DRUG REPURPOSING AND OPTIMISATION TO IMPROVE TUBERCULOSIS TREATMENT

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes three papers published in peer-reviewed journals and two papers currently undergoing peer review. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the instances where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

CHAPTER 1

This chapter consists of a published manuscript, reporting on the results of a narrative review of rifampicin. I developed the systematic search strategy, screened the selected articles, extracted the data, and used R software to assess the relationship between rifampicin dose and exposure metrics in different patient populations. In addition, I wrote the manuscripts included in the chapter, and coordinated co-authors concerning the reporting and interpretation of our findings.

Clinical pharmacokinetics and pharmacodynamics of rifampicin in human tuberculosis.

Abulfathi AA, Decloedt EH, Svensson EM, Diacon AH, Donald P, Reuter H. *Clin Pharmacokinet*. 2019;58(9):1103-1129.

- EH Decloedt contributed to critical review of the manuscript and interpretation of the results.

- PR Donald supported with data extraction, critical review of the manuscript and interpretation of the results.
- EM Svensson, AH Diacon, and H Reuter contributed to critical review of the manuscript, interpretation of the results, and supervision.

CHAPTER 2

This chapter consists of a published manuscript, reporting on the result of a narrative review of para-aminosalicylic acid (PAS). I developed the systematic search strategy, screened the selected articles, and extracted the data. R software and Web Plot digitizer were used to recreate PAS plots from studies dating back to the 1950s and 1960s. In addition, I wrote the manuscript included in this chapter, contributed to the critical review and interpretation of the findings, and coordinated the contribution of co-authors concerning the reporting, and interpretation of our findings.

The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance.

Abulfathi AA, Donald PR, Adams K, Svensson EM, Diacon AH, Reuter H. *Br J Clin Pharmacol*. 2020;86(11):2123-2132. https://doi.org/ 10.1111/bcp.14395.

- PR Donald contributed to developing the study concept, literature search, selection of studies, data extraction, and critical review of the manuscript and interpretation of the results.
- K Adams contributed with literature search, selection of studies and revision of the manuscript.
- EM Svensson, AH Diacon and H Reuter contributed to study design, critical review of the manuscript, interpretation of the results, and supervision.

CHAPTER 3

This chapter consists of a published manuscript. I contributed to developing the research questions, data formatting, designing and execution of the modelling and simulation study, interpreting the results and drafting the manuscript. In addition, I coordinated co-authors concerning the reporting, and interpretation of our findings.

Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens.

Abulfathi AA, Assawasuwannakit P, Donald PR, Diacon AH, Reuter H, Svensson EM. *Eur J Clin Pharmacol*. 2020;76(11):1557-1565. doi:10.1007/s00228-020-02943-8.

- P Assawasuwannakit contributed to data formatting, designing the modelling study and critical review of the manuscript.
- PR Donald contributed to the conceptual design of the study, interpreting the results and critical review of the manuscript.
- AH Diacon and H Reuter contributed to the conceptual design of the study, interpreting the results, critical review of the manuscript, and co-supervision.
- EM Svensson contributed to developing the research questions, designing the modelling and simulation study, interpreting the results, critical review of the manuscript, and primary supervision.

CHAPTERS 4

This chapter consists of a revised manuscript undergoing second round of peer review in the American Journal of Respiratory and Critical Care Medicine (AJRCCM). The chapter describes first-time data on the pharmacokinetics of first-line anti-tuberculosis drugs in children with severe

intra-thoracic tuberculosis. I am joint first-author and contributed to the methodology, investigation, data formatting, visualisation, writing the original draft manuscript, interpretation of results and critical review of the manuscript.

Drug concentration at the site of disease in children with pulmonary tuberculosis.

Lopez-Varela E*, Abulfathi AA*, Strydom N, Goussard P, Van Wyk AC, Demers AM, Van Deventer A, Garcia-Prats AJ, Van der Merwe J, Zimmerman M, Carter CL, Janson J, Morrison J, Reuter H, Decloedt EH, Seddon JA, Svensson EM, Warren R, Savic RM, Dartois V#, Hesseling AC#

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- AC Van Wyk contributed to methodology, data curation, investigation, resources, and critical review of the manuscript.
- AM Demers, AV Deventer, AJ Garcia-Prats, and J Van der Merwer contributed to methodology, data curation, and critical review of the manuscript.
- M Zimmerman and CL Carter contributed to methodology, investigation, and critical review of the manuscript.
- J Janson contributed to data curation, resources, and critical review of the manuscript.

- J Morrison contributed to data curation, and critical review of the manuscript.
- H Reuter contributed to data curation, resources, co-supervision, and critical review of the manuscript.
- EH Decloedt contributed to data curation and critical review of the manuscript.
- JA Seddon contributed to study conceptualisation and critical review of the manuscript.
- EM Svensson contributed to methodology, critical review of the manuscript, and supervision.
- R Warren contributed to methodology and critical review of the manuscript.
- RM Savic contributed to study conceptualisation, modelling and simulations, resources, software, critical review of the manuscript, and supervision.
- V Dartois contributed to study conceptualisation, investigation, methodology, resources, critical review of the manuscript, and supervision.
- AC Hesseling contributed to study conceptualisation, resources, critical review of the manuscript, and supervision.

CHAPTER 5

This chapter consists of a submitted manuscript undergoing peer review in Frontiers in Pharmacology. I contributed in data formatting, explorative data analysis, designed the population pharmacokinetics study, wrote the NONMEM control stream, performed the NONMEM modelling, interpreted the results, wrote the original manuscript and critical review of the submitted manuscript.

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis.

Abulfathi AA, De Jager V, Van Brakel E, Reuter H, Gupte N, Vanker N, Barnes GL, Nuermberger E, Dorman SE, Diacon AH, Dooley KE, Svensson EM.

- V De Jager contributed to patient recruitment, investigation, critical review of the manuscript.
- E Van Brakel contributed to patient recruitment, investigation, critical review of the manuscript.
- H Reuter contributed to critical review of the manuscript and co-supervision.
- N Gupte contributed to the investigation, methodology, data formatting and critical review of the manuscript.
- N Vanker contributed to patient recruitment, investigation, critical review of the manuscript.
- GL Barnes contributed to methodology, investigation, and critical review of the manuscript.
- E Nuermberger and SE Dorman contributed to investigation, methodology, and critical review of the manuscript.
- AH Diacon contributed to investigation, methodology, critical review of the manuscript, interpretation of results and co-supervision.
- KE Dooley contributed to investigation, methodology, critical review of the manuscript and interpretation of results.
- EM Svensson contributed to designing the study, interpretation of results, initial review of the original draft, critical review of the manuscript and supervision.

DECLARATION BY CO-AUTHORS:

The undersigned hereby confirm that:

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to several chapters as stated.
2. No other authors contributed besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties.

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SUMMARY

DRUG REPURPOSING AND OPTIMISATION TO IMPROVE TUBERCULOSIS TREATMENT

Tuberculosis, a leading infectious cause of mortality, morbidity, and reduced quality of life with loss of income, remains a huge public health burden particularly in resource-limited settings. The golden thread throughout this dissertation, was improving tuberculosis treatment through repurposing and optimisation of existing medicines. For that purpose, we sought to improve our understanding of the pharmacokinetics and pharmacodynamics of selected anti-tuberculosis drugs.

CHAPTER 1

Clinical pharmacokinetics and pharmacodynamics of rifampicin in human tuberculosis

In this review, spanning 51 years, we summarised rifampicin pharmacokinetic and pharmacodynamic data across a range of doses in adult healthy volunteers, tuberculosis patients, and special patient populations. We included 170 articles, and pharmacokinetic data extracted from 69 studies that enrolled 3666 participants who received rifampicin over a dose range of 2-35 mg/kg. We found considerable inter- and intra-individual variability in rifampicin exposure, which can be reduced by administration under fasting conditions. Factors that alter rifampicin exposure and/or efficacy include malnutrition, human-immunodeficiency virus infection, diabetes mellitus, dosing, pharmacogenetic polymorphisms, hepatic cirrhosis, and substandard medicinal products. Area under the concentration-time curve (from time zero to 24 h) [AUC_{0-24}]/ minimum inhibitory concentration (MIC) is the pharmacokinetic/pharmacodynamic parameter that correlates best with rifampicin bactericidal activity. Higher rifampicin doses compared to the standard adult 10 mg/kg oral may be required for some indications such as tuberculous meningitis, where higher rifampicin exposure has been associated with reduced mortality. Therapeutic drug monitoring integrated with Bayesian priors could allow dose individualisation and attainment of optimal drug exposure quicker.

CHAPTER 2

The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance

This chapter expanded on a previous review of the development of para-aminosalicylic acid (PAS) regarding formulations, dosing practices and their relationship to the prevention of resistance in companion drugs and intolerance, and reviewed the pharmacokinetics of PAS in greater depth, in particular focusing on the lack of relationship between PAS plasma concentrations and intolerance. This chapter also presented previously unappreciated evidence that PAS may well have some bactericidal efficacy. Available evidence suggest PAS C_{max}, AUC, and, by extension C_{max}/MIC and/or AUC/MIC rather than proportion of time PAS concentration is above MIC (%T>MIC) of 1 mg/L are the most important determinants of efficacy and suppression of resistance development in companion drugs. Therefore, there is need for a prospective study to re-evaluate PAS pharmacokinetics/pharmacodynamics using modern methodologies.

CHAPTER 3

Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens

In chapter 2, we provided evidence to support high once-daily dosing of PAS. In particular, a granular slow-release PAS formulation (PASER® GRANULES [Aminosalicylic Acid Delayed-Release Granules], JACOBUS PHARMACEUTICAL COMPANY, INC.Princeton, NJ, USA), at the current dosing regimen of 8-12 g per day administered in 2-3 divided doses will not achieve PAS concentrations likely reached in the early clinical trials supporting the introduction of PAS. The optimal use of PASER requires adequate understanding of PAS dose-exposure-response relationship. We, therefore, established a representative population pharmacokinetics model for PASER and evaluated the probability of bactericidal and bacteriostatic target attainment with different dosing regimens. The pharmacokinetic model included both inter-individual and inter-occasion variability in PAS bioavailability and allometric scaling with total body weight on disposition parameters.

Assuming a PAS MIC of 1 mg/L, the proposed target C_{max}/MIC of at least 100 was achieved in 53%, 65%, 72% and 84% of the 1000 virtual patients each administered 12, 14, 16 and 20 g once-daily PASER, respectively. In addition, for the typical individual, the exposure remained above 1 mg/L for ≥98% of the dosing interval. A prospective study should evaluate the tolerability and early bactericidal activity of 14, 16, and 20 g once-daily dosing of PASER, and determine the pharmacokinetic/pharmacodynamic parameters linked to the bactericidal activity.

CHAPTER 4

Drug concentration at the site of disease in children with pulmonary tuberculosis

Effective tuberculosis treatment that results in cure and sterilisation of tuberculous lesions requires adequate exposure of antituberculosis drugs at the site of disease. Site of disease, also referred to as site of infection, is the compartments where the *Mycobacterium tuberculosis* (*M. tuberculosis)* bacilli reside and where the relevant antituberculosis drug exerts it antimycobacterial effect. There are no data for site of disease pharmacokinetics of antituberculosis drugs in children. In this chapter, we aimed to characterise the concentrations of first-line antituberculosis drugs at the site of disease in children with complicated intrathoracic tuberculosis. This was a prospective study in children with severe intrathoracic tuberculosis requiring bronchoscopy or transthoracic surgical lymph node decompression (SD). Patients administered rifampicin, isoniazid, pyrazinamide, with or without ethambutol for at least 10 days had plasma samples collected at approximately pre-dose, 2, 4, and 6 hours post-dose. Site of disease samples were targeted to be collected at 2, 4, or 6 hours post-dose for bronchoscopy, and at 2 hours post-dose for the SD group. A population pharmacokinetics modelling approach was used to reconstruct the entire plasma pharmacokinetic profiles. Similarly, site of disease data was modelled by an additional compartment for each site, and the rate and extent of distribution of each drug from plasma to the sites determined. To our knowledge, this is the first study to provide pharmacokinetic data in children with pulmonary tuberculosis, the most common form of tuberculosis in children and adults, on site of disease-focused exposure of antituberculosis drugs and addresses

important questions regarding site of disease drug-exposure relationships. We found the extent of distribution of first-line antituberculosis drugs to sites of disease to be drug- and site-specific, and that numerous bacilli in hard-to-reach lesions such centres of caseous granuloma are exposed to periods of monotherapy with key sterilising drugs rifampicin and pyrazinamide, despite being on combination treatment for at least a month. Overall, drug penetration of tuberculosis sites of disease seems to be better in children than in adults. In addition, we provided first time data in humans, on the ethambutol penetration of tuberculous sites of disease. Except for isoniazid, all first-line antituberculosis drugs had lower plasma exposure in children compared to adults. We, therefore, suggest that these drugs should be dosed at the higher end of the World Health Organisation's (WHO) dosing range to increase overall systemic exposures. Furthermore, higher doses of rifampicin should be explored, and the pharmacokinetic/pharmacodynamic parameter(s) of efficacy at site of disease be determined. This could allow treatment stratification or shortening of treatment duration depending on disease severity.

CHAPTER 5

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis

Meropenem, a carbapenem, is being investigated for repurposing as an antituberculosis drug. Understanding the pharmacokinetic/pharmacodynamic parameters of carbapenem efficacy, is key to its optimal use, and to the programmatic translation of research findings using parenteral formulations of carbapenems to novel, oral carbapenems in development. A population pharmacokinetics model of meropenem in patients with pulmonary tuberculosis is the first step in performing an integrated pharmacokinetics/pharmacodynamics analysis linking carbapenem exposure to early bactericidal activity. This chapter aimed to develop such a model and identify covariates improving predictive performance. A 2-compartment model with first-order elimination process adequately described the observed meropenem concentration-time data from a phase 2a study. The model was parameterised

with clearance from the central compartment, intercompartmental clearance, central and peripheral volumes of distribution. The uncertainty in parameters estimates were low (3.8-35%). Similarly, the combined additive and proportional errors were low. Furthermore, covariates found to improve model fit were size-standardised creatinine clearance on clearance and total body weight on all disposition parameters. Rifampicin and age had no significant influence on meropenem clearance. The final model can be used as the pharmacokinetic component of future exposure-response analyses of early bactericidal activity data.

OPSOMMING

HEROORMERKING EN OPTIMISERING VAN MIDDELS VIR DIE VERBETERING VAN TUBERKULOSE BEHANDELING

Tuberkulose, ‘n hoofoorsaak van infeksiesiekte mortaliteit, morbiditeit en verlaagte lewenskwaliteit met verlies aan inkomste, bly ‘n enorme publieke gesondheidslas, veral in omgewings met beperkte hulpbronne. Ons het met hierdie tesis deurlopend beoog om tuberkulose behandeling deur die heroormerking en optimisering van bestaande medisynes te verbeter. Ten einde hierdie doel te bereik, het ons dit ten doel gestel om ons begrip van die farmakokinetika en farmakodinamika van gekose teen-tuberkulose middels te bevorder.

HOOFSTUK 1

Kliniese farmakokinetika en farmakodinkamika van rifampisien in menslike tuberkulose

In hierdie oorsig, wat 51 jaar strek, word die data rakende rifampisien farmakokinetika en farmakodinamika oor ‘n reeks dosisse in gesonde vrywilligers, tuberkulose pasiënte en spesiale pasiëntpopulasies opgesom. Ons het 170 artikels ingesluit en farmakokinetika data is ontgin uit 69 studies waarby 3666 deelnemers betrek is, en wat rifampisien teen ‘n doseringsreeks van 2-35 mg/kg ontvang het. Ons het ‘n beduidende inter- en intra-individuele veranderlikheid in rifampisien blootstelling gevind, wat deur toediening onder vastende toestande beperk kan word. Faktore wat rifampisien blootstelling en/of effektiwiteit kan verander, sluit wanvoeding, menslike immuniteitsgebreksvirusinfeksie, diabetes mellitus, dosering, farmakogenetiese polimorfismes, lewersirrose en ondergeskikte medisinale produkte in. Area onder die konsentrasie-tydkurwe (vanaf tydpunt 0 tot 24h) [AOK_{0-24}]/ minimum inhiberende konsentrasie (MIK) was die farmakokinetiese/farmakodinamiese parameter wat die beste met rifampisien bakteriedodende aktiwiteit gekorrelleer het. Rifampisien dosisse hoër as die standaard dosis van 10mg/kg oraal mag benodig word vir sekere indikasies soos tuberkulose breinvliesontsteking, waar verhoogde rifampisien blootstelling geassosieer word met verlaagde mortaliteit. Terapeutiese middelmonitering

geïntegreer met ‘n Bayes-verspreidingsvooruitskatting mag vinniger dosis individualisering en die verkryging van optimale middelblootstelling bewerkstellig.

HOOFSTUK 2

Die farmakokinetika van para-aminosalisielsuur ten opsigte van effektiwiteit en onverdraagsaamheid

Hierdie hoofstuk het uitgebrei op ‘n vorige oorsig van die ontwikkeling van para-aminosalisielsuur (PAS)-verwante formulasies, doseringspraktyke en hul verwantskap met voorkoming van weerstandigheid in metgeselmiddels en onverdraagsaamheid, en evalueer die farmakokinetika van PAS in groter diepte, met spesifieke fokus op die gebrek aan verwantskap tussen PAS plasmakonsentrasies en onverdraagsaamheid. Hierdie hoofstuk lê ook voorheen ongewaardeerde bewyse voor wat toon dat PAS wel ‘n mate van bakteriedodende effektiwiteit het. Beskikbare bewyse dui aan dat PAS maksimale konsentrasies (K_{maks}), AOK, en, ter uitbreiding, K_{maks}/MIK en/of AOK/MIK, eerder as die proporsie van tyd wat PAS konsentrasie bo MIK (%T>MIK) van 1mg/L is, die belangrikste bepalers van effektiwiteit en onderdrukking van weerstandigheidsontwikkeling tot metgeselmiddels is. Daarom is daar ‘n behoefte vir ‘n prospektiewe studie om PAS farmakokinetika/farmakodinamika te ondersoek met moderne metodologie.

HOOFSTUK 3

Waarskynlikheid van mikobakteriedodende aktiwiteit van para-aminosalisielsuur met nuwe doseringstrategieë

In hoofstuk 2 het ons bewyse voorgelê wat hoë een keer per dag dosering met PAS ondersteun. In die besonder, ‘n korrelvormige, stadig-vrystellingsformulasie van PAS (PASER® GRANULES [Aminosalicylic Acid Delayed-Release Granules], JACOBUS PHARMACEUTICAL COMPANY, INC.Princeton, NJ, VSA) teen die huidige doseringstrategie van 8-12 g per dag, toegedien in 2 tot 3 verdeelde dosisse, sal heel moontlik nie PAS konsentrasies wat in vroeër kliniese proewe bereik is,

en wat die gebruik van PAS ondersteun het, tot gevolg hê nie. Die optimale gebruik van PASER is afhanklik van ‘n omvattende begrip van PAS se dosis-blootstelling-respons verhouding. Ons het daarom ‘n verteenwoordigende bevolkingsfarmakokinetiese model vir PASER ingestel en die waarskynlikheid vir bakteriedodende- of bakteriostatiese effek teikenbehaling met verskillende doseringstrategieë ge-evalueer. Die farmakokinetiese model het beide inter-individuele en inter-geleentheid veranderlikheid in PAS biobeskikbaarheid in ag geneem, asook ‘n allometriese glyskaal vir totale liggaamsgewig in geneigtheidsparameters. Met die veronderstelling van ‘n PAS MIK van 1 mg/L, is die voorgestelde teiken K_{maks}/MIK van minstens 100 behaal in onderskeidelik 53%, 65%, 72% en 84% van die 1000 virtuele pasiënte aan wie elk 12, 14, 16 en 20 g PASER as eenmalige daaglikse dosis toegdien is. Verder, vir die tipiese individu, het die blootstelling bo 1 mg/L gebly vir ≥98% van die doseringsinterval. ‘n Prospektiewe studie moet poog om die verdraagsaamheid en vroeë bakteriedodende aktiwiteit van 14, 16 en 20 g PASER as een keer per dag daaglikse dosis te evalueer, en die farmakokinetiese/farmakodinamiese parameters wat met bakteriedodende aktiwiteit verbind word, te bepaal.

HOOFSTUK 4

Middelkonsentrasie by die siekteteikenareas in kinders met pulmonêre tuberkulose

Effektiewe tuberkulose behandeling wat genesing en sterilisering van tuberkuloseletsels tot gevolg het, is afhanklik van voldoende blootstelling aan teen-tuberkulose middels by die siekteteikenarea. Siekteteikenarea, ook genoem area van infeksie, is die kompartemente waar die *Mycobacterium tuberculosis* (*M. tuberculosis)* basille voorkom en waar die relevante teen-tuberkulose middels hulle teen-tuberkulose effek moet uitoefen. Daar is tans geen data vir siekteteikenareafarmakokinetika vir teen-tuberkulose middels in kinders nie. In hierdie hoofstuk het ons gepoog om die konsentrasies van eerste-linie teen-tuberkulose middels by die siekteteikenarea in kinders met gekompliseerde intratorakale tuberkulose uit te beeld. Hierdie was ‘n prospektiewe studie in kinders met erge intratorakale tuberkulose wat brongoskopie of transtorakale chirugiese limfnode dekompressie (SD

groep) benodig het. Plasmamonsters is van pasiënte geneem aan wie rifampisien, isoniasied en pirasinamied, met of sonder etambutol, vir minstens 10 dae toegedien is, by tydpunte ongeveer voor-dosis, 2, 4 en 6 uur na dosering. Siekteteikenareabiopsies is sover moontlik geneem teen 2, 4, of 6 uur na dosering vir brongoskopie pasiënte, en 2 uur na dosering vir die SD groep. 'n Bevolkingsfarmakokinetiese modelleringsaanslag is gebruik om die algehele plasma farmakokinetiese profiel weer op te bou. Op 'n soortgelyke manier is siekteteikenarea data gemodelleer deur 'n ekstra kompartement vir elke area, asook die spoed en omvang van verspreiding van elke middel vanaf plasma na hierdie areas, te bepaal. Sover ons kennis strek, is hierdie die eerste studie wat farmakokinetiese data verskaf in kinders met pulmonêre tuberkulose, die mees algemene vorm van tuberkulose in beide kinders en volwassenes, rakende siekteteikenarea-gefokusde blootstelling aan teen-tuberkulose middels, en spreek ons belangrike vrae aan omtrent siekteteikenarea-middel blootstellingverhoudings. Ons het bevind dat die omvang van verspreiding van eerste-linie teen-tuberkulose middels na siekteteikenareas beide middel- en area-spesifiek is, en dat meertallige basille in moeilik bereikbare letsels (soos die kern van kaasagtige granulome) blootgestel is aan tydperke van monoterapie met sleutelmiddels rifampisien en pirasinamied, ten spyte daarvan dat kombinasie behandeling vir minstens 'n maand toegedien is. Oor die algemeen blyk dit of middel deurdringbaarheid in die tuberkulose siekteteikenareas beter is in kinders as in volwassenes. Verder verskaf ons ook vir die eerste keer data uit menslike studies aangaande die deurdringbaarheid van etambutol na siekteteikenareas. Met die uitsondering van isoniasied het alle getoetsde teen-tuberkulose middels laer plasma blootstelling in kinders as in volwassenes getoon. Ons stel daarom aan die hoër kant van die huidig voorgestelde doseringsreikwydte, om algehele sistemiese blootstelling te verhoog. Verder behoort die gebruik van hoër dosisse van rifampisien ondersoek te word en die farmakokinetiese/farmakodinamiese parameters van effektiwiteit by elke siekteteikenarea bepaal te word. Dit mag verskillende behandelingstrategieë of verkorte behandlingstydperke toelaat, afhangende van die erns van die siektegraad.

