Model-Based Assessment of Variability in Isoniazid Pharmacokinetics and Metabolism in Patients Co-Infected With Tuberculosis and HIV: Implications for a Novel Dosing Strategy

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Tuberculosis is the most common cause of death in HIV-infected patients. Isoniazid is used as a first-line drug to treat tuberculosis infection. However, variability in isoniazid pharmacokinetics can result in hepatotoxicity or treatment failure. Determination of clinical factors affecting isoniazid pharmacokinetics and metabolic pathways in HIV co-infected patients is therefore critical. Plasma levels of isoniazid, acetyl-isoniazid, and isonicotinic acid from 63 patients co-infected with tuberculosis and HIV were analyzed by liquid chromatography with tandem mass spectrometry followed by nonlinear mixed-effects modeling. Patients were genotyped to determine acetylator status. Patients were either on concomitant efavirenz-based antiretroviral therapy or HIV treatment naïve. Clearances of isoniazid were 1.3-fold and 2.3-fold higher in intermediate and rapid acetylators, respectively, compared with slow acetylators. Patients on concomitant efavirenz-based antiretroviral therapy had 64% and 80% higher population predicted clearances of acetyl-isoniazid and isonicotinic acid, respectively, compared with patients who were HIV treatment naïve. Both sex and CD4 cell count affected the bioavailability of isoniazid. Variability in isoniazid exposure could be reduced by dose adaptions based on acetylator type and sex in addition to the currently used weight bands. A novel dosing strategy that has the potential to reduce isoniazid-related toxicity and treatment failure is presented.

Mycobacterium tuberculosis continues to be a global health concern, and tuberculosis (TB) infection is the most common cause of death in HIV co-infected patients. Isoniazid (INH) is part of a first-line multidrug regimen in the treatment of TB. INH primarily undergoes metabolic elimination resulting in the formation of acetyl-isoniazid (ACINH) and isonicotinic acid (INA).1,2 INH is also eliminated through other pathways, such as hydrazine formation and renal excretion. ACINH is formed via acetylation of INH by polymorphic N-acetyltransferase 2 (NAT2) and is further metabolized to acetyl-hydrazine and INA.3 INA and hydrazine are formed via hydrolysis of INH.4 Both hydrazine and acetyl-hydrazine have been proposed to be involved in the hepatotoxicity related to INH administration.5,6

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The pharmacokinetics of isoniazid have previously been described. Due to the high variability in isoniazid exposure, dosing based on acetylator status has been clinically tested to decrease the incidence of treatment failure and toxicity.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study investigated the clinical factors responsible for the variability in isoniazid pharmacokinetics and metabolism in patients co-infected with tuberculosis (TB) and HIV. Furthermore, the study investigated how the current dose regimen can be modified to reduce the variability in isoniazid exposure.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This is the first population pharmacokinetic study of isoniazid and its major metabolites in patients co-infected with TB and HIV. Several factors affecting exposure of drug and metabolites were identified. Acetylator status and sex were identified as clinically significant, and a new individual-based dose regimen is proposed.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The suggested dose regimen may reduce incidence of treatment failure and adverse events during first-line TB treatment in patients co-infected with TB and HIV.
Population pharmacokinetic modeling is an effective tool to evaluate potential clinical factors which can explain pharmacokinetic variability. Previously, one population pharmacokinetic model simultaneously describing the pharmacokinetics of INH, ACINH, and INA has been published. However, the study was conducted in healthy Asians, and population differences such as genetics, disease, and drug–drug interactions can affect pharmacokinetic parameters and result in differences in both drug exposure and metabolic pathways. A study conducted in African patients showed a reduction in INH exposure when INH was administered together with antiretroviral therapy (ART). Results from a study by Chirchwa et al. indicated that concomitant treatment with ART only has an effect on INH exposure in NAT2 rapid metabolizers. Furthermore, weight, disease, and sex have been described to contribute to variability in INH pharmacokinetics.

Due to hepatotoxicity, treatment failure, and risk of drug resistance, a target INH maximum concentration of 3–6 mg/L (22–44 µM) has been suggested. Furthermore, 90% of early bactericidal activity has been associated with an area under the concentration-time curve from time zero to 24 hours (AUC0-24h) above 10.5 hour*mg/L. A study conducted in healthy Asians, and population differences such as genetics, disease, and drug–drug interactions can affect pharmacokinetic parameters and result in differences in both drug exposure and metabolic pathways. A study conducted in African patients showed a reduction in INH exposure when INH was administered together with antiretroviral therapy (ART). Results from a study by Chirchwa et al. indicated that concomitant treatment with ART only has an effect on INH exposure in NAT2 rapid metabolizers. Furthermore, weight, disease, and sex have been described to contribute to variability in INH pharmacokinetics.

