Population Pharmacokinetics and Pharmacogenetics of Ethambutol in Adult Patients Coinfected with Tuberculosis and HIV

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ABSTRACT This study aimed to characterize the population pharmacokinetics and pharmacogenetics of ethambutol in tuberculosis-HIV-coinfected adult patients. Ethambutol plasma concentrations, determined by liquid chromatography-tandem mass spectrometry, in 63 patients receiving ethambutol as part of rifampin-based fixed-dose combination therapy for tuberculosis were analyzed using nonlinear mixed-effects modeling. A one-compartment disposition model with first-order elimination and four transit compartments prior to first-order absorption was found to adequately describe the concentration-time profiles of ethambutol in plasma. Body weight was implemented as an allometric function on the clearance and volume parameters. Estimates of oral clearance and volume of distribution were 77.4 liters/h and 76.2 liters, respectively. A G/A mutation with regard to CYP1A2 2159 G>A was associated with a 50% reduction in relative bioavailability. Simulations revealed that doses of 30 mg/kg of body weight and 50 mg/kg for G/G and G/A carriers, respectively, would result in clinically adequate exposure. The results presented here suggest that CYP1A2 polymorphism affects ethambutol exposure in this population and that current treatment guidelines may result in underexposure in patients coinfected with tuberculosis and HIV. Based on simulations, a dose increase from 15 to 20 mg/kg to 30 mg/kg is suggested. However, the 50-mg/kg dose required to reach therapeutic exposure in G/A carriers may be inappropriate due to the dose-dependent toxicity of ethambutol. Additional studies are required to further investigate CYP450 polymorphism effects on ethambutol pharmacokinetics.

KEYWORDS antimicrobial agents, clinical therapeutics, pharmacology, population pharmacokinetics

Approximately one-third of the world's population is estimated to be infected by *Mycobacterium tuberculosis*. Tuberculosis (TB) is the most common cause of death among HIV-infected people, and in 2017, 27% of TB incidences were in HIV-infected individuals (1).

Ethambutol (EMB) in combination with rifampin, isoniazid, and pyrazinamide is used as a first-line therapy to combat TB infection. EMB inhibits bacterial growth and offers protection against the development of resistance to coadministered TB drugs in the first-line antitubercular regimen (2). EMB mainly undergoes renal elimination, with a half-life of approximately 4 h in adults (3). EMB is metabolized by alcohol dehydrogenase to an aldehyde intermediate, which is further oxidized to a dicarboxylic metabolite (4). After oral administration, 50 to 70% of the EMB dose is excreted unchanged in the urine (5), with 12 to 19% being excreted as metabolites (4). EMB has been shown to inhibit cytochrome P450 enzymes (CYPs) *in vitro*, with strong inhibition of CYP1A2 and

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FIG 1 Observed plasma concentrations after an oral dose of 550 mg (circles), 825 mg (triangles), or 1,100 mg (squares) of ethambutol in 63 adult Rwandan HIV-TB-coinfected patients. Time points have been changed by -0.1 h and $+0.1$ h for the 1,100-mg and 825-mg doses, respectively, to improve visualization of plasma concentrations after the different doses.

CYP2E1 (6), but little is known about whether CYP polymorphism affects EMB pharmacokinetics (PK) in humans.

The exposure profiles of EMB in TB patients have previously been described in several reports (3, 7–19). However, only a few of those involve TB-HIV-coinfected patients, and despite the genetic diversity among different African populations (20), no pharmacokinetic studies of antitubercular drugs have been performed in Rwandan patients. Furthermore, no previous reports have included potential CYP450 polymorphism effects on EMB exposure. The present study aimed to characterize the population pharmacokinetics of EMB in adult TB-HIV-coinfected patients. Moreover, the study aimed to assess the impact of CYP genotypes on EMB pharmacokinetics.

RESULTS

A total of 436 observations were used from 63 patients coinfecting with HIV and TB. Observed data are illustrated in Fig. 1, and patient demographics are summarized in

TABLE 1 Demographics of adult Rwandan HIV-TB-coinfected patients on a rifampin-based fixed-dose combination and concurrent HIV treatment or who were HIV treatment naïve^a

Parameter	Value for group	
	Concurrent HIV treatment ^b	HIV treatment naïve
No. of patients	23	40
Median age (yr) (range)	40 (26–57)	38 (21–52)
Median wt (kg) (range)	48 (35–65)	50 (30–68)
Median serum creatinine concn ($\mu\text{mol/liter}$) (range)	71 (44–159)	66 (35–159)
Median creatinine clearance rate (ml/min) (range)	81 (30–155)	84 (38–155)
Median aspartate aminotransferase concn (U/ml) (range)	33 (11–248)	34 (11–131)
Median alanine aminotransferase concn (U/ml) (range)	36 (9–126)	30 (5–101)
Median CD4 cell count (mm^3) (range)	230 (21–716)	240 (6–524)
No. of female patients/no. of male patients	10/13	16/24
Doses (mg/day) (no. of patients)	550 (1), 825 (13), 1,100 (9)	550 (4), 825 (22), 1,100 (14)

^aContinuous data are given as medians (ranges). Categorical data are given as numbers of patients.^bHIV treatment includes efavirenz, lamivudine, and zidovudine or tenofovir.