HOOFSTUK 5

Die bevolkingsfarmakokinetika van meropenem in volwasse pasiënte met rifampisien-sensitiewe pulmonêre tuberkulose

Meropenem, ‘n karbapenem-klas geneesmiddel, word tans ondersoek vir heroormerking as teen-tuberkulose middel. Begrip van die farmakokinetiese/farmakodinamiese parameters van karbapenem effektiwiteit is die sleutel tot optimale gebruik daarvan, en vir die programmatiese vertaling van navorsingsbevindinge vir nie-orale formulasies van karbapenems, na nuwe, orale karbapenems wat ontwikkel word. ‘n Bevolkingsfarmakokinetiese model van meropenem in pasiënte met pulmonêre tuberkulose is die eerste stap na die uitvoering van ‘n geïntegreerde farmakokinetiese/farmakodinamiese ontleding wat karbapenemblootstelling aan vroeë bakteriedodende effektiwiteit verbind. Die werk in hierdie hoofstuk het ten doel gehad om so ‘n model te ontwikkel, en kovariate wat voorspellingsprestasie verbeter te identifiseer. ‘n Twee-kompartement model met eerste-orde eliminasie het die waargenome meropenem konsentrasie-tyd data uit ‘n fase 2a studie voldoende beskryf. Die model is geparametriseer met opruiming uit die sentrale kompartement, interkompartementele opruiming en sentrale en perifere volumes van verspreiding. Die onsekerheidsvlak in parameter skattings was laag (3.8-35%). Soortgelyk hieraan was die gekombineerde bygevoegde en proporsionele foute ook klein. Kovariate wat die model se pas verbeter het was grootte-gestandaardiseerde kreatinienopruiming vir opruiming en totale liggaamsmassa vir alle geneigtheidsparameters. Rifampisien en ouderdom het geen betekenisvolle invloed op meropenem opruiming gehad nie. Die finale model kan gebruik word as die farmakokinetiese komponent in toekomstige blootstelling-respons analises van vroeë bakteriedodende aktiwiteitsdata.

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INTRODUCTION

Background

Tuberculosis (TB) is a leading infectious cause of morbidity, mortality, and poor quality of life in the world.[1,2] According to the 2020 WHO global TB report, TB incidence in relation to population size varies widely between countries, and with disproportionately higher burden in low and middle income countries (LMIC) than in high-income countries.[2] Only 4.9% of the 2019 global TB cases occurred in the WHO regions of Europe and the Americas, whereas the remaining 95.1% were reported in the WHO regions of South East Asia, Africa, Western Pacific and the Eastern Mediterranean, mainly affecting LMIC.[2] In addition, two-thirds of the 2019 global TB cases occurred in eight countries: India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa.[2] In the same year, 10 million (range, 8.9-11.0) new cases of TB were estimated to have occurred.[2] The major TB risk factors identified were undernourishment, infection with the human immunodeficiency virus (HIV), alcohol, smoking, and diabetes mellitus (DM), which in 2019 predisposed an estimated 2.2 million, 0.76 million, 0.72 million, 0.7 million, and 0.35 million people, respectively, to develop TB.[2] In 2019, approximately 8.2% of new TB cases globally were estimated to be HIV positive.[2] Africa accounted for the vast majority of the global HIV-associated TB, with the proportion coinfected exceeding 50% in some parts of South Africa.[3] The WHO Africa and South-East Asia regions accounted for 85% of the combined total of global TB deaths in HIV-negative and HIV-positive people.[2] It is however reassuring that the global TB mortality rate reduced by 45% between 2000 and 2019.[2] Use of multidrug regimens is a vital component of the currently adopted strategy in ensuring successful outcome (cure and prevention of relapse) for not only the patient but also public health benefit of TB control and suppression of emergence of resistant strains.[2]

The choice of treatment regimens and their individual drug dosages are dependent on drug susceptibility, site of infection, age, comorbidities such as HIV, DM, malnutrition, renal or hepatic impairment, and the severity of disease. Therefore, understanding the dose-exposure and exposure-

response properties of antituberculosis drugs and the factors that influence them is critical to successful TB therapy, and in the future construction of shorter regimens.

The traditional industry-sponsored drug discovery and development process is expensive, time-consuming (may take up to 15 years from discovery to obtaining marketing authorisation), and carries huge risks.[4,5] On the contrary, drug repurposing and optimisation of existing drugs deals with drugs already marketed for some indications. Because the repurposed drugs have been in clinical use for years or decades, there is safety and efficacy data for labelled or off-label indications available.[5,6] In addition, it is relatively cheaper and faster to repurpose and optimise existing drugs than to develop new ones.[6] Drug repurposing and optimisation holds the promise as an alternative to the costly, high-risk drug development process, or could be used to construct new regimens in combination with old or novel agents.

Rifampicin

Rifampicin, a rifamycin in clinical use for over 50 years, is a key drug in the treatment of drug-sensitive TB.[7] The excellent sterilizing property of rifampicin at standard adult dose of about 10 mg/kg per day permits TB treatment duration shortening to 9 months and when combined with pyrazinamide, to 6 months.[7–9] The 10 mg/kg per day rifampicin dosing is not optimal, and was chosen not based contemporary methods of assessing antimycobacterial effects of TB drugs, but based on considerations of cost and toxicity.[10,11] Studies evaluating rifampicin at doses higher than 10 mg/kg per day, indicate that at this dose, rifampicin efficacy is at the lower end of the dose-exposure-response curve.[10,11]

Para-aminosalicylic acid (PAS)

PAS is an important reserve drug for patients with highly resistant TB. The recommended dose of a widely available slow-release, granular PAS formulation (PASER) is "8-12 g daily in two to three divided doses".[12–14] However, there has been little consideration to the optimal dosage of PAS and limited research has been performed on the optimal application of PASER, a gastro-friendly formulation. Jindani *et al* found substantial 2-day early bactericidal activity (EBA) of a PAS preparation (15 g once daily), which was comparable to the 2-day EBA of rifampicin at 10 mg/kg body weight.[15] This "suggests that, although usually thought bacteriostatic, PAS has meaningful bactericidal activity if high enough concentrations are reached".[16] In addition, early British Medical Research Council (MRC) trials from the 1950s showed that PAS, when given in individual doses of no less than 5 g at least twice a day, prevents resistance to companion drugs.[17–21] The mean peak concentrations (C_{max}) likely observed in those studies was approximately 100 mg/L and the lowest C_{max} around 50 mg/L.[22] The authors suggested these to be the concentration ranges to be targeted in order to prevent resistance to companion drugs and/or possibly provide bactericidal activity.[16]

While the exposure levels mentioned above were achieved with older PAS salt preparations, PAS is currently available in a granular, slow-release formulation, including an enteric coating, PASER, which seems better tolerated than the salt preparations.[23] Approximately 12 g of PASER corresponds to 14.5 g and 20 g of acid PAS and sodium PAS, respectively.[23] The antituberculosis activity of this formulation has never been formally assessed, but it is unlikely that the maximal concentrations achieved in the historical trials can be matched with the current dosing schemes that are the same as previously used with the salt formulations. The PASER formulation has the advantages of better tolerability on the account of its slow release, and the consequent prolonged period that PAS concentrations remain above the MIC of 1-2 mg/L.[16] Theoretically, once daily dosing with high-dose PASER might achieve higher C_{max} with associated bactericidal activity, while retaining a long time above MIC (%T>MIC) for bacteriostatic activity, with the advantage of simplified supervision of drug intake and reduced side effects from the enteric formulation.

Much has been written about gastro-intestinal intolerance of PAS and yet very little is known about the contribution of different drug formulations and drug metabolites to intolerability. A highly relevant finding is that better or equivalent tolerance with PAS once daily, at higher single doses, was noted by several studies compared with the same dose divided across the day.[24–27] This may be related to saturation of acetylation of the amino group of the PAS molecule to acetyl-PAS by *N*-acetyl transferase 1 (*NAT1)*, hypothesizing that the metabolite is an important contributor to development of gastro-intestinal intolerance and that once daily dosing may lead to a smaller proportion of drug being metabolized due to the mentioned saturation.[16,28]

The optimal dose and dosing regimen of PASER needs to be determined, and will require an urgent investigation of the safety, tolerability, and efficacy of high once daily dosing of PASER.

First-line antituberculosis drug concentrations at the site of disease in children

For the successful treatment of TB, drugs used in antituberculosis regimens need to reach their targets at the site of disease at adequate concentrations and for the duration of time required to exert their intended bactericidal or bacteriostatic effect, as well as protecting companion drugs from development of resistance.[29] The complex and heterogeneous nature of TB pathology in humans, poses a barrier to antituberculosis drugs' access to bacilli at sites of disease especially cavitary lesions.[29] In adults, most antituberculosis drugs poorly penetrate cavitary lesions resulting in suboptimal drugs concentrations at site of disease and a risk of poor TB outcomes.[29] However, little is known regarding the pharmacokinetics (PK) of antituberculosis drugs at the target site of disease in children, including tissue penetration in the lung and intrathoracic lymph nodes. Indeed, there are no data regarding site of disease PK of first-line antituberculosis drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) in children with tuberculosis, and no data on site of disease PK of ethambutol in humans. Understanding the rate and extent of drug distribution at the site of infection

could lead to better dosing of current antituberculosis drugs and facilitate the design of more effective drug regimens relevant to the spectrum of TB disease in children.

Carbapenem drug repurposing

M. tuberculosis, is historically considered intrinsically resistant to β-lactam antibiotics, because of chromosomally encoded production of a broad-spectrum β-lactamase BlaC.[30] It was recently shown that *M. tuberculosis* contains L,D-transpeptidases that catalyses 3->3 linkages (rather than the 4->3 linkages catalysed by classical D,D-transpeptidases) and are critical for its cell wall biosynthesis.[31] Carbapenems including meropenem and ertapenem have in vitro activity against *M. tuberculosis.*[32,33]This activity is potentiated in combination with clavulanate, which has been found to irreversibly inhibit BlaC.[30,32] In addition, unlike penicillins and cephalosporins, carbapenems are relatively resistant to BlaC.[32] The combination of carbapenems and clavulanate have MIC of < 1 mg/L against *M. tuberculosis,* and this combination is active against both fast-growing metabolically active organisms, and slow-growing persisters.[32] The addition of amoxicillin to meropenem/clavulanate resulted in further reduction in MIC by an average of 3.2 dilutions.[34]

Rifampicin combination with meropenem exhibited synergistic effect on rifampicin-sensitive *M. tuberculosis.*[35] Besides, in rifampicin-resistant *M. tuberculosis,* the MICs for rifampicin were lowered by 10-100 fold in the presence of sub-inhibitory concentration of meropenem.[35]

A recent individual patient data meta-analysis of observational studies in patients with multidrug-resistant TB (MDR-TB, defined as resistance to at least rifampicin and isoniazid) found a significant association of carbapenem use with treatment success compared to non-use of the drug.[36] Further evidence of carbapenem use in TB patients is provided by Diacon *et al*, who conducted a randomized controlled study of the 14-day EBA of meropenem plus amoxicillin/clavulanate in adults with newly diagnosed sputum smear-positive, drug-sensitive pulmonary TB as part of NCT02349841.[37] Participants randomized to the meropenem plus amoxicillin/clavulanate arm received meropenem 2

g thrice daily by intravenous bolus, with amoxicillin 500 mg/clavulanate 125 mg administered orally thrice daily, for 14 days.[37] Participants randomized to the control arm received standard antituberculosis therapy with daily oral isoniazid, rifampicin, pyrazinamide, and ethambutol according to South African National Treatment Programme guidelines.[37] Meropenem plus amoxicillin/clavulanate reduced the mean mycobacterial load by 0.11 (95% CI 0.09 to 0.13) $\log_{10}$ colony forming units (CFU) per mL of sputum per day and thereby exhibited robust 14-day EBA (reference control regimen reduced mean mycobacterial load by 0.17 (95% CI 0.15 to 0.19)).[37] A carbapenem formulated for oral administration would have the greatest impact on feasibility provided adequate drug exposures can be reached. To this end, Diacon *et al* also randomized participants in another arm of the same trial (NCT02349841) to receive faropenem sodium 600 mg with amoxicillin 500 mg/clavulanate 125 mg, each administered orally thrice daily, for 14 days.[37] In contrast to meropenem, faropenem plus amoxicillin/clavulanate did not reduce the mean mycobacterial load at all (unpublished data). A better understanding of the PK drivers of activity and the optimal, as well as minimal PK targets would inform the development of more feasible meropenem dosing strategies in the short term, as well as appropriate dosing strategies for novel oral carbapenems used for TB treatment in the medium and longer term.

Introduction to pharmacometrics

Pharmacometrics is a relatively new discipline that can be used to solve therapeutic challenges of the huge burden of diseases facing Africa.[38] About four decades ago Sheiner *et al* contributed significantly to the literature about a new method of PK data analysis that was eventually called population PK.[39] In contrast to PK analysis using traditional SHAM (Slope, Height, Area, Moments) or non-compartmental analysis (NCA), pharmacometrics methods allow for better understanding of clinical pharmacology.[38] Nonlinear mixed-effect (NLME) modelling, also known as population modelling, has the advantage of not only providing typical population parameters represented as

fixed-effects, but also estimating the influence of individual-specific characteristics, and the random variability with specific distributions to describe individuals.[40] It allows identification and quantification of the sources of variability in drug exposure and response, even when data is sparse (however, more than one sample per patient is generally needed to separate levels of variability).

In the early 1980s, Sheiner *et al* introduced a computer software called NONMEM, an acronym for NON-linear Mixed-Effects Modelling, which has capability for performing complex statistical analysis.[39,41] The command line is used to invoke NONMEM through a control file. This is written in a unique modelling language that NONMEM translator (NM-TRAN) converts to Fortran code understood and implemented by NONMEM.[41]

NLME modelling using NONMEM and auxiliary software will be exploited in our study to (a) characterize, understand, and predict a drug's PK and pharmacodynamic (PD) behaviour, (b) quantify uncertainty of information about that behaviour, and (c) arrive at rational decision in drug development process and pharmacotherapy of TB. This will ultimately provide improved clarity on how best to optimize antituberculosis drug dosing regimens for the best clinical outcome with an acceptable safety margin in the future. By using pharmacometrics, researchers can model the characteristics of new, existing and repurposed drugs to simulate and predict their behaviour, which can enable more efficient and optimal drug development.[38] Model-building strategy generally entails sequential development of the structural model, stochastic model and covariate model.[39,40] The structural model is a framework or function that describes the time-course of drug concentration or effect, which is represented by algebraic or differential equations.[42] The stochastic model quantify the extent of random variability in the pharmacokinetic parameters, e.g. between-subject variability and between-occasion variability.[39,42] Population models unlike classical linear regression can have several levels of variability, commonly two. The first level is then the individual and the second level the samples variability (residual unexplained variability).[39,42] Covariates are characteristics such as age, body weight, renal function that can explain variability in drug exposure or response. A covariate

model quantifies the influence of covariates (example body weight) on a population parameter (example clearance).[39,40,42] A full model with the structural, stochastic and statistical components can be used to define the optimal dosing regimen of existing or repurposed antituberculosis drugs.

HYPOTHESES OF THE DISSERTATION

i. Several factors influence rifampicin's PK and PD.

ii. PAS is not optimally used in the treatment of TB.

iii. A population pharmacokinetics approach could assist in optimising PAS dosing.

iv. Understanding the concentrations of first-line antituberculosis drugs at site of disease in children could assist in optimising TB treatment.

v. A population pharmacokinetics approach could be used to repurpose meropenem use in TB patients.

AIM AND OBJECTIVES OF THE DISSERTATION

The overall aim of the dissertation was to improve TB treatment by accelerating repurposing and optimisation of existing antituberculosis drugs.

The objectives of the dissertation include the following:

i. To conduct a review summarizing rifampicin pharmacokinetic and pharmacodynamic data across a range of doses in adult healthy volunteers, tuberculosis patients, and special patient populations (Chapter 1).

ii. To review the development of PAS regarding formulations, dosing practices and their relationship to the prevention of resistance in companion drugs and intolerance (Chapter 2).

iii. To review the PK of PAS in greater depth, focusing on the relationship of PAS concentrations to intolerance (Chapter 2).

iv. To externally validate a previously published PAS population PK model,[43] (Chapter 3)

v. To optimize this PAS population PK model if needed (Chapter 3) and

vi. To evaluate the probability of target attainment with once-daily PASER regimens that could achieve bactericidal targets while not dropping below the bacteriostatic target during the dosing interval (Chapter 3).

vii. To characterise the concentrations of first-line anti-tuberculosis drugs at the site of disease in children with complicated intrathoracic tuberculosis (Chapter 4).

viii. To develop meropenem population pharmacokinetics model and identify covariates improving predictive performance of the model (Chapter 5).

METHODOLOGY AND RESULTS

Detailed methodology as well as results of this dissertation are submitted in the format of five chapters / manuscripts.

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CHAPTER 1

Clinical pharmacokinetics and pharmacodynamics of rifampicin in human tuberculosis

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REVIEW ARTICLE

Clinical Pharmacokinetics and Pharmacodynamics of Rifampicin in Human Tuberculosis

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Abstract

The introduction of rifampicin (rifampin) into tuberculosis (TB) treatment five decades ago was critical for shortening the treatment duration for patients with pulmonary TB to 6 months when combined with pyrazinamide in the first 2 months. Resistance or hypersensitivity to rifampicin effectively condemns a patient to prolonged, less effective, more toxic, and expensive regimens. Because of cost and fears of toxicity, rifampicin was introduced at an oral daily dose of 600 mg (8–12 mg/kg body weight). At this dose, clinical trials in 1970s found cure rates of ≥ 95% and relapse rates of < 5%. However, recent papers report lower cure rates that might be the consequence of increased emergence of resistance. Several lines of evidence suggest that higher rifampicin doses, if tolerated and safe, could shorten treatment duration even further. We conducted a narrative review of rifampicin pharmacokinetics and pharmacodynamics in adults across a range of doses and highlight variables that influence its pharmacokinetics/pharmacodynamics. Rifampicin exposure has considerable inter- and intra-individual variability that could be reduced by administration during fasting. Several factors including malnutrition, HIV infection, diabetes mellitus, dose size, pharmacogenetic polymorphisms, hepatic cirrhosis, and substandard medicinal products alter rifampicin exposure and/or efficacy. Renal impairment has no influence on rifampicin pharmacokinetics when dosed at 600 mg. Rifampicin maximum (peak) concentration (C_{max}) > 8.2 µg/mL is an independent predictor of sterilizing activity and therapeutic drug monitoring at 2, 4, and 6 h post-dose may aid in optimizing dosing to achieve the recommended rifampicin concentration of ≥ 8 µg/mL. A higher rifampicin C_{max} is required for severe forms TB such as TB meningitis, with C_{max} ≥ 22 µg/mL and area under the concentration–time curve (AUC) from time zero to 6 h (AUC_6) ≥ 70 µg·h/mL associated with reduced mortality. More studies are needed to confirm whether doses achieving exposures higher than the current standard dosage could translate into faster sputum conversion, higher cure rates, lower relapse rates, and less mortality. It is encouraging that daily rifampicin doses up to 35 mg/kg were found to be safe and well-tolerated over a period of 12 weeks. High-dose rifampicin should thus be considered in future studies when constructing potentially shorter regimens. The studies should be adequately powered to determine treatment outcomes and should include surrogate markers of efficacy such as C_{max}/MIC (minimum inhibitory concentration) and AUC/MIC.

1 Introduction

Tuberculosis (TB) is associated with significant morbidity, mortality, and poor quality of life [1, 2]. The use of multidrug regimens is a vital strategy for ensuring relapse-free cure for patients as well as ensuring TB control and suppression of resistant strains [2]. The choice of treatment regimens and the dosage of their components is dependent on drug susceptibility, site of infection, patient age, and co-morbidities such as HIV infection, diabetes mellitus (DM), malnutrition, renal impairment, hepatic impairment, and TB severity [3–6].

Rifampicin (rifampin) was first introduced into clinical use in 1968 and remains a key drug for the treatment of TB disease caused by bacilli susceptible to it [2, 7, 8]. Rifampicin was introduced at a relatively low dose of 600 mg (about 8–12 mg/kg body weight). This choice of a low dose of rifampicin can be explained by the exorbitant cost at the time, dose-dependent toxicity concerns, and evidence that rifampicin concentrations were achieved above the minimum inhibitory concentrations (MICs) of *Mycobacterium tuberculosis* (*M. tuberculosis*) [7, 8]. Several decades ago, a series of clinical trials evaluated rifampicin in treatment regimens for drug-sensitive pulmonary TB (PTB),

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Key Points

AUC_{24} (area under the concentration–time curve [AUC] from time zero to 24 h)/MIC (minimum inhibitory concentration) is the pharmacokinetic/pharmacodynamic parameter that correlates best with rifampicin (rifampin) bactericidal activity.

Therapeutic drug monitoring integrated with Bayesian priors could allow dose individualization and attainment of optimal pharmacokinetic/pharmacodynamic parameters quicker.

Higher rifampicin doses may be required at least for some indications such as tuberculous meningitis where a clear rifampicin exposure–response relationship exists, with AUC from time zero to 6 h (AUC_6) of ≥ 70 µg·h/mL and maximum (peak) concentration (C_{max}) of ≥ 22 µg/mL associated with reduced mortality.

which ultimately allowed shortening of the treatment duration to 6 months when combined with pyrazinamide in the first 2 months, with a success rate in excess of 95% and a relapse rate of less than 5% [9–11]. However, outside the clinical trial environment, the treatment success rate is less impressive. The standard short course based on rifampicin is estimated to cure 83% of HIV-negative patients and only 78% of patients with HIV-associated TB [2]. Preclusion of rifampicin because of either resistance or hypersensitivity effectively condemns a patient to prolonged multidrug regimens that are expensive, toxic, and less effective than rifampicin-containing regimens.

