Role of Pharmacogenetics in Rifampicin Pharmacokinetics and potential effect on TB –rifampicin Sensitivity among Ugandan Patients.

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Abstract

Introduction: Suboptimal antituberculosis drugs exposure may cause multi-drug resistant tuberculosis. The role of African predominant *SLCO1B1* variant alleles on rifampicin pharmacokinetics and subsequent effect on occurance of *M. tuberculosis* – rifampicin sensitivity requires to be defined. We described rifampicin population pharmacokinetics profile and investigated the relevance of *SLCO1B1 genotypes* on rifampicin pharmacokinetics and rifampicin-TB sensitivity status.

Methods: Fifty patients with tuberculosis (n=25 with Rifampicin resistant TB and n=25 with rifampicin susceptible TB) were genotyped for *SLOC1B1* rs4149032 (g.38664C>T), *SLOC1B1*1B (c.388A>G)*, and *SLOC1B1**5 (*c.521T>C)*. Steady state plasma rifampicin levels were determined among patients infected with rifampicin sensitive TB. Data were analysed using NONMEM to estimate population rifampicin pharmacokinetics as well as the effect of *SLOC1B1* genotype on rifampicin pharmacokinetics and TB-rifampicin sensitivity status.

Results: Overall allele frequencies of *SLOC1B1* rs4149032, **1B* and *5 were, 0.66, 0.90 and 0.01 respectively. Median (IQR) C_{max} and T_{max} were 10.2 (8.1-12.5) mg/L and 1.7 (1.125- 2.218) hours respectively. Twenty four percent of patients exhibited C_{max} below the recommended 8-24 mg/l range. *SLOC1B1* genotypes, sex and age did not influence rifampicin pharmacokinetics or TB-rifampicin sensitivity.

Conclusions: Although *SLOC1B1* genotype, age and sex influence neither rifampicin pharmacokinetics nor rifampicin-TB sensitivity status, one of every four Ugandan TB patients achieve sub-therapeutic plasma rifampicin concentrations.

Keywords: SLCO1B1 polymorphism, MDR-TB, Rifampicin, pharmacokinetics, Treatment outcomes, Sub-Saharan Africa

Introduction

Tuberculosis (TB) is still a major global cause of morbidity and mortality. Being ranked together with HIV as a leading cause of death worldwide (1). In 2017, the WHO estimated 10.0 million TB cases globally, of which there were 1.3 million deaths In Uganda, the prevalence of TB was reported to be 201/100,000 population in 2017 and that of rifampicin- or multidrug -resistant TB (RR/MDR-TB) being 4,8/100,000 (1). Standard WHO recommended TB treatment regimens (2) achieve a treatment success rate of 86% (1). However tolerability, adherence and suboptimal bioavailability of TB drugs remain significant obstacles to achieving higher treatment success rates (3). Black African patients with TB seem to have low rifampicin concentrations (4) and poorer early TB treatment outcomes (5, 6). Possible explanations include differences in severity of tuberculosis, comorbidities such as HIV infection, and inter-ethnic variability in pharmacokinetics of anti-tubercular drugs including rifampicin (3, 4).

Rifampicin is a critical and potent component of the first-line combination drug TB therapy because of its early sterilizing activity against *Mycobacterium tuberculosis* in the intensive phase, and throughout the continuation phase of TB treatment (7). Rifampicin is mainly metabolized in liver where it induces several microsomal enzymes. About 13% to 24% is excreted in urine as unchanged drug (8, 9). Rifampicin uptake into the liver is largely mediated by an organic anion transporter polypeptide (OATP1B1) coded for by the *SLCO1B1* gene (10). Antimicrobial effect of rifampicin is concentration dependent (11). Anti-TB activity of rifampicin and development of resistance have been linked to drug

exposure, with concentrations of 8 to 24µg/ml being the expected peak concentrations at the currently recommended rifampicin dose (12, 13).

Polymorphism in *SLCO1B1* has previously been associated with low plasma concentrations (4)

There is an association between low plasma concentrations of rifampicin and isoniazid and delayed culture conversion rate (14). Hence genetic variation affecting rifampicin pharmacokinetics and disposition may determine anti-TB treatment response.

SLCO1B1 rs4149032 (g.38664C>T) exhibits a high allelic frequency of 76 % among Africans (15-17). Existing research findings on the effect of *SLCO1B1* polymorphism on rifampicin pharmacokinetics are conflicting. *SLCO1B1 rs4149032* polymorphism is associated with reduced rifampicin exposure (15). Patients heterozygous and homozygous for this polymorphism had reductions in the bioavailability (and, thus, the area under the curve [AUC]) of rifampicin of 18% and 28%, respectively (3). Recent studies however, demonstrated absence of any significant role of *SLCO1B1 (rs4149032, rs4149033* and *rs 11043819)* on rifampicin pharmacokinetics accross several populations (18)

Despite having a generally high level of genetic diversity (16, 19), and also shouldering the greater burden of TB incidence and mortality (1), very limited data is available about the pharmacogenetic determinants of anti-TB drug pharmacokinetics in African patients.

