Pharmacokinetics of anti-tuberculosis drugs in HIV-positive and HIV-negative adults in Malawi

J.J. van Oosterhout[1,2], F.K. Dzinjalamala[3], A. Dimba[4], D. Waterhouse[5], G. Davies[6,7], E.E. Zijlstra[2], M.E. Molyneux[5,6], E.M. Molyneux[8], S. Ward[5]

Institution(s):
1 Dignitas International, Medical and Research Department, Zomba, Malawi,
2 College of Medicine, Department of Medicine, Blantyre, Malawi,
3 College of Medicine, Department of Pharmacy, Blantyre, Malawi,
4 National Tuberculosis Control Program, Lilongwe, Malawi,
5 Liverpool School of Tropical Medicine, Liverpool, United Kingdom,
6 Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi,
7 University of Liverpool, Liverpool, United Kingdom,
8 College of Medicine, University of Malawi, Department of Pediatrics, Blantyre, Malawi

Word count: 2669 Refs: 23 Abstract 247

Corresponding author: Joep J van Oosterhout
Address: Dignitas International, PO Box 1071, Zomba, Malawi
Telephone: +265-999922682
Email: joepvanoosterhout@gmail.com
Fax: +265-1527558

Abstract

Limited data address the impact of HIV co-infection on the pharmacokinetics of anti-tuberculosis drugs in Sub-Saharan Africa. 47 Malawian adults underwent rich pharmacokinetic sampling at 0-0.5-1-2-3-4-6-8 and 24 hours post-dose. 51% were male; mean age was 34 years. 65% were HIV-positive with a mean CD4 count of 268 cells/µL. Anti-tuberculosis drugs were administered as fixed-dose combinations (rifampicin150mg/isoniazid75mg/pyrazinamide400mg/ethambutol275mg) according to recommended weight bands. Plasma drug concentrations were determined by high-performance liquid chromatography (rifampicin and pyrazinamide) or liquid chromatography-mass spectrometry (isoniazid and ethambutol). Data were analysed by non-compartmental methods and analysis of variance of log-transformed summary parameters. Pharmacokinetic parameters were: rifampicin C_{max} 4.129 (2.474-5.596)µg/mL, AUC_{0-24} 21.32 (13.57-28.60)µg/mL*h, half-life 2.45 (1.86-3.08)h; isoniazid C_{max} 3.97 (2.979-4.544)µg/mL, AUC_{0-24} 22.5 (14.75-34.59)µg/mL*h, half-life 3.93 (3.18-4.73)h.; pyrazinamide C_{max} 34.21 (30.00-41.60)µg/mL, AUC_{0-24} 386.6 (320.0-463.7)µg/mL*h, half-life 6.821 (5.71-8.042)h; ethambutol C_{max} 2.278 (1.694-3.098)µg/mL, AUC_{0-24} 20.41 (16.18-26.27)µg/mL*h, half-life 7.507 (6.517-8.696)h. Isoniazid PK data analysis suggested that around two-thirds 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 tuberculosis drugs in Malawian adults, measures of pharmacokinetic exposure were comparable with other studies for all first line drugs except for rifampicin, for which C_{max} and AUC_{0-24} were notably lower. Contrary to some earlier observations, HIV status did not significantly affect AUC of any of the drugs. Increasing the dose of rifampicin could be beneficial in African adults, irrespective of HIV status. Current co-trimoxazole prophylaxis was associated with an increase in half-life of isoniazid of 41% (p=0.022). Possible competitive interactions between isoniazid and sulphamethoxazole mediated by the N-acetyltransferase pathway should therefore be explored further.