Exceeding the current standard rifampicin dose range might result in better bactericidal and sterilizing activities with increased prevention of resistance. Preclinical data from experimental in vitro and in vivo TB models provide an indication that area under the concentration–time curve (AUC) from time zero to 24 h (AUC_{24}) over MIC (AUC_{24}/MIC) is the pharmacokinetic/pharmacodynamic parameter that correlates best with rifampicin's bactericidal effect [12, 13]. There is a heightened interest in studying the dose–exposure and exposure–response relationships of high-dose rifampicin and the factors that influence them. The objective of this review is to summarize rifampicin pharmacokinetic and pharmacodynamic data across a range of doses in adult healthy volunteers, TB patients, and special patient populations.

2 Methods

2.1 Search Strategy and Study Selection

We searched PubMed from inception to 12 April 2018 in order to identify studies in adults that report on the pharmacokinetics and/or pharmacodynamics of rifampicin in healthy volunteers, patients, and special populations (such as those with malnutrition, HIV co-infection, DM, and hepatic and renal impairment). The search terms used in various combinations were: Rifampin*[Mesh]* OR rifampicin OR rifampin OR antitubercul* OR antimycobacterial OR antimycobacterial activit* OR Antitubercular Agents*[Mesh]* AND Pharmacokinetics*[Mesh]* OR PK OR pharmacokinetic* OR PK PD OR pharmacodynamic* OR Pharmacology*[Mesh]* OR Treatment Outcome*[Mesh]* OR Tuberculosis*[Mesh]* OR Tubercul* OR Critical Illness*[Mesh]* OR Hepatic Insufficiency*[Mesh]* OR Renal Insufficiency*[Mesh]* OR Renal Insufficiency, chronic*[Mesh]* OR Acute Kidney Injury*[Mesh]* OR renal failure OR chronic renal failure OR Diabetes Mellitus*[Mesh]* OR Diabetes Mellitus OR Malnutrition*[Mesh]* OR malnutrition.

Studies were included if one or more of the following metrics were reported: rifampicin maximum (peak) plasma or serum concentrations (C_{max}), or concentrations at any time, AUC from time zero to time t (AUC_t), and an outcome measure such as time to sputum culture conversion, cure rate, relapse rate, rate of treatment failure, sterilizing activity, or early bactericidal activity (EBA) defined as the daily $\log_{10}$ decline in viable colony-forming units (CFU) of *M. tuberculosis* per mL of sputum collected overnight within up to 14 days. The identified articles were screened by title and abstract. We identified additional articles from related citations in PubMed and referenced articles.

3 Results and Discussion

One hundred and seventy articles were included in this review. Pharmacokinetic data were extracted from 69 studies with 3666 participants with a dosage of rifampicin ranging from 2 to 35 mg/kg (Table 1, Figs. 1, 2). A considerable body of data relating to rifampicin pharmacokinetics and pharmacodynamics in both healthy volunteers and patients have been accumulated since its introduction into clinical use. Pharmacokinetic data interpretations are, however, complicated by different laboratory analytical methods, uncertainty as to whether or not the studied individuals were established or not on rifampicin (defined as being on daily rifampicin for at least 3 days or fewer than 3 days, respectively) and the lack of uniformity in data presentation. While some papers report results as rifampicin AUC_t, and

C_{max}, others provide only serum concentrations at certain timepoints after dosing. Similar challenges were experienced when pharmacodynamics were reported. Differences in EBA study reports included duration from 2 to 14 days; however, many of the 14-day EBA studies include 2-day reports. Other pharmacodynamic outcome measures were time to sputum culture conversion reported on solid or liquid media, and inconsistencies in definitions of cure rate, relapse rate, and rate of treatment failure.

4 Pharmacokinetics

4.1 Dose, Serum Concentrations, and Sources of Variability

Hepatic esterases are responsible for rifampicin metabolism into desacetyl rifampicin [14]. Both rifampicin and its metabolite undergo biliary excretion [14, 15], with only about 17% of rifampicin 600 mg recovered unchanged in urine [15]. Organic anion-transporting polypeptide 1B1 (OATP1B1) is primarily responsible for the hepatocellular uptake of rifampicin [14].

Rifampicin exhibits a dose-dependent increase in serum concentration [16–20]. In 1967, Furesz et al. [20] reported patients on rifampicin for less than 3 days to have an increase in rifampicin C_{max} from 0.94 to 27.7 µg/mL over a dose range of 100 mg (2 mg/kg) to 900 mg (18 mg/kg) [20]. Other studies have replicated these findings [15–17, 19, 21–24]. A near quadrupling of AUC was observed when the rifampicin dose was increased from 300 to 600 mg [25, 26]. Ruslami et al. [27] confirmed the dose-dependent increase in rifampicin AUC (79.7 vs. 48.5 µg·h/mL; $p < 0.001$) and C_{max} (15.6 vs. 10.5 µg/mL; $p < 0.001$) values [27]. Of interest is that beyond a 300–450 mg dose in individuals established on rifampicin, the serum rifampicin concentration assumes a non-linear increase [17, 22]. Recent evaluation of high-dose rifampicin by Boeree et al. [16] found an almost ten-fold increase in average rifampicin exposure with dose increase from 10 to 35 mg/kg. This more than dose-proportional increase in rifampicin exposure is probably due to saturable biliary excretion or a transport saturation of rifampicin across the liver being reached and a dose-dependent increase in rifampicin bioavailability [19, 22, 28]. The non-linear rifampicin concentrations when increasing doses should be taken into account while dose adjusting rifampicin in response to a sub-therapeutic concentration during therapeutic drug monitoring (TDM) (see Sect. 6).

Constans et al. [29] reported one of first studies that demonstrated auto-induction with a reduction in the rifampicin serum concentration over time. This might be explained by rifampicin's potent induction of drug-metabolizing enzymes and transporters that results in increased rifampicin clearance [15, 17–19, 22, 30–33]. Rifampicin activates nuclear pregnane X receptors (PXRs) that in turn lead to amplified gene transcriptions [19]. Thus, compared to serum rifampicin concentrations on day 1, concentrations are expected to be lowest from day 15 onwards, when maximum induction is expected to have been attained [19, 29, 34]. Smythe et al. [34] and Svensson et al. [19] have demonstrated that maximum induction of drug-metabolizing enzymes/transporters is achieved within 24–40 days using rifampicin pharmacokinetic-enzyme turnover models. Svensson et al. [19] also evaluated the impact of rifampicin auto-induction on its apparent clearance (CL/F) over a wide range of doses. Compared to subjects not established on rifampicin, the CL/F of rifampicin upon repeated daily dosing was found to increase by 1.73-, 1.89-, 1.91-, 1.94-, 1.97-, and 1.99-fold at doses of 10, 20, 25, 30, 35, and 40 mg/kg of rifampicin, respectively [19]. The authors found the magnitude of rifampicin auto-induction to be dose and concentration dependent [19, 35]. The lag time to achieve full enzyme induction with additional dose- and concentration-dependent induction is important to take into consideration when co-administering other medicines with rifampicin. Rifampicin drug interaction studies are needed to provide clarity on the impact of high doses of rifampicin on co-administered medicines. In addition, rifampicin's induction of its own metabolism progressively shortens the half-life ($t_{½}$) with repeated daily dosing [17, 18, 36]; this is already evident after the first few days of treatment but continues up to day 24 when maximum induction is anticipated [19, 22, 37]. The $t_{½}$in patients with normal liver function is 2–5 h, and appears to be dose dependent with the lower and upper end of the spectrum seen with a dose range of 8 and 16 mg/kg, respectively [17, 32]. Return of enzymes and transporters to pre-treatment levels is anticipated within 24 days after the end of treatment [19]. Co-administered medicines induced by rifampicin may therefore require dose adjustment up to 3–4 weeks after discontinuation of rifampicin.

In an attempt to evaluate sources of pharmacokinetic variation of anti-TB drugs, McIlleron et al. [38] enrolled 142 patients with PTB into a pharmacokinetic study and found wide variations in plasma rifampicin concentrations. The authors also brought to the fore the menace of substandard medicinal products. Fifty-four (38%) of the patients received rifampicin batches that were later withdrawn from the market by the local medicine regulatory authority on the basis of insufficient bioavailability data being submitted [38, 39]. The median rifampicin C_{max} values in patients who received the substandard batches versus approved batches were 3.8 and 5.9 µg/mL, respectively, while the median rifampicin AUC from time zero to 8 h (AUC_8) in patients who received the substandard batches versus approved batches were 13.7 and 21.5 µg·h/mL, respectively [38]. Thus, the rifampicin manufacturing process can be an important factor contributing to

Table 1 Rifampicin pharmacokinetics

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (µg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (µg·h/mL)	HIV + participants (%)
Furesz et al. [20], 1967	Microbiological	Not established	2.0	Pts and fasting	6				0.94 (± 0.18)[a]				0.94		0.5		0.1			0
	Microbiological	Not established	3.0	Pts and fasting	27				1.87 (± 0.20)[a]				1.87		1.2		0.4			0
	Microbiological	Not established	5.0	Pts and fasting	10				3.15 (± 0.62)[a]				3.15		3.0		1.1	0.3		0
	Microbiological	Not established	6.0	Pts and fasting	5				5.68 (± 1.62)[a]				5.68		3.5		1.6	0.4		0
	Microbiological	Not established	9.0	Pts and fasting	13				7.92 (± 1.52)[a]				7.92		6.8		5.4	2.1		0
	Microbiological	Not established	12.0	Pts and fasting	12				8.80 (± 1.48)[a]				8.80		7.4					0
	Microbiological	Not established	15.0	Pts and fasting	10				20.87 (± 3.25)[a]				20.87		17.6		10.3	4.9		0
	Microbiological	Not established	18.0	Pts and fasting	9				27.7 (± 4.16)				27.7		22.5		15.4	8.3		0
Verbist and Gyselen [21], 1968	Microbiological	Not established	9.0	Pts and fasting	15	1.5			6.88 (2.76–9.16)					5.9		1.3				0
	Microbiological	Not established	12.0	Pts and fasting	5	1.5			7.23 (3.43–16.9)					4.9		3.7				0
Verbist [30],1969	Microbiological	Not established	12.0	Pts and fasting	41				7.76 (5.83)[b]					6.3		3.6		0.6		0
	Microbiological	Established	12.0	Pts and fasting	41				6.85 (5.24)[b]					5.3		2.1		0.2		0
Verbist [144], 1971	Microbiological	Not established	30.0	Pts	55				16.71				12.7		15.2		10.9	7.5		0
	Microbiological	Not established	30.0	Pts	55				16.65				13.7		14.7		9.6	5.7		0
	Microbiological	Not established	30.0	Pts	45				18.56				12.9		17.8		11.2	7.1		0
	Microbiological	Established	30.0	Pts	45				15.47				15.1		15.4		9.8	5.4		0
Acocella [80], 1971	Microbiological	Not established	10.0	HVs	6	2.0			8.5				8.5		7.6		4.1	1.7		0
	Microbiological	Not established	10.0	HVs	6	2.0			9.2				9.2		7.4		4.1	1.8		0

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
	Microbiological	Not established	10.0	Pts with liver disease	7	4.0			12				10.3		12.0		8.6	6.9		0
	Microbiological	Not established	10.0	Pts with liver disease	7	2.0			9.8				9.8		9.2		7.7	5.5		0
Acocella et al. [22], 1971	Microbiological	Not established	18.0	HVs	6		0.1	5.1	24.12 (± 3.17)[a]				22.7		15.7		11.2	5.9		0
	Microbiological	Not established	10.0	HVs	6		0.2	3.4	22.70 (± 3.18)[a]				22.5		16.2		7.9	3.0		0
	Microbiological	Not established	5.0	HVs	5			2.7	4.35 (± 0.68)[a]				3.9		3.2		1.3	0.4		0
	Microbiological	Established	18.0	HVs	6		0.3	2.7	19.11 (± 3.21)[a]				17.8		13.3		4.9	2.0		0
	Microbiological	Established	10.0	HVs	6		0.3	2.1	15 (± 2.06)[a]				12.0		10.8		2.6	0.4		0
	Microbiological	Established	5.0	HVs	5			1.9	6.41 (± 1.81)[a]				3.4		2.4		0.5	0.2		0
Curci et al. [17], 1972	Microbiological	Not established	8.0	Pts	12		0.3	2.6	11.54 (± 0.81)[a]				11.5		8.7	5.2		0.9		0
	Microbiological	Not established	12.0	Pts	12		0.2	2.9	16 (± 0.86)[a]				16.0		12.6	9.5		1.6		0
	Microbiological	Not established	16.0	Pts	12		0.1	6.5	24.58 (± 2.06)[a]				24.6		20.1	15.4		7.1		0
	Microbiological	Not established	8.0	Pts	6		0.2	3.4	11.17 (± 0.91)[a]				11.2		9.7	5.5		1.8		0
	Microbiological	Established	8.0	Pts	6		0.4	1.8	9.17 (± 1.05)[a]				9.2		6.3	2.7		0.3		0
	Microbiological	Not established	12.0	Pts	6		0.2	3.5	14.4 (± 1.99)[a]				14.4		14.4	9.4		3.8		0
	Microbiological	Established	12.0	Pts	6		0.4	1.8	13.83 (± 2.34)[a]				13.8		13.5	5.9		0.4		0
	Microbiological	Not established	16.0	Pts	6		0.2	4.1	26.11 (± 3.73)[a]				24.8		26.1	20.7		9.0		0
	Microbiological	Established	16.0	Pts	6		0.2	2.9	22.17 (± 2.47)[a]				22.2		21.2	9.3		1.7		0
Boman [145], 1974	Microbiological	Not established	10.0	Pts	68		0.2	3.8	7.6				7.6		6.4	4.6	3.1			0

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Garnham et al. [23], 1976	Microbiological	Not established	8.4	HVs and fasting	10	2.0			11.1 (± 1.3)[a]	2.1	8.0		11.1		10.1	7.3	5.0	2.5		0
	Microbiological	Not established	8.4	HVs and fasting	10	2.0			11.7 (± 1.0)[a]	4.6	9.8		11.7		10.3	7.1	4.9	1.8		0
	Microbiological	Not established	6.3	HVs and fasting	10	4.0			8.2 (± 0.8)[a]	1.4	5.1		7.2		8.2	5.8	3.5	1.2		0
Dickinson et al. [24], 1977	Microbiological	Established	22.5	Pts	10				23.7 (13.50–48.5)											0
	Microbiological	Established	20.0	Pts	9				20.6 (16.50–33.0)											0
	Microbiological	Established	12.4	Pts	10				14.9 (7.40–24.60)											0
Polasa and Krishnaswamy [55], 1983	Microbiological	Not established	10.0	HVs and fasting	6	2.0	0.2	3.5	11.8 (±1.03)[a]	4.5	6.7	8.7	11.9		7.2	5.4	3.9		76.88 (±9.45)[a,c]	0
	Microbiological	Not established	10.0	HVs and fed	6	4.0	0.2	4.0	8.4 (±1.07)[a]	1.4	1.9	2.4	3.6		8.4	5.4	4.2		64.83 (±6.87)[a,c]	0
Polasa et al. [68], 1984	Microbiological	Not established	10.0	HVs and well-nourished	8		0.2	4.6	11.09 (± 1.27)[a]										59.12 (± 4.91)[a,c]	0
	Microbiological	Not established	11.3	HVs and Malnourished	10		0.2	4.6	5.61 (± 0.50)[a]										42.24 (± 4.22)[a,c]	0
	Microbiological	Established	10.7	Pts and malnourished	10		0.2	4.2	4.13 (± 0.53)[a]										24.11 (± 3.28)[a,c]	0
Polasa and Krishnaswamy [69], 1986	Microbiological	Not established	9.5	HVs and well-nourished	6		0.2	4.1	12.7 (± 1.24)[a]										61.3 (± 5.83)[a,c]	0
	Microbiological	Not established	17.0	HVs and malnourished	6		0.2	2.9	14.2 (± 1.38)[a]										73.3 (± 10.9)[a,c]	0
Gurumurthy et al. [49], 1990	Microbiological	Established	12.0	Pts	30	3.0			7		3.1		6.5	7.0		4.7	3.0			0
Bhatia et al. [146], 1991	Spectrophoto	Established	9.0	Pts	15	2.0			5.7		3.0		5.7		4.2	1.5			20.7[c]	0

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Chan et al. [117], 1992	HPLC	Not established	3.0	Pts	15				1.96 (0.79–4.87)				2.0		1.8	1.4				0
	HPLC	Not established	6.0	Pts	10				3.27 (1.34–8.01)				3.2		3.3	3.0				0
	HPLC	Not established	12.0	Pts	11				9.53 (7.81–11.63)				9.3		9.5	6.9				0
Acocella et al. [147], 1993	Microbiological	Not established	10.0	HVs	6	2.3	0.2	3.8	8.2 (±0.9)[a]										56.3 (±11.6)[a,c]	0
	Microbiological	Not established	10.0	HVs	6	2.2	0.2	4.5	6.6 (±0.7)[a]										59.1 (±11.2)[a,c]	0
Ellard et al. [43], 1993	Microbiological	Established	10.7	Pts and fasting	19				11.5 (± 1.0)[a]				11.5		10.6	4.7				0
Choudhri et al. [78], 1997	HPLC	Established	8.6	Pts with HIV	15		0.3	2.0	4.3										19.6[d]	48
	HPLC	Established	8.6	Pts without HIV	14		0.2	2.8	4.1										25.2[d]	0
Sahai et al. [74], 1997	HPLC	Established	8.1	HVs	12	0.9	0.2	2.9	9.29										44.2[e]	0
	HPLC	Established	7.5	Asymptomatic HIV+	12	1.3	0.3	2.6	5.92										31.5[e]	100
	HPLC	Established	8.1	Symptomatic HIV +	12	0.5	0.2	3.2	5.3										30.7[e]	100
	HPLC	Established	9.2	Symptomatic HIV + with diarrhea	12	1.4	0.2	3.1	5.24										28.8[e]	100
Peloquin et al. [148], 1997	HPLC	Not established	7.8	HVs	24	1.6			11.80 (9.65–24.99)										79.79 (47.40–142.73)[c]	100
Jaruratanasirikul [79], 1998	HPLC	Established	11.3	Pts with HIV	8	2.3			9.81											100
Taylor and Smith [72], 1998	HPLC	Established	10.0	Pts with HIV and fasting	13	2.7			12.3											100
	HPLC	Established	10.0	Pts without HIV and fasting	14	2.0			7.4											0

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (µg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (µg·h/mL)	HIV + participants (%)
Zwolska et al. [149], 1998	Microbiological	Not established	9.0	HVs and fasting	16	1.9			9.61											0
	Microbiological	Not established	9.0	HVs and fasting	16	2.0			9.39											0
Gurumurthy et al. [150], 1999	Microbiological	Not established	11.9	HVs	18	2.3			9.21				8.2	8.5		5.5		2.1		0
	Microbiological	Not established	11.9	HVs	18	3.0			8.2				7.6	7.5		5.5		2.2		0
Peloquin et al. [57], 1999	HPLC	Not established	7.6	HVs and fasting	14	2.4			10.54 (30)[f]										57.15 (23)[f]	0
	HPLC	Not established	7.6	HVs and fasting	14	2.2			11.32 (27)[f]										58.98 (27)[f]	0
	HPLC	Not established	7.6	HVs and antacid	14	2.4			10.89 (48)[f]										58.37 (32)[f]	0
	HPLC	Not established	7.6	HVs and fed	14	4.4			7.27 (31)[f]										55.2 (26)[f]	0
Pargal and Rani [151], 2001	HPLC	Not established	10.0	HVs	12	1.6			8.59 (2.68–16.47)										49.54 (61.79)[c,f]	0
	HPLC	Not established	10.0	HVs	11	1.5			14.76 (8.60–21.06)										119.12 (28.24)[c,f]	0
Prakash et al. [152], 2003	HPLC	Established	10.0	Pts and fasting	77				11.15				11.2		7.5					
Agrawal et al. [153], 2004	HPLC	Not established	10.0	HVs	22	2.3	0.2	4.3	11.5 (± 2.7)[b]										87.9 (± 23.7)[b]	0
	HPLC	Not established	10.0	HVs	22	2.1	0.1	4.9	10.2 (± 2.4)[b]										80.1 (± 16.9)[b]	0
Gurumurthy et al. [154], 2004	HPLC	Established	11.0	Pts without HIV	13	2.4			7.2 (5.5–8.9)										44.6 (36.3–52.9)[c]	0
	HPLC	Established	11.0	HIV infected	13	3.9			3.4 (2.5–4.3)										21.2 (14.8–27.6)[c]	100
	HPLC	Established	11.0	Pts with HIV	15	3.6			3.4 (2.7–4.5)										28.2 (18.1–38.4)[c]	100
van Crevel et al. [155], 2004	HPLC	Not established	9.0	HVs and fasting	12	1.9			8.98 (7.43–10.87)[g]										44.83 (38.29–52.48)[g,h]	0
Perlman et al. [156], 2005	HPLC	Established	9.6	Pts with HIV	34	6.0			5.49 (1.06–11.21)[i]				3.3			3.7			17.01 (3.68–47.67)[i,j]	100
	HPLC	Established	8.7	Pts with HIV	21	2.2			5.44 (2.24–13.94)[i]				4.7			4.1			22.33 (4.64–49.07)[i,j]	100