The purpose of this study was to investigate i) the pharmacokinetic and pharmacogenetics of rifampicin in Ugandan population, ii) to determine the allele frequency of the *SLCO1B1*

rs4149032 and rs2306283 (**1B)* and rs4149056 (**5*, *c.521T>C* encoding V174A) in African TB patients, and iii) to investigate whether *SLCO1B1* variant alleles predict rifampicin pharmacokinetics and multi-drug resistant tuberculosis (MDR-TB)

Materials and Methods

*E*thics approval:

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from Makerere University College of Health Sciences School of Biomedical Sciences Institutional Review Board and The Uganda National Council for Science and Technology.

Study site and population:

Upon written informed consent, 25 patients with rifampicin sensitive pulmonary TB and

25 patients with rifampicin resistant TB were enrolled at Mulago National referral Hospital Kampala Uganda. All participants were confirmed HIV negative as part of the study screening process. Rifampicin-resistant TB was determined using the GeneXpert MTB/RIF assay on sputum samples and subsequent laboratory confirmation. Both DS-TB and MDR-TB were treated following WHO and Uganda's National Treatment Guidelines. DS-TB patients received rifampicin-based fixed dose combination from Sandoz Limited 200 Frimley Carmberley Surrey UK. Demographic data including body weight in kilograms, age in years and sex and medication information were collected. All study participants were genotyped for *SLCO1B1 (rs 4149032* and **1B* and **5*). Plasma rifampicin pharmacokinetics blood samples (3 mls) were collected at zero (pre-dose), 1, 2, 4, 6, 12-hour post dose at steady state (>21 days from anti-TB treatment initiation) from

rifampicin–sensitive TB infected participants who were treated with rifampicin- based anti-tubercular therapy

SLCO1B1 Genotyping

Genomic DNA was isolated from whole blood samples using QIAmp DNA Blood Midi Kit (QIAGEN GmbH, Hilden, Germany) according to manufacturers' instructions. *SLCO1B1* genotyping for rs2306283 (**1B*) rs4149056 (**5*) and rs4149032. were done by real time PCR using pre-developed Taqman assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) as described previously (16, 17). The final volume for each reaction was 10 µL, consisting of TaqMan Fast advanced master mix (Applied Biosystems, Foster City, CA), TaqMan 20X drug metabolism genotyping assay mix (Applied Biosystems), and 10 ng genomic DNA. The PCR steps were set to an initial step at 60°C for 30 sec, hold at 95°C for 10 min and amplifications for 40 cycles, consisting of 95°C for 15 min, 60°C for 1 min, and a read stage at 60°C for 30 sec.

Rifampicin concentration determination

Rifampicin concentrations were determined using a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method at the routine therapeutic drug-monitoring laboratory, Department of Clinical Pharmacology, Karolinska University Hospital, in Stockholm, Sweden. The method was validated according to European Medicines Agency (EMA) guidelines (20) and was based on protein precipitation with methanol containing deuterated rifampicin as internal standard. After centrifugation, a supernatant aliquot was injected onto LC/MS/MS system equipped with a C18 reversed phase column. Elution was achieved with a mobile phase gradient of water and methanol, both

2 mmol/L ammonium formate and 0.1% formic acid. Quantification range was 0.2 to 25 mg/mL and concentrations were calculated by linear regression from a six-point calibration curve. Accuracy and precision was determined according to EMA, Spikedsamples at 4 levels including Lower limit of quantification were analyzed in pentaplicate at three different occasions. Within-run accuracy was determined to be <4.6% for all levels and between-run to <6.5%. Within-run precision was determined to be -6.4 to

+3.8 over the quantification range and between-run to -4.8% to -2.0%. All analytical runs were monitored with internal quality control samples.

Pharmacokinetic-Pharmacodynamic modelling

A population pharmacokinetic model was built in NONMEM version 7.3 (21, 22) and Xpose4 (23) were used during data set construction, graphical inspection, as well as statistical analysis. A two-compartment model specified in NONMEM by the ADVAN2 and TRANS2/ADVAN4 and TRANS4 subroutines, which described the data best was assumed. Inter individual variability was modelled on each parameter by assuming the exponential error model. Covariance between parameters was not modelled initially but was tested in the final structural models. The residual variability was modelled as additive plus proportional. The First Order conditional estimation with interaction (FOCEI) method was used to estimate the parameters. The first-order absorption rate constant (KA), clearance (CL), central volume of distribution (V1), peripheral volume of distribution (V2), inter-compartmental clearance (Q), and absorption lag time (ALAG) were estimated. Choice of the models was based on lowest objective function value (OFV), best goodness of fit plots, precision of parameter estimates and biological plausibility.

In order to optimize the selected structural models, absorption lag time was tested. The likelihood ratio test and improvement in the goodness of fit plots were used as criteria for retention of these parameters in the final model. An automated call in Perl Speaks NONMEM (PsN) software (24) was used to perform a covariate analysis on clearance (CL), central volume of distribution (V2), peripheral volume of distribution (V3) and absorption rate constant (ka) in a stepwise approach using the likelihood ratio test at a 5% significance threshold followed by backward elimination at a 1% significance threshold.