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METHODS
Patients and study design
The open-label, observational clinical study was conducted in four different sites in Rwanda. Patient inclusion criteria defined in the study protocol were 21–65 years of age, HIV antibody positive, TB drug naïve, and metabolic pathways. A study conducted in African patients showed a reduction in INH exposure when INH was administered together with antiretroviral therapy (ART). Results from a study by Chirchwa et al. indicated that concomitant treatment with ART only has an effect on INH exposure in NAT2 rapid metabolizers. Furthermore, weight, disease, and sex have been described to contribute to variability in INH pharmacokinetics.

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Dose intake and sample collection
Blood samples from patients were collected predose and at 1, 2, 3, 4, 6, and 8 hours after dose. No restriction regarding food intake in proximity to dose was defined in the study protocol. Plasma was harvested by centrifugation of the samples and stored at −30°C for 1 week in the clinic before being transferred to −80°C.

Genotyping
Genotyping of cytochrome P450 (CYP) single nucleotide polymorphisms (SNPs) CYP1A2 (739 T>G, 163 C>A, and 2159 G>A), CYP2A6 (1436 G>T, 1093 G>A, and 48 T>G), CYP2B6 (516 G>T and 983 T>C), CYP3A4 (392 A>G), and CYP3A5 (6986 A>G) was performed by polymerase chain reaction, and the results have been described elsewhere.

DNA extraction from blood samples was carried out using QIAamp DNA blood kit (Qiagen, Hilden, Germany) for genotyping of NAT2 (282 C>T, 803 A>G, 481 C>T, 590 G>A, 857 G>A, and 341 T>C) SNPs. Genotyping was performed by multiplexed primer extension chemistry of an iPLEX assay (Agena Bioscience, San Diego, CA) with detection of the incorporated allele by mass spectrometry with a MassARRAY analyzer (Agena Bioscience). The output data was converted to genotype data using Typser software (Agena Bioscience). The genotyped SNPs were all in Hardy Weinberg equilibrium (P > 0.05) and the genotyping success was >99%. Based on NAT2 genotypes, patients were classified into slow, intermediate, or rapid acetylators using an algorithm published by Kuznetsov et al.

Drug quantification
Plasma concentrations of INH, ACINH, and INA were quantified simultaneously by liquid chromatography with tandem mass spectrometry. In short, the method was validated according to US Food and Drug Administration (FDA) guidelines in the concentration range of 80–10,000 ng/mL for INH and INA and 40–5,000 ng/mL for ACINH. The method exhibited adequate accuracy (89–110%) and precision (<10 % relative standard deviation) for INH and the two metabolites. No signal interference from coadministered drugs rifampicin, pyrazinamide, ethambutol, efavirenz, lamivudine, zidovudine, or tenofovir was detected.

Pharmacokinetic analysis
Pharmacokinetic data of INH, ACINH, and INA was analyzed using nonlinear mixed effects modeling in NONMEM software, version 7.4.3

| No. of patients | Concurrent HIV treatment | HIV treatment naive |
|-----------------|-------------------------|-------------------|
| 23              | 40                      |
| Age (years)     | 40 (26–57)              | 38 (21–52)        |
| Weight (kg)     | 48 (35–65)              | 50 (30–68)        |
| Serum creatinine (µmol/L) | 71 (44–159) | 66 (35–159) |
| Creatinine clearance (mL/min) | 81 (30–155) | 84 (38–155) |
| Aspartate aminotransferase (U/mL) | 33 (11–248) | 34 (11–131) |
| Alanine aminotransferase (U/mL) | 36 (9–126) | 30 (5–101) |
| CD4 cell count (/mm³) | 230 (21–716) | 240 (6–524) |
| Sex (female/male) | 10/13                  | 16/24             |
| Dose (mg/day)   | 150 (n = 1); 150 (n = 4); 225 (n = 13); 225 (n = 22); 300 (n = 9) | 300 (n = 14) |

Continuous data given as median (range). Categorical data given as counts. TB, tuberculosis.
First-order conditional estimation with interaction was used to fit the models to observed concentration-time data. Observations below the lower limit of quantification (n = 2, 15, and 7 for INH, ACINH, and INA, respectively) were excluded. Perl-Speaks-NONMEM version 4.8.1 (Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) with Xpose4 package version 4.6.1 (Uppsala University) were used for interaction with NONMEM, model automation, model development tracking, and model diagnostics. The structural identifiability analysis of the pharmacokinetic model was done in Wolfram Mathematica version 11.2 (Champaign, IL).