Table 1. Fifty-six of the patients were genotyped for CYP2E1 single-nucleotide polymorphisms (SNPs). The results are presented in Table 2.

The pharmacokinetics of EMB in the studied population was well described by a one-compartment model with first-order absorption, including four transit compartments and first-order elimination. One covariate was found to influence the pharmacokinetics of EMB: CYP1A2 2159 G>A, where patients with the G/A mutation had a relative F reduced by 45% in comparison to G/G wild-type carriers (difference in objective function value [dOFV] = -7.0 ; 1 degree of freedom; $P < 0.01$). The addition of the covariate reduced interindividual variability (IIV) in F from 57% to 34%. Additionally, the 163 C>A genotype influenced relative clearance (CL/ F) and F ($P < 0.05$), and 1436 G>T had a significant effect on relative F ($P < 0.05$). However, both were eliminated in the backward step during covariate analysis.

In the final model, CL/ F , the relative volume of distribution (V/F), the absorption rate constant (k_a), and the mean transit time (MTT) were estimated to be 77 liters/h, 76 liters, 0.3 h^{-1} , and 1.2 h, respectively. IIV was applied to CL/ F , V/F , MTT, and F . Primary parameters are summarized in Table 3. Epsilon shrinkage was low (14%), indicating reliable model diagnostics involving individual predictions. The predictive performance of the model is illustrated in Fig. 2.

The area under the concentration-time curve from 0 to 8 h (AUC_{0-8}) values (means $\pm$ standard deviations) for G/G and G/A carriers were $10.8 \pm 7.6 \text{ h} \cdot \text{mg/liter}$ and $6.3 \pm 5.5 \text{ h} \cdot \text{mg/liter}$, respectively. In the studied population, 59% of patients had low peak plasma concentrations (maximum concentration of drug in serum [C_{max}] of $<2 \text{ mg/liter}$) of EMB. With regard to CYP1A2 2159 G>A polymorphism, 53% (27 out of 51 patients) of G/G carriers and 83% (10 out of 12 patients) of G/A carriers had a C_{max} below the recommended threshold. Simulations of higher doses of EMB showed that increasing the dose from 15 to 20 mg/kg of body weight to 30 and 50 mg/kg for G/G and G/A carriers, respectively, would result in adequate exposure to EMB in the majority of patients. Observed and simulated C_{max} values are presented in Fig. 3.

TABLE 2 Distribution of CYP2E1 SNPs in Rwandan TB-HIV-coinfected patients

CYP2E1 genotype	Allele	No (%) of patients with SNP
1053 C>T	C/C	56 (100)
1293 G>C	G/G	54 (96)
	G/C	2 (4)
71 G>T	G/G	56 (100)
7632 T>A	T/T	52 (93)
	T/A	4 (7)

TABLE 3 Primary pharmacokinetic parameters of ethambutol in adult Rwandan HIV-TB-coinfected patients on rifampin-based fixed-dose antitubercular treatment estimated by the final pharmacokinetic model^a

Parameter	Population estimate	95% confidence interval ^b	% RSE ^b
CL/F (liters/h)	77.4	62.0 to 95.4	11.2
V/F (liters)	76.2	46.9 to 117.9	23.7
k_a (h ⁻¹)	0.30	0.26 to 0.34	6.9
MTT (h)	1.22	0.91 to 1.61	15.0
Relative bioavailability (F)	1 fix		
Effect of G2159 G/A on F	-0.45	-0.12 to -0.67	32.3
IIV for CL/F (%)	90.6		38.9
IIV for V/F (%)	106.7		42.5
IIV for MTT (%)	94.4		25.5
IIV for F (%)	34.4		66.0
Residual variability (μg/liter)	0.47		13.4

^aIIVs are presented as standard deviations. CL/F, relative clearance; V/F, relative volume of distribution; k_a , absorption rate constant; MTT, mean transit time; RSE, relative standard error.