Age, body weight, and the two *SLCO1B1* SNPs (*rs4149032* and **1B)* were tested in the drug model. Due to very low allele frequency (0.01), *SLCO1B1*5* genotype data was not included in the model. In addition to the likelihood ratio test, improvement in the goodness of fit plots was also inspected. The most conservative models became our final covariate models. A bootstrap of the stepwise covariate modelling was performed to eliminate any spurious covariates. This was carried out by creating 1000 new datasets through resampling with replacement from the original dataset and repeating the covariate step on each new dataset. The inclusion frequency and stability were calculated for each covariate–parameter relationship. A covariate with inclusion frequency of 50% or more was considered non-spurious and retained in the final conservative model.

The antimicrobial effect of rifampicin is concentration dependent, and Cmax is the most frequently used PK parameter for therapeutic drug monitoring (11, 13, 25). For rifampicin, Cmax correlates well with AUC and has been reported as a suitable functional proxy (26). Simulation was therefore used to estimate peak rifampicin plasma concentrations

(Cmax). The final model obtained from the above data was used to simulate 100 datasets of 25 individuals each using a more intense sampling scheme. For every virtual individual in each simulated dataset, sampling was done every 10 minutes for 24 hours post dose. The maximum simulated concentration in the 24 hours was obtained for all individual in each simulation replicate. A median Cmax for the simulated data was obtained and plotted on a scatterplot including the recommended range (8-24 mg/l) (12, 13). A Chi square test was done to analyse any significant differences in SNP allele frequencies between the patients with MDR TB and those with drug susceptible TB.

Results

Of the 50 participants, 39 (78%) were male. Their median (IQR) age and body weight were 25 (21 to 37.25) years and weight 53 (50.75 to 60) kg respectively. All study participants were genotyped for *SLCO1B1 c.521T>C* rs4149056 (**5*). Only one study participant (2.3%) was found to be heterozygous and the remaining were homozygous wild type. The observed *SLCO1B1* genotype and allele frequencies stratified by rifampicin sensitivity are presented in **Table 1.** The overall allele frequencies of *SLOC1B1* rs4149032, **1B* and *5 were, 0.66, 0.90 and 0.01 respectively. Due to very low allele frequency (0.01), *SLCO1B1*5* genotype data was excluded from analysis. Neither *SLCO1B1 rs4149032* nor *SLCO1B1*1B* genotypes predict rifampicin sensitivity status ($p > 0.05$).

Rifampicin pharmacokinetics

Rifampicin plasma concentration were determined in rifampicin sensitive cohort who received rifampicin based anti-TB therapy. Majority (84%, n=21) of the participants were men with a median (IQR) body weight and age of 54 kg (53 to 60) and 24 years (20 to

39) respectively. During exploratory data analysis, peak concentrations occurred between 2 and 3 hours of post rifampicin administration. The log-concentration plots had two phases in the post-absorption phase, suggesting two compartment kinetics for rifampicin as seen in figure 1 (panel A). The model pharmacokinetic parameter estimates are indicated in **Table 2.**

Structural model optimization

Estimation of a lag time resulted in improvement in fit as measured by change in OFV (p=0.067). The DV vs IPRED plot, as shown in figure 1 (panel B), also showed marked improvement, indicating good prediction of observations by the model in figure 1 (panel C).

Covariate Analysis

At univariate level sex, *SLCO1B1 rs4149032* genotype and weight significantly affected rifampicin clearance, CL. Age and *SLCO1B1 (*1B*, *rs4149032* genotype) affected absorption constant, KA. The volume of distribution of the central compartment, V1 was significantly affected by age and *SLCO1B1 rs4149032* genotype whereas that of the peripheral compartment, V2, was significantly affected by *SLCO1B1 rs4149032* genotype only. However, upon adjustment for all factors only sex had significant effect on both CL and V2 at $p<0.05$. Inclusion of sex as a covariate on both V1 and CL resulted in over-parameterization of the model leading to instability. The base model therefore became the final model. Basic goodness-of-fit plots for the final model are shown in the **figure 1 (panel D),** whereas a visual predictive check of the final model is presented in **figure 2**. Parameter estimates for the final model are listed in **Table 3**.The median (IQR)

of simulated Cmax was 10.12mg/L (8.12-12.47) occurring at Tmax 1.7 (1.125 to 2.218) hours. About 24% and 0.04% of the simulated patients were observed to have peak plasma rifampicin concentrations that are below and above the recommended therapeutic range of 8 to 24 mg/L respectively, **figure 3**.

Discussion

To our knowledge this is the first study that investigated genetic associations between rifampicin-TB sensitivity status using candidate gene approach in a black African population. Our result may serve as a preliminary finding for future large sample size studies. Sex or other demographic factors did not significantly affect rifampicin PKs or rifampicin-TB sensitivity status. Considering that consecutive sampling technique was employed to recruit study participants, the skewness of study participation to men (78%) is in agreement with previous findings that revealed male gender being a risk factor for TB infection infection(36, 28-30).