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Tappero et al. [157], 2005	HPLC	Established	10.0	Pts with HIV and fasting	59	2.0			5.60 (0–13.71)[i]										24.0 (7.0–52.4)[i,j]	100
	HPLC	Established	10.0	Pts without HIV and fasting	28	2.0			5.96 (2.16–14.63)[i]										21.7 (7.1–52.9)[i,j]	0
Sirgel et al. [26], 2005	HPLC	Not established	3.0	Pts and fasting	8	3.1			2.53 (± 0.69)[a]										13.1 (± 1.6)[a,c]	24
	HPLC	Not established	6.0	Pts and fasting	8	3.7			3.19 (± 0.56)[a]										24.5 (± 3.1)[a,c]	24
	HPLC	Not established	12.0	Pts and fasting	8	2.5			13.0 (± 1.6)[a]										100.1 (± 7.5)[a,c]	24
	HPLC	Established	3.0	Pts and fasting	8	2.8			1.49 (± 0.35)[a]										7.8 (± 1.6)[a,c]	24
	HPLC	Established	6.0	Pts and fasting	8	3.4			2.89 (± 0.53)[a]										15.5 (± 2.6)[a,c]	24
	HPLC	Established	12.0	Pts and fasting	8	2.1			9.53 (± 1.8)[a]										65.2 (± 9.6)[a,c]	24
McIlleron et al. [38], 2006	HPLC	Established	10.9	Pts and fasting	88	2.5			5.9 (1.3–14.9)[i]				4.4						25.6 (5.6–80.1)[c,i]	10
Pinheiro et al. [158], 2006	HPLC	Established	12.6	Pts and fasting	18				6.5				6.5							0
Nijland et al. [95], 2006	HPLC	Established	8.1	Pts with DM and fasting	17	4[g]			3.49 (2.4–6.3)										12.3 (8.0–24.2)[j]	0
	HPLC	Established	9.7	Pts and fasting	17	2[f]			6.74 (5.6–10.1)										25.9 (21.4–40.2)[j]	0
Weiner et al. [159], 2007	HPLC	Established	12.0	HVs and fasting	16		0.4	1.9	9.9										44.0 (18.6)[b]	0
Diacon et al. [119], 2007	HPLC	Not established	3.0	Pts and fasting	8	3.1			2.53 (1.95)[b]										13.1 (4.5)[b,c]	0
	HPLC	Not established	6.0	Pts and fasting	8	3.7			3.19 (1.58)[b]										24.5 (8.8)[b,c]	0
	HPLC	Not established	12.0	Pts and fasting	8	2.5			13.0 (4.5)[b]										100.0 (21)[b,c]	0
	HPLC	Not established	20.0	Pts and fasting	13	3.3			14.0 (4.7)[b]										171.0 (56)[b,c]	0

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Um et al. [160], 2007	LC–MS/MS	Established	10.5	Pts and fasting	68				9.7 (4.6)[b]											0
Ruslami et al. [27], 2007	HPLC	Established	9.5	Pts and fasting	24	2.0	0.4	1.9	10.5 (6.2–16.6)										48.5 (26.7–72.8)[e]	4
	HPLC	Established	12.9	Pts and fasting	23	1.0	0.3	2.2	15.6 (5.1–26.6)										79.7 (38.7–138.1)[e]	0
McIlleron et al. [161], 2007	HPLC	Not established	9.6	HVs and fasting	20				13.9 (11.7–15.2)[k]										95.59 (83.18–114.44)[c,k]	0
Weiner et al. [66], 2010	HPLC	Established	7.3	HVs and fasting	16		0.4	1.9	9.9 (3.6)[b]										44.0 (18.6)[b,e]	0
	HPLC	Established	10.9	Pts and fasting	37		0.3	2.4	8.0 (5.0)[b]										43.2 (22.3)[b,e]	3
	HPLC	Established	9.8	Pts and fasting	35				8.2 (5.0)[b]										50.1 (27.8)[b,e]	6
Ruslami et al. [94], 2010	HPLC	Established	9.7	Pts with DM and fasting	17	2[f]		2.1	10.5 (9.0–12.3)										49.0 (40.9–58.7)[e]	0
	HPLC	Established	9.6	Pts and fasting	17	2.5[f]		2.2	9.6 (8.4–11.0)										50.6 (42.9–59.8)[e]	0
McIlleron et al. [63], 2012	HPLC	Established	10.8	Pts with HIV and fasting	51				7.2											100
Babalik et al. [96], 2013	HPLC	Established	11.0	Pts	56				5.1 (0.5)[a]				5.1							0
	HPLC	Established	9.0	Pts with DM	14				2.9 (0.2)[a]				2.9							0
	HPLC	Established	11.0	Pts	56				5.4 (0.5)[a]				5.4							0
	HPLC	Established	9.0	Pts with DM	14				3.2 (0.5)[a]				3.2							0
Burhan et al. [136], 2013	HPLC	Established	9.8	Pts and fasting	181				6.5 (0.1–19.7)				6.5							1
	HPLC	Established	9.8	Pts and fasting	9	3.0	0.4	1.6	9 (6.6–12.8)				6.6						35.4 (26.8–49.9)[e]	1
Ruslami et al. [5], 2013	HPLC	Not established	10.0	Pts and fasting	26	2.0			6.3 (4.9–8.3)[g]				6.3						26.0 (19.0–35.6)[g,j]	12
	HPLC	Not established	13.0	Pts and intravenous	26	2.0			22.1 (19.9–24.6)[g]				22.1						78.7 (71.0–87.3)[g,j]	12

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Pasipanodya et al. [137], 2013	HPLC	Established	10.9	Pts and fasting	142		1.2	0.6	5.72											15
Gengiah et al. [62], 2014	HPLC	Established	10.5	Pts with HIV	57				3.6 (2.8–5.0)[k]											100
Medellín-Garibay et al. [162], 2014	HPLC	Not established	9.2	HVs and fasting	24	1.8			11.9 (6.4–18.9)[i]										93.6 (40.4–136.5)[c,i]	
	HPLC	Not established	10.9	Pts and fasting	24	1.6			11.62 (7.01–19.9)[i]										76.25 (40.20–218.5)[c,i]	0
Bhatt et al. [163], 2014	HPLC	Established	10.0	Pts with HIV and fasting	21	2.0			6.59 (3.49–14.07)[i]				6.4						29.71 (12.45–109.75)[d,i]	100
	HPLC	Established	10.0	Pts with HIV and fasting	16	2.0			6.69 (2.85–12.29)[i]				5.0						31.15 (13.99–57.71)[d,i]	100
Lin et al. [59], 2014	LC–MS/MS	Established	12.0	Pts and fasting	16	1.4			15.92 (8.98)[b]										75.13 (63.53)[b,l]	0
	LC–MS/MS	Established	12.0	Pts and fed	16	3.5			9.52 (6.93)[b]										55.31 (57.14)[b,l]	0
Kwara et al. [164], 2014	HPLC	Established	7.8	HVs and fasting	11				8.9 (7.3–13.8)[k]										48.8 (63.53)[e,k]	0
Chigutsa et al. [123], 2015	LC–MS/MS	Established	10.0	Pts	54				7.3 (3.7–11.0)[i]										48 (18.0–126.0)[e,i]	13
Boeree et al. [16], 2015	UPLC	Established	10.0	Pts and fed	8				7.4 (6.1–9.9)										26.3 (21.3–40.9)[e]	13
	UPLC	Established	20.0	Pts and fed	15				21.6 (16.0–31.9)										113 (77.5–162.0)[e]	0
	UPLC	Established	25.0	Pts and fed	15				25.1 (16.3–34.6)										135 (91.5–228.0)[e]	0
	UPLC	Established	30.0	Pts and fed	15				33.1 (17.6–55.8)										190 (84.7–436.0)[e]	0
	UPLC	Established	35.0	Pts and fed	15				35.2 (28.6-44.2)										235 (166.0–321.0)[e]	0
Heinrich et al. [165], 2015	HPLC	Established	10.0	Pts and fasting	14				8.0 (5.1–16.2)										32.7 (20.3–58.6)[e]	7

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Te Brake et al. [6], 2015	HPLC	Not established	10.0	Pts	18				14 (9–19)										55 (43.0–67.0)[j]	1
	HPLC	Not established	10.0	Pts	18				4.9 (0.3–8.7)										19 (1.5–41.0)[j]	4
	HPLC	Not established	13.0	Pts and intravenous	16				26 (21–35)										90 (69.0–136.0)[j]	2
Shenje et al. [50], 2015	LC–MS/MS	Established	10.2	Pts	11				6.8											8
van Oosterhout et al. [166], 2015	HPLC	Established	10.0	Pts and fed	41	3.0	0.3	2.5	4.13 (2.47–5.60)[k]										21.3 (13.57–28.60)[k]	65
Hemanth Kumar et al. [167], 2015	HPLC	Established	9.9	Pts with HIV and fasting	26	4.0	0.3	2.2	6.4 (5.6–7.6)[k]						6.4				29.4 (20.6–41.0)[e,k]	100
	HPLC	Established	9.8	Pts with HIV and fasting	15	2.0	0.3	2.6	3.7 (2.1–4.6)[k]				3.7						20.7 (14.3–29.3)[e,k]	100
te Brake et al. [65], 2015	HPLC	Established	10.3	Pts and fasting	25	1.5	0.3	2.0	10.9 (7.1–16.3)										54.6 (35.0–88.8)[e]	0
	HPLC	Established	10.3	Pts and malnourished, fasting	11	2.0	0.3	2.1	10.9 (6.4–16.6)										54.8 (32.6–85.0)[e]	0
Hemanth Kumar et al. [168], 2016	HPLC	Established	9.6	Pts and fasting	101	2.0	0.1	4.7	5.0 (3.8–6.9)[k]										45.0 (29.8–68.0)[c,k]	2

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Participants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (μg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (μg·h/mL)	HIV + participants (%)
Yunivita et al. [48], 2016	UPLC	Not established	13.0	Pts and intravenous	9	1.5	0.1	4.9	24.7 (13.9–37.8)										145.7 (77.7–430.2)[e]	40
	UPLC	Not established	16.7	Pts and fasting	10	2.1	0.1	5.4	14.3 (6.1–22.2)										131.4 (38.1–275.1)[e]	9
	UPLC	Not established	18.0	Pts and fasting	9	2.3	0.1	6.1	16.2 (5.7–28.3)										164.8 (66.9–291.2)[e]	11
	UPLC	Established	13.0	Pts and intravenous	8	1.5	0.3	2.1	23.9 (14.9–36.4)										94.9 (59.7–161.2)[e]	40
	UPLC	Established	16.7	Pts and fasting	8	2.0	0.3	2.6	18.1 (10.8–32.8)										100.1 (46.1–162.3)[e]	9
	UPLC	Established	18.0	Pts and fasting	7	2.0	0.3	2.4	17.9 (12.8–29.6)										101.2 (41.7–167.8)[e]	11
Saktiawati et al. [169], 2016	LC–MS/MS	Not established	10.0	Pts and intravenous	20	1.8			12.3 (7.5–17.3)										79.6 (36.9–162)[e]	10
	LC–MS/MS	Not established	10.0	Pts and fasting	20	2.3			10.7 (7.1–15.1)										71.8 (36.0–129.0)[e]	10
	LC–MS/MS	Not established	10.0	Pts and fed	20	3.6			8.3 (4.1–13.3)										58.2 (29.0–115.0)[e]	10
Sloan et al. [14], 2017	LC–MS/MS	Established	8.6	Pts with HIV and fasting	174				4.8 (1.4–10.9)[f]				4.8						29.9 (19.7–63.4)[c,i]	56
Peloquin et al. [170], 2017	HPLC	Established	10.0	Pts and fasting	58	2.0	0.3	2.3	6.2 (5.1–8.7)[i]				6.2						24.9 (17.6–32.1)[j,k]	3
	HPLC	Established	15.0	Pts and fasting	57	2.0	0.3	2.5	10.2 (7.8–14.1)[k]				10.2						43.1 (30.3–57.5)[j,k]	3
	HPLC	Established	20.0	Pts and fasting	53	2.0	0.3	2.3	13.3 (8.9–17.1)[k]				13.3						55.5 (35.7–73.2)[j,k]	2
Boeree et al. [100], 2017	HPLC	Established	10.0	Pts and fed	19	3.1	0.4	1.8	5.8 (2.1–12.0)										24.2 (11.9–52.5)[e]	7
	HPLC	Established	20.0	Pts and fed	19	3.1	0.4	1.8	11.3 (2.3–25.5)										57.8 (15.0–121.0)[e]	5
	HPLC	Established	35.0	Pts and fed	20	4.0	0.3	2.5	26.7 (15.0–39.1)										170.0 (15.0–39.1)[e]	6

Table 1 (continued)

Study, year	Method	Dosing	Dose (mg/kg)	Partici-pants	Sample size	t_{max} (h)	k_{el} (h^{-1})	$t_{½}$ (h)	C_{max} (µg/mL)	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	AUC_t (µg·h/mL)	HIV + partici-pants (%)
Aarnoutse et al. [132], 2017	UPLC	Established	10.7	Pts and fed	23	4.0	0.4	1.9	5.3 (2.0–23.3)						5.3				23.9 (9.1–118.5)[e]	12
	UPLC	Established	16.7	Pts and fed	21	4.0	0.2	2.8	9.1 (4.9–15.4)						9.1				50.8 (18.9–153.6)[e]	10
	UPLC	Established	21.4	Pts and fed	19	4.0	0.3	2.4	14.1 (8.1–29.0)						14.1				76.1 (43.5–167.0)[e]	12

C_{max} and AUC_t values are given as mean (range) unless otherwise stated

AUC_∞ area under the concentration–time curve from time zero to infinity, *AUC_t* area under the concentration–time curve from time zero to time *t*, *C_{max}* maximum (peak) concentration, *DM* diabetes mellitus, *HIV* + HIV positive, *HPLC* high-performance liquid chromatography, *HVs* healthy volunteers, *k_{el}* elimination rate constant, *LC–MS/MS* liquid chromatography–tandem mass spectrometry, *Pts* patients, *$t_{½}$* half-life, *t_{max}* time to C_{max}, *UPLC* ultra-performance liquid chromatography

[a]Mean (standard error)

[b]Mean (standard deviation)

[c]AUC_∞

[d] AUC_{12}

[e]AUC_{24}

[f]% Coefficient of variation

[g]Mean (95% confidence interval)

[h]AUC_8

[i]Median (range)

[j]AUC_6

[k]Median (interquartile range)

[l]AUC_{10}

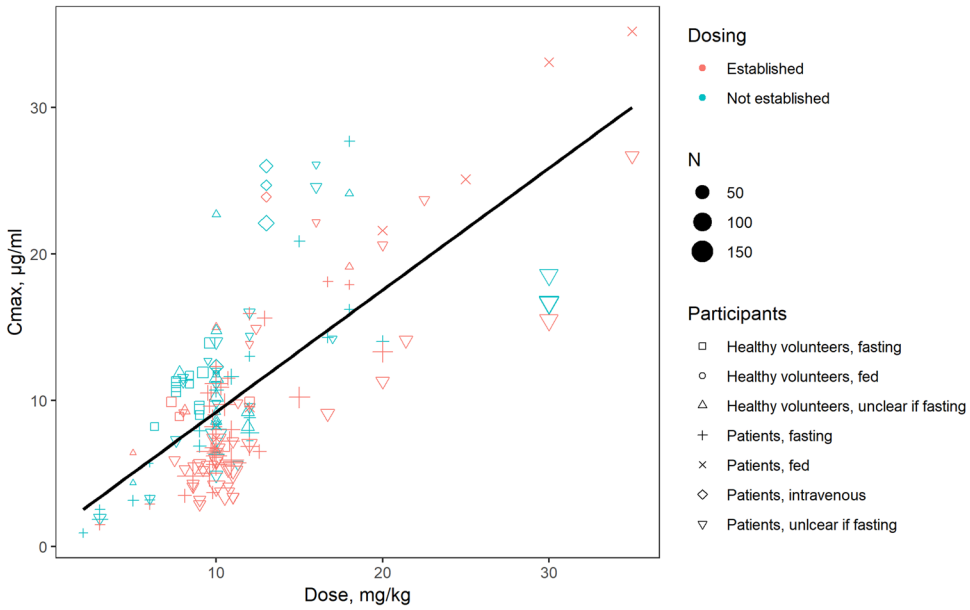

Fig. 1 Straight-line regression, weighted for number of study participants, of rifampicin C_{max} in healthy volunteers and tuberculosis patients, established and not established on rifampicin, administered with or without food. C_{max} maximum (peak) concentrations

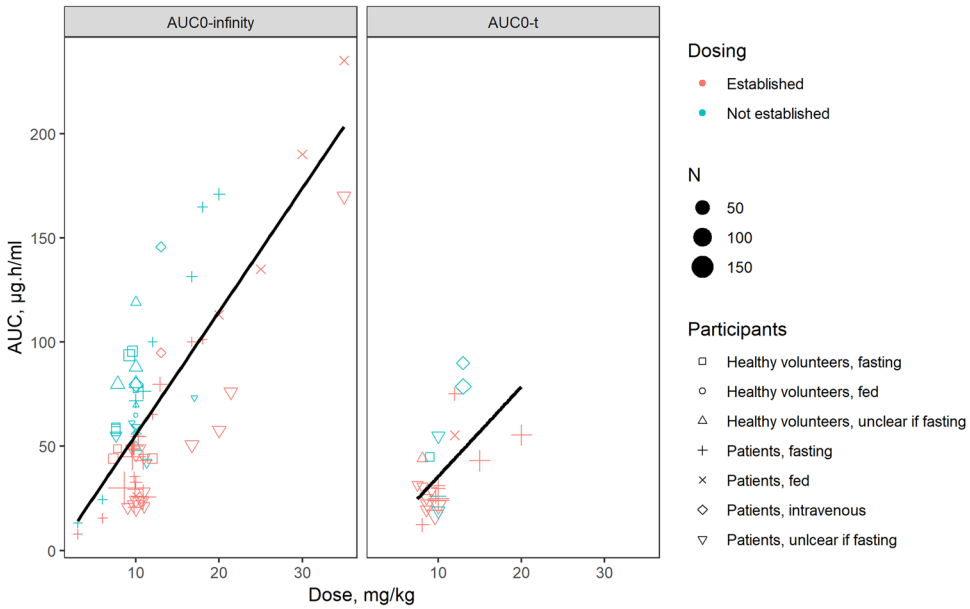

Fig. 2 Straight-line regression, weighted for number of study participants, of rifampicin AUC in healthy volunteers and tuberculosis patients, established and not established on rifampicin, administered with or without food. *AUC* area under the concentration–time curve, AUC_{∞} AUC from time zero to infinity (comprised of both AUC_{24} and AUC_{∞}), AUC_t AUC from time zero to time *t* (comprised of AUC_6, AUC_8, AUC_{10}, and AUC_{12})

not only low concentrations but also the variations in plasma rifampicin concentrations. Medicine regulatory authorities have a crucial role in protecting public health by ensuring only good-quality medicines are marketed.

4.2 Protein Binding

Rifampicin has a high plasma protein binding, with about 70–80% plasma protein bound [40, 41], but at doses ≥ 600 mg, the free rifampicin C_{max} is usually higher than the MIC [44].

4.3 Concentrations in Compartments Other than Serum

Free serum rifampicin distributes well throughout body tissues [36] but concentrations in various tissues vary, with concentrations often lower than those in blood. Compared to serum, average cerebrospinal fluid (CSF) rifampicin concentrations rarely exceed 1 µg/mL in patients with TB meningitis (TBM) [20, 42–48], which is only slightly above the rifampicin MIC of *M. tuberculosis* [43]. Studies by Ruslami et al. [5] and Te Brake et al. [6] found lower mortality in TBM patients when high-dose rifampicin was administered intravenously for the first 14 days of treatment than with

orally administered standard dose rifampicin. The rifampicin plasma and CSF exposures in patients receiving high-dose intravenous rifampicin dose of 13 mg/kg were approximately three times that of those receiving an oral standard dose of 10 mg/kg ($p < 0.0001$) [5]. The rifampicin AUC from time zero to 6 h (AUC_6) had a significant correlation with the highest CSF concentrations (Spearman's $\rho = 0.720$; $p < 0.001$) [6]. Future studies should assess whether rifampicin administered intravenously should be the preferred option for TBM. Nau et al. [47] reported that in patients with "uninflamed" meninges, the CSF rifampicin $t_{½}$ was 9–21 h, which is significantly longer than that in serum. The clinical relevance of the increased rifampicin $t_{½}$ in CSF of patients with intact meninges is unclear.

Gurumurthy et al. [49] studied patients with pulmonary and intestinal TB and found the mean rifampicin C_{max} following a dose of 12 mg/kg to be 7 µg/mL, while the corresponding salivary rifampicin concentration was 0.8 µg/mL. Patients with intestinal TB may have impaired absorption of rifampicin, requiring TDM of rifampicin and dose adjustment.

A sparse and intensive pharmacokinetic sampling of pericardial fluid and plasma were undertaken as substudies of the IMPI (Investigation of the Management of Pericarditis) trial [50]. In this study of patients with TB pericarditis, Shenje et al. [50] found only about 20% of the total plasma rifampicin concentration is achieved in pericardial fluid. In addition, the median free rifampicin concentrations found in pericardial fluid were lower than the median rifampicin MIC (0.125 vs. 0.208 µg/mL, respectively; $p < 0.001$) [50]. Higher than current standard rifampicin doses may be required to achieve higher exposure in pericardial fluid.

In contrast, rifampicin concentrations closer to those in blood are achieved in walls of tuberculous cavities and fibrous tissues [51, 52]. A pulmonary pharmacometrics model was developed by Clewe et al. [35] in order to determine the rate and extent of rifampicin distribution from plasma to epithelial lining fluid (ELF) and alveolar cells (ACs) [35, 53]. Forty participants without TB were enrolled and pharmacokinetic samples taken at 2 and 4 h post dosage of rifampicin, while bronchoalveolar lavage (BAL) was performed at only 4 h post rifampicin dosage [35, 53]. The model predicted the extent of rifampicin distribution into ELF as the ratio of the rifampicin concentration in the ELF/plasma ($R_{ELF/plasma}$), which was 0.26, while that into AC is the ratio of the rifampicin concentration in the AC/plasma ($R_{AC/plasma}$), which was 1.1 [35]. However, when rifampicin protein binding in the different compartments was taken into account, the model predicted a higher extent of rifampicin distribution into ELF and AC: 1.28 for unbound $R_{ELF/plasma}$ and 5.5 for unbound $R_{AC/plasma}$ [35].

4.4 Impact of Food

Ingestion of rifampicin with food results in delayed absorption with prolongation of the time to C_{max} (t_{max}) to 3–4 h compared with 1.5–2 h when given without food [21, 54–57]. Food decreases the rifampicin C_{max} [54, 55, 57] by up to 36–40% [57–59] and decreases the rifampicin AUC by about 6–26% [57, 59]. The concentration-lowering influence of food on rifampicin pharmacokinetics seems to be more likely with high-carbohydrate than high-lipid food [56]. Rifampicin is therefore recommended to be taken without food, to ensure not only optimal absorption is achieved but also a reduction in variability in absorption.

4.5 Impact of Antacids

In 1968 Vello and Vittori [60] reported that gastric pH has considerable influence on rifampicin absorption as serum rifampicin concentrations after gastric acidification were twice those found after alkalinization with sodium bicarbonate; however, more recent studies have found that the antacids aluminium/magnesium hydroxide and ranitidine do not alter rifampicin pharmacokinetics significantly [57, 61].

4.6 Impact of Sex

Males are more likely to have lower plasma rifampicin concentrations than females [62–65]. McIlleron et al. [63] suggested that "higher lean-body/total-weight ratios in males might partly account for this finding". However, lean body mass, or fat-free mass, appears to correlate with rifampicin clearance and volume of distribution [19] and sex is not taken into account when dosing rifampicin.