Model development was performed in a stepwise manner where the structural model for INH was developed before adding the metabolites. One-compartmental, two-compartmental, and three-compartmental disposition models with first-order absorption and elimination were applied to INH observations. Discrimination between nested models was based on a drop in objective function values (OFVs). OFV is considered χ² distributed, and a decrease in OFV by −3.84 and −6.63 implies a significant model improvement by P < 0.05 and P < 0.01, respectively. Lag time, an estimated number of transit compartments, and fixed numbers of transit compartments with and without an estimated absorption rate constant were tested to describe the absorption of INH. Due to the impact of NAT2 on INH clearance, acetylator type (slow, intermediate, or rapid) was incorporated during the development of the structural model. Allometric scaling by body weight normalized by the population median was applied to all clearance and volume parameters by a factor of 0.75 and 1, respectively. INH was assumed to be eliminated via either two or three metabolic pathways where two parallel pathways were responsible for the formation of ACINH and INA. The fraction of total INH clearance responsible for the respective pathways was estimated. Bioavailability was added to the models fixed at 1 with an estimated interindividual variability.

One-compartment and two-compartment disposition models were tested to describe the pharmacokinetics of ACINH and INA. ACINH was assumed to be eliminated via two pathways with one pathway leading to the formation of INA. Separate residual errors were estimated for INH, ACINH, and INA. Additive, proportional, or additive and proportional error models were tested to describe the residual errors. Interindividual variabilities were added as exponential random effects on all parameters of the final model followed by stepwise exclusion of interindividual variabilities that could not be estimated. The exact arithmetic rank (EAR) approach was used to determine whether the final model, both with and without fixed volumes of ACINH and INA, was structurally identifiable.23

Following the development of a structural model, the effects of potential covariates were tested on the pharmacokinetic parameters of INH, ACINH, and INA. Covariates were added in a stepwise manner followed by a stepwise elimination of the included covariates. Covariates were included in the model if OFV was reduced by 3.84 in the addition step and retained during the elimination step if the OFV increased by 10.8 corresponding to a χ² value of < 0.001 for a χ² distribution. Continuous covariates tested were alanine aminotransferase, aspartate aminotransferase, age, serum creatinine, creatinine clearance as determined by the Cockcroft and Gault equation,24 and CD4 cell count. The continuous covariates were centered on their respective median. Categorical covariates tested were sex, the presence or absence of HIV drugs (study arm), and CYP1A2 (739 T>G, 163 C>A and 2159 G>A), CYP2A6 (1436 G>T, 1093 G>A and 48 T>G), CYP2B6 (516 G>T and 983 T>C), CYP3A4 (392 A>G), and CYP3A5 (6986 A>G) genotypes.

The final model was evaluated by visual predictive check (n = 1000), goodness-of-fit plots, and parameter plausibility. Sampling importance resampling (M/m = 5000/1000)25 was performed to determine precision of the parameter estimates and to calculate 95% confidence intervals. The covariance output was used as proposal without an inflation factor. Stochastic simulations (n = 200) based on estimated parameters and variabilities of the final model were performed to predict the influence of significant covariates for the typical individual weighting 50 kg (the median weight in the data set) on INH exposure. An area under the concentration-time curve from time zero to 24 hours (AUC₀₋₂₄h) of 10.5 hour·mg/L was used as a cutoff value for adequate therapeutic effect.

RESULTS

In total, 432, 412, and 423 plasma concentration–time observations of INH, ACINH, and INA, respectively, from 63 individuals were used in the present study. Fifty-six patients were genotyped for NAT2 SNPs. Results from the genotyping are summarized in Table 2. Acetylator phenotypes for the remaining seven patients were determined by AUC_{ACINH,0-8h}/AUC_{INH,0-8h} ratio and post-checked for acetylator type plausibility depending on INH oral clearance (CLp). The distribution of slow, intermediate, and rapid acetylators in the present population were 28 (44%), 30 (48%), and 5 (8%), respectively.