We report high allelic frequencies of *SLCO1B1 rs.4149032 (*66%) and *SLCO1B1*1B (*90%) among Ugandans. *SLCO1B1* (*rs.4149032, *1B)* which affects neither the rifampicin PKs nor rifampicin-TB sensitivity status. This finding is in agreement with results of a recent study, that evaluated the effect of *SLCO1B1* (*rs. 4149032,11043819,4149033 and 2306283)* on rifampicin pharmacokinetics in a South Indian population (18). Likewise no effect of genotype on rifampicin PK among South African patients with recurrent tuberculosis has been reported **(27).**

The simulated median peak plasma rifampicin concentration for our study population, Cmax, (10.12mg/L) was well within the currently recommended therapeutic range (8-24mg/L) (13), unlike other reports where majority of the TB patients were found to have lower Cmax values (3, 28). Differences between extremely low Cmax values previously reported and findings of this study might in part be explained by study design differences. For instance, while Gengiah et al (3) collected samples for rifampicin Cmax determination at 2.5hrs post rifampicin dose, according to our study, the median (IQR) rifampicin Tmax was 1.7 (1.125 to 2.218) hours implying that Gengiah et al (3) could have collected rifampicin sample during the elimination phase. Other reasons might include formulation factors (29) as well as inter-ethnic factors. The other possible source of variation and difference in results and observation between the similar studies could be the study population, whereas the study in South Africa was done amongst HIV positive patients who had TB co-infection, our rifampicin pharmacokinetic study was conducted among HIV negative patients. HIV disease has been reported to affect drug pharmacokinetics (30) while possible drug interaction with some drugs that comprise of cART and other co-treatments including herbal preparations given to HIV patients could significantly affect rifampicin pharmacokinetics.

Since there was no observed significant effect of the *SLCO1B1* genotype on the above primary parameters, it is plausible that neither would they affect the secondary/derived pharmacokinetic parameters including volume of distribution or peak concentrations. Interestingly, a significant 1 in every 4 TB patients in our study population exhibited plasma rifampicin concentrations below the minimum recommended therapeutic

concentration. Development of drug resistance in *Mycobacterium tuberculosis* (*Mtb*) has been ascribed to inadequate treatment, insufficient dose or dosing frequency, non-adherence to the regimen, and PK variability (31). PK variability becomes even more important given that emergence of drug resistance during the course of therapy is a major problem and sub-therapeutic drug exposure may further select for the drug resistant mutants (32). Sub-optimal rifampicin concentrations exhibition by a significant proportion of TB patients in Uganda and other settings might therefore be a key driver for emergence and transmission of multi–drug resistant TB. Notable, there are suggestions for an upward adjustment of rifampicin dose **(33)**. A chi square test was done and no significant differences in SLCO1B1 variant allele frequencies between the two study patient populations. Given the finding of no significate effect of *SLCO1B1* genotype on rifampicin pharmacokinetics, it is plausible that the genotype also may not be a driving factor for the development of rifampicin resistant tuberculosis phenotype. However, our sample size may not be adequate enough to draw conclusion therefore future large sample size studies are needed.

As reported in the results, several factors had significant influence on the rifampicin pharmacokinetics in the base model during univariate analysis. However, these effects were seen to diminish to near insignificance upon adjusting for other covariates. This implies that when considered alone, the polymorphism of *SLCO1B1* affects rifampicin primary pharmacokinetics parameters like clearance, volume of distribution and absorption constant. Unlike what was reported by some studies from South Africa and elsewhere (3, 15, 28), polymorphism of *SLCO1B1 rs.4149032* does not affect the above primary PK parameters upon adjusting for other patient factors like weight and sex that

were seen to also significantly affect rifampicin pharmacokinetics individually. However, the result obtained for *SLCO1B1*1B* was consistent with that reported another study (4). The 46 minutes absorption lag time reported by this study was found to be slightly higher than the 30 to 40 minutes and 37 minutes lag time reported by Medellin et al in Mexican patients (34) and Denti et al in South African patients (35) respectively. This could have been because of sparse sampling in the absorption phase or also because of the larger percentage of patients who are mutant for the above transporter gene.

The observed overall high frequency of *SLCO1B1* rs4149032 (66%) in Ugandans is similar to previous reports from South Africans (70%) (15) and Tanzanians (52%). *SLCO1B1*1B* is the most common variant allele in Ugandans (90%) which is similar to Tanzanians (87%) and in Ethiopians (60%) (16). However *SLCO1B1*5* is a rare variant allele (1.1%) in Ugandans, which is a similar finding from Tanzanian population (3.2%) but this variant allele is more common in Ethiopians (19%) (16).

There are some limitations in this study. Our study was conducted in Kampala a cosmopolitan setting in Uganda, one of the 22 highest burden countries globally (WHO, 2018 Report). The selective focus of the study may not be representative of the rest of Uganda, whole East Africa region or other sub-Saharan countries. As MDR/rifampicin resistant TB is not frequent in Uganda occurring at a rate of 4.8/100,000 population (1), getting large number of cases was challenging, which subsequently resulted in a small sample size. However our population PK modelling and simulation approach to data analysis may minimize the impact of low sample size.

