)	0.46
Injection drug use	13/40 (33)	11/34 (32)	0.99	17/93 (18)	168/836 (20)	0.68
Underweight at enrollment	24/40 (60)	20/34 (59)	0.92	26/93 (28)	236/836 (28)	0.96
Chest radiographic features						
Cavitation	34/40 (85)	28/34 (82)	0.76	47/91 (52)	415/810 (51)	0.94
Bilateral involvement	30/40 (75)	26/34 (77)	0.88	51/92 (55)	452/830 (55)	0.86
Sputum culture-positive after 2 months of treatment	22/37 (59)	15/33 (46)	0.24	12/87 (14)	131/729 (18)	0.33

*Definition of abbreviations:* IQR = interquartile range; PK = pharmacokinetic.

Underweight = weight 10% or more below ideal body weight (3).

Mann–Whitney or  $\chi^2$  statistic for comparisons of continuous or nominal values, respectively.

dose of the regimen received during the study. In the prospective phase, 50 HIV-seronegative patients were enrolled while still on study treatment (outcomes later determined for these patients). In addition, 33 HIV-seropositive patients were enrolled. The pharmacokinetic substudy was approved by the Institutional Review Boards of the CDC and of 26 participating TBTC sites.

### Sample Collection, Drug, and Genotype Analyses

Blood samples for analyses were collected just before an observed dose and then 1, 2, 5, and 24 hours afterward (and 48 hours for rifampentine). Patients received medications under the same conditions (fasting or fed) that they received their TBTC Study 22 therapy. Standard techniques were used for sample preparation (8), HPLC analyses of plasma drug concentrations (MDS Pharma Services, Montreal, QC), and *N*-acetyltransferase genotyping (9).

### Statistical and Pharmacokinetic Analyses

All primary analyses were performed in 133 HIV-seronegative patients. The primary null hypothesis was that there were no differences in isoniazid or rifampicin pharmacokinetic parameters in HIV-seronegative patients with the endpoints of cure versus failure/relapse.

After establishing the similarity of pharmacokinetic parameters among retrospectively and prospectively sampled patients, we combined these two groups, and for the remainder of the analyses compared all patients with failure/relapse with those who had been cured. We adjusted for rifampin autoinduction. In patients who had not received a dose of rifampin within 14 days before sampling, the estimated rifampin area under the concentration–time curve ( $AUC_{0-12}$ ) was decreased by 24%, the serum half-life by 44%, and the maximal concentration ( $C_{max}$ ) by 4% (10). Isoniazid and rifampentine (11–13) parameters were not adjusted. Analyses of pharmacokinetic parameters were performed using non-compartmental techniques (14, 15). Because a sparse sampling method was used, pharmacokinetic parameters (half-life and  $AUC_{0-12}$ ) approximate what would have been obtained with more frequent sampling.

Data analyses were performed using SAS software (Cary, NC). Differences between groups were determined using the  $\chi^2$  statistic. The Mann–Whitney *U* test was used for non-normally distributed data. Differences between groups or correlations between covariates were considered statistically significant at *p* values less than 0.05. To control for potential confounding factors, proportional hazards analyses were performed using the five factors (3) independently associated with fail-

ure/relapse in proportional hazards analysis of the parent trial (positive sputum culture at 2 months of treatment, cavitation on chest radiograph, greater than or equal to 10% under ideal body weight, bilateral disease on chest radiograph, and non-Hispanic white race).

## RESULTS

### Subjects

The demographics and clinical characteristics of the 133 HIV-seronegative patients in the pharmacokinetic substudy were similar to those of all HIV-seronegative patients in Study 22 (Table 1). In the retrospective phase, 4 patients with failure, 33 patients with relapse, and 46 control patients with cure were enrolled. In the prospective phase 50 patients were enrolled, of whom one had failure, two had relapse, and 47 were cured. Combining the retrospective and prospective phases of the study, 54% of the patients (40 of 74) who failed or relapsed in the parent trial underwent pharmacokinetic sampling.