4.7 Pharmacogenetics

The inter-individual variability in plasma rifampicin exposure may be partly explained by single nucleotide polymorphism (SNP) of genes that encode for influx or efflux transporters of rifampicin into the liver or bile, respectively. Weiner et al. [66] performed a multivariate analysis of rifampicin blood samples obtained from 72 adult patients with PTB and 16 healthy volunteers, and found individuals with *SLCO1B1* genotype c.463CA (rs11045819) to have a lower mean rifampicin AUC_{24} than those with *SLCO1B1* genotype c.463CC (29.8 vs. 46.7 µg·h/mL, respectively; $p = 0.001$). Similarly, Chigutsa et al. [67] found lower rifampicin AUC_{24} values in patients homozygous or heterozygous for *SLCO1B1* rs4149032 polymorphism than in those with the wild-type genotype (43 vs. 56 µg·h/mL, respectively; $p < 0.05$). The genotypes associated with lower rifampicin exposure were more frequently found in Black African participants [66, 67].

Sloan et al. [14] recently used a population pharmacokinetics model to evaluate the influence of *SLCO1B1* SNPs on the variability of the rifampicin AUC from time zero to infinity (AUC_{∞}) [14]. In this Malawian study, 174 adult patients with PTB were sampled at 2 and 6 h post-dose [14]. The *SLCO1B1* rs4149032 polymorphism tested could not account for the observed inter-individual variability in rifampicin exposure [14]. This is contrary to the findings in South African populations where the same SNP was found to be associated with risk of low plasma rifampicin exposure [62, 66, 67]. The frequency of *SLCO1B1* rs4149032 polymorphism is high (70%) in the South African study [67] but low (32%) in the Malawian study [14]. Thus, a large sample size may be required in order to determine the impact of this genotype in the Malawian population. In addition, the genetic determinants of variability in rifampicin plasma exposure may differ considerably amongst Black Africans or the plasma rifampicin variability may be due to presence of unknown confounders.

A rifampicin dose higher than the current doses is required in patients with *SLCO1B1* rs4149032 polymorphism if exposures similar to those with wild-type genotypes are to be achieved. Evidence from population pharmacokinetic model simulations found giving an additional 150 mg of rifampicin will result in doubling of the number of patients reaching the target C_{max} of ≥ 8 μg/mL [67].

4.8 Malnutrition

Malnutrition, defined as a body mass index (BMI) < 18.5 kg/m^2 [65], may result in low, high, or no change in rifampicin plasma concentrations. Malnutrition can result in low rifampicin C_{max}, AUC, and protein binding in both 'healthy' volunteers and patients [68]. Impaired absorption can lead to low rifampicin plasma concentrations and a risk of treatment failure, relapse, or development of resistance. This might not be limited only to rifampicin, but might also affect companion drugs. Low BMI combined with normal absorption may result in patients receiving relatively higher mg/kg in the dose range and subsequently higher plasma concentrations [69]. Malnutrition may be accompanied by low plasma protein concentrations resulting in higher free rifampicin plasma concentrations and a subsequent increase in its CL/*F* [68, 69], equilibrium reached, and normal concentrations. In practice, the impact of malnutrition on rifampicin pharmacokinetics can be challenging to determine as a combination of these scenarios may occur. Rifampicin TDM can identify low plasma rifampicin exposure and inform subsequent dose adjustment in a patient with suspected malabsorption.

4.9 HIV Infection

Several studies [62, 70–74] and case reports [75, 76] indicate that HIV-positive patients are likely to have low serum concentrations of anti-TB drugs, which may be attributed to gastrointestinal factors such as gastric hypoacidity, enteropathy, opportunistic bowel infections, or diseases that may predispose to malabsorption or to drug–drug interactions (DDIs) [77]. A study by Sahai et al. [74] evaluated the pharmacokinetics of isoniazid, ethambutol, pyrazinamide, and rifampicin in healthy volunteers and HIV-positive patients. In this study, the serum rifampicin C_{max} in participants with HIV was lower than 8 μg/mL [64, 71]. Rifampicin was given at a dose of 600 mg daily, corresponding to 7.5–9.2 mg/kg in both groups. In a South African study, the multiple linear regression model used explained 36% variability in plasma rifampicin AUC_8 and found HIV infection to reduce the AUC_8 by 8.34 μg·h/mL ($p = 0.051$) [38]. This result should be interpreted with caution given the small number of HIV-positive patients (14/141) in the study [38]. A Kenyan study in 29 TB patients, 14 of whom were HIV positive, found serum rifampicin C_{max} to be uniformly low (4.1–4.3 μg/mL) following a dose of 600 mg/day (8.6 mg/kg) [78], irrespective of HIV status. Similarly, a study conducted in Thailand in eight patients with AIDS administered rifampicin 600 mg/day (11.3 mg/kg) had a mean (± standard deviation [SD]) serum rifampicin C_{max} of 9.81 (± 4.41) μg/mL and a mean (± SD) AUC_{24} of 60.25 (± 36.88) μg·h/mL [79]. However, their findings may be confounded by lower body weight and higher mg/kg dosing. Even though both the Thai and Kenyan patients received rifampicin 600 mg/day, the Thai patients received higher mg/kg doses because of their lower body weight [78, 79]. This could, at least in part, explain the higher rifampicin C_{max} seen in the Thai study than in the Kenyan study [78, 79]. In summary, when weight is taken into account, rifampicin concentrations in HIV-positive patients without malabsorption are likely similar to concentrations in those without HIV. A high index of suspicion is therefore required to identify subsets of HIV-positive patients at risk of low rifampicin exposure that may benefit from TDM.

4.10 Hepatic Impairment

Serum concentrations of rifampicin were significantly higher in patients with liver disease than in healthy volunteers [37, 80]. Acocella et al. [37, 80] reported findings of repeated administration of rifampicin 600 mg for 7 days in patients with hepatic cirrhosis and healthy volunteers. Serum rifampicin concentrations on day 7 were lower than those on day 1 in healthy volunteers, whereas day 7 rifampicin concentrations in hepatic cirrhotic patients were higher than concentrations on day 1, suggesting impaired rifampicin

clearance and possibly rifampicin accumulation [37, 80]. The finding of higher serum rifampicin concentrations in patients with hepatic cirrhosis were replicated by Capelle et al. [81]. This may imply that hepatic cirrhosis counteracts the anticipated time-dependent reduction in rifampicin concentrations due to auto-induction.

Liver disease poses a significant challenge for combination treatments including potentially hepatotoxic agents such as isoniazid and pyrazinamide combined with rifampicin. Guidelines for management of TB in patients with pre-existing liver disease aim to maintain rifampicin as part of treatment but replace pyrazinamide and isoniazid with less hepatotoxic agents [3]. The risk of drug-induced hepatitis is amplified in the presence of pre-existing hepatitis [3, 82]. Treating TB in patients with liver disease requires consultation with experts and careful monitoring for drug toxicity utilizing both clinical and laboratory parameters such as alanine aminotransferase (ALT), total bilirubin, and the international normalized ratio (INR) every 1–4 weeks for at least the first 2–3 months of treatment [3, 83, 84]. In addition, rifampicin TDM offers a unique opportunity to ensure inadvertent toxic concentrations are detected or avoided [3]. It is, however, important to note that rifampicin toxic concentrations are yet to be defined. No specific stopping rule based on ALT or total bilirubin elevation is available to decide when to interrupt or stop therapy in patients with severe pre-existing hepatic disease, including cirrhosis or encephalopathy [3]. However, some authors recommend this threshold to be a three-fold increase in ALT [3].

Regimens are suitable for patients with pre-existing hepatic impairment when pyrazinamide or isoniazid are omitted, as follows:

- Regimen without pyrazinamide: isoniazid–rifampicin–ethambutol for 2 months, followed by 7 months of isoniazid–rifampicin [3, 85, 86].
- Regimen without isoniazid and pyrazinamide: rifampicin–ethambutol with a fluoroquinolone, aminoglycosides, or cycloserine for 12–18 months depending on disease extent and response [3, 87].
- Regimen without isoniazid: rifampicin–ethambutol–pyrazinamide with or without a fluoroquinolone could be considered for a total duration of 6 months [3, 88].
- In severe cases of liver disease rifampicin might have to be left out altogether [3, 89].

4.11 Renal Impairment

In patients with renal impairment no dose adjustment is necessary with the rifampicin 600 mg daily dose in the presence of normal liver function [3, 90, 91]. It may be reasonable to consider TDM when rifampicin is administered at doses beyond 600 mg, since the renal excretion of unchanged rifampicin appears to be dose dependent, at least between the 450 and 600 mg dose range [15].

4.12 Diabetes Mellitus

DM is an important risk factor for the development of TB, accounting for about 7.7% of the global incident cases in 2016 [2, 92]. TB patients with DM have higher treatment failure or relapse rates than non-diabetic patients [93, 94]. This negative impact of DM on TB treatment outcomes might in part be due to altered pharmacokinetics of rifampicin and other anti-TB drugs. There are, however contrasting reports regarding low plasma rifampicin exposure in patients with DM [94, 95]. Ruslami et al. [94] compared the pharmacokinetics of rifampicin during the intensive phase of TB treatment in patients with and without DM. Both groups had similar oral bioavailability of rifampicin of 69% versus 74% ($p = 0.41$), with no delay in absorption as indicated by a t_{max} of 0.5–4 h ($p = 0.28$) [94]. In addition, rifampicin the AUC_{24}, C_{max}, $t_{½}$, clearance, and volume of distribution were similar in TB patients with or without DM ($p = 0.81$) [94].

While Nijland et al. [95] and Babalik et al. [96] found plasma rifampicin exposure in TB patients with DM to be reduced by about two-fold compared with those without DM, Ruslami et al. [94] did not find any association between the presence of DM and altered rifampicin pharmacokinetics. In the studies by Nijland et al. [95] and Babalik et al. [96], TB patients with DM have a higher body weight than those without DM, whereas in the Ruslami et al. [94] study the two groups were matched for weight, thus avoiding this bias. The observed differences might be explained by the fact that the studies were conducted at different phases of TB treatment. Rifampicin administration was daily during the intensive phase followed by three times per week during the continuation phase according to the Indonesian National Tuberculosis program [94, 95]. Hence, in these settings one would expect a larger magnitude of rifampicin auto-induction of its clearance and a consequent lower plasma exposure at steady state during the intensive phase than during the continuation phase. In addition, commencement of insulin in TB patients with DM could result in weight gain which is likely to be more marked with the passing of time [94]. Therefore, an increased rifampicin dose may be required. More prospective studies with a design similar to that of Ruslami et al. [94]. but with additional pharmacokinetic samplings during the continuation phase of TB treatment and in different patient populations are needed to better assess the clinical relevance and further management.

In summary, DM may have negative impacts on TB treatment. We therefore recommend meticulous evaluation of TB patients with DM complications predisposing to malabsorption in order to detect and avoid low rifampicin concentrations. Rifampicin dosing in DM patients should be based on

mg/kg and weight gain as a result of insulin administration should be taken into account.

5 Pharmacodynamics

5.1 Mechanism of Action

At recommended doses rifampicin is a bactericidal drug that inhibits DNA-dependent RNA polymerase in *M. tuberculosis* [97–99]. It binds to the β-subunit of this enzyme and suppresses RNA synthesis [13, 97]. Rifampicin is active against both extracellular and intracellular organisms even when replication is slow [11]. The desacetyl-rifampicin metabolite retains about 20% of rifampicin's activity against *M. tuberculosis* [98].

5.2 Physiological Changes

Several physiological changes may occur during rifampicin administration, including orange discoloration of body fluids, particularly at high doses [100], and non-pathological changes of biochemical markers of liver function [101]. A study by McColl et al. [101] in seven healthy volunteers given rifampicin 600 mg daily for 4 weeks showed that serum total bilirubin increased to 31 ± 5.2 μmol/L from pre-treatment levels of 9.4 ± 1.4 μmol/L within the first 24 h of treatment (three-fold increase; $p < 0.01$) [101]. This was due to an increase in the unconjugated bilirubin fraction. But even with continued rifampicin dosing, the serum total bilirubin level decreased to the pre-treatment level by day 7 and to below pre-treatment levels during the third and fourth week of treatment [101]. One healthy volunteer was studied more extensively, and following each dose of rifampicin the serum total bilirubin peaked at about 12 h post-dose but returned to pre-treatment level within 24 h [101]. Competition of rifampicin for binding to plasma proteins, hepatic uptake, and conjugation in the liver might be responsible for the initial rise in serum unconjugated bilirubin. The finding that serum unconjugated bilirubin decreases during the third and fourth weeks of rifampicin use (when maximum induction of drug-metabolizing enzymes and transporters occur) suggests increased activity of a rate-limiting step in bilirubin clearance. Similar findings of a rifampicin-induced increase in serum total bilirubin at the beginning of therapy, which declines over time, have been reported [17, 37, 81, 101], and are considered reversible with discontinuation of treatment [81]. Hence, isolated elevation of unconjugated serum bilirubin is most likely rifampicin induced, perhaps by competition for binding to serum albumin or hepatic uptake, but importantly may not require treatment cessation or interruption.

Verbist and Rollier [102] evaluated the effect of intermittent high doses of rifampicin 30 mg/kg administered with isoniazid 15 mg/kg (group A, treatment-naïve patients) or with ethambutol 100 mg/kg (group B, previously treated patients) on days 1, 3, 5, and 8, and once weekly thereafter. The authors reported that approximately 55, 30, and 15% of group A patients had serum total bilirubin of < 20.5, 20.5–30.8, and > 30.8 μmol/L, respectively on the first day of treatment, while approximately 37, 42, and 21% of group B patients had serum total bilirubin of < 20.5, 20.5–30.8, and > 30.8 μmol/L, respectively, also on the first day of treatment [102]. This increase in serum bilirubin occurred in the first week and was almost entirely due to the conjugated bilirubin, suggesting that rifampicin (or rather desacetyl-rifampicin) competes for excretion [102]. Compared to group A, the higher proportion of patients in group B with abnormal serum total bilirubin might be explained by previous treatment with multiple drugs (in some for up to 5 years), and perhaps less capacity for liver adaptation [102].

Similar to the findings of McColl et al. [101], the C_{max} of total and conjugated bilirubin levels attained in serum occurred between 8 and 12 h after rifampicin administration, and did not correlate with the rifampicin t_{max} of 2–4 h [102]. The distribution of serum total bilirubin levels on day 10 of treatment (i.e., 48 h after the previous dose of rifampicin) were no different from those at baseline.

Serum concentrations of γ-glutamyl transpeptidase (GGT) progressively increased during the first 3 weeks of rifampicin administration from 18 ± 5 to 35 ± 3.9 IU/L (1.9-fold increase; $p < 0.02$) [101]. Furthermore, elevated aspartate transaminase (AST) were seen in 44% of patients taking a rifampicin and isoniazid combination, but these were typically transient and of no clinical significance [103]. The various changes described in this section are physiological and should be differentiated from those that are drug induced.

5.3 Toxicity

The first-line regimen for treatment of drug-sensitive TB includes rifampicin, isoniazid, and pyrazinamide, all of which are potentially hepatotoxic, making it difficult to identify the causative agent. Rifampicin at a dose of 450–750 mg daily appears to be well-tolerated, with only 3.3% of adverse reactions reported to require discontinuation, even when combined with isoniazid [103]. Clinical jaundice or hepatitis are seen in < 0.6 to 11.5% of patients treated with rifampicin, isoniazid, and pyrazinamide [36, 82, 104–106]; they seem to be idiosyncratic but could be dose related in the presence of pre-existing liver disease [104]. The rate at which drug-induced hepatitis develops can vary considerably between countries, with higher rates observed in resource-limited countries than in high-income countries [82, 106]. A study

reported by Bright-Thomas et al. [82] found drug-induced hepatitis to be more likely in White patients than those of Asian origin (adjusted odds ratio [aOR] of 2.13; $p < 0.008$). It is, however, important to note that the study population comprised about 75% Asians, 21% Whites, and < 1.7% Africans [82]. This study also identified advancing age as a risk factor of drug-induced hepatitis (aOR 1.16; $p = 0.02$) [82]. Other risk factors for drug-induced hepatitis include pre-existing liver disease, alcoholism, malnutrition, female sex, HIV, and slow acetylator status [3, 105–107]. In the study by Kaneko et al. [105], the incidence of drug-induced hepatitis in patients with chronic hepatitis taking a rifampicin, isoniazid, and pyrazinamide combination ranged from 11.1% to 27.8% depending on the etiology, while the incidence in the control group without liver disease was 6.9%. However, in patients taking a rifampicin and isoniazid combination without pyrazinamide, the incidence of drug-induced hepatitis was 4.1%, irrespective of the presence or absence of chronic hepatitis [105].

Adverse effects due to hypersensitivity are idiosyncratic and may be minor (cutaneous, gastrointestinal, or influenza-like syndrome) or major (anaphylaxis, serum sickness, hemolytic anemia, thrombocytopenia, shock, acute interstitial nephritis, or acute renal failure) [3, 104, 108, 109]. Rifampicin can trigger drug rash with eosinophilia and systemic symptoms (DRESS) syndrome, a Type 4b delayed hypersensitivity reaction [110, 111]. Re-challenge should not to be attempted in patients experiencing severe cutaneous adverse reactions with or without internal organ involvement, and such cases should be discussed with specialists. Flu-like symptoms seen with rifampicin have been attributed to intermittent regimens rather than high daily dosing [112, 113], and were associated with the presence of circulating rifampicin-dependent antibodies [112]. A cholestatic pattern with elevated alkaline phosphatase may occur [36, 114].

5.4 Pharmacokinetic–Pharmacodynamics Considerations and Target Concentrations

Shortly following the introduction of rifampicin into clinical use, animal experiments found it to have exceptional sterilizing activity [115] and dose-dependent bactericidal activity [21]. The work of Jayaram et al. [12] on a murine TB model indicated a potential link between rifampicin exposure and bactericidal activity. More recently, Gumbo et al. [13] evaluated the pharmacokinetic–pharmacodynamic index of rifampicin using an in vitro pharmacokinetic–pharmacodynamic TB model, and found that rifampicin AUC_{24}/MIC correlates best with bactericidal activity, while free rifampicin C_{max}/MIC and post-antibiotic effect were associated with resistance prevention.

The bactericidal activity of rifampicin in EBA studies is dose related, with a continuous trend to higher activity with increasing doses [16]. EBA studies of rifampicin at doses of 150–1200 mg, corresponding to 3–20 mg/kg, found an increase of EBA with increasing dose [26, 116–119]. In one such 2-day EBA study by Chan et al. [117], doubling of the rifampicin dose from 300 to 600 mg resulted in increased EBA and a more than dose-proportional increase in serum rifampicin concentrations. When the dose was further increased to 1000 mg (20 mg/kg) in studies by Jindani et al. [116] and Diacon et al. [119], a greater increase in 2-day EBA was found (0.41 and 0.44 $\log_{10}$ CFU/mL/day; $p < 0.05$ and $p < 0.01$, respectively) [116, 119]. Replication of these findings was observed in a recent phase II clinical trial by Boeree et al. [16], which evaluated the 14-day EBA of rifampicin at doses of 10, 20, 25, 30, and 35 mg/kg.

In a Hong Kong study rifampicin in doses of 150, 300, and 600 mg given on day 1 of a 2-day EBA study were associated with mean 2 h rifampicin concentrations of 1.96, 3.21, and 9.25 µg/mL, respectively [117]. In a South African study in which the same doses were used as in the Hong Kong study, serum rifampicin concentrations were determined not only on day 1 of treatment but also on day 5 [26]. This yielded C_{max} values of 2.53, 3.19, and 13 µg/mL, respectively on day 1 of treatment, and by day 5 following rifampicin auto-induction, 150, 300, and 600 mg of rifampicin resulted in lower C_{max} values of 1.49, 2.89, and 9.53 µg/mL, respectively. The associated EBA was none at a dose of 150 mg, but for a dose of 300 mg the EBA was 0.16 $\log_{10}$ CFU/mL/day in Hong Kong and 0.12 $\log_{10}$ CFU/mL/day in South Africa, and for a dose of 600 mg the EBA was 0.29 and 0.22 $\log_{10}$ CFU/mL/day in Hong Kong and South Africa, respectively [26, 117]. Thus, although a detectable EBA was associated with a rifampicin C_{max} of approximately 3 µg/mL, a much higher EBA is found with concentrations of approximately 9 µg/mL. Little bactericidal activity was observed at a rifampicin dose of 5 mg/kg, but an appreciable increase was seen when the rifampicin dose was increased to 10 and 20 mg/kg (0.19 and 0.41 $\log_{10}$ CFU/mL/day, respectively; $p < 0.05$) [116]. The study by Diacon et al. [119] found the mean 2-day EBA of rifampicin at a dose of 20 mg/kg to be almost double that found at a dose of 12 mg/kg (0.43 and 0.221 $\log_{10}$ CFU/mL/day, respectively; $p = 0.02$). Thus, bactericidal activity is dose dependent.

Diacon et al. [119] noted that since EBA studies reflect bactericidal and not necessarily sterilizing activity, more studies are needed to evaluate the potential of a higher dose of rifampicin to further reduce treatment duration to less than 6 months. A very early study by Kreis et al. [120] lends credence to this suggestion. This study evaluated a 3-month regimen with daily rifampicin 1200 mg, isoniazid 900 mg, and streptomycin 1000 mg, and achieved near-complete sputum culture negativity after 90 days, but with a recurrence rate of 11.4% during the first year after treatment [120]. Drawing on data from studies in both healthy volunteers and

TB patients receiving standard doses of rifampicin, some authors have suggested that a serum rifampicin concentration 2 h post-dose of between 8 and 24 μg/mL is desirable for successful TB treatment and 2 h concentrations of < 4 μg/mL were identified as very low [121, 122]. Rifampicin pharmacokinetic studies carried out in association with EBA studies provided guidance as to the desirable serum concentrations.

Several studies gave an early indication that a rifampicin dose < 9 mg/kg/day may be inadequate for the treatment of PTB. At a dose < 9 mg/kg, the rifampicin C_{max} is likely < 8 μg/mL [26, 116, 117]. Although a number of early studies used a daily rifampicin dose of 900 mg, the majority of later studies used a daily dose of 600 mg. Several studies have provided evidence of a dose-related clinical response to rifampicin [103, 120, 121]. In one such study by Long et al. [103], rifampicin was given in dosages of 450, 600, or 750 mg in combination with isoniazid [103]. This study showed that weekly sputum cultures not only became negative, significantly faster in patients receiving 600 mg than amongst those receiving 450 mg, but also that the rate of treatment failure was higher in those receiving 450 mg: at 20 weeks 7.7% versus 0.5% of patients on rifampicin 450 and 600 mg had positive cultures, respectively ($p < 0.01$) [103]. However, no significant difference was found between 600 and 750 mg of rifampicin [103]. In 1972 Jeanes et al. [15] reported their evaluation of two rifampicin doses: 600 or 450 mg in combination with ethambutol in PTB patients resistant to all three major anti-TB drugs of the time. Faster sputum culture conversion rate was achieved with the 600 mg dose of rifampicin [15]. Boeree et al. [100] recently reported that compared to standard dosing of 10 mg/kg, daily rifampicin at 35 mg/kg resulted in a more than dose-proportional increase in plasma rifampicin C_{max} and AUC_{24} values with a consequent shortening of time to stable culture conversion on liquid media from 62 to 48 days (adjusted hazard ratio [HR] 1.78; 95% confidence interval [CI] 1.22–2.58).