Conclusions

Following the recommended rifampicin dosing, a significant 1 in 4 of TB patients taking rifampicin were predicted to have sub-optimal drug concentrations, i.e., below 8mg/L. Whether this might be one of the factors driving rifampicin resistance needs to be further investigated so that a higher evidence based rifampicin dose might be recommended in consideration of possible varying population needs. Despite the high allelic frequencies of *SLCO1B1 rs*. *4149032 (*66%) and *SLCO1B1*1B (*90%) among in the Ugandan population*, SLCO1B1* genotypes effected neither the rifampicin PKs nor rifampicin-TB sensitivity status. Additionally, rifampicin pharmacokinetics and TB-sensitivity do not dependent upon sex or other demographic factors.

Authors contributions:

JKM- Conceptualization, supervision of research, manuscript writing and approval

K- Conceptualization, data collection, laboratory analysis, manuscript writing and approval

SN- Supervision of research, manuscript writing and approval

BK- Data analysis, manuscript review and approval

AP- Laboratory analysis, manuscript review and approval

TDM- Manuscript review and approval

AZ- Manuscript review and approval

EA- Conceptualization, supervision of research, manuscript writing and approval

Acknowledgements: Authors hereby acknowledge all study participants

Funding: This work was supported: The International AIDS Society (CIPHER) [Grant Number kij-216] and The Swedish Research Council (Vetenskapsrådet) [Grant number: 2015-03295].

Conflict of interest: Authors have no conflict to declare.

Ethical Approval: The study was approved by Makerere University, School of Biomedical Sciences Institutional Review Board & Higher Degrees Committee

References

1. World Health Organization: Global tuberculosis report 2018. Geneva. Available at:https: www.who.int/tb/publications/global_report/en/ 2018 [Available from: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1.

2. World Health Organization: Guidelines for treatment of tuberculosis: fourth edition: WHO Library Cataloguing-in-Publication Data:WHO/HTM/TB/2009.420. 2010.

3. Gengiah TN, Botha JH, Soowamber D, Naidoo K, Abdool Karim SS. Low rifampicin concentrations in tuberculosis patients with HIV infection. J Infect Dev Ctries. 2014;8(8):987-93.

4. Weiner M, Peloquin C, Burman W, Luo CC, Engle M, Prihoda TJ, et al. Effects of tuberculosis, race, and human gene SLCO1B1 polymorphisms on rifampin concentrations. Antimicrob Agents Chemother. 2010;54(10):4192-200.

5. Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, et al. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. Am J Respir Crit Care Med. 2006;174(3):331-8.

6. Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, Bozeman L, et al. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. Am J Respir Crit Care Med. 2009;180(3):273-80.

7. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. Int J Tuberc Lung Dis. 2000;4(9):796-806.

8. Burman WJ, Gallicano K, Peloquin C. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin Pharmacokinet. 2001;40(5):327-41.

9. Acocella G. Clinical pharmacokinetics of rifampicin. Clin Pharmacokinet. 1978;3(2):108-27.

10. Baciewicz AM, Chrisman CR, Finch CK, Self TH. Update on rifampin and rifabutin drug interactions. Am J Med Sci. 2008;335(2):126-36.

11. Gumbo T, Louie A, Deziel MR, Liu W, Parsons LM, Salfinger M, et al. Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. Antimicrob Agents Chemother. 2007;51(11):3781-8.

12. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs. 2014;74(8):839-54.

13. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs. 2002;62(15):2169-83.

14. Sekaggya-Wiltshire C, von Braun A, Lamorde M, Ledergerber B, Buzibye A, Henning L, et al. Delayed Sputum Culture Conversion in Tuberculosis-Human Immunodeficiency Virus-Coinfected Patients With Low Isoniazid and Rifampicin Concentrations. Clin Infect Dis. 2018;67(5):708-16.

15. Chigutsa E, Visser ME, Swart EC, Denti P, Pushpakom S, Egan D, et al. The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is

associated with reduced rifampin concentrations: dosing implications. Antimicrob Agents Chemother. 2011;55(9):4122-7.

16. Aklillu E, Habtewold A, Ngaimisi E, Yimer G, Mugusi S, Amogne W, et al. SLCO1B1 Gene Variations Among Tanzanians, Ethiopians, and Europeans: Relevance for African and Worldwide Precision Medicine. OMICS. 2016;20(9):538-45.

17. Aklillu E, Mugusi S, Ngaimisi E, Hoffmann MM, Konig S, Ziesenitz V, et al. Frequency of the SLCO1B1 388A>G and the 521T>C polymorphism in Tanzania genotyped by a new LightCycler(R)-based method. Eur J Clin Pharmacol. 2011;67(11):1139-45.