### Comparison of Pharmacokinetic Parameters among Retrospectively Sampled versus Prospectively Sampled Patients

To determine if time of sampling affected isoniazid or rifampicin pharmacokinetics, pharmacokinetic parameters were compared among control cases sampled retrospectively to study therapy versus control cases sampled prospectively (Table 2). The control cases were compared in this analysis because only three patients with failure or relapse were sampled prospectively. On initial analysis, all rifampin pharmacokinetic parameters were lower in prospectively sampled patients compared with retrospectively sampled patients. However, after adjustment for rifampin autoinduction, these differences narrowed. The only significant difference in pharmacokinetic parameters was a shorter rifampentine half-life in patients sampled prospectively compared with those sampled retrospectively (median half-life of 14.5 versus 19.5 hours, *p* = 0.006).

**TABLE 2. COMPARISON OF PHARMACOKINETIC PARAMETERS OF ISONIAZID, RIFAMPIN, AND RIFAPENTINE FROM THE CASES WHO WERE CURED AND EITHER SAMPLED RETROSPECTIVELY (AFTER COMPLETING STUDY THERAPY) OR SAMPLED PROSPECTIVELY (DURING STUDY TREATMENT)**

Pharmacokinetic Parameter	Retrospectively Sampled	Prospectively Sampled	p Value*
Isoniazid	n = 43	n = 39	
Median AUC <sub>0-12</sub> (IQR)	54.6 (41.0–86.6)	52.9 (32.2–67.8)	0.22
Median maximal concentration (IQR)	11.9 (9.6–17.7)	10.4 (7.3–17.0)	0.14
Median serum half-life (IQR)	2.8 (1.6–3.8)	2.3 (1.5–3.8)	0.59
Rifampin (no adjustment for autoinduction)	n = 21	n = 19	
Median AUC <sub>0-12</sub> (IQR)	71.3 (45.1–108.7)	49.7 (43.6–61.4)	0.07
Median maximal concentration (IQR)	10.7(5.6–14.0)	6.4 (5.3–10.8)	0.18
Median serum half-life (IQR)	4.5 (3.1–11.0)	3.8 (3.4–7.0)	0.71
Rifampin (adjusted for autoinduction <sup>†</sup> )	n = 21	n = 19	
Median AUC <sub>0-12</sub> (IQR)	53.8 (34.0–82.1)	49.7 (43.6–61.4)	0.84
Median maximal concentration (IQR)	10.3 (5.4–13.4)	6.4 (5.3–10.8)	0.23
Median serum half-life	2.9 (1.8–6.5)	3.7 (3.2–7.0)	0.19
Rifapentine	n = 24	n = 26	
Median AUC <sub>0-24</sub> (IQR)	207 (139–236)	188 (156–274)	0.88
Median maximal concentration (IQR)	12.2 (8.0–13.6)	12.0 (9.4–16.5)	0.70
Median serum half-life (IQR)	19.5 (14.9–24.1)	14.5 (12.2–17.6)	0.006

Definition of abbreviations: AUC<sub>0-12</sub> = area under the concentration–time curve for 12 hours after the dose; IQR = interquartile range.

\* p Value by Mann–Whitney rank test; IQR indicates interquartile range.

† Adjustments made to rifampin pharmacokinetic parameters for autoinduction according to Acocella (10).

