Meta-Analysis of Clinical Studies Supports the Pharmacokinetic Variability Hypothesis for Acquired Drug Resistance and Failure of Antituberculosis Therapy

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(See the Editorial Commentary by Egelund and Peloquin, on pages 178–9.)

Background. Using hollow-fiber tuberculosis studies, we recently demonstrated that nonadherence is not a significant factor for ADR and that therapy failure only occurs after a large proportion of doses are missed. Computer-aided clinical trial simulations have suggested that isoniazid and rifampin pharmacokinetic variability best explained poor outcomes. We were interested in determining whether isoniazid pharmacokinetic variability was associated with either microbiological failure or ADR in the clinic.

Methods. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed. Prospective, randomized, controlled clinical trials that reported isoniazid acetylation status and microbiological outcomes were selected. The main effects examined were microbiological sputum conversion, ADR, and relapse. Effect size was expressed as pooled risk ratios (RRs) comparing rapid with slow acetylators.

Results. Thirteen randomized studies with 1631 rapid acetylators and 1751 slow acetylators met inclusion and exclusion criteria. Rapid acetylators were more likely than slow acetylators to have microbiological failure (RR, 2.0; 95% confidence interval [CI], 1.5–2.7), ADR (RR, 2.0; CI, 1.1–3.4), and relapse (RR, 1.3; CI, .9–2.0). Higher failure rates were encountered even in drug regimens comprising >3 antibiotics. No publication bias or small-study effects were observed for the outcomes evaluated.

Conclusions. Pharmacokinetic variability to a single drug in the regimen is significantly associated with failure of therapy and ADR in patients. This suggests that individualized dosing for tuberculosis may be more effective than standardized dosing, which is prescribed in directly observed therapy programs.

Tuberculosis has ravaged humans since antiquity. Fortunately, the development of short-course chemotherapy regimens comprised of isoniazid, rifampin, and pyrazinamide has led to considerable success in treating tuberculosis [1]. Although program-defined measures such as completion of therapy are said to be about 95% successful, the microbiological success is

only 50%–60% in many high-burden countries such as those in Africa [2–5]. In addition, acquired drug resistance (ADR) continues to be a major problem despite the use of the directly observed therapy, short course (DOTS) program [6]. The DOTS program was developed to improve outcomes such as microbiological failure, relapse, and ADR, with a focus on ensuring adherence; it was formally adopted by all countries that were being served by the World Health Organization at the end of the 1990s.

We recently examined the effect of adherence in hollow-fiber studies in which degrees of noncompliance were varied from missing 0% of doses to missing 100% of doses [7]. Microbiological failure was only encountered when nonadherence was >60%. Even more surprising was the fact that no ADR emerged with any extent

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of nonadherence. In addition, common factors such as pharmacokinetic mismatching of isoniazid and rifampin have also been found not to account for ADR [8]. However, pharmacokinetic mismatch seen with intermittent regimens using isoniazid and long half-life rifamycin has yet to be studied. We then used computer-aided clinical trial simulations to examine other reasons for therapy failure and ADR [7]. Pharmacokinetic variability was most commonly responsible for ADR and microbiological failure in the *in silico* analysis. Nevertheless, this has been properly identified as a Gedanken experiment that allows for generation of a hypothesis but still needs to be confirmed by clinical studies [9].

The first step in isoniazid metabolism is hepatic acetylation by N-acetyl transferase 2 (NAT2) [10]; NAT2 has several alleles associated with rapid and slow acetylation, which are associated with 88% of all variability in isoniazid systemic clearance [11]. It is known that the microbial kill and drug-resistance suppression of isoniazid best correlate with the ratios of both the 24-hour area under the concentration time curve (AUC) and the peak concentration to minimum inhibitory concentration across many animal species and in patients [12–14]. Because AUC is proportional to dose divided by clearance, variability in isoniazid clearance could lead to rapid acetylators with decreased sputum conversion rates, poorer microbiological outcomes, and increased ADR. Nevertheless, it is widely believed that acetylation status does not affect efficacy of isoniazid in modern combination therapy regimens [1, 15]. Here, we tested this pharmacokinetic variability hypothesis for isoniazid alone in a meta-analysis of prospective clinical studies that compared outcomes in patients with different acetylation statuses.