Introduction

Tuberculosis (TB) and human immunodeficiency virus (HIV) co-infection 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 TB sufferers are HIV seropositive. Treatment guidelines for TB do not recommend any adjustments in TB treatment in the presence of HIV co-infection, unless protease inhibitors are also being administered. Though a recent systematic review concluded that longer duration of rifamycin-based treatment reduces recurrence rates of TB after treatment in HIV-positive people, this appeared not to be true when antiretroviral therapy (ART) was co-administered (1). Concern has nonetheless lingered as to the reliability of TB treatment in this context with repeated reports of lower plasma concentrations of anti-tuberculosis drugs, particularly rifampicin, in HIV co-infected TB patients (2–11) and 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 inter-individual variability in PK and evaluate the impact of important covariates such as HIV co-infection and co-administration 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, HIV prevalence in adults is around 11% and 70% of TB patients are HIV infected. HIV 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 programmes. TB treatment is provided through a well-established community DOT system. Infrastructure to support therapeutic drug monitoring of TB drugs and PK data of tuberculosis drugs from 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 high rates of HIV co-infection 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) with a diagnosis of sputum microscopy acid fast bacilli positive pulmonary TB and who provided informed consent were enrolled into a pharmacokinetic study from January 2007 to February 2008. Sampling took place after a minimum of two weeks of TB treatment and before the end of the intensive treatment phase. Eligibility for enrollment was irrespective of current antiretroviral therapy (ART) for HIV. 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: Hb of less than 8 g/dL, vomiting within the preceding 72 hours, diarrhoea 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 WHO criteria, malnutrition was defined as a BMI of <18.5 kg/m2. We collected a venous sample for CD4 count, and concentrations of creatinine, Hb and ALT. HIV status was determined by rapid tests using HIV DETERMINE (Invernos Med, Japan Co Ltd) and confirmed by a second assay with UNIGOLD (Trinity Biotech PK Ireland) as per the national protocol at the time.

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

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

Bio-analytical methods

Rich pharmacokinetic sampling took place during the intensive phase of tuberculosis therapy. The first sample was taken at 0 hours (pre-dose) after which the patient had a light breakfast and then took the TB drugs. Dosing was administered under observation by the research nurse. An intravenous cannula was inserted and maintained using heparin-saline flushes. Samples were then drawn 0.5-1-2-3-4-6-8 and 24 hours post-dose 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 degrees Celsius until transportation on dry ice to Liverpool, UK.

Rifampicin and pyrazinamide plasma concentrations were determined using high-performance liquid chromatography on a Shimadzu LC 2010 HT system (Shimadzu, Manchester, UK). Isoniazid and ethambutol concentrations were determined simultaneously using LC-MS/MS on a triple-quadruple TSQ Quantum Access mass spectrometer (Thermo Scientific, Hemel Hempstead, UK). All methods incorporated appropriate internal standards (13) and were validated to internationally recognised 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 rifampicin, pyrazinamide, isoniazid and ethambutol respectively (13).

Statistical methods

Non-compartmental PK analysis of plasma concentration-time data was performed using Kinetica 4.1.1 (Adept Scientific Ltd, Armor Way, Letchworth Garden City, UK) 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 checked using routine graphical diagnostics. Generalised additive models were used to evaluate continuous covariate relationships using the package **mgc**V and clustering analysis for subpopulation detection in the parameter distributions was performed using the package **mclus**t.

Results

Forty-seven patients were enrolled (Table 1). Mean age was 34 years, 24 (51%) were male. Thirty (65%) were HIV-positive with a mean CD4 count of 268 cells/µL. Thirteen were on nevirapine-based and one

on efavirenz-based antiretroviral therapy. All HIV-positive participants received co-trimoxazole prophylaxis and none had chronic diarrhoea. The mean weight-adjusted dose received for each of the drugs was rifampicin 10.03, pyrazinamide 5.01, isoniazid 26.58, and ethambutol 18.38 mg/kg. Plasma concentration curves for each of the drugs are shown in Figure 1. Summary PK parameters derived from non-compartmental analysis are presented in Table 2. PK parameters for rifampicin, isoniazid, pyrazinamide and ethambutol could be computed for 41, 46, 46 and 47 participants respectively, due to missing observations or a non-credible 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, 3.68 contributing to the analysis and a percentage extrapolation on area under the curve ($AUC_{0-\infty}$) of 13.49, 5.31, 28.10 and 14.80 for the four drugs respectively.