### Association of Pharmacokinetic Parameters with Treatment Outcome

Among patients treated with once-weekly isoniazid/rifapentine, all isoniazid pharmacokinetic parameters (area under the concentration–time curve during 12 hours after dosing [AUC<sub>0-12</sub>], maximal concentration [C<sub>max</sub>] and half-life) were lower among patients with failure or relapse compared with control cases (Table 3). For example, the median isoniazid AUC<sub>0-12</sub> in 22 patients with failure/relapse was 36.0 versus 55.9  $\mu\text{g} \cdot \text{hour}/\text{ml}$  in 49 patients with cure ( $p = 0.005$ , Mann–Whitney  $U$  test and Figure E1A in the online supplement). In contrast in 49 patients treated with twice-weekly therapy, isoniazid levels did not significantly differ between failure/relapse and cure (isoniazid AUC<sub>0-12</sub>  $p = 0.65$ , Table 3 and Figure E1A in the online supplement). Of note, isoniazid AUC<sub>0-12</sub> was not significantly different between all cases in the once-weekly treatment arm versus the twice-weekly treatment arm ( $p = 0.25$ ).

Rifampin half-lives by univariate analyses were shorter

among patients with failure or relapse compared with control cases. However, there was no association between the C<sub>max</sub> or AUC of either rifampin and treatment outcome. If these analyses were repeated in only patients sampled retrospectively, the same associations between isoniazid and rifampin pharmacokinetic parameters versus treatment outcome were found (data not shown). Finally, these results were similar when restricted to patients with relapse and cure (i.e., patients with failure excluded; data not shown).

In proportional hazards regression analyses, we adjusted for demographic and clinical factors associated with treatment outcome in the parent trial (a positive sputum culture at 2 months of treatment, cavitation on chest radiograph, being underweight, having bilateral disease on chest radiograph, or being of non-Hispanic white race). In these analyses, isoniazid pharmacokinetic parameters retained their association with failure or relapse, whereas no rifampin pharmacokinetic parameter was associated with treatment outcome (Table 4). To illustrate the

**TABLE 3. COMPARISON OF PHARMACOKINETIC PARAMETERS OF ISONIAZID, RIFAMPIN, AND RIFAPENTINE IN ALL CASES WITH FAILURE/RELAPSE COMPARED WITH CONTROL CASES WITH CURE**

Pharmacokinetic Parameter	Twice-Weekly Isoniazid/Rifampin		p Value*	Once-Weekly Isoniazid/Rifapentine		p Value
	Failure/Relapse	Cure (Control Cases)		Failure/Relapse	Cure (Control Cases)	
Isoniazid	n = 16	n = 33		n = 22	n = 49	
Median AUC <sub>0-12</sub> (IQR)*	43.3 (23.1–81.5)	48.8 (30.9–66.7)	0.65	36.0 (25.2–56.2)	55.9 (41.1–86.6)	0.005
Median maximal concentration (IQR)	11.9 (6.1–14.6)	10.0 (7.4–14.5)	0.90	11.1 (8.2–13.4)	11.9 (9.5–18.6)	0.08
Median serum half-life (IQR)	2.1 (1.6–3.5)	2.3 (1.8–4.0)	0.42	1.4 (1.2–2.8)	2.4 (1.5–3.7)	0.02
Rifampin <sup>†</sup>	n = 16	n = 40		n = 23	n = 50	
Median AUC <sub>0-12</sub> (IQR) <sup>‡</sup>	46.1 (35.8–49.3)	50.5 (38.2–72.9)	0.23	211 (149–273)	196 (144–251)	0.47
Median maximal concentration (IQR)	8.3 (6.1–10.2)	7.5 (5.3–11.9)	0.96	12.3 (9.3–17.9)	12.2 (8.1–13.8)	0.31
Median serum half-life (IQR)	2.2 (1.6–3.8)	3.4 (2.2–6.9)	0.11	14.6 (12.3–17.4)	16.8 (13.8–19.8)	0.04

Definition of abbreviations: AUC<sub>0-12</sub> = area under the concentration–time curve during 12 hours after dosing; IQR = interquartile range.

\* p Value by Mann–Whitney rank test; IQR indicates interquartile range.

† Adjustments made to rifampin pharmacokinetic parameters for autoinduction according to Acocella (10).

‡ AUC<sub>0-12</sub> for rifampin and AUC<sub>0-24</sub> for rifapentine.