METHODS

Null Hypothesis

Isoniazid acetylation status is not associated with microbiological failure of antituberculosis therapy, ADR, or relapse. The alternative is that for a similar given dose, microbiological failure and ADR are as likely to occur among slow acetylators as among rapid acetylators.

Standards

The study methods and reporting followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [16].

Search Strategy

The following medical subject heading terms and strategies were used; “tuberculosis” AND “arylamine N-acetyltransferase” OR “isoniazid N-acetyltransferase” OR “isoniazid acetyltransferase” OR “hydrazine acetyltransferase” OR “acetylation status” AND “treatment outcomes” to search the MEDLINE

and Cochrane databases for titles, abstracts, and texts. We used the Ovid interface to search MEDLINE (1948 to December 2010), OLDMEDLINE, and MEDLINE In-Process citations. A separate search was made in the Cochrane Library, the online archives of the *International Journal of Tuberculosis and Lung Disease* and *Tubercle*, for applicable studies not identified in previous searches. We did not exclude any articles on the basis of publication date or language. The electronic search was supplemented with manual examination of references from the selected articles and key reviews [1, 15].

Study Selection Criteria

Selection was limited to randomized, controlled clinical trials in which microbiological and clinical outcomes were reported by acetylation status in table, graph, or text format. Studies were required to clearly define treatment outcome as an end point specified by mycobacterial cultures during therapy, at the end of therapy, and during follow-up to identify relapses after therapy. Studies in which patients had prior tuberculosis treatment for ≥ 2 weeks, primary drug-resistant tuberculosis, or extrapulmonary tuberculosis and studies for early bactericidal effect were excluded. Studies were independently assessed and reviewed by T. G. and J. G. P., with disagreements resolved by a third party (S. S.).

Data Abstraction

For each study, we obtained patient data, including estimate of disease burden, as measured by microbiological and radiological examinations; the place where the study was conducted; the date patient enrollment started; and data on acetylation status assays or NAT2 alleles, when available. Given that dose size and dosing schedule have an effect on isoniazid efficacy and resistance suppression, we obtained the isoniazid dose and dosing schedule used in both the intensive and continuation phases. Because the primary aim of these clinical trials was to compare various antituberculosis regimens and not to compare outcomes between acetylation phenotypes, complete details about the characteristics of the trials were sometimes reported in different studies addressing related hypothesis. As a result, we abstracted data for each trial from those multiple publications. We recorded all patients that were considered microbiological failures or relapses during treatment for slow and rapid (also called *fast*) acetylators.

Definition of Terms

For the purpose of this study, we maintained the terms and definitions as intended in each original study; inconsistencies that could potentially bias study effects were highlighted. Microbiological failure was defined as remaining culture positive at the end of therapy (ie, patients produced 3 consecutive monthly examinations at the end of therapy with ≥ 20 colonies). Relapse was restricted to patients who were followed up after cure and

completion of therapy. No efforts were made to distinguish reinfection from relapse. This is not likely to have great impact on the findings, except in studies of those populations with a high prevalence of human immunodeficiency virus infection.

Quantitative Data Synthesis

Acetylation status group comparisons were performed for the following outcomes: (1) microbiological failure during therapy, (2) relapse after therapy, and (3) ADR. Isoniazid dose, dosing intervals, and isoniazid combination regimens used in intensive and continuation phases were also examined. We analyzed outcomes on the basis of a modified intention-to-treat principle using the data from patients with initial drug susceptible pulmonary tuberculosis, as stated in each original trial protocol. Because multiple regimens and dosing schedules were used in different studies, we computed risk ratios (RRs) with 95% confidence intervals (CIs) and assigned regimen as the unit of analysis. Random-effects models, the DerSimonian and Laird method, and mixed-effects modeling were used to calculate overall pooled RRs of failure outcomes when rapid and slow acetylators were compared [17]. Heterogeneity across studies was examined by calculating I^2 , an index of the proportion of total variation across studies that is due to heterogeneity rather than to chance [16, 17]. Random-effects models were used to combine studies or regimens within each subgroup when the I^2 statistic was $>50\%$ and the P value $<.05$; otherwise, fixed-effects models were used to combine the subgroups and obtain a combined RR.