^bGenerated by 601 nonparametric successful bootstrap runs ($n = 1,000$).

DISCUSSION

We characterized the population pharmacokinetics of EMB in adult TB-HIV-coinfected patients. To the best of our knowledge, the present study is the first assessment of the possible effects of CYP polymorphisms on exposure to EMB. In this study, the effect of 12 CYP SNPs and efavirenz-based antiretroviral therapy on EMB pharmacokinetics was evaluated. A 45% decrease in F was found in carriers of CYP1A2 G2159 G/A compared to G/G wild-type carriers.

The significant effect of CYP1A2 on EMB bioavailability in the model was unexpected since EMB is eliminated primarily through renal excretion. The results suggest close to a 50% increase in hepatic extraction among G/A carriers. EMB has been reported to exhibit a high, but surprisingly variable, absolute oral bioavailability of about 85% (13), indicating a low degree of hepatic first-pass extraction, wherefore such a difference in metabolic capacity seems unlikely. Moreover, carriers of CYP wild types are commonly extensive metabolizers, which contradicts their higher EMB exposure in the present study (21). On the other hand, the decreased exposure to EMB in G/A carriers is in intriguing agreement with lower exposure to the CYP1A2 substrate agomelatine reported in individuals carrying the G/A variant (22).

Isoniazid has been shown to inhibit CYP1A2, and EMB has been shown to strongly inhibit CYP1A2 and CYP2E1 *in vitro*, demonstrating an interaction between EMB and CYP enzymes (6, 23). An alternative hypothesis could be variant-specific inhibition of the CYP1A2 G/G wild type by isoniazid resulting in lower EMB exposure in uninhibited G/A carriers. However, such variant-specific inhibition would assume EMB metabolism by CYP1A2, which, to the best of our knowledge, has not been previously reported.

A one-compartment model was found to most adequately describe systemic exposure to EMB in Rwandan TB-HIV-coinfected adults. The number of compartments in the model is in agreement with a model for North American TB patients (3) but is in contrast to the two-compartment models used for South African TB patients (12) and healthy volunteers (24). In the present study, plasma concentrations were observed over a duration of 8 h after dosing, in comparison to 12 h and 48 h after dosing in the studies by Jonsson et al. and Peloquin et al., respectively. This could plausibly explain the difference in numbers of compartments between the studies.

The estimated CL/F is similar to the clearance value (90 liters/h) of EMB in healthy volunteers (24), whereas it is twice the estimated CL/F (40 liters/h) reported in South African TB patients (12). The demographics of the South African cohort and those of the population in this analysis were similar except for the number of HIV-positive patients, which was only 13% (24 out of 189 patients) in the South African cohort. HIV status has been shown to affect EMB clearance in a pediatric population (25), but no significant effect on EMB pharmacokinetics in adults has been observed (14). The difference in CL/F despite population similarities is indicative of the importance of local population

FIG 2 Prediction-corrected visual predictive check ($n = 1,000$) for the final pharmacokinetic model for ethambutol in adult Rwandan HIV-TB-coinfected patients stratified by CYP1A2 G2159 genotypes G/G (left) and G/A (right). Open circles are observed data points, solid lines are the medians of the observed concentrations, dashed lines are the 2.5th and 97.5th percentiles of observed concentrations, and shaded areas are the 95% confidence intervals of the 2.5th, 50th, and 97.5th percentiles of the simulated data.

studies in areas of high endemicity, like sub-Saharan Africa. Moreover, no effect of HIV treatment on EMB pharmacokinetics was observed. These results are in line with those of a previous noncompartmental analysis of EMB pharmacokinetics in HIV-infected patients (7).

The absorption phase of EMB was best described by a transit compartment absorption model with four transit compartments, similarly to the three transit compartments used in the model describing EMB pharmacokinetics in South African TB patients (12). Four patients had a significant delay in absorption; there was no observed concentration of EMB in plasma before 2 h, compared to 1 h in the rest of the population. There were no covariates significantly affecting absorption. The time to the maximum concentration of EMB has been shown to be affected by concomitant food intake (24), which could explain the absorption delay. However, no information regarding food intake in proximity to dosing was recorded during this study, and potential food interactions could therefore not be evaluated.

IIV was applied to all parameters except for k_a . IIV on k_a prevented the model from successful minimization, most likely due to the lack of data in the absorption phase.

FIG 3 Observed and simulated maximum concentrations after a single dose of ethambutol in patients coinfecting with TB and HIV stratified by CYP1A2 G2159 G>A genotype. Simulations (90% range of the simulation output) are based on the typical individual in the studied population weighing 50 kg ($n = 200$). Boxes represent the 25th to 75th percentiles, and the solid lines in the boxes are the arithmetic means. Dashed lines represent the clinical target interval.

