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PHARMACOLOGY

Low Antituberculosis Drug Concentrations in HIV-Tuberculosis-Coinfected Adults with Low Body Weight: Is It Time To Update Dosing Guidelines?

Christine Sekaggya-Wiltshire,[a] Maxwell Chirehwa,[b] Joseph Musaazi,[a] Amrei von Braun,[c] Allan Buzibye,[a] Daniel Muller,[d] Ursula Gutteck,[d] Ilaria Motta,[e] Andrea Calcagno,[e] Jan S. Fehr,[c] Andrew Kambugu,[a] Barbara Castelnuovo,[a] Mohammed Lamorde,[a] Paolo Denti[b]

[a]Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda
[b]Department of Pharmacology, University of Cape Town, Cape Town, South Africa
[c]Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Zurich, University of Zurich, Zurich, Switzerland
[d]Department of Clinical Chemistry, University Hospital Zurich, University of Zurich, Zurich, Switzerland
[e]Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy

ABSTRACT Antituberculosis drugs display large pharmacokinetic variability, which may be influenced by several factors, including body size, genetic differences, and drug-drug interactions. We set out to determine these factors, quantify their effect, and determine the dose adjustments necessary for optimal drug concentrations. HIV-infected Ugandan adults with pulmonary tuberculosis treated according to international weight-based dosing guidelines underwent pharmacokinetic sampling (1, 2, and 4 h after drug intake) 2, 8, and 24 weeks after treatment initiation. Between May 2013 and November 2015, we enrolled 268 patients (148 males) with a median weight of 53.5 (interquartile range [IQR], 47.5 to 59.0) kg and a median age of 35 (IQR, 29 to 40) years. Population pharmacokinetic modeling was used to interpret the data and revealed that patients weighing <55 kg achieved lower concentrations than those in higher weight bands for all drugs in the regimen. The models predicted that this imbalance could be solved with a dose increment of one fixed-dose combination (FDC) tablet for the weight bands of 30 to 37 and 38 to 54 kg. Additionally, the concomitant use of efavirenz increased isoniazid clearance by 24.1%, while bioavailability and absorption of rifampin and isoniazid varied up to 30% in patients on different formulations. Current dosing guidelines lead to lower drug exposure in patients in the lower weight bands. Simply adding one FDC tablet to current weight band-based dosing would address these differences in exposure and possibly improve outcomes. Lower isoniazid exposures due to efavirenz deserve further attention, as does the quality of currently used drug formulations of anti-TB drugs. (This study has been registered at ClinicalTrials.gov under identifier NCT01782950.)

KEYWORDS antitubercular drugs, Monolix, pharmacokinetics, pharmacometrics

In 2017, 10 million people worldwide were diagnosed with tuberculosis (TB), and 1.5 million died (1). Over 25% of these deaths were among HIV-infected patients, who have a higher risk of more severe disease and worse treatment outcomes. Several studies have reported suboptimal rifampin and isoniazid concentrations and a high level of variability in the pharmacokinetics (PK) of anti-TB drugs among HIV-infected patients on TB treatment. This may contribute to unfavorable treatment responses, including death, relapse, and the emergence of resistant strains, which threaten the control of the TB pandemic (2–5). Anti-TB drug PK variability may be influenced by several factors, including age, body weight, sex, and genetic polymorphisms, such as

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Address correspondence to Christine Sekaggya-Wiltshire, csekaggya@idi.co.ug.

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TABLE 1 Baseline characteristics of TB-HIV-coinfected patients[a]

Characteristic	Value ($n = 254$)
No. of male patients (%)	157 (58.6)
Median age (yr) (IQR)	35 (29, 40)
Median wt (kg) (IQR)	52 (47.5–59)
Median BMI (kg/m^2) (IQR)	19.2 (17.7–21.5)
No. of patients with BMI $<$ 18 (in kg/m^2) (%)	74 (32.6)
Median CD4 count (cells/μl) (IQR)	163 (46, 298)
No. (%) of patients with NAT2 *rs1799930* SNP	
NAT2 *rs1799930* GG	106 (41.2)
NAT2 *rs1799930* GA	94 (37)
NAT2 *rs1799930* AA	9 (3.5)
Unknown	45 (17.7)
No. (%) of patients on formulation	
RHZE (intensive phase)	254
HR1 (continuation phase)	37 (14.6)
HR2 (continuation phase)	44 (17.3)
HR3 (continuation phase)	106 (41.7)

[a]RHZE, rifampin, isoniazid, pyrazinamide, and ethambutol; HR, isoniazid-rifampin fixed-dose combination; NAT2, *N*-acetyltransferase 2.

the *N*-acetyltransferase (NAT2) gene, which encodes the acetylation and, therefore, elimination of isoniazid (6, 7). In addition, TB-HIV-infected patients are often on several other comedications, especially antiretroviral therapy (ART), which may influence adherence and cause drug-drug interactions (8). The World Health Organization (WHO) recommends the use of fixed-dose combination (FDC) anti-TB medication to simplify treatment, encourage compliance, and ensure adequate dosage to limit the emergence of drug-resistant strains of TB (9). Some authors suggest that the use of FDCs from different manufacturers may contribute to the large PK variability observed within and between clinical studies; this may be due to differences in physiochemical properties of the FDCs that may lead to variation in absorption and bioavailability, even though the formulations have been certified to be bioequivalent (10–13). We set out to describe the pharmacokinetics of first-line anti-TB drugs in TB-HIV-coinfected patients and determine the factors that may influence inter- and intraindividual variability.