Sensitivity Analyses

An estimate of potential publication bias was carried out using the funnel plot in which the standard error of the log (odds ratio) of each study was plotted against its log (odds ratio). An asymmetric plot was interpreted as a possible publication bias. Funnel plot asymmetry was assessed using Egger's linear regression test [18]. The significance of the intercept was set at $P < .05$ [17], which is representative of significant publication bias. To examine bias due to change in treatment regimens, tuberculosis disease diagnostics, and terminology over time, an influence analysis of change in effect size was performed.

Software

Comprehensive Meta Analysis software, version 2 (Biostat Inc), was used for the pooled parameter estimate for the meta-analysis data. GraphPad Prism software, version 5, and Stata software, version 12, were used to generate graphs.

RESULTS

The electronic search of selected databases using the predefined criteria identified 16 studies; of these, only 3 met inclusion and

exclusion criteria on closer examination [19–21]. Manual search of the references of the selected studies and review articles identified an additional 10 studies. Thus, 13 randomized clinical trials met selection criteria, comprising 6510 patients randomly assigned to receive 26 separate antituberculosis regimens that included isoniazid, between 1957 and 2003 [19–31]. The selected studies plus the antituberculosis regimens are shown in Table 1. None of the selected studies used crossover design. Different assays were used to define acetylation status (Table 1). Sputum microbiological burden at presentation and disease burden by radiological appearance were reported in all studies, but only 3 reported both measures by acetylation status [19, 22, 25]. The proportion of patients with moderate or severe disease on microscopy (ie, 2-plus or higher) was 68% in rapid acetylators versus 63% in slow acetylators ($P = .03$); the presence of cavitation on chest radiographs was similar between acetylation phenotypes ($P = .35$). Patients were prospectively randomly assigned to antituberculosis regimens for all studies; however, 2 studies used retrospective controls to compare clinical outcomes between acetylation status groups [21, 23]. In all studies, patients took medications under the direct supervision of a healthcare worker; patients who failed to come to their appointments received home visits from either a social worker or a healthcare worker.

All 13 studies examined for microbiological failure. Three studies (23%) combined microbiological failure and relapse as a single outcome [21, 23, 24]. Of the 6510 patients enrolled in the 13 clinical trials, 3471 (53%) presented with both acetylation phenotype data and microbiological outcomes. They consisted of 1631 rapid acetylators (47%), 89 intermediate acetylators (3%), and 1751 slow acetylators (50%). Because few patients were classified as intermediate acetylators, this group was excluded from further analysis. Figure 1 is a forest plot of studies evaluated for microbiological failure showing a significant heterogeneity of RRs for microbiological failure between rapid and slow acetylators ($I^2 = 39\%$; $P = .035$). Therefore, mixed-effects modeling was used. Overall, rapid acetylators were more likely to have microbiological failure (RR, 2.0; CI, 1.5–2.7) than were slow acetylators.

In view of the heterogeneity of studies, we performed extensive sensitivity analysis on the microbiological failure outcome. We used mixed-effects methods to combine data across trial regimens based on dosing schedule, number of drugs used in the regimens, study location, and acetylation status assays. First, microbiological failure was higher in the once-a-week dosing schedule compared with the daily or every-other-day dosing schedule (RR, 4.1; CI, 2.9–5.8). Nevertheless, the risk of microbiological failure was still significantly higher among rapid acetylators within each dosing schedule, as shown in Figure 2.

