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Pharmacokinetics of Antituberculosis Drugs in HIV-Positive and HIV-Negative Adults in Malawi

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Limited data address the impact of HIV coinfection on the pharmacokinetics (PK) of antituberculosis drugs in sub-Saharan Africa. A total of 47 Malawian adults underwent rich pharmacokinetic sampling at 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 h postdose. Of the subjects, 51% were male, their mean age was 34 years, and 65% were HIV-positive with a mean CD4 count of 268 cells/μl. Antituberculosis drugs were administered as fixed-dose combinations (150 mg rifampin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol) according to recommended weight bands. Plasma drug concentrations were determined by high-performance liquid chromatography (rifampin and pyrazinamide) or liquid chromatography-mass spectrometry (isoniazid and ethambutol). Data were analyzed by noncompartmental methods and analysis of variance of log-transformed summary parameters. The pharmacokinetic parameters were as follows (median [interquartile range]): for rifampin, maximum concentration of drug in plasma (C_{max}) of 4.129 μg/ml (2.474 to 5.596 μg/ml), area under the curve from 0 to 24 h ($AUC_{0-\infty}$) of 21.32 μg/ml · h (13.57 to 28.60 μg/ml · h), and half-life of 2.45 h (1.86 to 3.08 h); for isoniazid, C_{max} of 3.97 μg/ml (2.979 to 4.544 μg/ml), AUC_{0-24} of 22.5 (14.75 to 34.59 μg/ml · h), and half-life of 3.93 h (3.18 to 4.73 h); for pyrazinamide, C_{max} of 34.21 μg/ml (30.00 to 41.60 μg/ml), AUC_{0-24} of 386.6 μg/ml · h (320.0 to 463.7 μg/ml · h), and half-life of 6.821 h (5.71 to 8.042 h); and for ethambutol, C_{max} of 2.278 μg/ml (1.694 to 3.098 μg/ml), AUC_{0-24} of 20.41 μg/ml · h (16.18 to 26.27 μg/ml · h), and half-life of 7.507 (6.517 to 8.696 h). The isoniazid PK data analysis suggested that around two-thirds of the participants were slow acetylators. Dose, weight, and weight-adjusted dose were not significant predictors of PK exposure, probably due to weight-banded dosing. In this first pharmacokinetic study of antituberculosis drugs in Malawian adults, measures of pharmacokinetic exposure were comparable with those of other studies for all first-line drugs except for rifampin, for which the C_{max} and AUC_{0-24} values were notably lower. Contrary to some earlier observations, HIV status did not significantly affect the AUC of any of the drugs. Increasing the dose of rifampin might be beneficial in African adults, irrespective of HIV status. Current co-trimoxazole prophylaxis was associated with an increase in the half-life of isoniazid of 41% ($P = 0.022$). Possible competitive interactions between isoniazid and sulfamethoxazole mediated by the *N*-acetyltransferase pathway should therefore be explored further.

Tuberculosis (TB) and HIV coinfection remains a challenging public health problem in sub-Saharan Africa. TB is the most common serious opportunistic infection in people living with HIV/AIDS, while a majority of individuals with TB are HIV seropositive. Treatment guidelines for TB do not recommend any adjustments in TB treatment in the presence of HIV coinfection, unless protease inhibitors are also being administered. Although a recent systematic review concluded that a longer duration of rifamycin-based treatment reduces the recurrence rates of TB after treatment in HIV-positive people, this appeared not to be true when antiretroviral therapy (ART) was coadministered (1). Concern has nonetheless lingered as to the reliability of TB treatment in this context, with repeated reports of lower plasma concentrations of antituberculosis drugs, particularly rifampin, in HIV-coinfected TB patients (2–11) and the association of these findings with worse treatment outcomes in some studies (11, 12).

Due to their logistical complexity, however, few intensive pharmacokinetic (PK) studies of HIV-infected tuberculosis patients have been performed in the sub-Saharan region, with the bulk of the published data originating from South Africa (7, 9, 10, 13). Consequently, larger and more detailed field PK studies are required in order to obtain accurate estimates of key PK and pharmacodynamics parameters, define the full extent of the interindividual variability in PK, and evaluate the impact of important covariates such as HIV coinfection and coadministration of antiretroviral therapy. Such studies may also assist in determining the possible impact of local treatment practices, drug formulation, nutritional factors, and pharmacogenetic differences on PK parameters in these different settings.