A two-compartment disposition model with first-order elimination modeled as three elimination pathways described the pharmacokinetics of INH adequately. The addition of a third elimination pathway (F_{other}) calculated from the results published by Peretti et al.26 did not significantly improve the model. However, due to the existing knowledge on INH elimination1,2,26 it was included in the final model since simulations with F_{other} fixed at 0 did not significantly impact the AUCs derived by the model and therefore

| Genotype | Allele | No. (%) |
|----------|--------|---------|
| 282 C>T² | C/C    | 24 (44) |
|          | C/T    | 26 (48) |
|          | T/T    | 4 (8)   |
| 341 T>C  | T/T    | 16 (28) |
|          | T/C    | 30 (54) |
|          | C/C    | 10 (18) |
| 418 C>T  | C/C    | 18 (32) |
|          | C/T    | 31 (55) |
|          | T/T    | 7 (13)  |
| 590 G>A  | G/G    | 34 (60) |
|          | G/A    | 21 (38) |
|          | A/A    | 1 (2)   |
| 803 A>G  | A/A    | 8 (14)  |
|          | A/G    | 31 (55) |
|          | G/G    | 17 (31) |
| 857 G>A  | G/G    | 55 (100)|

TB, tuberculosis. *n = 54, **n = 55.
did not affect dose optimization. The absorption of INH was best described by first-order absorption with one transit compartment. ACINH and INA observations were adequately fitted by one-compartment disposition models with first-order elimination. The distribution volumes of both metabolites were fixed to 17 L, a previously estimated volume for ACINH, to ensure identifiability of the structural model. Results and code from the exact arithmetic rank evaluation are attached in the Supplementary Material S1. Residual errors were best modeled as proportional errors for INH, ACINH, and INA. A schematic diagram of the final model is depicted in Figure 1, and the NONMEM code for the final model is attached in Supplementary Material S2.

Rapid and intermediate metabolizers with regard to NAT2 had a 2.3-fold and 1.3-fold higher conversion of INH to ACINH, respectively, compared with slow metabolizers. The presence of ART significantly increased the population predicted clearances of ACINH (CL_A) and INA (CL_I) by 64% and 80%, respectively. Furthermore, bioavailability of INH (F) was affected by both sex and CD4 cell count where females had 37% higher relative F compared with males. The estimated parameters of the final model are summarized in Table 3.

The visual predictive check in Figure 2 illustrates the predictive adequacy of the model. Both the population prediction and the individual predictions described the observations well (Supplementary Material S3). The epsilon-shrinkage was 22%, indicating reliable assessment of model diagnostics.

### Table 3 Primary parameters of isoniazid, acetyl-isoniazid, and isonicotinic acid in adult TB/HIV co-infected patients estimated by the final pharmacokinetic model

| Parameter | Population mean (%RSE) | 95% Confidence interval | % IV (%RSE) |
|-----------|------------------------|------------------------|-------------|
| **Isoniazid** |                         |                        |             |
| CL_p slow acetylator (L/h) | 9.2 (9.6) | 7.7–11.2 | 82.7 (17.5) |
| F_s slow acetylator | 0.17 (11.1) | 0.14–0.21 | 72.9 (21.1) |
| Effect of intermediate acetylator on CL_p and F_s | 0.32 (40.1) | 0.08–0.58 |             |
| Effect of rapid acetylator on CL_p and F_s | 1.29 (34.1) | 0.56–2.28 |             |
| V_c (L) | 41.3 (7.2) | 36.1–47.5 | – |
| MTT (hour) | 0.58 (15.5) | 0.41–0.78 | 180.6 (21.7) |
| F | 1 fix | – | 27.2 (23.2) |
| Q (L/h) | 10.8 (18.7) | 7.4–15.4 | 120.6 (38.2) |
| V_p (L) | 42.8 (17.3) | 30.3–59.1 | – |
| F_other rapid acetylator | 0.1 fix | – | – |
| F_other intermediate acetylator | 0.13 | – | – |
| F_other slow acetylator | 0.2 | – | – |
| Effect of CD4 on F | −0.0009 (21.7) | −0.0005 to −0.001 | – |
| Effect of female sex on F | 0.37 (28.0) | 0.20–0.61 | – |
| **Acetyl-isoniazid** |                         |                        |             |
| CL_A (L/h) | 5.5 (6.3) | 4.8–6.2 | – |
| Effect of ART on CL_A | 0.64 (23.5) | 0.38–0.96 | – |
| V_A (L) | 17 fix | – | – |
| F_A | 0.19 (49.0) | 0.05–0.40 | – |
| **Isonicotinic acid** |                         |                        |             |
| CL_I (L/h) | 7.2 (10.8) | 6.0–8.9 | 38.5 (34.6) |
| Effect of ART on CL_I | 0.8 (30.0) | 0.39–1.34 | – |
| V_I (L) | 17 fix | – | – |
| Residual errors |                        |                        |             |
| Proportional, isoniazid | 0.34 (5.8) | 0.31–0.38 | – |
| Proportional, acetyl-isoniazid | 0.12 (11.8) | 0.09–0.14 | – |
| Proportional, isonicotinic acid | 0.15 (7.3) | 0.13–0.18 | – |