**TABLE 4. ASSOCIATION BETWEEN PHARMACOKINETIC PARAMETERS AND OUTCOME BY TREATMENT ARM WITH AND WITHOUT ADJUSTMENT FOR OTHER RISK FACTORS FOR TREATMENT FAILURE/RELAPSE IN HUMAN IMMUNODEFICIENCY VIRUS–SERONEGATIVE PATIENTS**

	Univariate (Continuous PK Parameters and Dichotomous Other Risk Factors)		Multivariate (Continuous PK Parameters and Dichotomous Other Risk Factors)	
	Hazard Ratio (95% CI)	p Value	Hazard Ratio (95% CI)	p Value
<b>Model with isoniazid AUC<sub>0-12</sub> and rifapentine half-life, n = 76</b>				
Isoniazid AUC <sub>0-12</sub>	1.02 (1.01–1.04)	0.009	1.03 (1.01–1.05)	0.001
Rifapentine half-life	1.14 (1.02–1.27)	0.02		0.46
Culture (+) at 2-months	7.16 (2.93–17.46)	< 0.0001	3.08 (1.07–8.87)	0.04
Lung cavity	5.60 (1.66–18.89)	0.006	4.22 (1.06–16.79)	0.04
Underweight	2.73 (1.17–6.35)	0.02	3.89 (1.45–10.44)	0.007
White non-Hispanic	2.16 (0.94–4.95)	0.07	2.84 (1.13–7.17)	0.03
Bilateral lung disease	1.30 (0.55–3.06)	0.55		0.34
<b>Model with isoniazid AUC<sub>0-12</sub> and rifampin AUC<sub>0-12</sub>, n = 57</b>				
Isoniazid AUC <sub>0-12</sub>	1.00 (0.99–1.02)	0.82		0.65
Rifampin AUC <sub>0-12</sub>	1.12 (0.90–1.39)	0.30		0.34
Culture (+) at 2-months	4.17 (1.54–11.31)	0.005	4.52 (1.52–13.47)	0.007
Lung cavity	3.03 (0.87–10.56)	0.08		0.58
Underweight	3.08 (1.17–8.10)	0.02	3.30 (1.12–9.74)	0.03
White non-Hispanic	2.04 (0.78–5.37)	0.15		0.74
Bilateral lung disease	5.98 (1.37–26.21)	0.02		0.18

Definition of abbreviations: AUC<sub>0-12</sub> = area under the concentration–time curve during 12 hours after dosing; CI = confidence interval.

In the multivariate proportional hazards models, covariates with a probability < 0.15 were entered in a stepwise procedure with the isoniazid AUC<sub>0-12</sub> (continuous variable), rifampin AUC<sub>0-12</sub> adjusted for autoinduction (continuous variable) or rifapentine half-life (continuous variable), sputum culture after 2-months treatment for *Mycobacterium tuberculosis* (positive or negative), underweight at 2 months (yes or no), radiographic lung cavities at baseline or 2-months treatment (present or none), white non-Hispanic race (yes or no) and bilateral lung disease (yes or no). Hazard ratios expressed for the continuous AUC<sub>0-12</sub> parameter for a decrease 1 µg · hour/ml, and half-life for a decrease of 1 hour.

magnitude of the effect of isoniazid pharmacokinetic parameters relative to the previously identified demographic and clinical risk factors for failure or relapse, we dichotomized isoniazid pharmacokinetic parameters at the median value and repeated the multivariate analyses (data not shown). When analyzed in this manner, low isoniazid AUC<sub>0-12</sub> among HIV-seronegative patients treated with the once-weekly regimen was associated with failure or relapse with a hazard ratio (5.99) similar to clinical risk factors such as cavitation on chest radiograph or having a positive sputum culture at 2 months of therapy (Table 4).