Chigutsa et al. [123] studied the pharmacokinetic–pharmacodynamic relationship of 54 patients with PTB [124] and two distinct slopes, α (bactericidal activity) and β (sterilizing activity), were observed following analysis of the rate of decline in sputum bacillary load. The authors used multivariate adaptive regression splines (MARS) analyses that simultaneously performed linear and non-linear analyses in order to identify relationships between predictors and sterilizing activity [123]. Chigutsa et al. [123] found a rifampicin C_{max} of > 8.2 μg/mL, but not AUC, to be an independent predictor of rifampicin's sterilizing activity. This is consistent with a rifampicin C_{max} of 8–24 μg/mL, which is often cited as the recommended target concentration to be achieved 2 h post-dose [98, 122]. Interestingly, a reduction in sterilizing activity was seen with an increase in the isoniazid C_{max} in patients with rifampicin AUC_{24} < 35.4 μg·h/mL [123]. This suggests that in the face of low rifampicin exposure, a higher isoniazid C_{max} may further compromise sterilizing activity by antagonism [123]. This is in keeping with isoniazid dose-dependent antagonism to the sterilizing activity of rifampicin and pyrazinamide in murine TB [125, 126].

Studies have found no mortality benefit following the introduction of rifampicin-containing regimens at standard dose of about 10 mg/kg in adults with TBM and this might be explained by the low rifampicin concentrations achieved in CSF, and, thus, higher doses of rifampicin or intravenous administration may be necessary to improve treatment outcome in TBM [51, 127–130]. This exposure–response relationship was found in clinical trials evaluating high-dose versus standard-dose rifampicin in patients with TBM [5, 6]. These clinical trials found reduced mortality in patients with TBM that achieved high plasma and CSF rifampicin concentrations [5, 6]. In a phase II trial Ruslami et al. [5] randomized patients to receive high-dose intravenous rifampicin (13 mg/kg) or standard-dose rifampicin (10 mg/kg), and found a reduction in mortality in the high-dose group (adjusted HR 0.42; 95% CI 0.20–0.87). Each patient in the group with reduced mortality had a rifampicin C_{max} of at least 8 μg/mL, while in the group with higher mortality only half of the patients had a C_{max} of at least 8 μg/mL ($p < 0.0001$) [5]. In line with this exposure–response relationship of rifampicin and TBM outcome, Te Brake et al. [6] concluded that there should be a target rifampicin AUC_{24} of at least 116 μg·h/mL (equivalent to AUC_6 of 70 μg·h/mL) and C_{max} of 22 μg/mL in patients with TBM. The limitation of this study is that rifampicin MIC was not determined, and, thus, C_{max}/MIC and AUC/MIC were not calculated. In contrast, Heemskerk et al. [131] found no mortality benefit of intensified treatment of orally administered rifampicin 15 mg/kg/day and levofloxacin 20 mg/kg/day compared with standard-dose rifampicin 10 mg/kg/day (HR 0.94; 95% CI 0.73–1.22). This study did not report on rifampicin exposure [131]; it is likely that the exposure with 15 mg/kg/day of orally administered rifampicin is lower than that with 13 mg/kg/day of intravenously administered rifampicin.

Considering the increasing body of evidence for the need to increase rifampicin doses, at least for some indications such as TBM [5, 6], it is reassuring that the safety and tolerability of high doses of daily rifampicin up to 35 mg/kg for up to 12 weeks are comparable to standard dose of 10 mg/kg [16, 48, 100, 132]. In addition, the plasma rifampicin exposure in patients who experienced adverse effects were not different from those without adverse effects [27, 48, 132].

5.5 Therapeutic Drug Monitoring

TDM is a standard clinical technique that measures plasma or serum concentrations of drugs and thus provides an objective measure to inform dose adjustments. There are no prospective clinical trials showing rifampicin TDM improves TB outcomes. Nevertheless, TDM may be useful in patients who fail or are slow to respond to treatment with or without co-morbidities (such as HIV and DM) that predispose to malabsorption; patients with malnutrition; those with drug-resistant TB; anticipated DDIs that may put patients at risk of low plasma concentrations; or in those at risk of toxicity (such as hepatic impairment) [3, 77].

There are indications that most patients with a slow response to anti-TB treatment had low 2 h post-dose concentrations of rifampicin and isoniazid [70, 77, 133], and appropriate dose adjustment based on TDM can aid in attaining the therapeutic concentrations and may shorten the period of non-response and ultimately result in faster cure [77, 134, 135]. Studies have found an association between low plasma concentrations of rifampicin, isoniazid, ethambutol, and pyrazinamide and poor clinical outcomes such as treatment failure, relapse, or acquired drug resistance [70, 77, 123, 136, 137].

For practical purposes, some authors recommend a two-point sample collection; the first is a 2 h post-dose sample which will approximate the C_{max} of rifampicin and most anti-TB drugs, and the second is a 6 h post-dose sample which will provide information on delayed drug absorption [77]. The target rifampicin concentration should be ≥ 8 µg/mL. When both 2 and 6 h post-dose samples are subtherapeutic, this may imply malabsorption, and increasing rifampicin dose may be required. However, when the 2 h post-dose sample is low, but the 6 h post-dose sample is 'therapeutic', this implies delayed absorption as seen in subset of patients, and requires no dose adjustment.

Drawing from the body of evidence in preclinical experiments, AUC_{24}/MIC is probably the most important pharmacokinetic/pharmacodynamic parameter linked to rifampicin bactericidal activity. In the absence of prospectively validated AUC_{24} target in humans, some authors suggest using an average AUC_{24} of 41.1–41.5 µg·h/mL as the "target" AUC_{24} [138, 139]. These AUC_{24} 'targets' were obtained from rifampicin population pharmacokinetics studies [138, 139]. A higher AUC_{24} target of at least 116 µg·h/mL is recommended in TBM [6]. In general, the intensive pharmacokinetic sampling required to obtain AUC_{24} is cumbersome and not suitable for routine clinical care. One way of addressing this problem is to use limited (or optimal) plasma sampling and integrating it with Bayesian-TDM tools to estimate the AUC_{24} [140]. Magis-Escurra et al. [138] provided an alternative approach of using limited sampling strategy to obtain the AUC_{24}. The authors conducted an intensive pharmacokinetic sampling at two Dutch centers to obtain the rifampicin AUC_{24} [138]. This was followed by multiple linear regression analyses being performed to derive limited sampling equations [138]. The best performing limited sampling equations were $AUC_{24} = -4.75 + 1.74 \times C1 + 3.76 \times C4 + 4.83 \times C6$ for C1, C4, and C6 timepoints, followed by $AUC_{24} = -2.22 + 2.05 \times C2 + 2.25 \times C4 + 4.93 \times C6$ for C2, C4, and C6, where C1, C2, C4, and C6 are rifampicin pharmacokinetic samples collected at 1, 2, 4, and 6 h post-dose, respectively [138]. Patients were required to fast prior to rifampicin administration [138]. The limited sampling equation is implementable through Microsoft Excel®, which is widely available even in low-income countries. Therefore, when AUC_{24} is to be estimated, the three-point sample collection (C2, C4, C6) should be preferred to C1, C4, and C6 in order to estimate the C_{max} [138].

Conventionally, TDM samples are collected by venous blood sampling that requires appropriate processing: prompt centrifugation, harvesting, and freezing of serum or plasma [77], followed by analysis using high-performance liquid chromatography (HPLC) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) that are prohibitively expensive. Cost is an important limitation to utilization of TDM in low- and middle-income countries that bear the greatest burden of TB—there is an urgent need for cheaper alternatives. Dried blood spot testing is a promising method that, when compared to conventional ones, requires a smaller blood volume of about 0.1 mL, has easier processing, storage, and transportation, and a lower cost and biohazardous risk [141, 142]. Vu et al. [141, 143] found no significant difference in rifampicin concentrations in dried blood spot testing and in plasma. This study validated dried blood spot testing as an alternative to plasma for rifampicin TDM, with a high correlation of 0.9076 [143].

6 Conclusion

Our review of the literature on rifampicin pharmacokinetics and pharmacodynamics in adults spanning 51 years included 170 articles involving pharmacokinetic data extracted from 69 studies that enrolled 3666 participants who received rifampicin over a dose range of 2–35 mg/kg. We found considerable inter- and intra-individual variability in rifampicin exposure, which can be reduced by administration in a fasting state. Several factors including malnutrition, HIV, DM, mg/kg dosing, certain pharmacogenetic polymorphisms, hepatic cirrhosis, and substandard medicinal products are factors that can alter rifampicin exposure and/or efficacy. TDM may aid in optimizing dosing in carefully selected scenarios.

We found dose–exposure–response relationships for rifampicin particularly in TBM, but more studies are needed

to confirm whether doses higher than the current standard of care could translate into a faster sputum conversion rate, higher cure rate, lower relapse and death rates, and potentially treatment shortening. Daily rifampicin doses up to 35 mg/kg for 12 weeks were safe and well-tolerated.

Compliance with ethical standards

Conflict of interest Ahmed A. Abulfathi has no conflict of interest to declare. Eric H. Decloedt has no conflict of interest to declare. Elin M. Svensson has no conflict of interest to declare. Andreas H. Diacon has no conflict of interest to declare. Peter Donald has no conflict of interest to declare. Helmuth Reuter has no conflict of interest to declare.

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CHAPTER 2

The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance

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REVIEW

The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance

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Following its introduction as an antituberculosis agent close to 75 years ago, the use of para-aminosalicylic acid (PAS) has been limited by gastrointestinal intolerance and multiple formulations were produced in attempts to reduce its occurrence. More recently, an enteric-coated, granular, slow-release PAS formulation (PASER) was introduced and is now in wide-spread use for the treatment of drug-resistant tuberculosis. The current PASER dosing regimen is based on recommendations derived from older studies using a variety of different PAS formulations and relegate PAS to a role as an exclusively bacteriostatic agent. However, there is ample evidence that if sufficiently high serum concentrations are reached, PAS can be bactericidal and that intolerance following once daily dosing, that aids the achievement of such concentrations, is no worse than that following intermittent daily dosing. In particular, prevention of resistance to companion drugs appears to be dependent on the size of the single dose, and hence the peak concentrations, and not on maintaining serum levels consistently above minimum inhibitory concentration. We present a narrative review of the development of PAS formulations, dosing practices, and published data regarding pharmacokinetics and pharmacodynamics and the relationship of PAS dosage to intolerance and efficacy. Our conclusions suggests that we are at present not using PAS to its maximum ability to contribute to regimen efficacy and protect companion drugs.

KEYWORDS
efficacy, intolerance, para-aminosalicylic acid, pharmacokinetics

1 | INTRODUCTION

In 1943, Jorgen Lehmann proposed that para-aminosalicylic acid (PAS) might have antituberculosis activity[1–4]; and, by 1944, PAS was successfully used in Sweden to treat pulmonary tuberculosis (PTB) patients.[5]

At almost the same time, streptomycin (SM) was discovered and its activity studied in the USA by Schatz, Bugie and Waksman and other clinicians[6–8] in parallel to a series of randomized controlled studies conducted by the British Medical Research Council (BMRC) between 1948 and 1952.[9–12] Although initially significant improvement in the condition of PTB patients was documented following SM monotherapy, SM resistance emerged within the first months of treatment, and, by 3 months, 85% (35/41) of the patients remaining sputum culture-positive were producing viable bacilli resistant to SM.[13] The combination of sodium PAS (NaPAS) with SM and, later isoniazid (INH),[9,11,12,14,15] inhibited the emergence of resistance to SM and INH and created an efficacious antituberculosis regimen that was the backbone of TB chemotherapy for approximately 30 years. PAS disappeared from many pharmacies after ethambutol was found to be effective and better tolerated.[16,17]

The emergence of human immunodeficiency virus infection, however, and the concomitant multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB epidemics, led to a renewed interest for PAS to protect companion drugs within regimens comprised of second- and third-line agents. Amongst the formulations introduced in response to this need was an enteric-coated PAS formulation, PASER (Jacobus Pharmaceuticals, Princeton, NJ, USA). Assuming that PAS is essentially a bacteriostatic agent, PASER was designed to provide a slow-release of PAS allowing a prolonged period of PAS concentrations above the minimum inhibitory concentration (MIC) of *Mycobacterium tuberculosis* of 1–2 μg/mL when given in divided daily doses,[18–23] and with less intolerance. This formulation is now widely used to manage certain forms of drug-resistant TB.[21,22,24,25]

Other older antituberculosis drugs such as rifampicin are now undergoing re-evaluation using modern techniques and parent molecules[26,27]; the recommendations for PAS dosing are seldom questioned and have not been reassessed by methodologies that might be now considered routine, such as determination of early bactericidal activity (EBA), evaluation of the maximum tolerated dose or the relationship between dosage, pharmacokinetics (PK), activity and intolerance. The knowledge from older studies regarding these aspects of PAS use is commonly ignored as is the fact that the introduction of PASER requires old dogmas to be challenged, because its PK is very different from the formulations used when the principles of PAS usage were established. Irrespective of the formulation used, it could be argued that PAS therapy with PASER should aim at providing exposures similar to those achieved by the most successful NaPAS treatments used in the BMRC studies.[9,11,12,14,15]

In 2018, the World Health Organization revised its treatment recommendation on MDR-TB, partly based on a recent meta-analysis investigating the relationship of individual antituberculosis agents with treatment success and death.[28–30] This meta-analysis found PAS to contribute little or nothing to treatment success in patients with MDR-TB susceptible to PAS,[28] but it should be noted that those patients with MDR-TB isolates resistant to PAS had worse outcome.[28] Other reports, however, do provide a more positive picture of the value of PAS in managing drug-resistant TB patients.[31] This emphasizes the need to re-examine all existing data on PAS to determine if we are currently using PASER optimally.

In this paper, we expand our previous review of the development of PAS regarding formulations, dosing practices and their relationship to the prevention of resistance in companion drugs and intolerance[22] and review the PK of PAS in greater depth and in particular regarding the lack of relationship of PAS concentrations to intolerance. We also present previously unappreciated evidence that PAS may well have some bactericidal efficacy.

2 | METHODS

We conducted a literature search in PubMed to identify articles on the PK, pharmacodynamics (PD), safety, and tolerability of PAS. The search terms used in various combinations were: "Aminosalicylic Acid"[MeSH], "para-aminosalicylic acid", "PAS", "efficacy", "intolerance", "dosage", "dose", "intravenous", "PK", "PD", "PK PD", "Pharmacokinetic*", "Pharmacokinetics"[MeSH], "Pharmacodynamic*", "Pharmacology"[MeSH], "Tuberculosis"[MeSH], "Tubercul*". The identified articles were screened by title and abstract. Additional articles were identified from referenced articles and related citations in PubMed. Data on PAS PK/PD including intolerance were assessed with particular emphasis on comparison between single-daily doses *vs* the same dose but in divided smaller doses; we also noted the PK and intolerance related to intravenous administration of NaPAS. R version 3.5.1 and WebPlotDigitizer version 4.2 were used to reconstruct figures from 2 studies.[32–35]

3 | RESULTS AND DISCUSSION

3.1 | PAS formulations

In the face of severe intolerance noted soon after its introduction various formulations of PAS were manufactured, including PAS acid and various PAS salts such as NaPAS, potassium PAS (KPAS), and calcium PAS (CaPAS).[22,36] Several granular and enteric coated formulations also became available in an attempt to reduce the gastrointestinal intolerance to PAS but were often associated with lower peak concentrations (C_{max}) and total exposure (area under the curve, AUC) compared to PAS salts.[22,37–39] Similarly, the PASER formulation of PAS was designed to be better tolerated than earlier formulations and does appear to cause less gastrointestinal intolerance.[20–23,40,41] Multiple other PAS formulations have been reported in the literature, few of which have less gastrointestinal intolerance when compared to NaPAS.[22,37–39,42–51]

3.2 | The dose of PAS

Several PAS dosing regimens use oral and intravenous routes of administration, ranging from once daily to multiple daily doses.

3.2.1 | Intravenous dosing regimens

As early as the 1950s, intravenous NaPAS at 24–25 g was utilized in patient care.[3,52]

3.2.2 | Oral dosing regimens

PAS in multiple daily doses has been in use from its first introduction into clinical use.[5,9,11,14,15,53] The dosage of NaPAS in the earliest studies was 20 g/dadministered orally in 4 divided 5-g doses.[9,11] Subsequent studies evaluated lower doses including NaPAS dosages of 5 g, and 10 g/d administered orally in 4 divided 1.25- and 2.5-g doses, respectively.[11] In a later BMRC study, NaPAS dose of 10 g/d was

administered orally but in 2 divided 5-g doses.[14] Other PAS regimens investigated in clinical studies are 3.3 g twice or thrice daily or 6.6 g twice daily, and 4 g PASER twice daily.[18,21,40,54]

Various researchers have studied single daily PAS administration. The doses include PASER at 6 g single dose and 8 g once daily[21,23,41]; NaPAS at 15 and 17 g once daily[39,55]; and, Neopasalate 12 g once daily.[47]

For use in the clinic, current recommendation is for PAS to be used as 4 g twice or thrice daily.[24,56] Similarly, the World Health Organization recommends 8–12 g/d in 2 or 3 divided doses.[30]

It is important to note that 1 g of PAS acid is equivalent to 1.19 g of NaPAS, 1.37 g of crystalline NaPAS and 1.12 g of CaPAS.[3] To complicate matters, these conversion factors could vary between formulations of the same generic. For example, 1 g of PAS acid is equivalent to 1.43–1.7 g of NaPAS,[44,45] 1.12–1.54 g of CaPAS and 1.54 g of KPAS.[3,44]

3.3 | PAS PK

The absorption of PAS salts including NaPAS and KPAS is rapid and complete following oral administration, which usually produces higher PAS concentrations than a PAS acid formulation.[44] PAS acid is poorly soluble in acidic environments, tends to be slowly released while still in the stomach, and is therefore readily acetylated during first-pass metabolism.[22] Compared to the PAS acid, NaPAS, KPAS and CaPAS formulations are more water soluble, and more easily absorbed and more easily saturate the *N*-acetyltransferase-1 (*NAT1*) acetylation capacity of the gut and liver.[22,44,51]

PASER administration with food results in 1.5 and 1.7-fold higher PAS C_{max} and AUC from time zero to infinity, respectively, compared to its administration when fasting.[41] In addition to the better absorption when given with food, intolerance to PASER might be less.[41] The plasma protein binding of PAS ranges between 50 and 73%.[2,22,57] PAS distribution to various sites of disease was shown in animal studies to depend on how high the concentrations were in the blood.[51,57]

The elimination half-life of PAS varies from about 0.5 to 2.5 hours depending on the PAS formulation and administration with or without food or antacid.[21,24,41,45] Following oral administration, PAS is metabolised in the gut to acetyl-PAS, and in the liver to both acetyl-PAS and glycine-PAS.[18,22,51] About 80–90% of an administered dose is excreted in urine following glomerular filtration and tubular secretion as PAS, glycine-PAS and acetyl-PAS.[2,18,22,44,45,51,58] Although, previously considered monomorphic, *NAT1* is now proven to be polymorphic just like *N*-acetyl transferase-2 (*NAT2*).[23,59,60] A South African study in patients with MDR- or XDR-TB, found *NAT1*4/*10* genotype in 44%, *NAT1*14A* genotype in 6%, *NAT1*10/*3* genotype in 3%, and heterozygotes or homozygotes *NAT2*5* genotype in 37% of the study population, all of which are slow acetylators. Whereas, 66% of the study population were heterozygotes or homozygotes for the rapid acetylator *NAT2*4* genotype.[23] A population PK modelling that included *NAT1* and *NAT2* alleles as covariates, found *NAT1*3*, *NAT1*14* and *NAT2*5* alleles to result in significant reduction in the oral clearance of PAS by 17, 14 and 27%, respectively.[23]

It is important to note that more PAS is inactivated when administered in repeated small doses in comparison to same daily dose administered as a single large dose. Lehmann in a 1969 paper, reported the PK of a microgranulate PAS formulation (PASolac, A/B Ferrosan) that was designed to be rapidly absorbed.[51] The microgranulate was administered orally either as 4 g thrice daily or as a single 12-g dose.[51] This study showed the excretion of acetyl-PAS was larger with repeated small doses (4 g × 3) than with a single large dose (12 g × 1).[51] This is probably because at high PAS concentrations, acetyl coenzyme A depletion contributes to the suppressed transformation to acetyl-PAS.[22,38,51]

Some PK parameters of various PAS formulations are provided in Table 1 and it is clear that the plasma PAS concentrations achieved following administration of different PAS formulations vary greatly. Furthermore, the considerable interindividual and interoccasion variability found with different PAS formulations contributes to the challenges of using PAS.[21,23,37,43–45]

PAS can be a subject of drug interactions at both PK and PD levels. PAS absorption could be interfered with by antacid such as aluminium hydroxide because of its adsorption effect,[58] a later study, however, showed negligible effect on overall PAS exposure when PASER formulation was administered with a combination of aluminium hydroxide and magnesium hydroxide/simethicone.[41] In addition, digoxin could reduce PAS absorption and vice versa.[62,63] PAS, by competing with INH for acetylation possibly through depletion of coenzyme A, could result in elevated INH concentrations.[51,58] The oral clearance of PAS is increased by efavirenz.[21,23] A recent in vitro study reported PAS to be a substrate of multiple organic and cation transporters.[64] In this study, nonsteroidal anti-inflammatory drugs such as diclofenac and indomethacin, and proton pump inhibitors such omeprazole inhibit PAS uptake through **OAT1** and **OAT3** inhibition.[64] Similarly, in vitro study showed metformin inhibits OCT1 and **OCT2** mediated PAS uptake. At the PD level, PAS combination with ethionamide/prothionamide could increase the risk of reversible thyroid dysfunction.[62,65]

3.4 | Efficacy

PAS is structurally related to para-aminobenzoic acid, a substrate for dihydropteroate synthase.[2,64] PAS mechanism of action is thought to involve PAS incorporation into the folate biosynthetic pathway by dihydropteroate synthase and dihydrofolate synthase to generate a hydroxyl dihydrofolate antimetabolite that in turn inhibits **dihydrofolate reductase**.[2,64] In addition, PAS is hypothesized to also inhibit synthesis of mycobactin, a mycobacterial cell wall component.[24]