In Malawi, the HIV infection prevalence in adults is around

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11%, and 70% of TB patients are HIV infected. HIV infection and TB treatment are provided according to a public health approach, using standardized combination regimens with generic drugs, which are provided free of charge in both programs. The TB treatment is provided through a well-established community directly observed treatment (DOT) system. Infrastructure to support therapeutic drug monitoring of TB drugs and PK data for antituberculosis drugs in Malawian adults are not available. The National TB Programme expressed a need to address concerns about the pharmacological robustness of TB treatment in the local context with the high rates of HIV coinfection and malnutrition. We therefore determined the PK profiles of the four drugs comprising the first-line TB treatment regimen in a representative cohort of Malawian TB patients to establish whether they attain optimal plasma TB drug concentrations and whether HIV status and other variables have an important impact on pharmacokinetics.

MATERIALS AND METHODS

Clinical protocol. The study took place at outpatient clinics and tuberculosis wards of Queen Elizabeth Central Hospital, a tertiary government hospital with around 1,000 beds in Blantyre, Malawi. Malawian adults (>16 years) who had a diagnosis of sputum microscopy acid-fast bacilli-positive pulmonary TB and who provided informed consent were enrolled in a pharmacokinetic study from January 2007 to February 2008. Sampling took place after a minimum of 2 weeks of TB treatment and before the end of the intensive treatment phase. Patients were eligible for enrollment irrespective of their current antiretroviral therapy (ART) for HIV infection. Patients with an unknown HIV status were encouraged to undergo HIV testing. Patients who were unwilling to be tested or have the result disclosed to the study team were excluded from the study. Other exclusion criteria were hemoglobin (Hb) of <8 g/dl, vomiting within the preceding 72 h, diarrhea more than 3 times per day during the preceding 3 days, and discontinuation of ART within the last 2 weeks.

We obtained demographic and clinical information, including gender, age, height, weight, HIV status, concomitant drug use, and ART duration. Following the WHO criteria, malnutrition was defined as a body mass index (BMI) of <18.5 kg/m^2. We collected a venous sample for the CD4 count and concentrations of creatinine, Hb, and alanine aminotransferase (ALT). HIV status was determined by rapid tests using HIV Determine (Invernos Med, Japan Co. Ltd.) and confirmed by a second assay with Uni-Gold (Trinity Biotech PK, Ireland), according to the national protocol at the time.

Antituberculosis drugs were administered as fixed-dose combination (FDC) tablets, approved by the National TB Programme and the WHO and dosed according to the recommended weight bands. Each tablet contained 150 mg rifampin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol. Doses were administered orally once daily within the following body weight bands: 25 to 37 kg, 2 tablets; 38 to 54 kg, 3 tablets; 55 to 74 kg, 4 tablets; and ≥75 kg, 5 tablets.

Ethical approval of the study protocol was obtained from the National Health Sciences Research Committee.

Bioanalytical methods. Rich pharmacokinetic sampling took place during the intensive phase of the antituberculosis therapy. The first sample was taken at 0 h (predose), after which the patient had a light breakfast and then took the TB drugs. Dosing was performed under observation by the research nurse. An intravenous cannula was inserted and maintained using heparin-saline flushes. Samples were then drawn at 0.5, 1, 2, 3, 4, 6, 8, and 24 h postdose and were immediately transported in a closed cooler box to the Malawi-Liverpool Wellcome Trust Clinical Research laboratory on the hospital premises, spun down, and snap-frozen at −80°C until transportation on dry ice to Liverpool, United Kingdom.

The rifampin and pyrazinamide plasma concentrations were determined using high-performance liquid chromatography on a Shimadzu LC 2010 HT system (Shimadzu, Manchester, United Kingdom). The isoniazid and ethambutol concentrations were determined simultaneously using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a triple-quadrupole TSQ Quantum Access mass spectrometer (Thermo Scientific, Hemel Hempstead, United Kingdom). All methods incorporated appropriate internal standards (13) and were validated to internationally recognized acceptance criteria (14). The lower limits of quantification for the assays were 0.5 μg/ml, 2.5 μg/ml, 20 ng/ml, and 10 ng/ml for rifampin, pyrazinamide, isoniazid, and ethambutol, respectively (13).