**N-acetyl-transferase Type 2 Genotype and Treatment Outcome**

Isoniazid acetylator status was determined by N-acetyl-transferase type 2 (NAT2) genotype in 89 HIV-seronegative cases (Table 5). NAT2 phenotype, as determined by genotype, was

associated with outcome in cases treated with once-weekly isoniazid/rifapentine compared with all control cases with cure (p = 0.03, χ<sup>2</sup>). By contrast, phenotype in cases treated with twice weekly isoniazid/rifampin was not significantly associated with outcome (p = 0.18). Of note, there was a highly significant association between NAT2 phenotype and isoniazid AUC<sub>0-12</sub> (p < 0.0001).

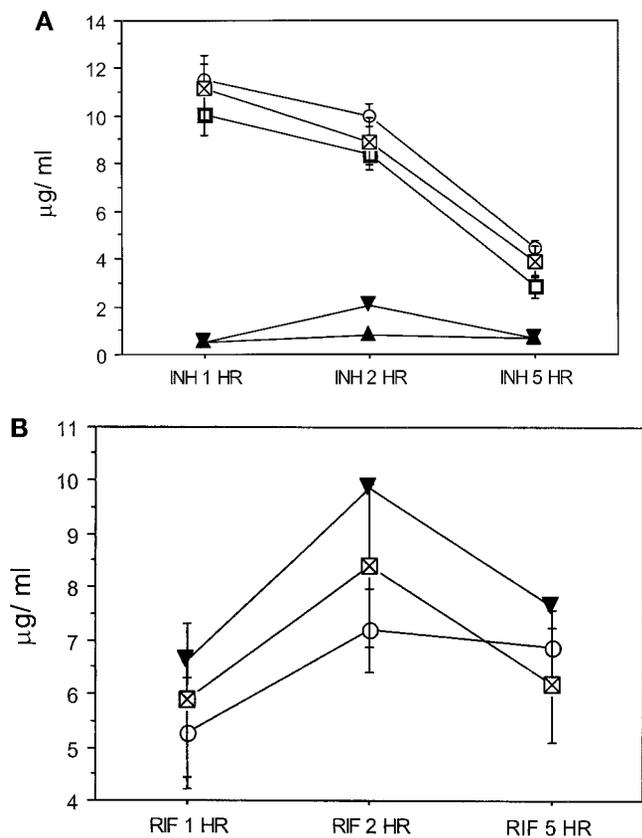
**Relapse of M. tuberculosis Mono-resistant to Rifamycin in Two Patients with Low Isoniazid Concentrations**

Five patients relapsed with *M. tuberculosis* mono-resistant to rifamycin in the TBTC Study 22 (3, 4). Four of these patients were coinfecting with HIV and were treated with once-weekly isoniazid/rifapentine; the fifth patient was HIV-seronegative and was treated with twice-weekly rifampin. Two of the five cases in TBTC Study 22 with acquired rifamycin-resistant relapse had

**TABLE 5. N-ACETYL-TRANSFERASE TYPE 2 PHENOTYPE (DETERMINED BY GENOTYPE) AND TREATMENT OUTCOME IN HUMAN IMMUNODEFICIENCY VIRUS–SERONEGATIVE PATIENTS WITH FAILURE/RELAPSE COMPARED WITH ALL CONTROL CASES WITH CURE**

Regimen	Outcome	N-acetyltransferase-2 Phenotype			p Value*
		Fast/Fast	Slow/Fast	Slow/Slow	
Once-weekly isoniazid/rifapentine	Failure/relapse	9 (45)	5 (25)	6 (30)	0.03
Twice-weekly isoniazid/rifampin	Failure/relapse	4 (31)	7 (54)	2 (15)	0.18
All cases with cure from once-weekly isoniazid/rifapentine and twice-weekly isoniazid/rifampin groups	Cure	9 (16)	24 (43)	23 (41)	

\* p Value by χ<sup>2</sup> for all cases with cure vs. cases of failure/relapse treated with either once-weekly isoniazid/rifapentine or with twice-weekly isoniazid/rifampin. Number (percent) of cases per group indicated.