RESULTS

Between May 2013 and November 2015, 268 patients were enrolled in the SOUTH (Study on the Outcomes Related to Tuberculosis and HIV) study (ClinicalTrials.gov identifier NCT01782950), and 254 had drug concentration data available. Of these patients, 148 (58.3%) were male, and the overall median weight and age were 52.0 (interquartile range [IQR], 47.5 to 59.0) kg and 35 (IQR, 29 to 40) years, respectively. Table 1 shows the baseline characteristics of the study population. The median number of pharmacokinetic sampling visits (profiles) was 3 (IQR, 3 to 3). A small number of profiles contained only concentrations below the limit of quantification (BLQ) and had to be excluded from the analysis. After these exclusions, the data consisted of 249 patients contributing 673 profiles for rifampin (1,805 samples, with 122 [6.8%] BLQ), 251 patients contributing 678 profiles for isoniazid (1,814 samples, with 164 [9%] BLQ), 251 patients contributing 493 profiles for pyrazinamide (1,209 samples, with 13 [1.1%] BLQ), and 250 patients contributing 452 profiles for ethambutol (1,206 samples, with 55 [4.6%] BLQ).

Rifampin. Rifampin demonstrated one-compartment disposition, with first-order elimination and first-order absorption with a lag time. The best body size descriptor for allometric scaling was fat-free mass (FFM), which was applied to both clearance (CL) and volume. The final parameter estimates for rifampin are reported in Table 2, and a visual predictive check (VPC) demonstrating satisfactory model fit is shown in Fig. 1. The values for apparent clearance and volume of distribution (V) for a typical participant with an FFM of 43 kg were 12.6 liters/h and 58 liters, respectively. Rifampin clearance

TABLE 2 Parameter estimates of rifampin pharmacokinetics in TB-HIV-coinfected patients

Parameter[c]	Estimated value	Relative SE (%)
CL[a] (at 2 wk) (liters/h)	12.2	5
Change in CL on wk 8 or at 24 wk (%)	+20	26
V^a (liters)	58.0	4
K_a (reference formulations, RHZE and HR1) (liters/h)	1.99	
Effect of formulation HR2 on K_a (%)	+20.7	33
Effect of formulation HR3 on K_a (%)	+97.8	19
T_{lag} (liters/h)	0.83	3
F (reference formulation, RHZE and HR1)	1 (fixed)	
Effect of formulation HR2 on F (%)	−15.2	47
Effect of formulation HR3 on F (%)	+17.8	28
Proportional error (%)	19.8	8
Additive error (mg/liter)	0.665	8
Between-subject variability of CL[b] (%CV)	25.7	12
Between-subject variability of V^b (%CV)	30.1	14
Between-occasion variability of $K_a{}^b$ (%CV)	50.7	19
Between-occasion variability of $T_{lag}{}^b$ (%CV)	48.2	6
Between-occasion variability of F^b (%CV)	36.4	4

[a]Allometric scaling was used for CL and V, and values are reported for an adult with an FFM of 43 kg.
[b]The between-subject and between-occasion variabilities were assumed to be log-normally distributed and are reported as approximate coefficient of variation (%CV).
[c]HR, isoniazid and rifampin fixed-dose combination; RHZE, isoniazid, rifampin, ethambutol, and pyrazinamide fixed-dose combination; T_{lag}, absorption lag time.

was 20% faster at weeks 8 and 24 than at week 2. Both bioavailability (F) and absorption varied in patients on different formulations: formulation HR2 (isoniazid-rifampin fixed-dose combination 2) caused a 15.2% reduction in bioavailability, while formulation HR3 caused a 17.8% increase in bioavailability, compared to the formulation used in the