Statistical methods. Noncompartmental PK analysis of plasma concentration-time data was performed with Kinetica 4.1.1 (Adept Scientific Ltd., Armor Way, Letchworth Garden City, United Kingdom) using the trapezoidal rule with the log up-linear down option and manual adjustment of the range of included time points. Data summaries, graphics, and analysis of variance of the summary PK parameters were performed in R 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria). Analysis of variance was performed with log-transformed PK parameters where appropriate and model assumptions were checked using routine graphical diagnostics. Generalized additive models were used to evaluate continuous covariate relationships using the package mgcV, and clustering analysis for subpopulation detection in the parameter distributions was performed using the package mclust.

TABLE 1 Patient characteristics (n = 47)

Characteristic	Value[a]
Age (yr)	34.4 (16–60)
Male sex	24 (52)
Weight (kg)	52.52 (35.80–74.30)
Height (m)	1.65 (1.51–1.81)
BMI (kg/m^2)	19.29 (13.92–28.39)
Malnutrition (BMI <18.5 kg/m^2)	19 (40)
TB retreatment regimen	6 (13)
HIV status	30 (65)
WHO stage 4	6 (13)
CD4 count (cells/μl)	268 (3–1,204)
Co-trimoxazole prophylaxis	19 (40)
Nevirapine-based ART	12 (26)
Efavirenz-based ART	1 (2)
Hb (g/dl)	11.39 (8.50–19.20)
Creatinine (mg/dl)	0.8 (0.4–1.9)
ALT (U/liter)	15.7 (5–44)

[a] Values are means (ranges) or no. (%).

RESULTS

Forty-seven patients were enrolled (Table 1). The mean age was 34 years, and 24 (51%) were male. Thirty (65%) were HIV-positive with a mean CD4 count of 268 cells/μl. Thirteen were receiving nevirapine-based and one was receiving efavirenz-based antiretroviral therapy. All HIV-positive participants received co-trimoxazole prophylaxis, and none had chronic diarrhea. The mean weight-adjusted dose received for each of the drugs was 10.03 mg/kg for rifampin, 5.01 mg/kg for pyrazinamide, 26.58 mg/kg for isoniazid, and 18.38 mg/kg for ethambutol. Plasma concentration curves for each of the drugs are shown in Fig. 1. The summary PK parameters derived from the noncompartmental analysis are presented in Table 2. The PK parameters for rifampin, isoniazid, pyrazinamide, and ethambutol could be computed for 41, 46, 46, and 47 participants, respectively, due to missing observations or a noncredible PK profile in some participants. Estimates of the apparent terminal elimination half-life (λ_z) were based on at least three data points in all cases with a mean number of data points of 3.4, 3.9, 3.9, and 3.68 contributing to the analysis and a percent