Second, because it has been noted that acetylation status is irrelevant in modern multiple-therapy regimens, we performed sensitivity analysis of the effect of monotherapy, dual

Table 1. Clinical Trials and the Characteristics of Antituberculosis Regimens Selected From Meta-Analysis

Reference	Study Location	Sample Size, No. of Patients ^a	Regimen No.	Regimen	Phenotyping Methods	Outcomes Examined
26	United Kingdom	481	1	3 mo: SHP daily/9 mo: SH twice weekly	Sulfadimidine	Failure, ADR, others
27	East Africa/Zambia	61	2	1 or 2 mo: STH daily/11 mo: TH twice weekly	Sulfadimidine	Failure, ADR, others
30	East Africa/Zambia	953	3	2 mo: SHRZ daily/4 mo: SHZ twice weekly	Matrix isoniazid	Failure, ADR, relapse
23	Hong Kong	672	4 ^b	2 mo: SHRZ daily/4 mo: HR or Rpt daily	NAT2 alleles	Failure and relapse
24	Hong Kong	620	5	9 mo: SHZ twice weekly	Matrix isoniazid	Failure and relapse (combined)
19	Chennai (Madras)	554	6	12 mo: SH twice weekly	Isoniazid ^c	Failure, ADR, others
			7	12 mo: SH weekly		
			8	12 mo: SH twice weekly		
			9	12 mo: SH weekly		
			10	12 mo: SHZ weekly		
26	Chennai (Madras)	359	11	1 mo: SH daily/11 mo: SH weekly	Isoniazid ^d	Failure, ADR, others
			12	1 mo: SPH daily/11 mo: SPH weekly		
31	Chennai (Madras)	220	13	12 mo: SPH twice weekly	Isoniazid ^d	Failure, others
			14	12 mo: SPH daily		
19	Chennai (Madras)	341	15	12 mo: P + H (100 mg twice daily)	Isoniazid ^e	Failure, ADR, others
			16	12 mo: H 400 mg daily		
			17	12 mo: H 200 mg twice daily		
			18	12 mo: H 100 mg twice daily		
20	Chennai (Madras)	484	19	0.5 mo: SHE daily/12 mo: EH daily	Isoniazid ^f	Failure, relapse
			20	0.5 mo: SHE daily/12 mo: EH twice weekly		
			21	0.5 mo: SHE daily/12 mo: E weekly, H twice weekly		
			22	0.5 mo: SHE daily/12 mo: EH weekly		
29	Prague	280	23	1 mo: SPH daily/12 mo: SH twice weekly	Isoniazid	Failure, relapse
			24	1 mo: SPH daily/12 mo: SH weekly		
28	Singapore	481	25	0.5 mo: SHR daily/12 mo: HR daily	Matrix isoniazid	Failure, relapse
21	United States	1004	26 ^b	2 mo: SHER daily/4 mo: HR or Rpt daily	NAT2 alleles	Failure, relapse (combined)

Abbreviations: ADR, acquired drug resistance; E, ethambutol; H, isoniazid; NAT2, N-acetyltransferase 2; P, para-aminosalicylic acid; R, rifampin; Rpt, rifapentine; S, streptomycin; T, thiacetazone; Z, pyrazinamide.

^a All patients were randomly assigned to regimen.

^b Randomized with retrospective controls. The threshold isoniazid concentration for determining acetylator phenotype varies with the method studied.

^c The threshold for determining slow acetylation status after a test dose of 3 mg/kg body weight of intramuscular isoniazid was a serum concentrations of 0.63 µg/mL from a blood specimen collected 4.5 hours after the dose.

^d Threshold: 0.90 µg/mL.

^e Threshold: 0.58 µg/mL.

^f Threshold: 0.80 µg/mL.

therapy, and ≥ 3 drug regimens (Figure 3). Rapid acetylators had a significantly higher risk of failure, and this risk was even more significant in the combination chemotherapy regimens compared with monotherapy (Figure 3).