This table summarizes the primary parameters of isoniazid, acetyl-isoniazid, and isonicotinic acid in adult TB/HIV co-infected patients estimated by the final pharmacokinetic model.
Simulations performed with the final model predicted rapid acetylators of both sexes to be underexposed, whereas female slow acetylators had an excessive exposure. Optimization of the dose regimen showed that a dose increase for intermediate and rapid acetylators would enable the majority of patients to reach therapeutic levels. Moreover, a dose decrease by 33% in females would reduce the risk of toxic exposure to INH. Figure 3 shows simulations of exposure following a standard INH dose and the optimized dose for a typical individual. The individual-based dose regimen can be described by the following equation:

$$D_{\text{opt}} = (\text{Sex} \times 5 \text{mg/kg} + \text{NAT2} \times 1.5 \text{mg/kg}) \times \text{WT}_{i}$$

where \( \text{Sex} \) is a factor of 1 for males and 0.67 for females, \( \text{NAT2} \) is a trichotomous value of 0 for slow acetylators, 1 for intermediate acetylators, and 3 for rapid acetylators, and \( \text{WT}_{i} \) is the individual weight.

**DISCUSSION**

The present study is the first study to evaluate the population pharmacokinetics of INH and its major metabolites in TB/HIV co-infected patients. Furthermore, this is the first study to simultaneously evaluate the effects of genetic polymorphisms in NAT2 and CYP450 enzymes, concomitant ART, and HIV on INH pharmacokinetics. Based on the present findings, a novel individual-based dosing strategy is suggested.

A two-compartment model including first-order absorption and elimination was found to adequately describe the population pharmacokinetics of INH. The pharmacokinetics of the two metabolites ACINH and INA were both best described by one-compartment models with first-order elimination, respectively. The results are in line with previous compartmental analysis of INH and INA pharmacokinetics.\(^8,11\) However, two-compartment models have previously been described for ACINH.\(^8,10\) A plausible
Acetylator phenotype is a well-established metabolic effector of INH clearance. Different CLP for slow, intermediate, and rapid acetylators were estimated in the model. Since only one metabolic pathway is affected by NAT2, the fraction of total CLP resulting in ACINH formation is expected to be higher in rapid acetylators, although our estimate is lower than previously reported. The results published by Ellard et al. indicated that the route of formation for INA is extensively via ACINH formation in rapid acetylators. This is contradictory to our estimated fraction of INH CLP forming ACINH ($F_r$), and in extension, $1 - (F_r + F_{\text{other}})$ representing the formation of INA from INH in our model. However, the quoted study was conducted in healthy volunteers, and drug metabolism is likely to differ between the populations due to genetic differences and HIV status.

The population CLP for rapid, intermediate, and slow metabolizers with regard to NAT2 were 21.1, 12.1, and 9.2 L/hour respectively. The clearances in this population are similar to INH clearances reported in South Africans (rapid: 21.6 L/hour, slow: 9.7 L/H) and West Africans (rapid: 35.9 L/hour, slow: 6.6 L/hour). However, clearances for rapid and slow acetylators in both the South African and West African cohorts were estimated with mixture models. INH has several elimination pathways that could potentially be affected by genetic diversity. Hence, there could be an overestimation of rapid acetylators due to extensive elimination other than acetylation when mixture models are used.

Females had 37% higher relative bioavailability ($F$) compared with males. These results are in agreement with higher INH concentrations in females previously reported. In addition, CD4 cell count had a significant effect on $F$. Studies have shown decreased plasma concentrations of antitubercular drugs in HIV-infected patients. However, in this study, $F$ for INH decreased with increasing CD4 cell count. Whether this could be explained by changes in absorption or in metabolic functions remains unclear.