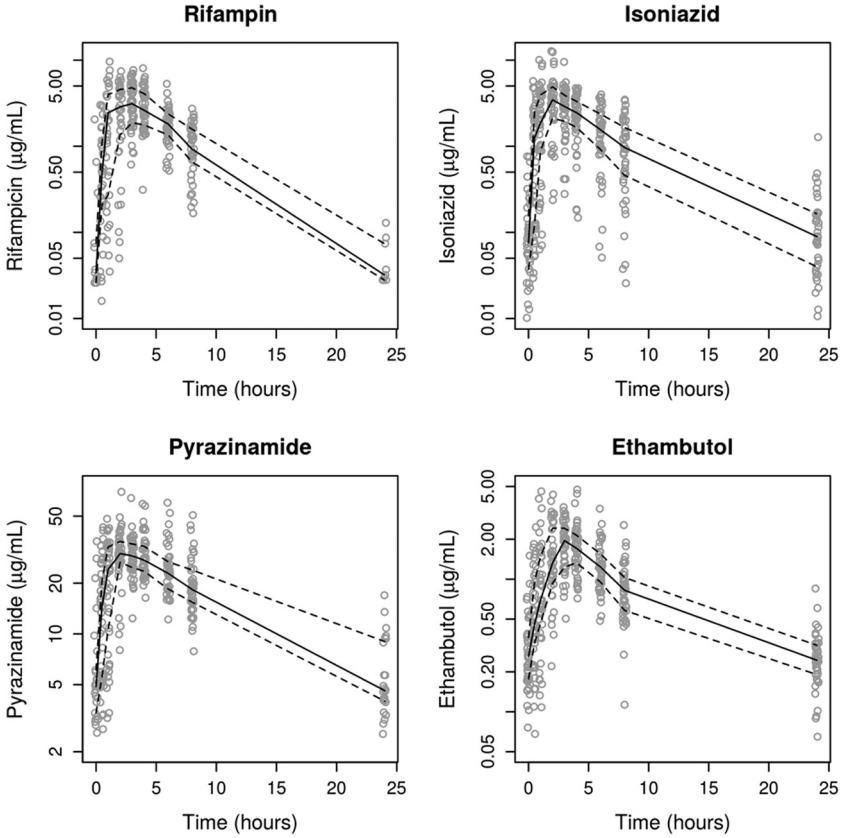

FIG 1 Semilogarithmic plots of the plasma concentrations of the four first-line drugs. The solid lines show the median concentrations, and the dashed lines show the upper and lower quartiles.

extrapolation to infinity on the area under the curve ($AUC_{0-\infty}$) of 13.49, 5.31, 28.10, and 14.80 for the four drugs, respectively.

For rifampin, the median observed maximum concentration of drug in plasma (C_{max}) was 4.13 μg/ml (interquartile range [IQR], 2.47 to 5.60 μg/ml) and the $AUC_{0-\infty}$ was 21.32 μg/ml · h (IQR, 13.57 to 28.60 μg/ml · h). In 14 of 47 (30%) participants, the C_{max} occurred at 4 h postdosing or later, representing delayed absorption, and in 4 of these cases the noncompartmental analysis failed to produce a meaningful estimate of the AUC. The C_{max} and AUC_{0-24} were not significantly associated with a weight-adjusted dose ($P = 0.10$ and 0.06), while the $AUC_{0-\infty}$ was negatively correlated with an absolute dose ($P = 0.048$). On closer examination, this finding appeared to be driven by exposure in participants with the lowest body mass. As expected, both measures of volume of distribution (apparent volume of distribution during terminal phase [V_z/F] and volume of distribution at steady state [V_{ss}/F]) tended to scale positively with both linear and power functions of weight ($P = 0.08$ and 0.09).

For isoniazid, the median observed C_{max} was 3.97 μg/ml (IQR, 2.98 to 4.54 μg/ml) and the $AUC_{0-\infty}$ was 22.5 μg/ml · h (IQR, 14.75 to 34.59 μg/ml · h). No relationship was observed between these parameters and the absolute or weight-adjusted dose. The

TABLE 2 Summary of pharmacokinetic parameters derived from noncompartmental analysis

Parameter[a]	Results (median [interquartile range]) for:			
	Rifampin ($n = 41$)	Isoniazid ($n = 46$)	Pyrazinamide ($n = 46$)	Ethambutol ($n = 47$)
C_{max} (μg/ml)	4.13 (2.47–5.60)	3.970 (2.979–4.544)	34.21 (30.00–41.60)	2.278 (1.694–3.10)
T_{max} (h)	3.00 (2.00–4.00)	2.00 (1.00–3.00)	2.00 (1.00–3.00)	3.00 (2.00–4.00)
$AUC_{0\text{-last}}$ (μg/ml · h)	16.62 (11.97–24.28)	21.83 (13.80–33.89)	273.10 (173.7–388.5)	16.72 (12.72–22.93)
$AUC_{0-\infty}$ (μg/ml · h)	21.32 (13.57–28.60)	22.50 (14.75–34.59)	386.6 (320.0–463.7)	20.41 (16.18–26.27)
$t_{1/2}$(h)	2.45 (1.86–3.08)	3.93 (3.18–4.73)	6.821 (5.71–8.04)	7.507 (6.517–8.69)
CL/F (liters/h)	25.11 (16.62–39.41)	11.60 (7.179–18.42)	3.577 (2.66–4.75)	48.39 (34.31–62.73)
V_z/F (liters)	83.96 (67.21–113.70)	66.67 (48.25–92.37)	35.03 (28.24–43.51)	488.40 (376.80–630.70)
V_{ss}/F (liters)	130.20 (90.75–202.40)	72.35 (59.56–98.33)	38.74 (31.44–46.13)	498.90 (410.60–722.80)