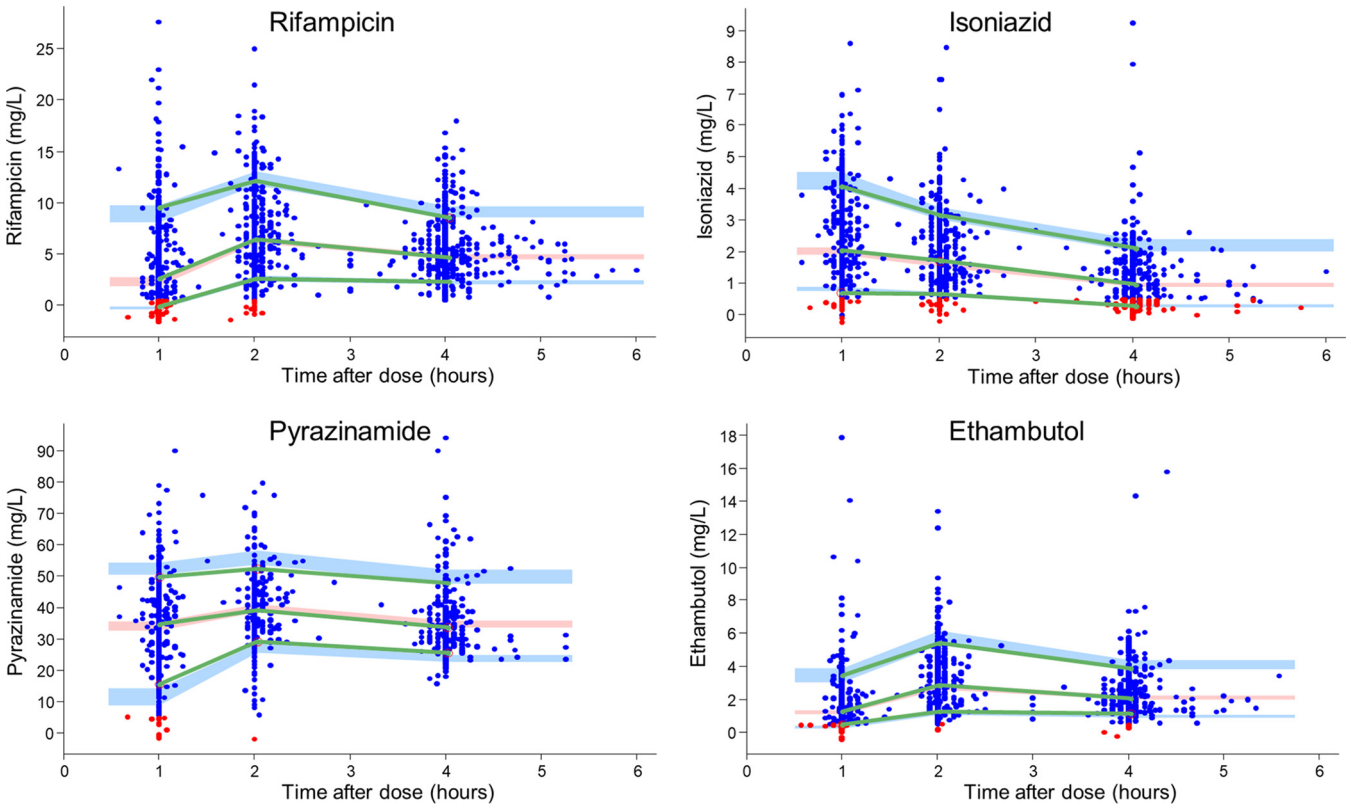

FIG 1 Visual prediction check for rifampin, isoniazid, ethambutol, and pyrazinamide. The dots represent the original data, and the middle line is the 50th percentile, while the upper and lower lines are the 5th and 95th percentiles of the original data. The shaded areas are the corresponding 95% confidence intervals for the same percentiles, as predicted by the model. The blue dots represent all observations above the limit of quantification, while the red ones represent the observation points that were below the limit of quantification (BLQ), as resimulated by the model.

TABLE 3 Parameter estimates of isoniazid pharmacokinetics in TB-HIV-coinfected patients

Description[d]	Estimated value	Relative SE (%)
CL[a] (liters/h) (reference group, NAT2 *rs1799930* GG)	22.8	8
Effect of NAT2 *rs1799930* GA on CL (%)	−26.3	36
Effect of NAT2 *rs1799930* AA on CL (%)	−74.6	28
Effect of efavirenz on CL (%)	+24.1	27
V_c[a] (liters)	64.1	7
K_a[a] (reference formulations, RHZE, HR1, and HR2) (liters/h)	1.73	13
Effect of formulation HR3 on K_a (%)	95.1	28
T_{lag} (liters/h)	0.25 (fixed)	
F (reference formulation, RHZE)	1 (fixed)	
Effect of formulation HR1 on *F* (%)	+26.9	30
Effect of formulation HR2 on *F* (%)	−15.2	49
Effect of formulation HR3 on *F* (%)	+19.3	23
V_p[a,b] (liters)	46.3	52
Q[a,b]	27.6	20
Proportional error (%)	30.6	5
Additive error (mg/liter)	0.172	24
Between-subject variability of CL[c] (%CV)	53.7	7
Between-subject variability of V[c] (%CV)	33.8	16
Between-occasion variability of K_a[c] (%CV)	73.1	12
Between-occasion variability of F[c] (%CV)	31.8	5
Between-occasion variability of T_{lag}[c] (%CV)	9.5	6

[a]Allometric scaling was used for CL, V_c, Q, and V_p, and values are reported for an adult with a weight of 52 kg.
[b]Q and V_p were estimated using Bayesian priors, with typical values of 16.1 liters/h and 16.5 liters, respectively, from a previous study (7), with 50% uncertainty.
[c]The between-subject and between-occasion variability were assumed to be log-normally distributed and are reported as approximate coefficients of variation (%CV).
[d]NAT2, *N*-acetyltransferase 2; K_a, absorption rate constant; HR, isoniazid and rifampin fixed-dose combination; RHZE, isoniazid, rifampin, ethambutol, and pyrazinamide fixed-dose combination.

intensive phase (RHZE [rifampin, isoniazid, pyrazinamide, and ethambutol]) and formulation HR1 (Δ−2LL [change in −2-log likelihood] = 96; 2 degrees of freedom [df]; $P < 0.001$). Formulations HR2 and HR3 were associated with an increased rate of absorption compared to formulations RHZE and HR1 (Δ−2LL = 83; 2 df; $P < 0.001$) (Table 2). SLCO1B1 (rs4149032) and HNF4A (rs1884613) polymorphisms had no significant effect on rifampin clearance. The model was also used to produce individual estimates of exposures, and the overall median area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) was 36.3 (IQR, 26.8 to 46.9) mg·h/liter.