Third, we examined the effect of different assays for acetylation status. Studies that used sulfadimidine assays ($I^2 = 0\%$) or matrix isoniazid assays ($I^2 = 32\%$) were significantly homogeneous, whereas those that used isoniazid concentration assays

Figure 1. Forest plot of studies analyzed for microbiological failure. Twenty-six antituberculosis regimens were administered, labeled regimens 1–26. Figure shows risk ratios and 95% confidence intervals for microbiological failure in fast versus slow acetylators, as well the percentage of weight contributed by each regimen toward the pooled estimate obtained using random-effects models. Abbreviations: CI, confidence interval; RR, risk ratio.

($I^2 = 54\%$) were heterogeneous. The acetylation status assay did not affect RRs of failure among rapid acetylators compared with slow acetylators. However, studies that included isoniazid assays used different drug concentration levels to determine acetylation status, a possible reason for the observed heterogeneity of effect. Supplementary Figure 1 shows influence analysis data for all regimens that examined failure. Exclusion of the inferior isoniazid monotherapy regimens did not significantly influence failure. Finally, the funnel plot did not suggest any publication bias or small-study effects for microbiological failure ($P = .58$). Thus, the sensitivity analysis confirmed that acetylation-defined pharmacokinetic variability was significantly associated with microbiological outcome, independent of dosing schedule, number of drugs in the regimen, or assay used to establish acetylation phenotype.

ADR was reported in a format that could be used in this meta-analysis in 5 studies, comprising 622 fast acetylators and 577 slow acetylators [25–29]. Of these studies, 2 [23, 27] contributed 80% to the pooled RR. The studies demonstrated significant homogeneity of effect ($I^2 = 0\%$), hence, fixed-effects modeling was used. Figure 4 shows the risk of ADR in each study. The pooled RR for ADR was 2.0 (CI, 1.1–3.4) in rapid acetylators compared with slow acetylators. However, in sensitivity analysis, when isoniazid monotherapy was excluded from computation of pooled estimates, the RR, though higher among rapid acetylators, just failed to attain statistical significance (RR, 2.3; CI, .9–6.2). The funnel plot did not suggest any publication bias or small-study effects for ADR ($P = .29$).

Relapse was assessed in 5 studies [20, 26, 28–30]; studies that combined relapse and failure were excluded from relapse

Figure 2. Effect of acetylation status on failure during different dosing schedules. Risk of failure among rapid acetylators compared with slow acetylators was examined for once-weekly, twice-weekly, and daily dosing schedules. “Overall” refers to circumstances in which patients receiving once-weekly dosing were pooled together with those receiving twice- or thrice-weekly dosing, including those receiving rifapentine regimens.

analysis. Because the studies were homogenous ($I^2 = 0\%$), fixed-effects models were used. The analysis included 557 rapid and 477 slow acetylators. Results are shown in Figure 5, which demonstrates that although the risk for relapse was higher among rapid acetylators, this difference did not achieve statistical significance. The funnel plot did not suggest any publication bias or small-study effects for relapse ($P = .08$).

DISCUSSION

Tuberculosis is currently treated with ≥ 3 drugs. The microbial kill of isoniazid is particularly important during early therapy, and pyrazinamide is important for its sterilizing effects. Consequently, together with the effects of pyrazinamide, the isoniazid effect is best reflected in the index of microbiological failure. The third pivotal drug in the regimen is rifampin, which exerts the most sterilizing effect for which relapse is the most important measure of efficacy. Isoniazid protects rifampin from emergence of resistance during the continuation phase. Thus, the effects of isoniazid failure are expected to best manifest as microbiological failure and ADR.