**Figure 1.** Drug concentrations in 154 patients with failure/relapse or cure compared with 2 patients with acquired rifamycin-resistant relapse. (A) Mean isoniazid concentrations ( $\pm$  SE  $\mu\text{g/ml}$ ) are depicted 1, 2, and 5 hours after oral dosing from patients ( $n = 111$ ) with cure (open circle); with drug-susceptible failure/relapse ( $n = 24$ ) treated once per week with rifapentine/isoniazid (square); with drug-susceptible failure/relapse ( $n = 17$ ) treated twice per week with rifampin/isoniazid (square with X); and from two patients with acquired rifamycin-resistant relapse (triangle, human immunodeficiency virus (HIV)-seropositive and inverted triangle, HIV-seronegative). (B) Mean rifampin concentrations ( $\pm$  SE  $\mu\text{g/ml}$ ) depicted from patients ( $n = 44$ ) with cure (open circle); with drug-susceptible failure/relapse ( $n = 16$ ) treated twice per week (square with X); and from a patient with acquired rifamycin-resistant relapse (inverted triangle).

pharmacokinetic sampling performed (Figure 1A) in the substudy; both had very low concentrations of isoniazid at all time points and low pharmacokinetic parameters. Of note, rifampin concentrations were obtained from the HIV-negative patient, and in this case rifampin concentrations were comparable with those obtained from all other patients (Figure 1B).

#### Association of Pharmacokinetic Parameters with Treatment Outcome in HIV-Seropositive Patients

Of the 33 HIV-seropositive patients in the pharmacokinetic study, 4 relapsed and 29 were cured. The median CD4 cell count at the time of the pharmacokinetic study was 240 cells/mm<sup>3</sup> (interquartile range 166–383), the median viral load was 11,244 copies/mm<sup>3</sup> (interquartile range 593–69,083).

Because prior studies of tuberculosis treatment outcomes and pharmacokinetic parameters were evaluated in HIV-seronegative patients and because the number of HIV-seropositive patients in this pharmacokinetic substudy was small, all primary analyses were done in the HIV-seronegative population. In secondary analyses with the 33 HIV-seropositive cases, the findings

remained the same. In 18 HIV-seropositive cases treated with once-per-week therapy, the median isoniazid AUC<sub>0–12</sub> was lower in 2 patients who relapsed versus 16 control cases (AUC<sub>0–12</sub> 23.8 vs. 60.6  $\mu\text{g} \cdot \text{hour/ml}$ ,  $p = 0.16$ , Mann–Whitney). By comparison, in the 15 HIV-seropositive patients treated twice per week with isoniazid and rifampin, the median isoniazid AUC<sub>0–12</sub> was similar in two cases with relapse versus the 13 control cases (41.9 versus 48.7  $\mu\text{g} \cdot \text{hour/ml}$ ,  $p = 0.87$ ). Furthermore, the association of pharmacokinetic parameters with treatment outcome remained the same if all HIV-seropositive and HIV-seronegative cases were analyzed together ( $n = 152$  with isoniazid AUC<sub>0–12</sub> determined). Specifically, median isoniazid AUC<sub>0–12</sub> in 88 cases receiving once-weekly isoniazid and rifapentine was lower in 24 patients with failure or relapse versus 64 cures (AUC<sub>0–12</sub> 34.9 versus 56.2  $\mu\text{g} \cdot \text{hour/ml}$ ,  $p = 0.002$ ). As before, the median isoniazid AUC<sub>0–12</sub> in the 64 cases receiving twice-weekly isoniazid and rifampin were similar in 18 patients with failure or relapse and the 46 cures (AUC<sub>0–12</sub> 43.3 versus 48.7  $\mu\text{g} \cdot \text{hour/ml}$ ,  $p = 0.7$ ).