Isoniazid. Isoniazid demonstrated two-compartment disposition with first-order elimination and first-order absorption with a lag time whose typical value was fixed at 0.25 h to improve on the stability of the model. Total body weight was the best body size predictor for allometric scaling, which was applied to all clearance and volume-of-distribution parameters. The estimates for the intercompartmental clearance (Q) and volume of the peripheral compartment (V_p) proved to be unstable; therefore, we used a weakly informative Bayesian prior based on results from a similar population (7, 14). The final parameter estimates for isoniazid are presented in Table 3, and the VPC is shown in Fig. 1. The typical value for clearance for a 52-kg participant who was a *NAT2 rs1799930* GG carrier and not on ART was 22.3 liters/h, and the volume of distribution of the central compartment (V_c) was 62.3 liters. Participants who were *NAT2 rs1799930* GA or AA carriers had 26.3% and 74.6% reductions in clearance, respectively (Δ−2LL = 49 points; 2 degrees of freedom; $P < 0.001$), compared to those who were *NAT2 rs1799930* GG carriers. Coadministration of efavirenz led to an increase in isoniazid clearance by 24% (Δ−2LL = 17; $P < 0.001$) regardless of the NAT2 genotype.

Formulations HR1 and HR3 used in the continuation phase had a 20 to 30% increase in bioavailability compared to formulation RHZE, while formulation HR2 had a

TABLE 4 Parameter estimates for pyrazinamide and ethambutol in TB-HIV-coinfected patients

Parameter	Pyrazinamide		Ethambutol	
	Estimated value	Relative SE (%)	Estimated value	Relative SE (%)
CL (liters/h)[a]	2.97	3	35.6	20
V (liters)[a]	33.0	3	80.5	17
K_a (h)	3.04	9	0.454	11
V_p (liters)[a,b]			649	110
Q[a,b]			43.7	32
T_{lag} (l/h)	0.419	12	0.81	6
F	1 (fixed)		1 (fixed)	
Proportional error (%)	0.05	18	31.7	4
Additive error (mg/liter)	2.49	12	0.25 (fixed)[d]	
Between-subject variability of CL[c] (%CV)	30.1	7	33.9	17
Between-subject variability of V_c (%CV)	15.1	11	77.2	12
Between-occasion variability of K_a[c] (%CV)	64.2	7	36.7	26
Between-occasion variability of T_{lag}[c] (%CV)	66.7	12	22.8	32
Between-occasion variability of F[c] (%CV)	16.7	4	30.6	9

[a]Allometric scaling was used for CL, V_c, Q, and V_p, and values are reported for the median weight of the cohort (52 kg).
[b]Q and V_p were estimated using Bayesian priors, with typical values of 64.4 liters/h and 420.7 liters, respectively, from a previous study (7), with 50% uncertainty.
[c]The between-subject and between-occasion variability values were assumed to be log-normally distributed and are reported as approximate coefficients of variation (%CV).
[d]The estimate of the additive error was approximating zero and was therefore fixed to half the value of the lowest limit of quantification (0.5 mg/liter).

13.6% decrease in bioavailability compared to formulation RHZE ($\Delta -2LL = 36$; 2 df; $P < 0.001$). Additionally, formulation HR3 demonstrated a slower absorption than the other three formulations ($\Delta -2LL = 12$; 1 df; $P < 0.001$). The median individual AUC_{0-24} observed was 18.6 (IQR, 17.0 to 22.8) mg·h/liter.

Pyrazinamide. The final model for pyrazinamide was a one-compartment disposition with first-order elimination and first-order absorption with a lag time. The PK parameter estimates are displayed in Table 4, and a VPC is shown in Fig. 1. The clearance and volume of distribution were allometrically scaled using total body weight, and their typical values for a 52-kg patient were 2.97 liters/h and 33.0 liters, respectively. The median individual AUC_{0-24} was 415 (IQR, 360 to 489) mg·h/liter.

Ethambutol. The best model for ethambutol was a two-compartment disposition with first-order absorption and elimination with a lag time. The PK parameter estimates for ethambutol are presented in Table 4, and a VPC is shown in Fig. 1. Clearance and volume of distribution were scaled for weight. The typical values for clearance and central volume for a patient with a body mass of 52 kg were 35.6 liters/h and 80.5 liters, respectively. Similar to the isoniazid model, the estimates for volume of the peripheral compartment and intercompartmental clearance were unstable, so we used a weakly informative Bayesian prior based on a similar population (7) to stabilize the model. The overall median AUC_{0-24} was 25.1 (IQR, 20.6 to 31.7) mg·h/liter.

Monte Carlo simulations of exposure. The simulated drug exposures obtained using the final PK models are shown in Fig. 2 and 3. When using the currently WHO-recommended weight band dosing, participants weighing <55 kg achieve lower AUC_{0-24} values for all four anti-TB drugs than those in the higher weight bands. We then aimed to find a simple dosing adjustment that would balance the median AUC_{0-24} in each weight band with the value achieved in the higher weight bands. A dose increment of 1 FDC tablet (150 mg for rifampin, 75 mg for isoniazid, 275 mg for pyrazinamide, and 400 mg for ethambutol) for the weight band categories 30 to 39 and 40 to 54 kg led to AUC_{0-24} values that are comparable to those in the higher weight band categories.