Sampling between 0 and 1 h postdose should be considered in future pharmacokinetic studies on EMB. In our study, however, selection of sampling times was based on compromise when simultaneously investigating the pharmacokinetics of seven HIV and TB drugs.

A $C_{\max}$ of 2 to 6 mg/liter has been proposed as a therapeutic target during EMB administration (26). Our simulations indicated that a dose increase from the currently used 15 to 20 mg/kg to 30 mg/kg and 50 mg/kg for G/G and G/A carriers of CYP1A2 G2159, respectively, would result in a significant improvement of EMB exposure among patients with TB-HIV coinfection. The incidence of EMB-related adverse events with regard to ocular toxicity is dose dependent (2). However, the risk of ocular toxicity is present at doses of 15 mg/kg. Since doses of up to 25 mg/kg are currently in clinical use, a regimen based on 30-mg/kg doses appears feasible. However, the use of 50-mg/kg doses may be undesirable with regard to the dose-dependent toxicity profile of EMB. Therefore, conclusions regarding potential dose increases in G/A carriers require further evaluation of the involvement of CYP in the variability of EMB pharmacokinetics.

There were some limitations to the present study. First, the time interval for blood sampling was relatively short. Since previous models have described a bimodal elimination of EMB, AUC based on a dosing interval would not be reliably derived by the model since the possibility of a second elimination phase could not be discarded. Furthermore, no response-to-treatment data, such as sputum conversion, was recorded during the conduction of the clinical study. However, a clinical threshold for treatment adequacy based on EMB C_{max} has been previously proposed (26).

In conclusion, the population pharmacokinetics and pharmacogenetics (PG) of EMB in TB-HIV-coinfected adults have been described. More than half of the cohort had peak plasma concentrations below the recommended threshold, indicating that the current dosing regimen may need modifications in patients coinfecting with HIV. Moreover, CYP1A2 polymorphism statistically affected EMB exposure in the present cohort. Simulations revealed that dose increases to 30 mg/kg and 50 mg/kg in G/G and G/A carriers, respectively, would improve exposure to EMB in patients coinfecting with TB and HIV. However, doses of 50 mg/kg may be inappropriate with regard to the dose-related adverse events of EMB. Further studies are required to investigate the potential involvement of CYP in EMB metabolism.

MATERIALS AND METHODS

Patients. An open-label, observational clinical study was conducted at four sites in Rwanda in patients coinfecting with HIV and TB (27). Patients were monitored before, during, and after initiation of intensive TB treatment.

Out of 105 recruited patients, 80 completed the study, and 63 were included in the analysis presented here. Patients were excluded from the analysis due to nevirapine-based antiretroviral treatment ($n = 4$), nondetectable levels of efavirenz ($n = 3$), or previous exhaustion of samples during bioanalysis of efavirenz (27). Demographics of the cohort are summarized in Table 1. EMB (550, 825, or 1,100 mg) was administered daily during 2 months as part of first-line initial intensive TB therapy. For each patient, the number of tablets (a fixed-dose combination of EMB together with rifampin, isoniazid, and pyrazinamide at 275/150/75/400 mg; Svizera, Almere, The Netherlands) was adjusted to reach a target dose of 15 to 20 mg/kg for EMB. Out of 63 participants included in the analysis, 8 were administered streptomycin in addition to the four primary TB drugs. Patients were either HIV treatment naive (arm A) or on treatment for HIV (arm B) when TB therapy was initiated. HIV treatment for patients in arm A was initiated between 2 and 8 weeks after TB treatment initiation. Patients included in the study received HIV and TB therapy, in agreement with current Rwandan guidelines for the management of HIV-TB coinfection (28).

The study was performed in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonization guidance for good clinical practice and received approval from the National Ethics Committee of the Ministry of Health in Rwanda. Each patient received full written and verbal information regarding the study. Participants were informed that inclusion was voluntary and that they could withdraw without prejudice at any time.

Dose intake and sample collection. Due to the observational nature of the clinical study, no restrictions regarding food or liquid intake in proximity to dose administration were specified in the study protocol.

Samples were drawn predose and at 1, 2, 3, 4, 6, and 8 h postdose at the initiation of enrollment, followed by sparse sampling once weekly during patient monitoring. Plasma was harvested after centrifugation of samples and stored at -30°C in clinics for 1 week before being transferred to -80°C . The plasma samples were airfreighted to Gothenburg, Sweden, in dry ice for quantification.

Genotyping. Genotyping of 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 using PCR. These results were described previously (29).