#### DISCUSSION

Three independent lines of evidence in this study suggest that isoniazid pharmacokinetics explain at least part of the decrease in efficacy of the once-weekly isoniazid/rifapentine regimen. First, there was a strong relationship between several isoniazid pharmacokinetic parameters and the occurrence of failure or relapse among patients treated with once-weekly therapy. This association remained strong after adjustment for demographic and clinical risk factors for failure or relapse. Second, an independently analyzed marker of isoniazid pharmacokinetics, the presence of NAT2 genotypes that more rapidly metabolize isoniazid, was also associated with failure or relapse among patients treated with once-weekly therapy. Finally, the finding that all cases of acquired resistance had acquired rifamycin-monoresistance is strong evidence of a deficiency of isoniazid. Although anecdotal, it is interesting that in the two such cases included in this study, isoniazid concentrations were very low.

These findings are somewhat surprising because isoniazid has long been believed to play a minor role in the efficacy of rifampin-based tuberculosis treatment, particularly after the first 2 months of therapy. In a variety of clinical trials in which standardized rifampin-based regimens were used without knowledge of initial drug-susceptibility patterns, isoniazid resistance appeared to have little effect on the rate of relapse. Specifically, although the number of patients was low, baseline isoniazid resistance was not a risk factor for failure or relapse among patients in the Hong Kong trial (1, 16, 17) of once-weekly isoniazid/rifapentine (2/16 [12.5%] having isoniazid-resistant isolates relapsed versus 38/346 [11.0%] of those with isoniazid-susceptible isolates). Furthermore, in the Hong Kong trial, rapid acetylators of isoniazid (determined by NAT2 genotyping) were not at higher risk of failure or relapse.

Despite the impressive evidence suggesting that isoniazid plays a minor role in standard twice-weekly rifampin-based therapy, evidence from several sources suggests that this conclusion may not be true for once-weekly therapy. In the British Medical Research Council Singapore trial of once-weekly regimens of isoniazid and rifampin (18), rapid acetylators of isoniazid were at greater risk of failure (12/155 rapid acetylators [8%], but 0/117 slow acetylators) but not of relapse. Furthermore, in a mouse model of tuberculosis treatment that has correlated closely with the results of human clinical trials, once-weekly therapy with isoniazid/rifapentine was associated with a low risk of acquired rifamycin-monoresistance (19). In further experiments (20) involving once-weekly rifapentine-based regimens, the occurrence of acquired rifamycin-monoresistance was prevented by supple-

menting the regimen with daily isoniazid or weekly moxifloxacin but not by increasing the dose of rifapentine.

Despite similar overall rates of failure/relapse in the two treatment arms, there were significant differences between the Hong Kong trial and TBTC Study 22 cohorts in risk factors for failure/relapse. Two-month culture positivity was detected in only 8.2% of 522 cases from Hong Kong (1, 16, 17) versus 20% of 886 in TBTC 22 ( $p < 0.0001$ ). Radiographic lung cavitation was identified in 37% of 592 cases from Hong Kong versus 54% of 975 in TBTC 22 ( $p < 0.0001$ ). These two differences suggest that patients enrolled in TBTC 22 may have had more advanced disease than the population in the Hong Kong trial. This difference in disease severity may, in part, account for the different conclusions about the role of isoniazid.

Finally, it is notable that the isoniazid pharmacokinetic parameter having the strongest association with treatment failure or relapse was AUC, not  $C_{max}$  (Table 3). This finding is consistent with the results in early studies of once-weekly isoniazid plus streptomycin, in which increasing the isoniazid dose among rapid acetylators (which increased the  $C_{max}$ , but had much less effect on AUC) did not alter their increased risk of treatment failure (21, 22). Both results suggest that time over a critical concentration (as approximated by AUC) is a more important determinant of the success of highly intermittent isoniazid therapy than is peak drug concentration ( $C_{max}$ ).