18. Ramesh K, Hemanth Kumar AK, Kannan T, Vijayalakshmi R, Sudha V, Manohar Nesakumar S, et al. SLCO1B1 gene polymorphisms do not influence plasma rifampicin concentrations in a South Indian population. Int J Tuberc Lung Dis. 2016;20(9):1231-5.

19. Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, et al. The genetic structure and history of Africans and African Americans. Science. 2009;324(5930):1035-44.

20. European Medicines Agency. Guideline on Bioanalytical Method Validation. London, 2011. Available at: https://www.ema.europa.eu/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf".

21. Baker SG, Xu JL, Hu P, Huang P. Vardeman, S. B. and Morris, M. D. (2013), "Majority Voting by Independent Classifiers can Increase Error Rates," The American Statistician, 67, 94-96: Comment by Baker, Xu, Hu, and Huang and Reply. Am Stat. 2014;68(2):125-6.

22. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing V, Austria 2013. Available online at https://www.R-project.org/.

23. Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed. 1999;58(1):51-64.

24. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. Comput Methods Programs Biomed. 2004;75 (2):85-94.

25. McCallum AD, Sloan DJ. The importance of clinical pharmacokinetic-pharmacodynamic studies in unraveling the determinants of early and late tuberculosis outcomes. Int J Pharmacokinet. 2017;2(3):195-212.

26. Hall RG, Leff RD, Gumbo T. Treatment of active pulmonary tuberculosis in adults: current standards and recent advances. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy. 2009;29 (12):1468-81.

27. Naidoo A, Chirehwa M, Ramsuran V, McIlleron H, Naidoo K, Yende-Zuma N, et al. Effects of genetic variability on rifampicin and isoniazid pharmacokinetics in South African patients with recurrent tuberculosis. Pharmacogenomics. 2019;20(4):225-40.

28. Kwara A, Cao L, Yang H, Poethke P, Kurpewski J, Tashima KT, et al. Factors associated with variability in rifampin plasma pharmacokinetics and the relationship between rifampin concentrations and induction of efavirenz clearance. Pharmacotherapy. 2014;34(3):265-71.

29. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob Agents Chemother. 2006;50(4):1170-7.

30. Mukonzo JK, Nanzigu S, Rekic D, Waako P, Roshammar D, Ashton M, et al. HIV/AIDS patients display lower relative bioavailability of efavirenz than healthy subjects. Clin Pharmacokinet. 2011;50(8):531-40.

31. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis. 2011;204(12):1951-9.

32. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis. 2012;55(2):169-77.

33. Seijger C, Hoefsloot W, Bergsma-de Guchteneire I, te Brake L, van Ingen J, Kuipers S, et al. High-dose rifampicin in tuberculosis: Experiences from a Dutch tuberculosis centre. PLOS ONE. 2019;14(3):e0213718.

34. Medellin-Garibay SE, Cortez-Espinosa N, Milan-Segovia RC, Magana-Aquino M, Vargas-Morales JM, Gonzalez-Amaro R, et al. Clinical Pharmacokinetics of Rifampin in Patients with Tuberculosis and Type 2 Diabetes Mellitus: Association with Biochemical and Immunological Parameters. Antimicrob Agents Chemother. 2015;59(12):7707-14.

35. Denti P SW, Simonsson U, Rustomjee R, Onyebujoh P, Smith P, et al., editors. A population pharmacokinetic model for rifampicin auto-induction. The 3rd international workshop on clinical pharmacology of TB drugs; 2010.

36. Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, et al. Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. Int J Epidemiol. 2005;34(4):914-23.

37. Neyrolles O, Quintana-Murci L. Sexual inequality in tuberculosis. PLoS Med. 2009;6(12):e1000199.

38. Boum Y, 2nd, Atwine D, Orikiriza P, Assimwe J, Page AL, Mwanga-Amumpaire J, et al. Male Gender is independently associated with pulmonary tuberculosis among sputum and non-sputum producers people with presumptive tuberculosis in Southwestern Uganda. BMC Infect Dis. 2014;14:638.

Figure legend

Figure 1: Log concentration plot of the data obtained during data analysis in panel A, DV vs IPRED before optimization in panel B, an improved DV vs IPRED after model optimization in panel C and conditional weighted residuals vs. time in panel D. = Conditional weighted residuals vs. time

Figure 2: Visual predictive check using the final rifampicin PK model. The shaded areas are the 95% confidence intervals of the simulated predictions.

Figure 3: A scatter plot of C_{max} for the 2400 simulated individuals. The figure also shows the documented therapeutic range of 8 to 24 mg/L, median of the population C_{max} as well individual data points within and outside the therapeutic range

Table 1: Distribution of *SLCO1B1* genotypes among study population.