DISCUSSION

We describe the population pharmacokinetics of first-line anti-TB drugs among TB-HIV-coinfected patients on ART. The key finding is the uneven exposure to anti-TB drugs using current WHO weight band-based dosing; patients with weights below 55 kg had lower exposure to all anti-TB drugs than those with weights above 55 kg.

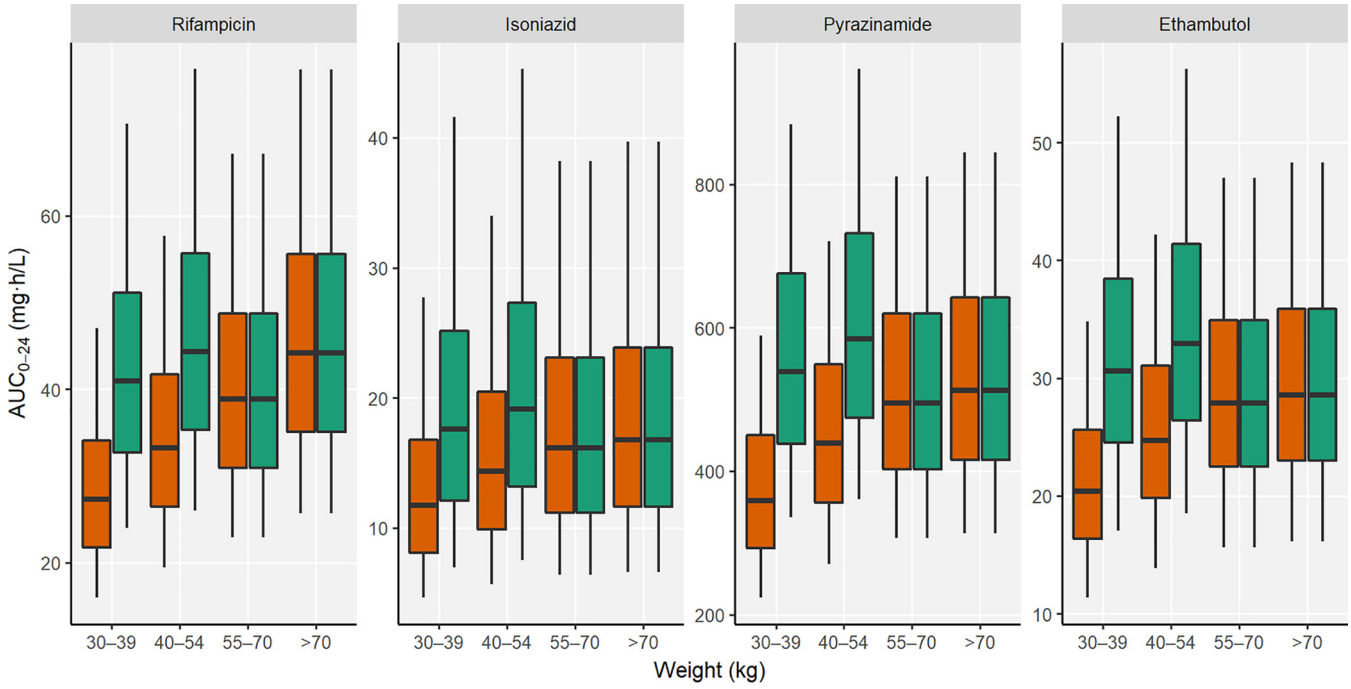

FIG 2 Comparison of simulated exposures using the current dosing strategy versus the suggested dose increment. Shown are box plots of simulated AUC_{0-24} values using the final models for rifampin, isoniazid, pyrazinamide, and ethambutol stratified by weight band. The orange boxes represent the exposure achieved with the currently WHO-recommended dose, while the green ones represent the adjusted dose. The box represents median (central line) and interquartile ranges (box boundaries), while the whiskers are the 2.5th and 97.5th percentiles.

These findings are consistent with previous reports which also demonstrated that patients with low body weight have reduced anti-TB drug concentrations (15, 16). In a similar cohort of South African HIV-infected patients on TB treatment, McIlleron et al. reported that a 10-kg change in body weight was associated with a 14% decrease in the isoniazid AUC, in spite of weight band dosing (15). Similarly, Rockwood et al. found lower anti-TB drug exposure in patients with lower body weight (17).

When a model-based approach is used to interpret the concentrations of anti-TB drugs, as in previous analyses by Chirehwa et al. and Rockwood et al. and the present analysis, the lower exposure in lower-weight patients has been explained as a direct consequence of allometric scaling. According to this well-established concept, corroborated by both empirical evidence and physiological/biological theory, the relationship between body size and drug clearance is nonlinear (18, 19). This means that weight-normalized clearance (liters per hour per kilogram of body weight) is higher in smaller individuals, who then need a larger milligram-per-kilogram dose to achieve concentrations comparable to those in larger individuals. This effect becomes more evident in children, whose weight is significantly lower than that of adults and who indeed need larger milligram-per-kilogram doses, as acknowledged in the new WHO pediatric recommendations, which were recently modified to advise larger milligram-per-kilogram doses for all anti-TB drugs in children than in adults (20). The current weight band dosing for adults is instead designed around the target of constant milligrams per kilogram in all patients, thus leaving lower-weight patients relatively underdosed.