3.4.1 | Early studies of PAS in the prevention of resistance in companion drugs

There is *in vitro* evidence of a concentration-related bacteriostatic effect of PAS; an early study by Singh and Mitchison demonstrated

TABLE 1 Para-aminosalicylic acid pharmacokinetics

Study	PAS formulation	Route of administration	Dose/dosing frequency	C_{max} (μg/mL)	AUC (h*μg/mL)	T_{max} (h)	Half-life (h)
Hollander 1955[46]	PAS-resin complex	Oral	8 g thrice daily	78–85			
Hollander 1955[46]	PAS-resin complex	Oral	16 g[#]	180			
Riska 1959[61]	NaPAS granules	Oral	19 g once daily	277			
Riska 1959[61]	NaPAS granules	Oral	22 g once daily	284			
Riska 1959[61]	NaPAS granules	Intravenous	24 g once daily	323			
Riska 1962[43]	NaPAS granules	Oral	16-g single-dose	240			
Riska 1962[43]	NaPAS granules	Oral	4 g 4 times daily	60–80			
Riska 1962[43]	PAS (free acid)	Intravenous	16-g single-dose over 1 h infusion	450			
Riska 1962[43]	PAS (free acid)	Intravenous	16-g single-dose over 2 h infusion	350			
Riska 1962[43]	PAS (free acid)	Intravenous	16-g single-dose over 3 h infusion	290			
Yue and Cohen 1966[47]	Neopasalate	Oral	6 g once daily	108.9		2	
Yue and Cohen 1966[47]	NaPAS	Oral	6 g once daily	136.2		2	
Yue and Cohen 1966[47]	Neopasalate	Oral	12 g once daily	169.8		2	
Yue and Cohen 1966[47]	NaPAS	Oral	12 g once daily	153.3		2	
Wan et al. 1974[44]	PAS acid	Oral	4-g single-dose	50	209	3.54	0.94
Wan et al. 1974[44]	NaPAS	Oral	4-g single-dose	155	313	0.83	0.91
Wan et al. 1974[44]	CaPAS	Oral	4-g single-dose	140	327	1.02	0.91
Wan et al. 1974[44]	KPAS	Oral	4-g single-dose	121	313	1.1	0.96
Peloquin et al. 1994[18]	PASER	Oral	4-g single-dose	20.23 (8.8)[a]	107.92 (52.45)[a,b]	7.95 (6.12)[a]	1.62 (0.85)[a]
Peloquin et al. 2001[41]	PASER	Oral, fasting	6-g single-dose	21.4 (11.4–79.3)[c]	140 (20.8–255)[b,c]	4.43 (2.09–6.64)[c]	1.88 (1.21–3.91)[c]
Peloquin et al. 2001[41]	PASER	Oral, food	6-g single-dose	32.5 (6.83–72.3)c	240 (71.1–472)[b,c]	6.56 (3.57–8.97)[c]	1.85 (1.10–6.0)[c]
Peloquin et al. 2001[41]	PASER	Oral, orange juice	6-g single-dose	24.7 (6.93–36.4)[c]	175 (29.2–295)[b,c]	4.57 (3.43–11.1)[c]	2.53 (0.95–4.09)[c]
Peloquin et al. 2001[41]	PASER	Oral, antacids	6-g single-dose	18.4 (5.22–46.0)[c]	124 (55.9–227)[b,c]	5.25 (2.7–13.0)[c]	2.1 (0.94–3.39)[c]
Liwa et al. 2013[40]	PASER	Oral, acidic food or beverage	4 g twice daily	51.3 (20.0)[a]	368 (194.0)[a,d]	5.2 (2.04)[a]	
Sy et al. 2015[23]	PASER	Oral, acidic food or beverage	4 g twice daily	61 (10–112)[c]	428 (119–934)[c,d]	4.0 (0.0–12.0)[c]	
Sy et al. 2015[23]	PASER	Oral, acidic food or beverage	8 g once daily	80 (21–135)[c]	652 (161–1055)[c,d]	8 (3.0–121.1)c	

All pharmacokinetics parameter values are given as mean unless otherwise stated. Para-aminosalicylic acid (PAS), sodium PAS (NaPAS), calcium PAS (CaPAS), potassium PAS (KPAS), para-aminosalicylic acid buffer complex (Neopasalate), granular slow-release PAS formulation (PASER), peak plasma concentration (C_{max}), time to C_{max} (T_{max})

[a]mean (standard deviation).

[b]area under the curve from time zero to infinity.

[c]median (range).

[d]area under the curve from 0 to 12 hours.

[#]Dosing frequency is unclear.

the failure of PAS in combination with either SM or INH to protect these companion drugs against resistance emergence at low PAS concentrations of 10 μg/mL, but that PAS concentrations of 100 μg/mL were effective in preventing emergence of resistant organisms.[66] Mitchison later described this effect attained at higher PAS dosages as bactericidal.[67] Other *in vitro* studies demonstrated a concentration-related bacteriostatic effect of PAS.[36,51,68,69] In addition, *in vivo* guinea pig and mouse TB models also showed a dose and concentration-related bacteriostatic effect of PAS.[68]

In early clinical studies of PAS, SM and INH, all the drugs were given in divided daily dosages as was then the practice with infectious diseases, it being considered necessary to maintain the constant presence of inhibitory concentration of the relevant drug.[9,11,14,15,53] The dosage of NaPAS in the earliest studies was 20 g/d but administered in 5-g doses.[9,11] In later studies, NaPAS dosages of 5, 10 and 20 g were evaluated, and a NaPAS dosage of 20 g (5 g × 4) used in combination with SM was more efficacious in the prevention of resistance to SM than 10 g (2.5 g × 4) or 5 g (1.25 g × 4) daily.[11] When used combined with INH, efficacy in prevention of INH resistance was similar whether a daily PAS dosage of 10 g (5 g × 2) or 20 g (5 g × 4) was used.[14] This suggests a dose-related gradient for PAS efficacy in the prevention of resistance developing in companion drugs, with a 5-g NaPAS single dose being more efficacious than 1.25- or 2.5-g doses. When discussing these studies Singh and Mitchison suggested that it was possible the doses used rather than the total daily dosage that was responsible for the improved prevention of resistance in companion drugs that accompanied the use of 5 g single doses of PAS. In other words, it is likely that the PAS C_{max} rather than just AUC or percentage of time above MIC (%T > MIC) was responsible for suppression of resistance in companion drugs.

3.4.2 | Studies documenting the efficacy of PAS monotherapy in reducing sputum acid-fast bacilli counts

It is important to note that a neglected, blinded randomized, placebo-controlled early study also evaluated the efficacy of PAS monotherapy in causing a fall in the percentage of patients coughing sputum containing acid-fast bacilli (AFB) seen on microscopy. In total, 176 patients were randomized to receive either placebo (n = 82) or an enteric-coated PAS granulate (n = 94).[34] The enteric-coated PAS granulate was given in 4 daily doses of 5, 2, 2 and 5 g.[34] In comparison to placebo, the percentage of PTB patients with sputum smears positive for AFB fell significantly from 62.2% (standard error ±5.35) to 18.9% (±3.27) during the first 8 weeks of PAS monotherapy; no similar response occurred in patients receiving placebo (Figure 1).[34]

In 1969, the results of 2 studies were published during which a research group in Berlin assessed the efficacy of the new potential antituberculosis agents, thiocarlide and morphazinamide, in comparison to the established agents such as PAS, isoniazid, rifampicin and ethambutol by measuring the fall in counts of AFB/mL of sputum

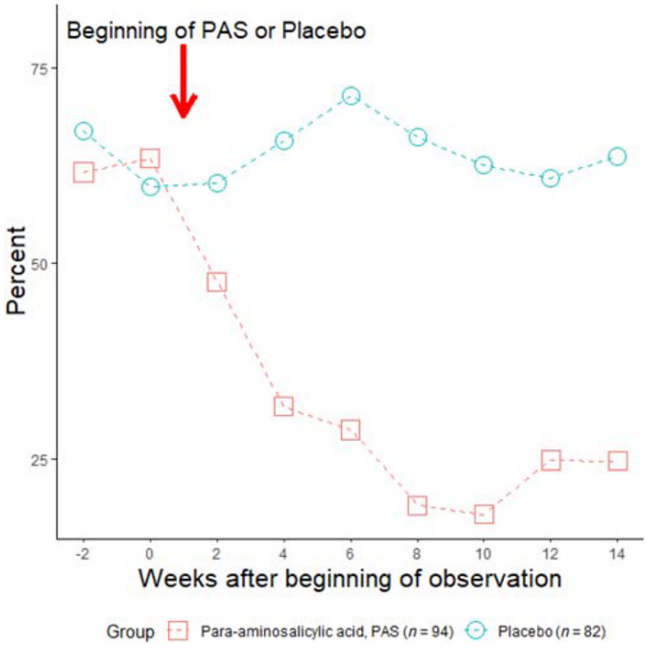

FIGURE 1 Percentage of patients with sputum smears positive for acid-fast bacilli after receiving a placebo or enteric-coated para-aminosalicylic acid (PAS) granulate given in 4 daily doses of 5, 2, 2, and 5 g (redrawn from[34])

quantified by Gaffky counts, a recognized manner of quantifying AFB/mL of sputum.[35] The response to treatment was thus assessed in a manner similar to that of today's EBA studies. In the first study, the activity of thiocarlide was compared to that of PAS at a NaPAS dosage of 12 g given orally 3 times daily in 4 g doses. During the second, similar study, morphazinamide, was assessed in comparison to INH, ethionamide and cycloserine and PAS given intravenously[35] and the results are illustrated in Figure 2.

Although PAS serum concentrations were not determined in either of the studies, the mean peak concentrations reached in patients during the first study (4 g × 3) would probably have been 50–100 μg/mL.[44,45] During both studies, a significant fall in log counts of AFB/mL of sputum in the patients receiving PAS was documented.[35] Remarkably, the fall in AFB in the second study after intravenous PAS, following which PAS concentrations of well over 200–300 μg/mL would probably be reached, matches the fall in AFB counts associated with INH the most bactericidal of our currently available antituberculosis agents.

During the well-known, first comprehensive study of the EBA of antituberculosis agents carried out in Nairobi by Jindani *et al*. in 1980, a small group of patients received NaPAS 15 g once daily for 14 days.[55] During the first 2 days (2-day EBA), a fall in $\log_{10}$ counts of viable colony forming units of 0.259 per mL of sputum per day was found, a value similar to that of 10 mg/kg rifampicin in this same study.[55] Plasma PAS concentrations were not measured in this study; but the mean C_{max} achieved was probably close to 190–240 μg/mL, based on the results of investigation by Frostad in few patients that were administered 12 g PAS formulation equivalent to 17 g NaPAS as a single dose.[39]

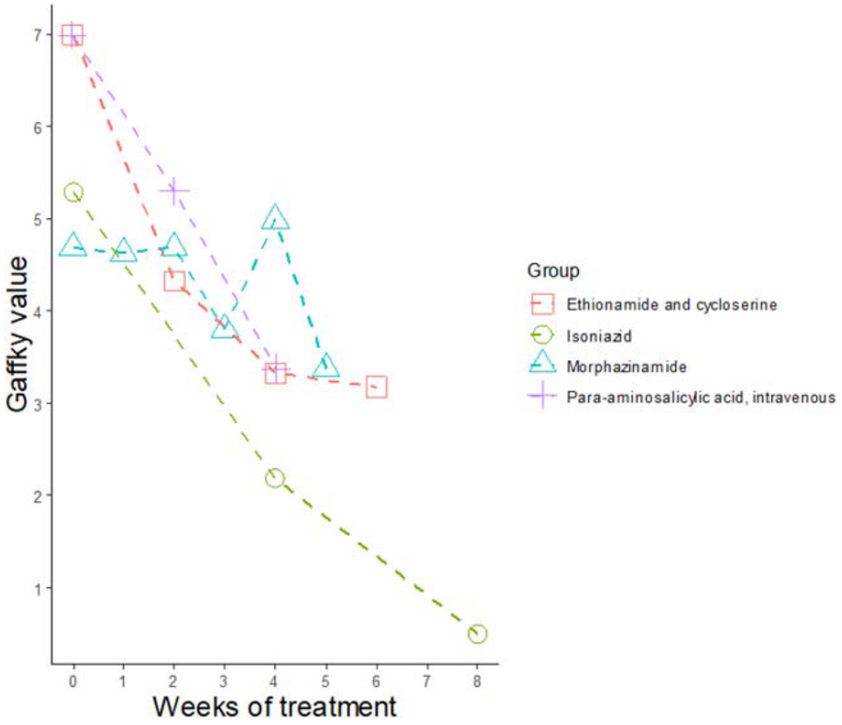

FIGURE 2 Behaviour of the Gaffky values in response to the treatment of cavitary pulmonary tuberculosis with different therapeutic modalities in the lung clinic Heckeshorn (redrawn from[35])

A study by Reisner over a 6-month period showed similar PAS efficacy was achieved irrespective of PAS administration as a single daily dose of 6 g, or as a 12-g daily dose (4 g × 3), in combination with INH.[70] Efficacy in this study was defined as improvement in chest X-ray abnormalities, cavity closure, and the disappearance of tubercle bacilli from previously positive sputum.[70] Even though plasma PAS concentrations were not measured in this study, one could assume that the C_{max} achieved with 6 g is probably higher than with 4 g PAS doses. Indeed, early researchers and clinicians, including Jorgen Lehmann, the discoverer of PAS recommended single daily dosing of PAS, if this could be tolerated.[39,43,61,71]

The usual PAS recommendation for divided daily doses is mainly to ensure that its plasma concentrations between dosing intervals are above the MIC of 1–2 μg/mL,[18–22,54] and to ensure that less intolerance is experienced. This is in keeping with the understanding that %T > MIC is the most important PAS PK/PD parameter of efficacy.[52] However, Charles, in 1955, suggested PAS C_{max} and/or its overall systemic exposure were more important determinants of efficacy, and not necessarily %T > MIC.[52] In his study, 25 g NaPAS solution administered as intravenous infusions 3 times a week, produced at least 10-fold higher PAS blood concentrations compared to oral PAS administration, and he claimed, with a better treatment outcome in the former.[52]

Furthermore, the designs of the aforementioned BMRC studies[9,11,14] suggest that individual PAS doses and, by extension C_{max}, might be linked to suppression of resistance emergence. Although, no PAS PK data are available from the studies,[9,11,14] Citron and Kuper later reported that a mean C_{max} of approximately 100 μg/mL was achieved following 5 g oral doses of NaPAS, this being the dose used in the most successful arms of the BMRC studies with the lowest C_{max} being 50 μg/mL.[22,37] It is reasonable to propose a clinical equipoise; that PAS C_{max} of at least 100 μg/mL may be desirable to achieve suppression of resistance-emergence in companion drugs, and that at high doses and by implication high PAS concentrations, bactericidal activity may be achieved as can be inferred from the study by Jindani *et al.*[55]

Studies by Peloquin *et al.*,[18,41] Liwa *et al.*[40] and Sy *et al.*[23] provided evidence that much lower plasma PAS C_{max} is achieved with PASER (Table 1). Indeed, we found that despite increasing PASER dose to 8 g once daily, the median C_{max} was 80 μg/mL.[23] PASER at the currently recommended dosing regimen of 8–12 g/d given in 2 or 3 divided doses of 4 g provides C_{max} values considerably lower than those reached with the NaPAS formulation used during the BMRC studies. There is, therefore, an urgent need to reconsider the current dosing of PASER, and to perform a prospective evaluation of its PK/PD using the current methodologies. We propose evaluating the PK/PD of high, once daily administration of PASER, as this facilitates use and simplifies supervision of drug intake.

3.5 | PAS intolerance

It is essential to distinguish between the toxicity and gastrointestinal intolerance of PAS. A major problem with intolerance is that it carries the liability of nonadherence and a risk of not completing treatment and following these, treatment failure, relapse and further development of drug resistance.

The frequency and severity of gastrointestinal intolerance including nausea, vomiting and diarrhoea vary with different PAS formulations, dose, route of administration and administration as a single daily dose or in divided daily doses, and, possibly, when taken with or without food.[3,9,11,41,47,52] In the BMRC studies, the gastrointestinal

intolerance to NaPAS administered orally in divided daily doses was reported to occur in 12–58% of patients, with higher NaPAS doses associated with more frequent intolerance.[9,11,22] By contrast, intolerance to PASER formulation seems to be low following its administration at dosing regimens of 8 g once daily or at 4 g once, twice, or thrice daily.[18,23,72] Peloquin *et al*. found that <4% of patients reporting intolerance with this formulation.[18] Other studies also reported PASER to be well tolerated by patients.[23,72] The better tolerability to PASER could be because of PAS release in the small intestine rather than in the stomach and the reduced production of meta-aminophenol.[20,24] Furthermore, the enteric-coating of PASER probably prevents direct gastric irritation by PAS.[20]

Yue and Cohen reported fewer patients having gastrointestinal symptoms when a Neopasalate PAS formulation was administered orally as a 12-g single daily dosage than following the same total daily dosage, but in divided doses.[47] Gastrointestinal symptoms were reported in 6.9% (5/72), 10.7% (3/28) and 4.5% (1/22) of patients administered oral Neopasalate at 4 g thrice daily, 6 g twice daily and 12 g once daily, respectively.[47] Similarly, gastrointestinal symptoms were reported in 14.8% (4/27), 12.5% (3/24), and 10.5% (2/19) of patients administered oral NaPAS at 4 g thrice daily, 6 g twice daily and 12 g once daily, respectively.[47] Importantly, a single daily dose of 12 g Neopasalate/NaPAS compared to the same total daily dosage in 2 or 3 divided doses did result in similar sputum culture conversion rate, despite failure to maintain PAS concentrations above 1–2 μg/mL throughout the dosing interval in those receiving single daily doses.[47] Similar observations were made by other investigators using various PAS formulations.[23,73]

Of further interest, the Yue and Cohen study provided evidence that intolerance to PAS is formulation related. Gastrointestinal symptoms were seen in 7.4% (9/112) of patients on Neopasalate, 12.3% (18/146) on Rezipas, 19.3% (23/119) on Phenyl PAS, 20.8% (35/168) on NaPAS, 24.8% (25/101) on CaPAS and in 50.9% (27/53) of patients on KPAS.[47]

Also in the study by Yue and Cohen, a mean C_{max} of 169.8 μg/mL was reached in patients administered 12 g once daily Neopasalate compared to a mean C_{max} of 40.6 μg/mL and 108.9 μg/mL in those administered 4 g thrice daily, and 6 g twice daily, respectively.[47] In spite of the higher C_{max} achieved with 12 g once daily, intolerance was lowest at 4.5% (1/22) compared to 6.9% (5/72) and 10.7% (3/28) in the 4 g × 3 (12 g/d) and 6 g × 2 (12 g/d) regimens, respectively.[47] Similarly, Riska also found NaPAS orally administered as a single daily dose rather than divided daily doses was associated with fewer side-effects and better tolerability, in spite of achieving very high PAS blood concentrations.[43,61] Earlier mentioned BMRC studies found an association between PAS dosage and intolerance. However, gastrointestinal intolerance to PAS seems to be independent of its plasma concentrations, as demonstrated in several PAS studies.[3,21,23,40,47,69] More recently, no association was found between PAS concentration and intolerance when PASER was given as 4 g twice daily or 8 g once daily.[21,23,40] Adams *et al*. found no relationship between plasma PAS, acetyl-PAS or glycine-PAS concentrations and gastrointestinal intolerance, irrespective of PASER administration as 4 g twice daily or 8 g once daily.[74]

In addition, Jones administered 4.8–5.0% solution of crystalline NaPAS (equivalent to 24-25 g NaPAS), intravenously to TB patients and found it well tolerated with only mild gastrointestinal symptoms seen in about 7.4% (2/27) of patients, despite very high plasma PAS concentrations.[3] Charles, in his 1955 paper, found no gastrointestinal intolerance in any of the 50 patients administered 25 g NaPAS intravenously.[52] In this study, each patient received on average, 50 infusions over a 4-month period. PAS concentrations reached were not provided, but based on similar dose and formulation used by Riska, the mean (range) PAS C_{max} would probably be about 323 μg/mL (190–570).[52,61] Therefore, PAS plasma concentrations probably play little role in gastrointestinal intolerance to PAS. Data from intravenous PAS administration show that if gastrointestinal tract is bypassed, intolerance to PAS is minimized; because gastrointestinal intolerance requires the presence of PAS in the gastrointestinal tract.[74]

3.6 | PAS toxicity

The overall incidence of adverse events (AEs) ascribed to PAS varies from 10 to 30%,[2] and several studies reported PAS to have minimal toxicity even at very high doses, with the most pressing concern being gastrointestinal intolerance.[3,21,40–43,51,61] PAS is associated with reversible hypothyroidism that is amplified when used at high doses and when combined with ethionamide or prothionamide.[3,75] Other potentially serious AEs associated with PAS include hepatitis, haemolytic anaemia, granulocytopenia, polyneuritis, psychosis and angioedema.[3] Furthermore, hypersensitivity reactions to PAS are experienced in about 5–10% of patients, but can be managed and PAS can usually be reintroduced.[2]

It is important to note that PAS overall appears to be safer than several other antituberculosis agents used in treatment of MDR- or XDR-TB. Potentially serious AEs such as central nervous system and haematologic disorders have been associated with other agents.[75,76] Moreover, serious toxicities such as peripheral neuropathy, optic neuritis, and hearing loss following the use of other agents can be irreversible.[75] Thus, if tolerated, PAS appears to have a safety profile comparable, if not better, than that of several other agents used in MDR- or XDR-TB treatment.

4 | CONCLUSION

In summary, available evidence summarized above suggests that PAS C_{max}, AUC and, by extension, C_{max}/MIC and/or AUC/MIC rather than %T > MIC are the more important determinants of efficacy and suppression of resistance development in companion drugs. Data obtained from studies using PASER in healthy volunteers and TB patients suggest that PAS exposures with currently used dosing regimens are lower than those expected to have been achieved in the BMRC studies of the 1950s and that we are not using the PASER formulation optimally.[18,21,40,41] Importantly it appears that PAS at higher

dosages that achieve higher blood concentrations has a hitherto unappreciated bactericidal effect that is not currently exploited.