For rifampicin, median observed maximum concentration (C_{max}) was 4.13 (inter-quartile range [IQR]: 2.47-5.60)µg/mL and $AUC_{0-\infty}$ 21.32 (IQR: 13.57-28.60)µg/mL*h. In 14 of 47 (39%) participants, C_{max} occurred at 4 hours post-dosing or later, representing delayed absorption and in 4 of these cases non-compartmental analysis failed to produce a meaningful estimate of AUC. C_{max} and AU_{Cl0-24} were not significantly associated with weight-adjusted dose (p=0.10 and 0.06) while $AUC_{0-\infty}$ was negatively correlated with 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, Vz and Vss 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 (IQR: 2.98-4.54)µg/mL and $AUC_{0-\infty}$ 22.5 (IQR: 14.75-34.59)µg/mL*h. No relationship was observed between these parameters and absolute or weight-adjusted dose. The median apparent terminal elimination half-life of isoniazid was 3.93 (IQR: 3.18-4.73)h. Seven of 46 (15%) had a half-life less than 130 minutes suggesting that 84.8% of participants would conventionally be classified as slow acetylator phenotype. However since the semi-logarithmic plot of the data suggested possible biphasic elimination with 38 of 46 participants (83%) showing detectable concentrations of isoniazid at 24 hours, these parameters were re-estimated with this data point omitted. The median elimination half-life was then 2.83 (IQR: 2.010-3.729)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 dataset with the 24 hour data point excluded, the algorithm predicted two subpopulations with estimated mean half-lives of 1.84 h 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 rifampicin, Vz and Vss 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 (IQR: 30.00-41.60)µg/mL and $AUC_{0-\infty}$ 386.6 (320.0-463.7)µg/mL*h. There was no consistent relationship between these parameters and absolute or weight-adjusted dose. C_{max} was reduced by 15% in HIV-positive participants though neither AUC_{last} or $AUC_{0-\infty}$ were affected. V_z increased by 0.42 L for each additional kilo of bodyweight (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 (IQR: 1.694-3.098)µg/mL and $AUC_{0-\infty}$ 20.41 (16.18-26.27)µg/mL*h. There was no observed relationship between these parameters and absolute or weight-adjusted dose. V_z increased by 5.18 L for each additional kilo of bodyweight (p=0.009) and this relationship accounted for an apparent univariate effect of sex on this parameter in multivariate analysis. Neither serum creatinine nor glomerular filtration rate was related to elimination half-life.

With the exception of the C_{max} of pyrazinamide, HIV co-infection did not significantly affect the PK parameters of any of the drugs studied. Age, gender and antiretroviral therapy also had no effect (see table S1).

Discussion

Since early reports of altered plasma concentrations of anti-tuberculosis drugs in HIV-positive patients, debate has continued as to the contribution of HIV co-infection to inter-patient PK variability in the treatment of TB. While Sub-Saharan Africa bears the brunt of HIV-associated tuberculosis and an increasing incidence of multi-drug resistance, surprisingly few PK studies have been performed in the region and fewer still have been able to employ intensive blood sampling. Though logistically simpler, sparse sampling can systematically underestimate key measures of exposure such as 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, 6, 16), HIV infection did not significantly or consistently affect the pharmacokinetics of any of the four first-line anti-tuberculosis drugs. The only exception was a

small reduction in C_{max} of pyrazinamide which did not affect overall plasma exposure as measured by AUC. These findings are reassuring and in accordance with other research supporting similar efficacy of TB treatment in HIV-positive patients (17). Early studies focused on patients with advanced HIV disease and associated diarrhoea prior to introduction of highly active antiretroviral therapy. About half of the HIV positive participants in our study were receiving ART according to national programme guidelines and all were on co-trimoxazole prophylaxis, which may have resulted in less advanced immunosuppression and fewer associated opportunistic infections. However, 50% had a CD4 less than 200 cells/mm3 with a median body mass of only 45kg, 12 kg less than those whose CD4 was greater than 200 cells/mm3. The cohort was thus representative of Malawian patients with HIV-TB co-infection in whom late presentation and advanced immunosuppression remain commonplace.

Thirty percent of subjects exhibited delayed absorption of rifampicin defined as a t_{max} of four hours or greater. While this phenomenon has been described previously (18), 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 PK parameters of rifampicin have been reported as low in many studies. However, despite the intensive sampling used in our study, rifampicin was the drug for which the AUC was lowest by comparison with other studies from the region. Though PK-pharmacodynamic targets in TB are not currently widely-agreed, using published sensitivity data (19) , the median AUC/minimum inhibitory concentration achieved by patients in this study would be 85.3 which appears less than optimal on the basis of *in vivo* and human data (12, 20). Our findings therefore add support to the rationale for ongoing clinical trials in which higher doses of rifampicin for treatment of tuberculosis are being evaluated.

Though the relationships expected between measures of volume of distribution and body mass could be estimated in this dataset, we did not find any clear relationship between weight-adjusted dose and either C_{max} or AUC. This is reassuring and perhaps not surprising due to the weight-banded approach to dosing that is now commonly used for anti-tuberculosis drugs and which is designed to achieve a narrow range of exposures irrespective of bodyweight. 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 weight-adjusted dose was a predictor of AUC for all the first-line drugs (7). However, a second study using a high-quality weight-banded fixed quadruple-drug combination found that weight-adjusted dose was only a significant predictor of AUC 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 collinearity among the dose and weight variables in the existing datasets.