In addition to the nonlinear effect of allometric scaling, a factor that further aggravates the underdosing of low-weight patents is that, for some drugs, the most appropriate determinant of drug clearance is not total body weight but rather ideal body weight, or fat-free mass, like in the case of rifampin in this analysis (21). In such cases, using total body weight leads to the miscategorization of overweight patients, who are then relatively overdosed, and most importantly of underweight patients, who end up underdosed. Most subjects in the lowest weight band (<39 kg) indeed have low weight not because of small body size but because they are wasted as a consequence

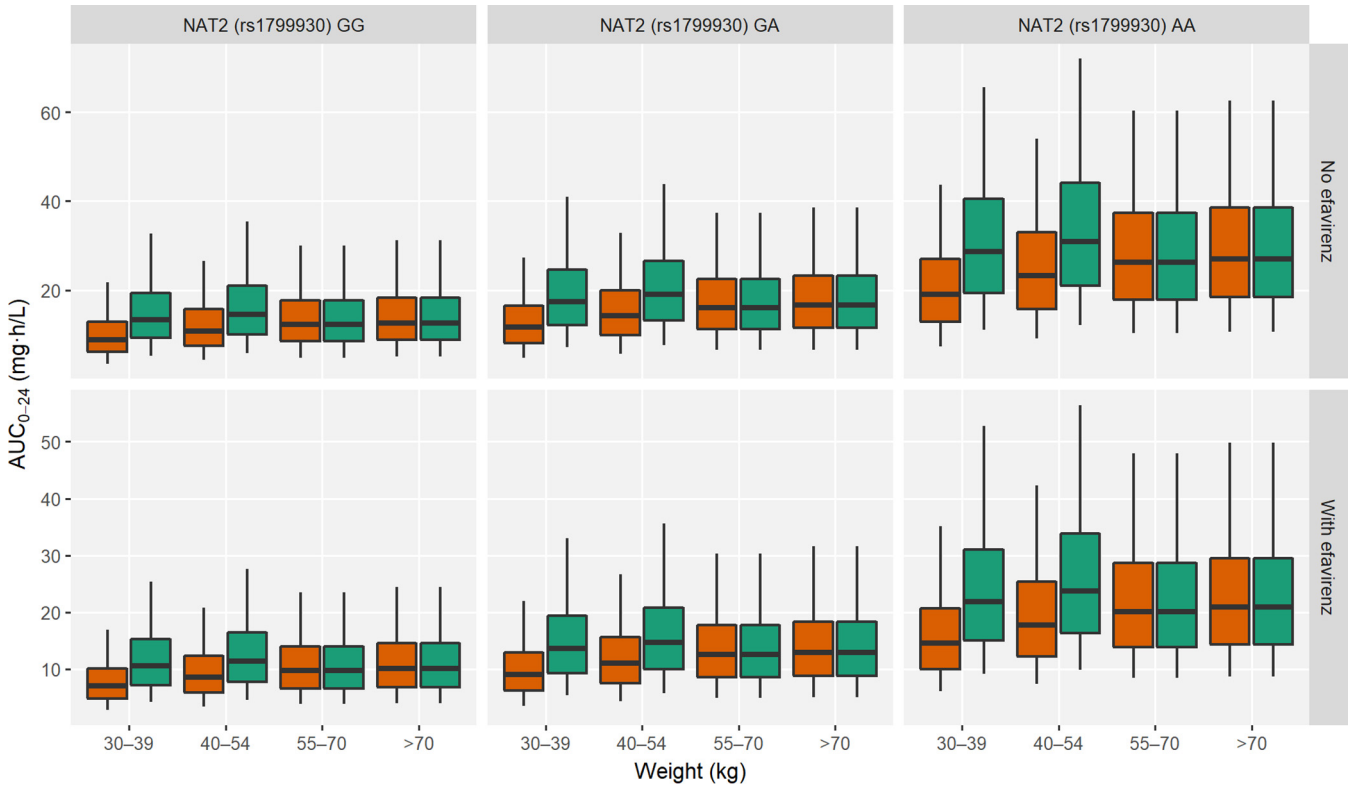

FIG 3 Isoniazid exposure across weight bands stratified by NAT2 rs1799930 genotype and efavirenz coadministration. Shown are box plots of simulated isoniazid AUC_{0-24} values using the final model. The panels show the values stratified by NAT2 rs1799930 genotype (left to right) and efavirenz coadministration (top to bottom). The orange boxes represent the exposure achieved with the currently WHO-recommended dose, while the green ones represent the adjusted dose. The box represents median (central line) and interquartile ranges (box boundaries), while the whiskers are the 2.5th and 97.5th percentiles.

of tuberculosis and other comorbidities. In addition, malnourished patients may be affected by malabsorption, even though no evidence of this was detected in this cohort. Malnourished patients have reduced albumin, leading to an increase in the free fraction of protein-bound drugs which can then be metabolized and eliminated.