SLCO1B1 Genotype		***Rifampicin resistance status***			
		Non-Resistant		***Resistant***	
		n	***Percentage***	***n***	***Percentage***
SLCO1B1 rs4149032 (g.38664C>T)	CC	4	16,0%	3	12,0%
	CT	9	36,0%	11	44,0%
	TT	12	48,0%	11	44,0%
*SLCO1B1 c.388A>G rs2306283 (*1B)*	AA	0		0	
	AG	5	20.0%	5	20.0%
Minor allele frequency					
rs4149032 (*g.38664C>T*)	T		66.0%		66.0%
rs2306283, c.388A>G (*1B)	G		88.5%		91.7%

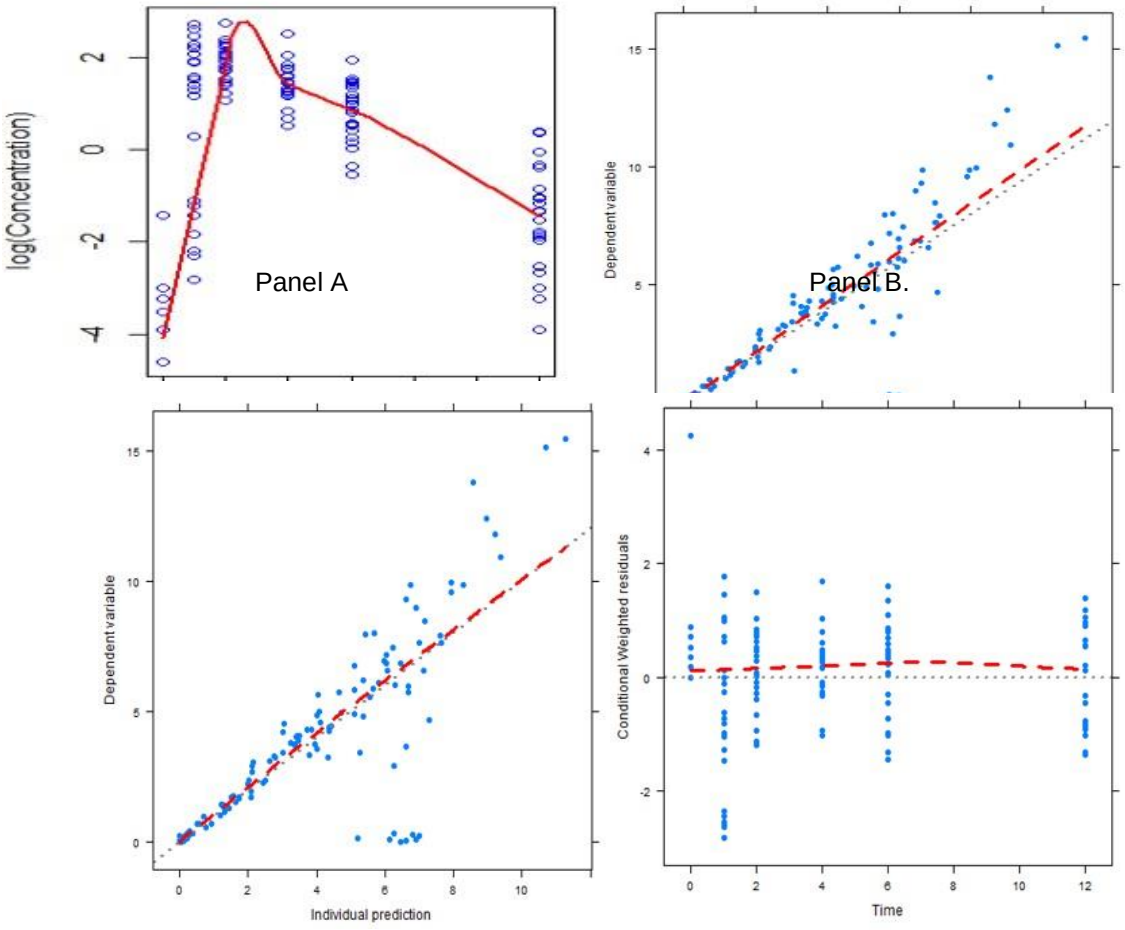
Table 2: Pharmacokinetic parameter estimates from the final model

Parameter/Units	**Value**	**Bootstrap 95% CI**
KA (/hr)	0.4682	0.39 – 0.91
V1 (L)	0.5383	0.09 – 1.72
CL (L/hr)	19.8831	16.06 – 23.43
V2 (L)	19.3284	10.29 – 37.98
Q (L/hr)	19.6854	9.38 -78.84
Lag time (hr)	0.7748	0.52 – 0.99
Inter-individual variability		
KA	0.0085	0.00013 – 0.23
V1	6.5359	3.32 – 20.17
CL	0.1461	0.06 – 0.3
V2	0	Fixed
Q	0	Fixed
Residual error		
Proportional component	0.1386	0.01 -0.18
Additive component (mg/L)	0.0032	0.0035 – 0.008

Table 3: The covariate effect on Pharmacokinetic parameters including Clearance (CL), absorption constant (KA), central volume of distribution (V1) and peripheral volume of distribution (V2). Baseline (base model) objective function value (OFV) was 80.93397.