[a] Abbreviations: C_{max}, observed maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC_{0-last}, area under the curve to last observed plasma concentration; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; $t_{1/2}$, apparent elimination half-life; CL/F, apparent clearance; V_z/F, volume of distribution; V_{ss}/F, volume of distribution at steady state.

median apparent terminal elimination half-life of isoniazid was 3.93 h (IQR, 3.18 to 4.73 h). Seven of 46 (15%) had a half-life of <130 min, suggesting that 84.8% of participants would conventionally be classified as the slow acetylator phenotype. However, since the semilogarithmic plot of the data suggested possible biphasic elimination with 38 of 46 participants (83%) showing detectable concentrations of isoniazid at 24 h, these parameters were reestimated with this data point omitted. The median elimination half-life was then 2.83 h (IQR, 2.010 to 3.729 h) with 67% classified as slow acetylators. These predictions of the proportion of slow acetylators were supported by finite normal mixture models of the distribution of the half-lives. For the data set with the 24-h data point excluded, the algorithm predicted two subpopulations with estimated mean half-lives of 1.84 and 3.64 h, comprising 36% (fast/intermediate acetylators) and 64% (slow acetylators) of the participants, respectively. A three-component mixture model did not convincingly discriminate between fast and intermediate acetylators. Similarly to the results for rifampin, V_z/F and V_{ss}/F tended to scale with a linear or power function of body mass ($P = 0.102$ and 0.053). Concomitant co-trimoxazole prophylaxis was associated with an increase in the half-life of isoniazid of 41% ($P = 0.022$).

For pyrazinamide, the median observed C_{max} was 34.21 μg/ml (IQR, 30.00 to 41.60 μg/ml) and the $AUC_{0-\infty}$ was 386.6 μg/ml · h (320.0 to 463.7 μg/ml · h). There was no consistent relationship between these parameters and the absolute or weight-adjusted dose. The C_{max} was reduced by 15% in HIV-positive participants, although neither the AUC_{last} nor $AUC_{0-\infty}$ was affected. V_z/F increased by 0.42 liters for each additional kilogram of body weight ($P < 0.001$), and this relationship accounted for an apparent univariate effect of sex on this parameter in multivariate analysis.

For ethambutol, the median observed C_{max} was 2.278 μg/ml (IQR, 1.694 to 3.098 μg/ml) and the $AUC_{0-\infty}$ was 20.41 μg/ml · h (IQR, 16.18 to 26.27 μg/ml · h). There was no observed relationship between these parameters and the absolute or weight-adjusted dose. V_z/F increased by 5.18 liters for each additional kilogram of body weight ($P = 0.009$), and this relationship accounted for an apparent univariate effect of sex on this parameter in multivariate analysis. Neither serum creatinine nor the glomerular filtration rate was related to the elimination half-life.

With the exception of the C_{max} of pyrazinamide, HIV coinfection did not significantly affect the PK parameters of any of the drugs studied. Age, gender, and antiretroviral therapy also had no effect (data not shown).

DISCUSSION

Since the early reports of altered plasma concentrations of antituberculosis drugs in HIV-positive patients, debate has continued as to the contribution of HIV coinfection to interpatient PK variability in the treatment of TB. While sub-Saharan Africa bears the brunt of HIV-associated tuberculosis and an increasing incidence of multidrug resistance, surprisingly few PK studies have been performed in the region and fewer still have been able to employ intensive blood sampling. Although logistically simpler, sparse sampling can systematically underestimate key measures of exposure such as the C_{max} and AUC and may not correctly define the characteristics of the elimination phase. Our comparatively intensive sampling allowed for parameter estimates with higher precision and less truncation of the AUC than some earlier studies. The values of the parameters from this first PK study of antituberculous drugs in Malawian adults are broadly comparable with data from two sites in South Africa (7, 10), one site from Tanzania (15), and one from Botswana (8), although some differences are worthy of comment.