Several others have demonstrated that patients with low body weight are more likely to have unfavorable TB treatment outcomes. In a recent large meta-analysis of patient-level data from regimens for drug-susceptible pulmonary tuberculosis, Imperial et al. reported that lower body mass index (BMI) was associated with worse outcomes (22). In the cohort of patients from this study, we previously demonstrated that patients with a weight of <55 kg had a 17 to 28% higher risk of unfavorable treatment outcomes, which included death, loss to follow-up, default, and TB treatment failure, than those in the higher weight bands (2). Even though it is unclear how much the lower concentrations in those with low body weight are contributing to worse outcomes, as opposed to the general clinical conditions of patients with low weight, recent research on the PK/pharmacodynamics (PD) of anti-TB drugs supports the notion that higher concentrations are beneficial toward improvements in outcome (23). For this reason, during the simulations, we decided to increase the concentrations in the patients in the lower weight bands to match the values achieved in patients in the higher weight bands. Our simulations revealed that increasing the doses by 1 standard FDC tablet in the patients in lower weight bands (<55 kg) led to higher drug exposures, which were closer to those in the higher weight bands. This conclusion is in accordance with the results of Chirehwa et al. (16) and a recent review by McIlleron and Chirehwa (24).

Another important finding of our analysis is that efavirenz, which is the recommended first-line ART in patients with TB, caused a 24% increase in isoniazid clearance, which could lead to suboptimal concentrations. Our findings are consistent with a

study by Chirehwa et al., who reported a 54% increase in isoniazid acetylation clearance in patients on efavirenz (25), and another by Bhatt et al., who demonstrated a 29% decrease in isoniazid concentrations in patients on efavirenz (8). Efavirenz concentrations in this cohort of SOUTH study patients have previously been reported to be higher than in other populations (26), so the effect of efavirenz coadministration may even be amplified compared to previous reports. The mechanism for this decrease in concentrations is still unclear and needs to be evaluated; it could be due to the induction of drug-metabolizing enzymes or the effect on an efflux pump or uptake transporter. Early initiation of ART is recommended in patients with TB and severe immunosuppression (27); however, our findings emphasize that we may need to reevaluate drug selection and dosing in this special population.

Finally, we found that the use of different FDC tablet formulations contributed to the interindividual variability in bioavailability and absorption in this population, although this variability was generally within the bioequivalence standard of 80 to 125%. Others have also demonstrated that pharmacokinetic variability may be derived from differences in FDC formulations (11, 28, 29). This reiterates the need for bioequivalence studies and stringent monitoring of the quality of FDC anti-TB drugs for new formulations or whenever there is a change in manufacturing processes.

This study has several strengths, including its large sample size, with blood sampling on multiple visits, and the inclusion of participants who were severely immunosuppressed, which represents the population that is most at risk for TB and in need of ART. One limitation was the lack of sampling beyond 4 h after the intake of TB drugs, which would have allowed for more precise estimation of clearance, especially for drugs with slower absorption and longer terminal half-life. Additionally, genetic information was available for only NAT2 single nucleotide polymorphisms (SNPs) (*rs1799930*), limiting our ability to accurately determine acetylator status. Finally, several other factors that may contribute to pharmacokinetic variability were not measured and could not be evaluated, for example, other causes of genetic variability that may affect drug absorption or elimination. However, the use of population modeling, which is the state-of-the-art technique for pharmacokinetic data, helped mitigate the limitations listed above by handling the limited sampling schedule and the concomitant effects of several covariates.

Conclusion. In summary, we identified and quantified the effects of several factors contributing to the pharmacokinetic variability of first-line TB drugs. We report a drug-drug interaction between efavirenz and isoniazid, which leads to lower isoniazid exposure, and the concerning effect of drug formulation on bioavailability and drug absorption. Most importantly, our findings show that current dosing guidelines lead to lower drug exposure to all four anti-TB drugs in patients with low weight, who are at higher risk of treatment failure. The addition of one FDC tablet to the current weight band dosing for patients weighing $<$55 kg is expected to achieve drug concentrations in line with those in the patients in the higher weight bands. This is a very simple dosing modification, which could be easily implemented, and has the potential to improve outcomes in a vulnerable segment of the patient population.

MATERIALS AND METHODS

From May 2013 to November 2015, we conducted a prospective observational study on the outcomes related to tuberculosis and HIV drug concentrations in Uganda (SOUTH study), which included HIV-infected adults $\geq$18 years of age with a new episode of drug-susceptible pulmonary TB at the Infectious Diseases Institute (IDI) in Kampala, Uganda. Patients who had a glomerular filtration rate of $<$50 ml/min or elevated alanine aminotransferase levels $>$5 times the upper limit of normal (40 U/liter) or were pregnant were excluded from the study. Details concerning the methods of this study have been reported previously in a cohort profile of this study (2, 30).