An important negative finding in this study was the lack of consistent association between pharmacokinetic parameters and the occurrence of failure or relapse in HIV-seronegative patients given twice-weekly isoniazid and rifampin. Prior uncontrolled studies (23, 24) have reported low serum concentrations of rifampin among patients who had failure or relapse with standard rifampin-based regimens. These studies have been interpreted as demonstrating that abnormal pharmacokinetics were an important cause of poor outcome with standard therapy. However, these studies had major methodological problems. The most serious problem was the lack of pharmacokinetic data from a control population of patients who were cured. In addition, these studies assessed only one or two time points and thus may have substantially underestimated the peak serum concentration, the pharmacokinetic parameter said to be abnormal. Our study—with more data points per patient and inclusion of pharmacokinetics among controls—found no consistent relationship between the pharmacokinetics of isoniazid and rifampin and failure or relapse. Although exceptions may occur at extreme values (as exemplified by the case of acquired rifamycin-resistance in an HIV-negative person treated with twice-weekly rifampin plus isoniazid), our data suggest that serum concentrations of isoniazid and rifampin achieved in the vast majority of patients are not associated with failure or relapse. Among patients treated with standard isoniazid and rifampin therapy, failure and relapse may reflect other host factors (pulmonary cavitation, for example) (3) and perhaps pathogen-related factors (such as drug tolerance) (25) rather than “low” serum concentrations of isoniazid and rifampin. Additional research is required to define the role of each of these variables, particularly during the first 2 months of treatment.

This pharmacokinetic substudy has several important limitations. Many of the patients had pharmacokinetic sampling performed after completing their initial course of treatment. It is possible that pharmacokinetic parameters among these patients would have been different if they had been sampled during the course of treatment. However, in the limited number of subjects in other studies who have had pharmacokinetic determinations on more than one occasion, there was relatively little inpatient variability (11–13, 26). In addition, after adjustment for the well-described effects of rifampin autoinduction, pharmacokinetic

parameters of patients sampled prospectively were quite similar to those of patients sampled retrospectively in this study. Thus, it was unlikely that our use of retrospective sampling resulted in a systematic bias in this analysis. Our sampling scheme (five time points per patient) was not sufficient to determine some pharmacokinetic parameters, like half-life, with a high degree of accuracy. However, the robustness of the findings suggests that this sampling scheme was sufficient to detect relevant differences in pharmacokinetic parameters. Residual confounding by measured or unmeasured variables is possible, but the risk factors for failure or relapse identified in the 1,004 patients in TBTC Study 22 were used to adjust relative risk in multivariate analyses. Finally, our study did not use all possible restriction enzymes to detect slow acetylator genotypes. Unfortunately, the NAT2 genetic analysis was incomplete; two mutations—G191A and A341C with frequencies in African Americans of 9 and 6%, respectively (9)—could not be detected. In the study, 4 of 22 African Americans were discordant between phenotype determined by drug pharmacokinetic sampling and by NAT2 genotype. However, detection of these mutations in the four discordant cases would have changed the genotype designations so as to further support the association of NAT2 genotypes with failure/relapse with once-weekly isoniazid/rifapentine but not twice-weekly isoniazid/rifampin.

In summary, our study offers strong evidence that isoniazid plays a significant role in once-weekly rifapentine/isoniazid therapy. Increasing the dose of rifapentine (27) may also increase the potency of once-weekly therapy, as was suggested by the mouse model, but is unlikely to result in highly active therapy that completely prevents acquired rifamycin-resistance. The implication of this finding is that efforts to strengthen once-weekly therapy should include some alteration to the nonrifamycin portion of the regimen. Interventions that may be effective include adding a drug with a longer half-life, such as moxifloxacin, or supplementing the regimen with daily isoniazid. Finally, these results illustrate the usefulness of incorporating pharmacokinetic measurements into studies evaluating new treatment regimens.

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