Contrary to some earlier observations (3, 4, 6), HIV infection did not significantly or consistently affect the pharmacokinetics of any of the four first-line antituberculosis drugs. The only exception was a small reduction in the C_{max} of pyrazinamide, which did not affect the overall plasma exposure as measured by the AUC. These findings are reassuring and in accordance with other research supporting similar efficacy of TB treatment in HIV-positive patients (16). Early studies focused on patients with advanced HIV disease and associated diarrhea prior to the introduction of highly active antiretroviral therapy. About half of the HIV-positive participants in our study were receiving ART according to national program guidelines, and all were receiving co-trimoxazole prophylaxis, which may have resulted in less advanced immunosuppression and fewer associated opportunistic infections. However, 50% had a CD4 level of <200 cells/mm^3 with a median body mass of only 45 kg, 12 kg less than those whose CD4 counts were >200 cells/mm^3. The cohort was thus representative of Malawian patients with HIV-TB coinfection in whom late presentation and advanced immunosuppression remain commonplace.

Thirty percent of subjects exhibited delayed absorption of rifampin defined as a time to maximum concentration of drug in plasma (T_{max}) of ≥4 h. While this phenomenon has been described previously (17), it also has practical consequences for sparse PK sampling strategies, which may produce falsely low or no estimates of AUC for these participants. This may be one reason why the PK parameters of rifampin have been reported as low in many studies. However, despite the intensive sampling used in our study, rifampin was the drug for which the AUC was lowest by comparison with other studies from the region. Although the PK-pharmacodynamic targets in TB are not currently widely agreed upon, with use of published sensitivity data (18), the median AUC/MIC achieved by patients in this study would be 85.3, which appears to be less than optimal on the basis of *in vivo* and human data (12, 19). Our findings therefore add support to the rationale for ongoing clinical trials in which higher doses of rifampin for treatment of tuberculosis are being evaluated.

Although the relationships expected between the measures of volume of distribution and body mass could be estimated in this data set, we did not find any clear relationship between the weight-adjusted dose and either the C_{max} or the AUC. This is reassuring and perhaps not surprising due to the weight-banded approach to dosing that is now commonly used for antituberculosis drugs and that is designed to achieve a narrow range of exposures irrespective of body weight. A larger and similarly intensive pharmacokinetic study in South Africa in which most patients received singly formulated drugs from different manufacturers rather than fixed-dose combinations reported that the weight-adjusted dose was a predictor of the AUC for all the first-line drugs (7). However, a second study using a high-quality weight-banded fixed quadruple-drug combination found that the weight-adjusted dose was a significant predictor of the AUC only for pyrazinamide and that there was an independent residual positive relationship with body mass alone (10). Other studies from the region used both singly formulated drugs and FDC tablets but did not present a detailed analysis of these covariates, so it remains unclear whether these findings relate to differences in formulation and dosing or to col-

linearity among the dose and weight variables in the existing data sets.

Due to the intensive sampling and sensitive bioanalytical method, it was possible to clearly demonstrate the biphasic elimination kinetics of isoniazid. This has been noted in some population PK studies (20) but is often not accounted for in noncompartmental analyses of sparse data and can result in inaccurate estimation of the terminal half-life and parameters derived from it. With use of a reduced data set, a distribution of half-lives and predicted acetylator phenotypes was observed that is similar to findings with other Central African populations, whether by an arbitrary cutoff or empirical clustering (21, 22). Of note, the proportion of slow acetylators was much higher in our study and that of Tostmann et al. (15) than in studies in the Western Cape (7, 23), emphasizing the need for more complete characterization of isoniazid PK and the diversity of the NAT2 genotype across the region.

The finding of a possible interaction between isoniazid and trimethoprim-sulfamethoxazole was unexpected and should be explored further. This might be a chance finding that is difficult to dissociate from the effect of HIV coinfection itself in our data set, but the *N*-acetylation pathway is known to be one of the primary routes of elimination of sulfamethoxazole (24) and is therefore a plausible target for competitive interactions with isoniazid.

In conclusion, in this first intensive PK study of first-line antituberculosis drugs in adult Malawians, HIV coinfection had no clinically significant effect on the key PK parameters that may be related to efficacy, supporting the current recommendations for use of similar regimens for HIV-positive and -negative individuals. Given the low exposure to rifampin, increasing its dose could be beneficial in African adults, irrespective of HIV status.

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