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 (21) but is often not accounted for in non-compartmental analyses of sparse data and can result in inaccurate estimation of the terminal half-life and parameters derived from it. Using a reduced dataset, a distribution of half-lives and predicted acetylator phenotypes similar to those observed from other Central African populations was observed, whether using an arbitrary cut-off or empirical clustering (22, 23). Of note, the proportion of slow acetylators was much higher in our study and that of Tostmann (14) than in studies in the Western Cape (7, 24), emphasizing the need for more complete characterization of isoniazid PK and diversity of the NAT2 genotype across the region.

The finding of a possible interaction between isoniazid and trimethoprim-sulphamethoxazole was unexpected and should be explored further. This could be a chance finding which is difficult to dissociate from the effect of HIV co-infection itself in our dataset but the N-acetylation pathway is known to be one of the primary routes of elimination of sulphamethoxazole (25) and is therefore a plausible target for competitive interactions with isoniazid.

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

Acknowledgements

The study was funded by the National Tuberculosis Control Programme, Lilongwe, Malawi and the Liverpool University, UK. Drug analysis was supported through the Wellcome Trust funded LOTLink initiative. We thank the staff of the Tuberculosis department and HIV clinic at Queen Elizabeth Central Hospital, especially Mrs Rosemay Sikwese and Mr Moffo Phiri. Lastly we are grateful to the patients who participated in this study.

References

1. **Khan FA, Minion J, Al-Motairi A, Benedetti A, Harries AD, Menzies D**. 2012. An Updated Systematic Review and Meta-analysis on the Treatment of Active Tuberculosis in Patients With HIV Infection. Clin. Infect. Dis. **55**:1154–1163.
2. **Peloquin C, Nitta A, Burman W, Brudney K, Miranda-Massari J, McGuinness M, Berning S, Gerena G**. 1996. Low antituberculosis drug concentrations in patients with AIDS. Ann. Pharmacother. **30**:919–925.
3. **Sahai J, Gallicano K, Swick L, Tailor S, Garber G, Seguin I, Oliveras L, Walker S, Rachlis A, Cameron D**. 1997. Reduced plasma concentrations of antituberculosis drugs in patients with HIV infection. Ann. Intern. Med. **127**:289–293.
4. **Choudhri SH, Hawken M, Gathua S, Minyiri GO, Watkins W, Sahai J, Sitar DS, Aoki FY, Long R**. 1997. Pharmacokinetics of antimycobacterial drugs in patients with tuberculosis, AIDS, and diarrhea. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. **25**:104–111.
5. **Kimerling ME, Phillips P, Patterson P, Hall M, Robinson CA, Dunlap NE**. 1998. Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. Chest **113**:1178–1183.
6. **Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Padmapriyadarsini C, Swaminathan S, Bhagavathy S, Venkatesan P, Sekar L, Mahilmaran A, Ravichandran N, Paramesh P**. 2004. Decreased bioavailability of rifampin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease. Antimicrob. Agents Chemother. **48**:4473–4475.
7. **McIlleron H, Wash P, Burger A, Norman J, Folb P, Smith P**. 2006. Determinants of rifampicin,isoniazid, pyrazinamide and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob. Agents Chemother. **50**:1170–1177.
8. **Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, Wells CD, Reingold AL, Kenyon TA, Moeti TL, Tappero JW**. 2009. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. **48**:1685–1694.
9. **Saleri N, Dembélé SM, Villani P, Carvalho ACC, Cusato M, Bonkoungou V, Nacanabo R, Kouanda S, Comelli M, Regazzi M, Matteelli A**. 2012. Systemic exposure to rifampicin in patients with tuberculosis and advanced HIV disease during highly active antiretroviral therapy in Burkina Faso. J. Antimicrob. Chemother. **67**:469–472.
10. **McIlleron H, Rustomjee R, Vahedi M, Mthiyane T, Denti P, Connolly C, Rida W, Pym A, Smith PJ, Onyebujoh PC**. 2012. 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.