TB medication and ART. Patients diagnosed with a first episode of TB were initiated on daily TB treatment as outpatients using FDC tablets approved by the national program using standard doses according to WHO weight bands: 3 tablets for patients with a weight of $<$55 kg, 4 tablets for those weighing $\geq$55 kg and $<$70 kg, and 5 tablets for patients with a weight of $\geq$70 kg (27). Each tablet contained 150 mg of rifampin (R), 75 mg of isoniazid (H), 400 mg of pyrazinamide (Z), and 275 mg of ethambutol (E) for the intensive phase (here referred as the RHZE formulation) and the same amounts of only rifampin and isoniazid for the continuation phase (HR). During the continuation phase, HR

formulations from three different local suppliers were used (here referred to as HR1, manufactured by Cosmos Pharmaceutical Limited; HR2, manufactured by Strides Arco Labs; and HR3, manufactured by Svizera Labs). RHZE was provided by the National TB Program. All patients were started on efavirenz-based antiretroviral therapy at 600 mg once daily, regardless of their weight, at least 2 weeks after initiation of TB treatment.

Pharmacokinetic and pharmacogenetic analysis. Blood sampling to quantify drug concentrations was performed 2, 8, 12, and 24 weeks after initiation of TB treatment, and patients were requested to attend the study visit after fasting for at least 8 h. Blood samples were drawn 1, 2, and 4 h after the observed intake of the anti-TB drugs, and serum was separated by centrifugation within 1 h of the blood draw; samples were then stored at −80°C within 30 min. Quantification of anti-TB drug concentrations was performed using high-performance liquid chromatography with ultraviolet detection (HPLC-UV) at the research translational laboratory of the IDI, as previously described (2). Drug concentration measurements were validated over the following measurement ranges: rifampin at 0.5 to 15 μg/ml, isoniazid at 0.5 to 10 μg/ml, pyrazinamide at 5 to 100 μg/ml, and ethambutol at 0.5 to 16 μg/ml. Concentrations below this range were considered below the limit of quantification and reported as such (censored).

Genotyping was also performed at the translational laboratory of IDI. Blood samples were stored at −80°C until the time of analysis. Single nucleotide polymorphisms, including *SLCO1B1* (*rs4149032*), *HNF4A* (*rs1884613*), and *NAT2* (rs1799930), were analyzed through real-time PCR by allelic discrimination.

Ethics. Ethical approval for this study was obtained from the Joint Clinical and Research Centre Council Research and Ethics Committee, and the study was registered at ClinicalTrials.gov (identifier NCT01782950). Written informed consent was obtained from all enrolled patients.

Data analysis. We carried out a population PK analysis using Monolix suite version 2016R1 (31). The modeling strategies were similar for all the drugs; we tested one- and two-compartment disposition models, with first-order elimination and absorption, possibly with delayed onset of absorption described by using either a lag time or a chain of transit compartments (32). Allometric scaling was applied using either total body weight or fat-free mass (FFM) (33) in order to adjust for the effect of body size and/or composition on the disposition parameters of clearance (CL and *Q*) and volume of distribution of the central and peripheral compartments (V_c and V_p, respectively) (33). We included random effects to describe the between-subject variability (BSV) for CL and *V* and between-occasion variability (BOV) for bioavailability (*F*), absorption rate constant (K_a), and other parameters related to absorption. The typical value of bioavailability was fixed to the reference value of 1, since only oral data were available. Parameter estimates from a previous study in a similar population were used as Bayesian priors to stabilize the estimation of parameters in our study (7). The uncertainty of these priors was set to 50%, a value selected so that the inclusion of the priors would not significantly bias the final parameter estimates but mostly prevent them from assuming values outside the plausible range.

The effect of covariates such as age, sex, use of efavirenz, formulation of anti-TB drugs, and genotype was investigated using a stepwise approach. Model development was guided by changes in −2-log likelihood (Δ−2LL) (estimated using importance sampling) with a drop of 3.84 points assumed to be statistically significant at a *P* value of 0.05 for the inclusion of 1 additional parameter. Covariates were included stepwise, at each step, including the one producing the largest drop in −2LL. The Wald test (34) (also with a *P* value of 0.05), based on precision of the estimates obtained using the stochastic approximation to the Fisher information matrix, was used at each step to assess whether previously included covariates remained significant or if their removal should be tested. In the final covariate model, the removal of each single covariate effect was tested to ensure its statistical significance. Additionally, inspection of goodness-of-fit plots, visual predictive checks (VPC), and physiological plausibility were considered to decide on covariate inclusion.

The final model was used to simulate the exposure to all four first-line drugs using the R package mlxR (35). Steady-state AUC_{0-24} values were computed using the function "exposure," which calculates secondary PK parameters by applying conventional noncompartmental analysis techniques to a simulated profile with a grid of 100 time points within the dosing interval (24 h). The demographic data from the patients included in the study were employed for Monte Carlo simulations, resimulating each *in silico* patient 1,000 times for each of the scenarios examined. Using the simulated data, we assessed exposure for the current WHO weight band dosing and explored alternative strategies. For isoniazid, we performed simulations for different NAT2 polymorphisms and for patients with and without efavirenz coadministration.

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M.L., B.C., A.K., J.S.F., and C.S.-W. contributed to the conceptualization of the original study protocol. C.S.-W. wrote the manuscript. C.S.-W., A.V.B., A.B., D.M., U.G., A.C., and I.M. contributed to the data collection and interpretation. C.S.-W., M.C., J.M., and P.D. contributed to the data management and analysis; P.D. supervised the analysis and writing of this paper; and all authors read and approved the final version of this paper.

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