We demonstrate that the pharmacokinetic variability of isoniazid, one drug alone in the combination, is associated with

microbiological failure and ADR. When different dosing schedules were examined, the more intermittent regimens led to even worse outcomes among fast acetylators. This is consistent with lower cumulative isoniazid AUCs in intermittent dosing compared with daily therapy, such that the effect of acetylation status becomes even more obvious in this group, given that this strategy is equivalent to reduction of total (cumulative) doses. This is consistent with findings of our hollow-fiber and in silico studies, which predicted that both poor adherence and pharmacokinetic variability would have even greater impacts on the more intermittent antituberculosis dosing strategies [7]. Finally, it is fascinating that the magnitude of the pooled RRs for ADR and microbiological outcomes are similar to the ratios of both AUC and peak concentration between rapid and slow acetylators, confirming that the increased risk for failure and ADR are due to pharmacokinetic-pharmacodynamic relationships [11].

Even though such categories as slow acetylators and rapid acetylators are useful in grouping patients, they are themselves summaries of distributions of acetylation status and thus lead to averaging out of AUCs. As an example, in Cape Town, South Africa, isoniazid peak concentrations and AUCs vary 7-fold and 25-fold, respectively [32]; however, the mean AUC differences between rapid and slow acetylators are <3 -fold.

Figure 3. Effect of acetylation status on failure with different numbers of drugs in combination. Shown are pooled risk ratios, number of regimens combined based on the number of different drugs in each regimen, and measures of heterogeneity (I^2) between the pooled regimens. Mixed-effects models were used to obtain effect estimates.

Figure 4. Forest plot for acquisition of drug resistance. Figure shows risk ratios and 95% confidence intervals, as well as the percentage of weight contributed by each regimen toward the pooled estimate obtained using fixed-effects models. Abbreviations: CI, confidence interval; RR, risk ratio.

Figure 5. Forest plot of studies that reported relapse. I^2 demonstrates good homogeneity. Results show a higher risk of relapse (risk ratio) for fast acetylators; however, the difference did not achieve statistical significance. Abbreviations: CI, confidence interval; RR, risk ratio.

Mathematically, this averaging out is equivalent to a misclassification bias for either category, which should lead to failure to reject the null hypothesis [33]. However, even with that limitation, the differences driven by pharmacokinetic variability in microbiological and ADR were sufficiently large to still be detected, despite the bias toward the null hypothesis. Moreover, our study examined pharmacokinetic variability of a single drug. There is also wide pharmacokinetic variability for both pyrazinamide and rifampin [32]. As an example, in Cape Town, the allele frequency of *SLCO1B1* rs4149032, which encodes for the rifampin organic anion-transporting polypeptide 1B1, is 0.70, indicating that low rifampin concentrations are extremely common [34]. If one considers that rapid acetylators comprise 0.56–0.67 of patients in Cape Town, it is clear even by simple conditional probabilities alone that a large proportion of patients will have low concentrations of both drugs. Thus, wide pharmacokinetic variability of the 3 principal drugs is expected to be common and, based on our current study, could be a powerful driver of microbiological failure and ADR.

There are several limitations to our current study. Assigning acetylation phenotypes to patients (Table 1) and those considered bacteriologically doubtful (ie, those who had ≥ 1 positive culture during the last 3 months of therapy) was inconsistently done because quality assurance was not standardized between studies. This, too, could lead to a misclassification bias. Moreover, in some studies, more slow acetylators developed neurotoxicity and were categorized as failures. Thus, the studies were biased against slow acetylators. Finally,

it has been argued that the “modern” regimen is optimized and the effects of acetylation status are thus unimportant. However, under the DOTS program, and even with stringent study conditions, therapy fails in many patients in high-burden countries in Africa [2, 3, 5]. For example, as many as half of all patients failed to convert after 2 months of DOTS in recent controlled trials [2, 3]. Clearly, the modern regimen is not optimized with respect to treatment of these patients. Moreover, our findings show that effects of acetylation status are relatively independent of the number or types of drugs in combination with isoniazid. Thus, despite these limitations, it can still be concluded from these meta-analyses that both microbiological failure and ADR were more frequently encountered at the end of therapy among rapid acetylators than among slow acetylators, regardless of the regimens compared.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/our_journal/cid). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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