MODEL	Drop in OFV	DOF	Threshold OFV drop for significance level at P value 0.01	P value
CL_AGE	0.05673	1	3.8415	0.81174
CL_SEX	78.47331	1	3.8415	8.11E-19
CL_SL1B	0.77427	1	3.8415	0.3789
CL_SL32	77.30208	3	7.8147	1.16E-16
CL_WT	5.29524	1	3.8415	0.021384
KA_AGE	75.6514	1	3.8415	3.38E-18
KA_SEX	0.07853	1	3.8415	0.7793
KA_SL1B	7.63844	1	3.8415	0.005714
KA_SL32	78.63132	3	7.8147	6.03E-17
KA_WT	0.01085	1	3.8415	0.91705
V1_AGE	5.32056	1	3.8415	0.021075
V1_SEX	0.45806	1	3.8415	0.49853
V1_SL1B	6.82588	1	3.8415	0.008985
V1_SL32	15.80594	3	7.8147	0.001243
V1_WT	0.19804	1	3.8415	0.65631
V2_AGE	0.07657	1	3.8415	0.782
V2_SEX	0.73814	1	3.8415	0.39026
V2_SL1B	76.09057	1	3.8415	2.71E-18
V2_SL32	77.80021	3	7.8147	9.10E-17

DOF-degrees of freedom, SL1B- SLCO1B1*1B genotype, SL32-SLCO1B1 (rs4149032) genotype



Panel C

Panel D

Figure 1

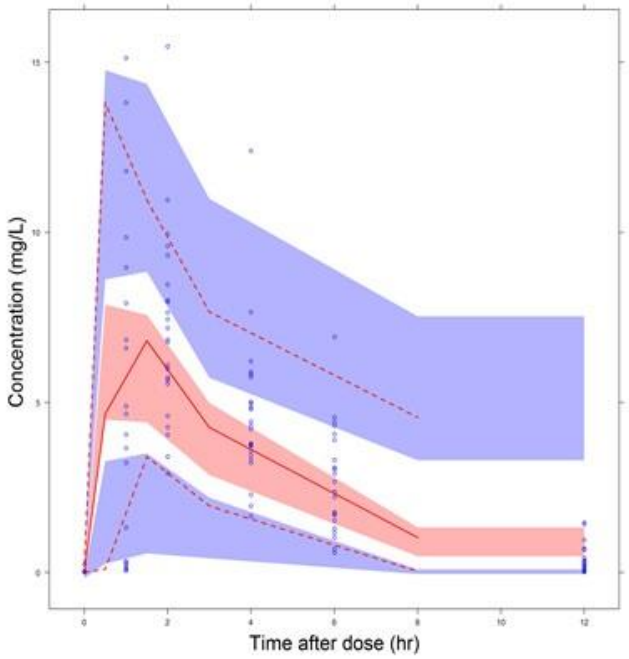


Figure 2:

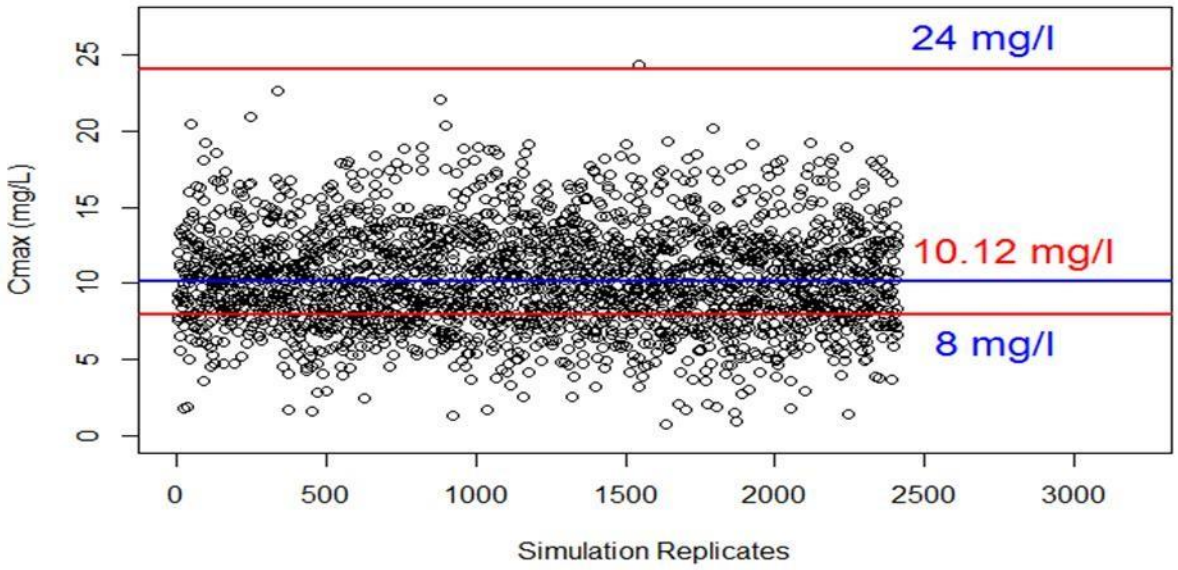

24 mg/l
10.12 mg/l
8 mg/l
Cmax (mg/L)
0
5
10
15
20
25
0
500
1000
1500
2000
2500
3000
Simulation Replicates

Figure 3