Efavirenz is mainly metabolized via CYP2B6 and CYP2A6, and slow metabolizers with regard to NAT2 and CYP2B6 have elevated plasma levels of efavirenz when TB drugs are concomitantly administered. INH has been shown to inhibit CYP2A6 resulting in increased exposure to efavirenz in CYP2B6 slow metabolizers. The results presented here suggest that efavirenz-based ART increases ACINH and INA clearances resulting in lower exposure of the metabolites. To our knowledge, no such effect has previously been reported. Hoffmann et al. reported a higher incidence of hepatotoxicity in an African cohort when HIV and TB were treated simultaneously compared with when HIV was treated alone. A mechanism has been proposed, advocating hydrazine as a main inducer of hepatotoxicity during concomitant therapy with efavirenz and INH. Since INH-induced hepatotoxicity is related to hydrazine and acetyl-hydrazine, products of INH, ACINH, and INA metabolism, changes in ACINH and INA elimination could result in different toxicity outcomes in patients treated for both HIV and TB. However, further studies are required to determine such effects on toxicity outcome.

A target AUC of 10.5 hour*mg/L for INH has been suggested since it achieves 90% of maximum bactericidal activity. Simulations predicted male intermediate acetylators and rapid acetylators of both sexes to be underexposed whereas female slow acetylators were predicted to be overexposed with regard to the suggested threshold. A randomized controlled trial showed that
NAT2 genotype-guided INH therapy reduces incidence of both hepatotoxicity and early treatment failure in patients. In the study, INH doses were based on acetylator type with a total body weight cutoff value of 40 kg. Jung et al. used a similar approach with fixed doses dependent on NAT2 type and weight. We present a new INH dose regimen for TB/HIV co-infected patients based on both acetylator type and sex incorporated in the currently used weight-guided dose regimen. Simulations of INH AUC0-24h showed that the proposed dose regimen was superior to standard weight-based dosing by lowering the dose in female patients in addition to adjusting the dose depending on NAT2 type. The described dose regimen requires clinical testing with recorded outcomes, such as hepatotoxicity and treatment failure. However, previous NAT2 genotype-guided dosing strategies have shown promising results.

The suggested dosing regimen has the potential to improve first-line TB therapy in TB/HIV co-infected patients and, in extension, patients infected with TB alone. Since it is based on prediction of acetylator phenotype in any given individual, it is independent of how its frequency distribution in various populations differ. Furthermore, since the described algorithm does not account for HIV treatment, it could be applied in TB/HIV patients independent of the ART used. The proposed dosing strategy is restricted by resources to determine acetylator status. However, genotype-based tailoring of antitubercular therapy could be used in hard-to-treat patients even if resources are limited.

The present study was limited by the relatively low number of patients, especially with regard to rapid acetylators. However, the population-estimated CLp for rapid and slow acetylators and the higher exposure in females are in line with previous studies. Hence, the suggested individual-based dosing strategy for INH offers a starting point for clinical testing but may require further adaptation.

In conclusion, acetylator type, sex, and CD4 cell count affected the pharmacokinetics of INH. ACINH and INA clearances were higher in patients on concomitant ART. A new optimized dose regimen for INH based on NAT2 type and sex is described. The optimized dose regimen could reduce the variability in INH exposure and therefore has the potential to increase therapeutic success rate and reduce the incidence of toxic effects of the first-line TB treatment in patients co-infected with TB and HIV.

Supporting Information
Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

Text S1.
Supplementary Material S1.
Figure S1–S3.

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Conflict of Interest
The authors declared no competing interests for this work.

Author Contributions
J.S., E.B., S.B., A.Ä., D.J., and M.A. wrote the manuscript. J.S., E.B., S.B., A.Ä., and M.A. designed the research. J.S., E.B., and D.J. performed the research. J.S., S.B., A.Ä., and M.A. analyzed the data.