A QIAamp DNA blood minikit was used to perform genomic DNA extraction from blood samples for genotyping of CYP2E1 (1053 C>T, 1293 G>C, 71 G>T, and 7632 T>A) SNPs. Genotyping was performed by multiplexed primer extension chemistry using an iPLEX assay, with detection of the incorporated allele by mass spectrometry with a MassArray analyzer (Agena Bioscience, San Diego, CA, USA) (30, 31). Raw data were converted to genotype data using Typer software (Agena Bioscience).

Drug quantification. EMB concentrations in plasma were quantified using a liquid chromatography-tandem mass spectrometry method described previously (32). Briefly, the method was validated over the range of 40 to 5,000 ng/ml for EMB. Inter- and intraday accuracy and precision (percent relative standard error [RSE]) were 87 to 115% and $<13\%$, respectively. No signal interference from isoniazid, pyrazinamide, rifampin, efavirenz, lamivudine, zidovudine, or tenofovir was observed. Samples with concentrations above the upper limit of quantification ($n = 17$) were diluted and reanalyzed. Samples with concentrations below the lower limit of quantification ($n = 6$) were ignored.

Pharmacokinetic analysis. Data were analyzed using nonlinear mixed-effects modeling in NONMEM version 7.4 (Icon Development Solutions, Ellicott City, MD, USA) (33). Log transformation on observations was performed to increase numerical stability during analysis. Models were fitted using a first-order conditional estimation method with interaction. Xpose library version 4.6.1 (Department of Pharmaceu-

tical Biosciences, Uppsala University, Uppsala, Sweden) in R version 3.4.1 (2017; R Foundation for Statistical Computing, Vienna, Austria) was used for model diagnostics. Pearl-speaks-NONMEM (PsN) version 4.8.0 (Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden) was used for model automation and diagnostics.

Model discrimination between nested models was based on OFVs. The OFV is considered χ^2 distributed, and decreases in the OFV by -3.84 and -6.63 are considered a significant model improvement at P values of 0.05 and 0.01 , respectively.

One-, two-, and three-compartment models with first-order absorption and elimination were fitted to the data. Different absorption models were tested, including adding a lag time, sequential absorption, and absorption with a fixed number of transit compartments. Log-normal distribution was assumed for all parameters, and interindividual variability was added to all parameters in a stepwise manner as an exponential random effect according to the equation $P_i = \theta_p \times e^{\eta_{i,p}}$, where P_i is the individual estimate of a parameter, θ_p is the population mean, and $\eta_{i,p}$ is a random variable assumed to be normally distributed with a mean of zero and a variance of ω^2 . Residual variability was evaluated as an additive error equivalent to an exponential error when the data are not log transformed.

Body weight normalized by the population median was applied as an allometrically scaled function on all clearance and volume parameters, with powers of 0.75 and 1 , respectively (34, 35). Bioavailability fixed at 1 with an estimated interindividual variability was introduced during the early development of the structural model.

Following the development of a structural model, covariates were added in a stepwise manner. Covariates were systematically added one by one during forward selection, followed by stepwise backward elimination. Covariates were selected to remain in the model if they improved the model by significance levels of 0.05 and 0.01 during the forward and backward selection steps, respectively. Categorical covariates tested were sex, the presence or absence of HIV drugs (i.e., study arm), and the effect of 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), CYP3A5 (6986 A>G), and CYP2E1 (1293 G>C and 7632 T>A) genotypes. Patients with missing genotypes were assigned the most common genotype for each SNP. Continuous covariates tested were age, serum creatinine level, creatinine clearance estimated with the Cockcroft and Gault equation (36), alanine aminotransferase level, aspartate aminotransferase level, and CD4 cell count. The tested continuous covariates were centered on their respective population median. All covariates were tested on clearance (CL/F) and relative bioavailability (F), and age, arm, gender, and CD4 cell count were tested on volume (V/F) and absorption mean transit time (MTT).

The adequacy of the final pharmacokinetic model was assessed by goodness-of-fit plots, visual predictive checks (VPCs) ($n = 1,000$), and parameter plausibility. To evaluate parameter precision, the model was bootstrapped to resample new sets of EMB plasma concentration-time profiles ($n = 1,000$).

Patient area under the plasma concentration-time curves (AUC_{0-8}) and maximum concentrations reached after dosing (C_{max}) were derived from the final model. C_{max} values were simulated after standard doses (15 to 20 mg/kg) and doses of up to 50 mg/kg for comparison with recommended clinical targets. The upper dose limit during the simulations was based on early clinical studies which included doses of up to 50 mg/kg (37).

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