11. **Burhan E, Ruesen C, Ruslami R, Ginanjar A, Mangunnegoro H, Ascobat P, Donders R, van Crevel R, Aarnoutse R**. 2013. Isoniazid, rifampin, and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. Antimicrob. Agents Chemother. **57**:3614–3619.
12. **Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T**. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J. Infect. Dis. **208**:1464–1473.
13. **Mlotha R, Waterhouse D, Dzinjalamala F, Ardrey A, Molyneux E, Davies GR, Ward S.** 2015 Pharmacokinetics ofanti-tuberculosis drugs in children : reconsidering the role of ethambutol. J Antimicrob Chemother. 70:1798-803. doi: 10.1093/jac/dkv039
14. **FDA.** Guidance for Industry, Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration and Center for Drug Evaluation and Research, 2001. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf. Accessed 3 July 2015
15. **Tostmann A, Mtabho CM, Semvua HH, van den Boogaard J, Kibiki GS, Boeree MJ, Aarnoutse RE**. 2013. Pharmacokinetics of first-line tuberculosis drugs in Tanzanian patients. Antimicrob. Agents Chemother. **57**:3208–3213.
16. **Choudri S, Hawken M, Gathua S, Minyiri G, Watkins W, Sahai J, Sitar D, Aoki F, Long R**. 1997. Pharmacokinetics of antimycobacterial drugs in patients with tuberculosis, AIDS, and diarrhea. Clin. Infect. Dis. **25**:104–111.
17. **Korenromp E, Scano F, Williams B, Dye C, Nunn P**. 2003. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifampin-based treatment:an analytical review. Clin. Infect. Dis. **37**:101–112.
18. **Peloquin CA, Jaresko GS, Yong CL, Keung AC, Bulpitt AE, Jelliffe RW**. 1997.Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide. Antimicrob. Agents Chemother. **41**:2670–2679.
19. **Schön T, Juréen P, Giske CG, Chryssanthou E, Sturegård E, Werngren J, Kahlmeter G, Hoffner SE, Angeby KA**. 2009. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for Mycobacterium tuberculosis. J. Antimicrob. Chemother. **64**:786–793.
120. **Jayaram R, Gaonkar S, Kaur P, Suresh B, Maresh B, Jayashree R, Nandi V, Bharat S, Shandil R, Kantharaj E, Balasubramanian V**. 2003. Pharmacokinetics-Pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrob. Agents Chemother. **47**:2118–2124.
21. **Wilkins JJ, Langdon G, McIlleron H, Pillai G, Smith PJ, Simonsson USH**. 2011. Variability in the population pharmacokinetics of isoniazid in South African tuberculosis patients. Br. J. Clin. Pharmacol. **72**:51–62.

22. **Dandara C, Masimirembwa CM, Magimba A, Kaaya S, Sayi J, Sommers DK, Snyman JR, Hasler JA**. 2003. Arylamine N-acetyltransferase (NAT2) genotypes in Africans: the identification of a new allele with nucleotide changes 481C>T and 590G>A. Pharmacogenetics **13**:55–58.

23. **Sabbagh A, Langaney A, Darlu P, Gérard N, Krishnamoorthy R, Poloni ES**. 2008. Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history. BMC Genet. **9**:21.

24. **Donald PR, Parkin DP, Seifart HI, Schaaf HS, van Helden PD, Werely CJ, Sirgel FA, Venter A, Maritz JS**. 2007. 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.

25. **McDonagh EM, Boukouvala S, Aklillu E, Hein DW, Altman RB, Klein TE**. 2014. PharmGKB Summary: Very Important Pharmacogenetic information for N-acetyltransferase 2. Pharmacogenet.

297

298

299

Figure legends

300 **Figure 1.** Semi-logarithmic plots of plasma concentrations of the four first-line drugs. Solid line shows
301 median concentration, the dashed lines the upper and lower quartiles.
302

Table 1. Patient characteristics (N=47)

Characteristic	**Mean[n] (range) or N (percentage)**
Age (years)	34.4 (16-60)
Sex (male)	24 (52%)
Weight (kg)	52.52 (35.80-74.30)
Height (m)	1.65(1.51-1.81)
BMI (kg/m2)	19.29 (13.92-28.39)
Malnutrition (BMI<18.5 kg/m2)	19 (40%)
TB retreatment regimen	6 (13%)
HIV status	30 (65%)
WHO stage 4	6 (13%)
CD4 count	268 (3-1204)
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/L)	15.7 (5-44)

Table 2. Summary of pharmacokinetic parameters derived from non-compartmental analysis. Data presented are median and interquartile range

	Rifampicin (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)
$AUC_{0\text{-}\infty}$ (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)
$t_{½}$ (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 (L/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 (L)	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 (L)	130.20 (90.75-202.40)	72.35 (59.56-98.33)	38.74 (31.44-46.13)	498.90 (410.60-722.80)

Abbreviations : C_{max} Maximum observed plasma concentration , T_{max} Time of maximum observed plasma concentration , $AUC_{0\text{-last}}$ area-under the curve to last observed plasma concentration , $AUC_{0\text{-}\infty}$ area under the curve extrapolated to infinity , $t_{½}$ apparent elimination half-life, Cl/F apparent clearance, V_z /F Volume of distribution , V_{ss} /F Volume of distribution at steady state.