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1. Ellard, G.A. & Gammon, P.T. Pharmacokinetics of isoniazid metabolism in man. J. Pharmacokinet. Biopharm. 4, 83–113 (1976).
2. Boxenbaum, H.G. & Riegelman, S. Pharmacokinetics of isoniazid and some metabolites in man. J. Pharmacokinet. Biopharm. 4, 287–325 (1976).
3. Wang, P., Pradhan, K., Zhong, X.-B. & Ma, X. Isoniazid metabolism and hepatotoxicity. Acta Pharm. Sin. B 6, 384–392 (2016).
4. Preziosi, P. Isoniazid: metabolic aspects and toxicological correlates. Curr. Drug Metab. 8, 839–851 (2007).
5. Woo, J., Chan, C.H., Walubo, A. & Chan, K.K. Hydrazine—a possible cause of isoniazid–induced hepatic necrosis. J. Med. 23, 51–59 (1992).
6. Woodward, K.N. & Timbrell, J.A. Acetylhylzine hepatotoxicity: the role of covalent binding. Toxicology 30, 65–74 (1984).
7. Tafazoli, S., Meshreghi, M. & O’Brien, P.J. Role of hydrazine in isoniazid-induced hepatotoxicity in a hepatocyte inflammation model. Toxicol. Appl. Pharmacol. 229, 94–101 (2008).
8. Seng, K.-Y., Hee, K.-H., Soon, G.-H., Chew, N., Kho, S.H. & Lee, L.-S.U. Population pharmacokinetic analysis of isoniazid, acetylisoniazid, and isonicotinic acid in healthy volunteers. Antimicrob. Agents Chemother. 59, 6791–6799 (2015).
9. Bhatt, N.B. et al. Pharmacokinetics of rifampin and isoniazid in tuberculosis-HIV-coinfected patients receiving nevirapine- or efavirenz-based antiretroviral treatment. Antimicrob. Agents Chemother. 58, 3182–3190 (2014).
10. Chirehwa, M.T. et al. Effect of efavirenz-based antiretroviral therapy and high-dose rifampin on the pharmacokinetics of isoniazid and acetylisoniazid. J. Antimicrobial Chemotherapy 74, 139–148 (2019).
11. Wilkins, J.J., Langdon, G., McIlerson, H., Pillai, G., Smith, P.J. & Simonsson, U.S.H. Variability in the population pharmacokinetics of isoniazid in South African tuberculosis patients. Br. J. Clin. Pharmacol. 72, 51–62 (2011).
12. McIlerson, H. et al. Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: implications for international dosing guidelines. Antimicrob. Agents Chemother. 56, 3232–3238 (2012).
13. Babalik, A. et al. Plasma concentrations of isoniazid and rifampin are decreased in adult pulmonary tuberculosis patients with diabetes mellitus. Antimicrob. Agents Chemother. 57, 5740–5742 (2013).
14. Alsultan, A. & Peloquin, C.A. Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs 74, 839–854 (2014).
15. Donald, P.R. et al. The influence of dose and N-acetyltransferase-2 (NAT2) genotype and phenotype on the pharmacokinetics and pharmacodynamics of isoniazid. Eur. J. Clin. Pharmacol. 63, 633–639 (2007).
16. Bienvenu, E., Ashton, M. & Abélo, A. Influence of CYP2B6 516G > T and long term HAART on population pharmacokinetics of
efavirenz in Rwandan adults on HIV and tuberculosis cotreatment. *Pharmacol. Pharm.* **6**, 533–546 (2015).

17. World Health Organization. Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. <https://www.who.int/hiv/pub/guidelines/arv2013/en/> (2013).

18. Bienvenu, E. et al. Frequencies of single nucleotide polymorphisms in cytochrome P450 genes (CYP1A2, 2A6, 2B6, 3A4 and 3A5) in a Rwandan population: difference to other African populations. *Curr. Pharm. Personal. Med.* **11**, 237–246 (2013).

19. Gabriel, S., Ziaugra, L. & Tabbaa, D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protocol. Human Genet.* **60**, (2009).

20. Kuznetsov, I.B., McDuffie, M. & Moslehi, R. A web server for inferring the human N-acetyltransferase-2 (NAT2) enzymatic phenotype from NAT2 genotype. *Bioinformatics* (Oxford, England) **25**, 1185–1186 (2009).

21. Sundell, J., Bienvenu, E., Birgersson, S., Ábeló, A., Ashton, M. & Hoffmann, K.J. Simultaneous quantification of four first line antitubercular drugs and metabolites in human plasma by hydrophilic interaction chromatography and tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **1105**, 129–35 (2019).

22. Beal, S., Boeckmann, A. & Sheiner, L. *NONMEM Users Guides* (Icon Development Solutions, Elicott City, MD, 1989–2009).

23. Karlsson, J., Angelova, M. & Jirstrand, M. An efficient method for structural identifiability analysis of large dynamic systems. *IFAC Proc.**45**, 941–946 (2012).

24. Cockcroft, D.W. & Gault, M.H. Prediction of creatinine clearance from serum creatinine. *Nephron* **16**, 31–41 (1976).

25. Dosne, A.G., Bergstrand, M., Harling, K. & Karlsson, M.O. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. *J. Pharmacokin. Pharmacodyn.* **43**, 583–596 (2016).

26. Peretti, E., Karlaganis, G. & Lauterburg, B.H. Increased urinary excretion of toxic hydrazine metabolites of isoniazid by slow acetylators. Effect of a slow-release preparation of isoniazid. *Eur. J. Clin. Pharmacol.* **33**, 283–286 (1987).

27. Rajman, I., Knapp, L., Morgan, T. & Masimirembwa, C. African genetic diversity: implications for cytochrome P450-mediated drug metabolism and drug development. *EBioMedicine* **17**, 67–74 (2017).

28. Jones, A.E. et al. Variability in drug metabolizing enzyme activity in HIV-infected patients. *Eur. J. Clin. Pharmacol.* **66**, 475–485 (2010).

29. Mcileron, H., Wash, P., Burger, A., Norman, J., Folb, P.I. & Smith, P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrob. Agents Chemother.* **50**, 1170–1177 (2006).

30. Ray, J., Gardiner, I. & Marriott, D. Managing antituberculosis drug therapy by therapeutic drug monitoring of rifampicin and isoniazid. *Intern. Med. J.* **33**, 229–234 (2003).

31. Peloquin, C.A. et al. Low antituberculosis drug concentrations in patients with AIDS. *Ann. Pharmacotherapy* **30**, 919–925 (1996).

32. Gurumurthy, P. et al. Decreased bioavailability of rifampin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease. *Antimicrob. Agents Chemother.* **48**, 4473–4475 (2004).

33. Kwara, A., Larney, M., Sagoe, K.W.C., Kenu, E. & Court, M.H. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS (London, England)* **23**, 2101–2106 (2009).

34. Ogburn, E.T., Jones, D.R., Masters, A.R., Xu, C., Guo, Y. & Desta, Z. Efavirenz primary and secondary metabolism in vitro and in vivo: identification of novel metabolic pathways and cytochrome P450 2A6 as the principal catalyst of efavirenz 7-hydroxylation. *Drug Metab. Dispos.* **38**, 1218–1229 (2010).

35. Luetkemeyer, A.F. et al. Combined effect of CYP2B6 and NAT2 genotype on plasma efavirenz exposure during rifampin-based antituberculosis therapy in the STRIDE study. *Clin. Infect. Dis.* **60**, 1860–1863 (2015).

36. Court, M.H. et al. Isoniazid mediates the CYP2B6*6 genotype-dependent interaction between efavirenz and antituberculosis drug therapy through mechanism-based inactivation of CYP2A6. *Antimicrob. Agents Chemother.* **58**, 4145–4152 (2014).

37. Hoffmann, C.J. et al. Hepatotoxicity in an African antiretroviral therapy cohort: the effect of tuberculosis and hepatitis B. *AIDS (London, England)* **21**, 1301–1308 (2007).

38. Lee, I.K. & Boelsterli, U.A. Bypassing the compromised mitochondrial electron transport with methylene blue alleviates efavirenz/isoniazid-induced oxidant stress and mitochondria-mediated cell death in mouse hepatocytes. *Redox. Biol.* **2**, 599–609 (2014).

39. Azuma, J. et al. NAT2 genotype guided regimen reduces isoniazid-induced liver injury and early treatment failure in the 6-month four-drug standard treatment of tuberculosis: a randomized controlled trial for pharmacogenetics-based therapy. *Eur. J. Clin. Pharmacol.* **69**, 1091–1101 (2013).

40. Jung, J.A. et al. A proposal for an individualized pharmacogenetic-guided isoniazid dosage regimen for patients with tuberculosis. *Drug Design Devel. Ther.* **9**, 5433–5488 (2015).

41. Calcagno, A. et al. The influence of pharmacogenetic variants in HIV/tuberculosis coinfected patients in Uganda in the SOUTH study. *Clin. Pharmacol. Ther.* **106**, 450–457 (2019).