Future PAS investigations should focus on exploring the relationships between PAS dose, PK and pharmacogenetics and the efficacy and tolerability of PAS in its different formulations. The slow-release formulation of PASER may well offer an acceptable compromise between intolerance and efficacy if dosed once daily. The demonstration of bactericidal activity by PAS, as is suggested by some of the studies summarised above and better knowledge of the relationship of PAS intolerance and efficacy to pharmacokinetic factors and dose might enable the far greater rational use of PAS in community-based programmes for patients otherwise therapeutically destitute. Resistance to several of the newly introduced drugs for the management of MDR- and XDR-TB has already been reported[77,78]; toxicity to several of the currently recommended agents for managing MDR- and XDR-TB patients is also not infrequent.[75,76] PAS remains an attractive orally administered, alternative agent for these patients, but we urgently need to be better informed regarding the most efficacious manner in which to administer PAS and the appropriate doses to use. Should further studies confirm that PAS once daily is acceptable, both as regards efficacy and intolerance, one might envisage a possible regimen where PAS is given once daily in the evening with the evening meal.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

COMPETING INTERESTS

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CONTRIBUTORS

P.R.D., A.H.D. and H.R. developed the study concept. A.A.A., E.M.S., A.H.D. and H.R. designed the study. A.A.A., P.R.D. and K.A. conducted electronic searches and selections of studies. A.A.A., P.R.D. and K.A. conducted data extraction. A.H.D., A.A.A., P.R.D., E.M.S. and H.R. were involved in interpretation of the results; A.A.A. and P.R.D. wrote the first draft of the manuscript. All authors were involved in critical review and revision of the manuscript. All authors approved the final version of the manuscript.

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CHAPTER 3

Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens

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PHARMACOKINETICS AND DISPOSITION

Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens

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Abstract

Purpose Para-aminosalicylic acid (PAS) is currently one of the add-on group C medicines recommended by the World Health Organization for multidrug-resistant tuberculosis treatment. At the recommended doses (8–12 g per day in two to three divided doses) of the widely available slow-release PAS formulation, studies suggest PAS exposures are lower than those reached with older PAS salt formulations and do not generate bactericidal activity. Understanding the PASER dose-exposure–response relationship is crucial for dose optimization. The objective of our study was to establish a representative population pharmacokinetics model for PASER and evaluate the probability of bactericidal and bacteriostatic target attainment with different dosing regimens.

Methods To this end, we validated and optimized a previously published population pharmacokinetic model on an extended dataset. The probability of target attainment was evaluated for once-daily doses of 12 g, 14 g, 16 g and 20 g PASER.

Results The final optimized model included the addition of variability in bioavailability and allometric scaling with body weight on disposition parameters. Peak PAS concentrations over minimum inhibitory concentration of 100, which is required for bactericidal activity are achieved in 53%, 65%, 72% and 84% of patients administered 12, 14, 16 and 20 g once-daily PASER, respectively, when MIC is 1 mg/L. For the typical individual, the exposure remained above 1 mg/L for ≥ 98% of the dosing interval in all the evaluated PASER regimens.

Conclusion The pharmacokinetic/pharmacodynamic parameters linked to bactericidal activity should be determined for 14 g, 16 g and 20 g once-daily doses of PASER.

Keywords Para-aminosalicylic acid · Tuberculosis · Pharmacokinetics · Modelling · PK/PD

Introduction

Para-aminosalicylic acid (PAS) is one of the essential add-on Group C medicines recommended by the World Health Organization for the treatment of drug resistant tuberculosis (TB) [1]. Early British Medical Research Council studies documented successful use of sodium PAS to treat pulmonary TB [2–5]. In addition, the combination of sodium PAS with streptomycin or isoniazid prevented resistance emergence in companion drugs [2–5]. Soon following PAS introduction to

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clinical use, however, the problem of gastrointestinal intolerance to PAS became apparent [2–4]. In attempts to address this problem, many different PAS formulations including phenyl PAS, Neopasalate, calcium, potassium or sodium salts of PAS were manufactured, to mention only but a few [6]. Compared to PAS salts, the granular slow-release PAS formulation (PASER, Jacobus, Princeton, USA) that became recently available is regarded to be better tolerated but the dosing required to achieve optimal antimycobacterial activity and protection of companion drugs still needs to be evaluated [7].

Mycobactericidal activity and protection of companion drugs have been linked to exceeding peak plasma PAS concentrations (C_{max}) of 100 mg/L, and thus, a C_{max} over minimum inhibitory concentration (MIC) of at least 100, assuming typical MIC of ≤ 1 mg/L. The C_{max} target is based on extrapolating concentrations from the British Medical Research Council studies where sodium PAS administered at 5 g individual doses could have resulted in an average C_{max} of 100 mg/L, and the lowest C_{max} reached was approximately 50 mg/L [8, 9]. A recent review supports the notion that PAS prevention of resistance emergence in companion drugs is concentration-related and that at high concentrations, PAS may exhibit bactericidal activity [10]. Several studies suggested that once-daily dosing regimen of PAS could have similar or better antimycobacterial activity than its administration in divided doses and with improved tolerability [10–13]. To date, PAS is considered a bacteriostatic agent and dosing regimens are designed to maintain plasma concentrations above MIC throughout the dosing interval [7]. The current dosing recommendation for PASER is to administer 8–12 g per day in two to three divided doses [14, 15]. Not surprisingly, available evidence suggests that much lower PAS concentrations are reached with PASER compared to PAS salts [16–19]. Importantly, the recommended C_{max}/MIC target of ≥ 100 is generally not achieved with the 4 g, 6 g or 8 g PASER doses [16–18, 20]. It is, therefore, crucial that the optimal PASER dosing regimen is determined to maximize the chances of mycobactericidal activity and cure while protecting companion drugs against the risk of resistance emergence, as was established for PAS salts in the past. Understanding the dose-exposure and exposure-response relationships of high-doses of PASER is critical for its optimization.

There is limited information on the pharmacokinetics (PK) of the now widely available PASER formulation. De Kock et al. present one of few investigations of the PK of PASER in adult TB patients with or without human immunodeficiency virus (HIV) co-infection. The authors developed a one-compartment disposition model with 3-transit absorption compartments in series to describe the population PK of PASER in South African patients with drug-resistant TB that received PASER together with acidic beverages [7]. The objectives of our study were to (i) externally validate the previously published PAS population PK model [7], (ii) optimize this PAS population PK model if needed and (iii) evaluate the probability of target attainment with once-daily PASER regimens that could achieve bactericidal targets while not dropping below the bacteriostatic target during the dosing interval.

Methods

External validation

We used the final parameter estimates from the PAS population PK model developed by de Kock et al. [7] and applied this model to a separate dataset obtained in a similar population [18]. Refer to the online methods (supplementary materials) for details on the external validation.

Model optimization

The PK information from De Kock et al. and Liwa et al. [7, 18] was pooled to be used for the model optimization. Table 1 summarizes the patient characteristics of the joint dataset. Model refinement was done using nonlinear mixed effects modeling (NONMEM) software (version 7.4) with first-order conditional estimation with interaction (FOCE-I), and the model parameters re-estimated on the joint dataset. The best model was chosen based on drop in objective function value (OFV) of at least 3.84 at a P value of 0.05 for nested models, or based on Akaike information criterion (AIC) for non-nested models, and by graphical techniques such as visual predictive checks (VPCs), goodness-of-fit plots, biological plausibility and parsimony. Allometric scaling of disposition parameters with body weight

Table 1 Patient characteristics in the joint dataset enrolled from studies of the pharmacokinetics of para-aminosalicylic acid in tuberculosis patients with or without HIV infection [7, 18]

	Value (N = 85)
Age (years)	
Median (Q1, Q3)	32 (27, 43)
Min-max	18–64
Sex	
Female	42 (49.4%)
Race/ethnicity	
Coloured	64 (75.3%)
Black	21 (24.7%)
Weight (kg)	
Median (Q1, Q3)	57 (47, 63)
Min-max	33.0–85.5
HIV status	
Positive	28 (32.9%)

HIV human immunodeficiency virus, *Q1* lower quartile, *Q3* upper quartile, *Min* minimum, *Max* maximum

was included. We sequentially explored the addition of inter-individual variability (IIV), inter-occasion variability (IOV) or both, on bioavailability. Additive, proportional and combined residual error models were assessed. Other aspects of the model optimization process included correlation between IIV in CL and V, covariate effect of study on residual error and different functions for including the effect of efavirenz (EFV) on CL. Finally, the performance of the optimized model was evaluated using posterior predictive checks of noncompartmental analysis (NCA) metrics. The NONMEM control stream of the optimized model is available in the supplementary material.

Probability of target attainment

The simulation dataset consisted of 1000 virtual patients created from the pooled dataset (random sampling with replacement) plus 10% extra uniform variability in body weight. Dosing and observation records were created using *dplyr* R package.

The optimized population PK model was used to simulate steady-state concentration-time profiles of PAS in the virtual patients, each administered 12, 14, 16 and 20 g once-daily PASER regimens. The simulation study was implemented through PsN [21]. A C_{max} of 100 mg/L and by extension C_{max}/MIC of at least 100 when MIC is 1 mg/L or lower were selected as the desired targets. Model-predicted plasma PAS C_{max} was estimated, and the probability of target attainment for the C_{max}/MIC target with varying PAS MIC was calculated for each dosing regimen [7, 8]. The typical PAS MIC is 1 mg/L [7, 22, 23]. The range of PAS MICs explored was that reported over the past seven decades, ranging from 0.25 to 4 mg/L [7, 22–28]. Concentration-time plots were created using R.

Results

External validation

The VPCs stratified on EFV administration showed that the de Kock et al. PAS population PK model [7] describes the external data [18] reasonably well (Fig. S1). However, the goodness-of-fit plots displayed in Fig. S2 and the posterior predictive checks demonstrated a bias in model predictions (Fig. 1).

Model optimization

The introduction of IIV and IOV on bioavailability resulted in a significantly better data fit and a reduction of both IIV and IOV on apparent oral clearance (CL/F), as well as IIV on

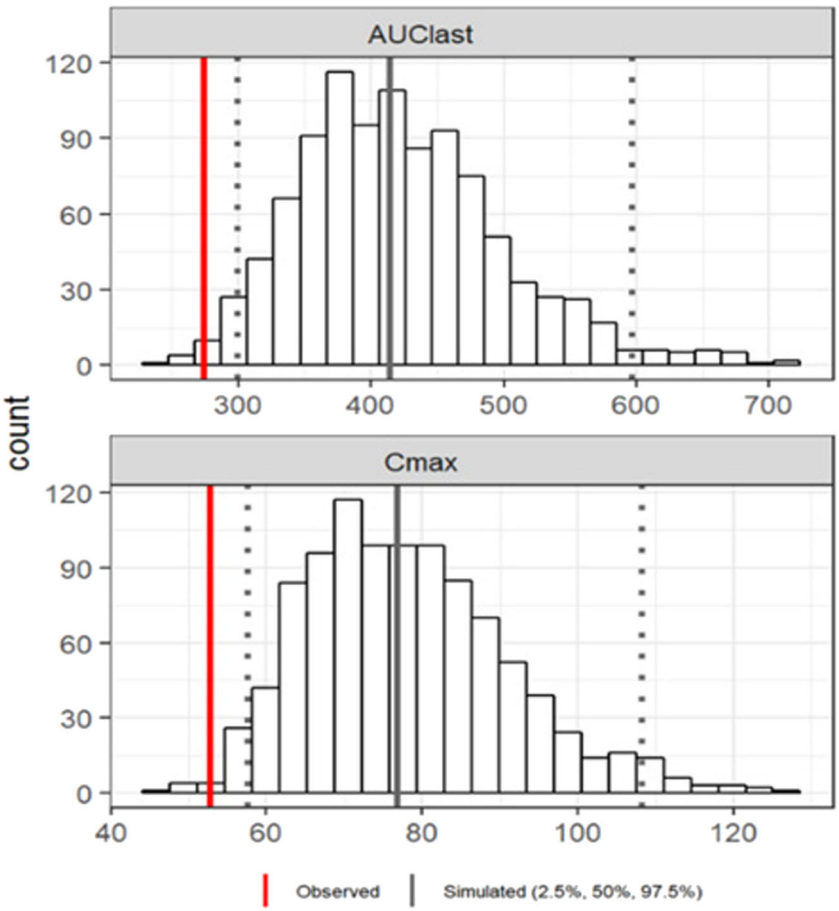

Fig. 1 Histogram of the population mean of AUC_{last} and C_{max} obtained from 1000 simulated studies using the base (published) model. The red and black solid vertical lines represent the population mean of the NCA metric obtained from the observed data ($n = 12$) and that of the same NCA metric obtained from the simulated data ($n = 12$, but the simulated study repeated 1000 times), respectively. The black dashed vertical lines represent the 95% nonparametric prediction interval for the population mean of the NCA metrics obtained from the simulated data. AUC_{last} is the area under the concentration-time curve from time 0 to time of the last measured concentration (that is 12 h) and C_{max} the peak plasma concentration

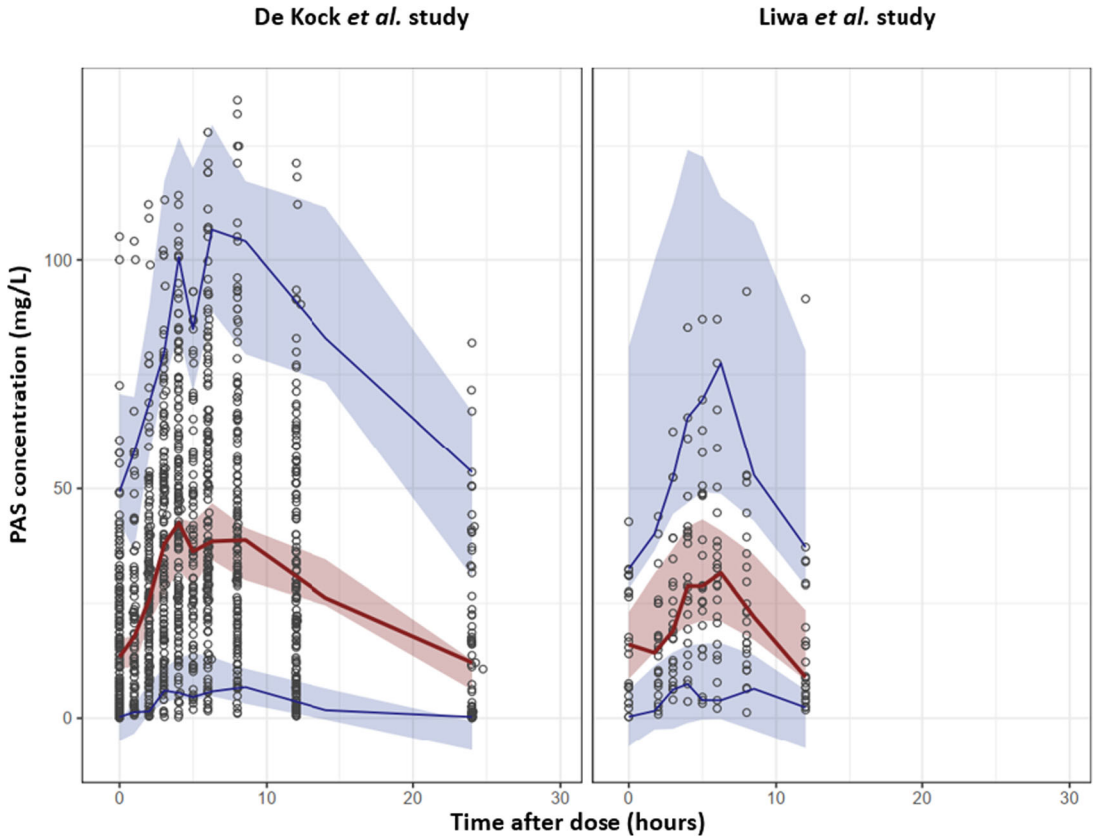

Fig. 2 Visual predictive check for the optimized model stratified by study. The solid blue lines represent the 97.5th and 2.5th percentiles of the observed para-aminosalicylic acid concentration data (open grey circles), the solid red line connects the median (50th percentile) of the observed data ($n = 85$). The blue shaded areas represent 95% confidence intervals of the 97.5th and 2.5th percentile of the predicted simulated data ($n = 1000$), whereas the red shaded area represents 95% confidence interval of the median (50th percentile) of the predicted simulated data

apparent volume of distribution (V/F). We found no impact of study on residual error magnitude. The final model incorporated allometric scaling with body weight and fixed theoretical exponents of 0.75 for CL and 1 for V, centred at 70 kg. The final optimized model had 90.1 points lower OFV compared to the published model [7]. Figure 2 and Fig. S3 display the VPC and basic goodness-of-fit plots of the optimized model (Table 2), respectively.

As expected, the posterior predictive checks with the optimized model found the mean C_{max} and AUC_{last} of the observed dataset fell within the 95% npi of the simulated datasets (Fig. 3).

Probability of target attainment

Plasma PAS exposure increased with dose, as expected (Fig. 4). The C_{max}/MIC target of ≥ 100 was reached in 53%, 65%, 72% and 84% of patients administered 12, 14, 16 and 20 g once-daily PASER, respectively, assuming the typical MIC of 1 mg/L (Fig. 5). At the lowest evaluated MIC of 0.25 mg/L, this target was reached in approximately 100% of patients irrespective of the dosing regimen received. On the contrary, the target was reached in < 6% of patients at

Table 2 Population pharmacokinetic model parameters of the final optimized model

Parameter	Population estimate (%RSE[a])
Structural model parameter	
CL/F[b] (L/h)	12.4 (5.5)
V/F[b] (L)	58.2 (7.6)
k_{tr} (h^{-1})	0.57 (5.9)
Number of transit compartments	3
Inter-individual variability (IIV) as %CV[c]	
IIV CL/F	24.4 (19.6)
IIV k_{tr}	39.1 (14.3)
IIV bioavailability (F)	29.6 (13.1)
Inter-occasion variability (IOV) as %CV[c]	
IOV CL/F	20.9 (28.6)
IOV k_{tr}	43.2 (12.7)
IOV F	30.6 (9.5)
Residual variability	
Proportional residual error	0.318 (12.2)
Additive residual error (mg/L)	5.36 (20.6)
Covariates	
Efavirenz on CL/F	0.401 (33.9)

Apparent oral clearance (CL/F), apparent volume of distribution (V/F), transit rate constant (k_{tr})

[a] Relative standard error (%RSE) was calculated as standard error/population estimate from the covariance step

[b] Population parameter estimates are centred on a typical patient weighing 70 kg

[c] Coefficient of variation (%CV) for IIV and IOV was calculated as (SQRT(EXP(OMEGA) − 1) * 100

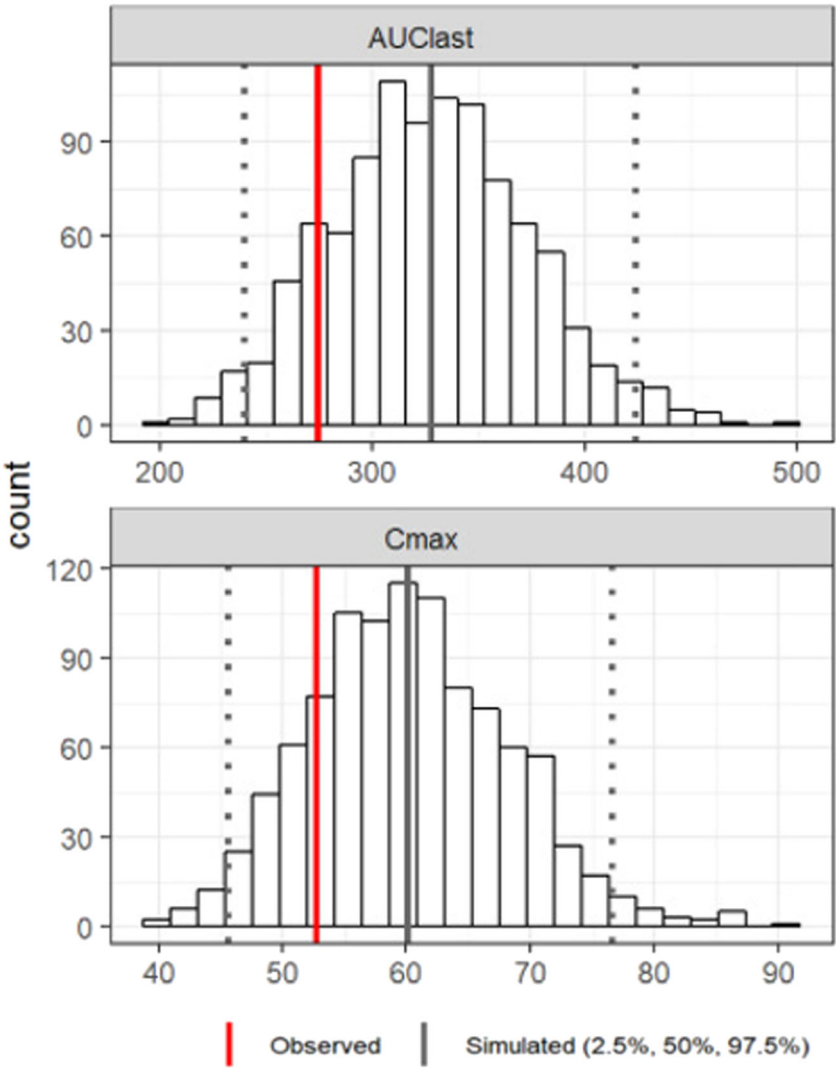

Fig. 3 Histogram of the population mean of AUC_{last} and C_{max} obtained from 1000 simulated studies using the optimized model. The red and black solid vertical lines represent the population mean of the NCA metric obtained from the observed data ($n = 12$) and that of the same NCA metric obtained from the simulated data ($n = 12$, but the simulated study repeated 1000 times), respectively. The black dashed vertical lines represent the 95% nonparametric prediction interval for the population mean of the NCA metrics obtained from the simulated data. AUC_{last} is the area under the concentration-time curve from time 0 to time of the last measured concentration (that is 12 h) and C_{max} the peak plasma concentration

MIC of 4 mg/L, notwithstanding the dosing regimen received. In addition, for the typical individual, the exposure remained above 1 mg/L for ≥ 98% of the dosing interval in all the evaluated PASER regimens (Fig. 6).

Discussion

Our modelling and simulation study demonstrates that bactericidal PAS exposures can be achieved in most patients when 14, 16 or 20 g once-daily PASER are administered. To allow optimal use of this long-established antituberculosis agent, these dosages should urgently be evaluated clinically to ensure that optimal tolerability, bactericidal activity and protection of companion drugs are achieved in clinical practice.

The PAS population PK model developed by de Kock et al. required optimization prior to performing simulation studies focused on maximal and minimal concentrations. The main differences between the original and the optimized models were the introduction of variability in bioavailability and allometric scaling on disposition parameters to account for variation in body size.

EFV resulted in 40.1% (95% confidence interval [CI] of 26.5 to 53.7%) increase in PAS CL with the optimized model compared to 52% (95% CI: 32.4 to 74.6%) found with the published model [7]. EFV, a known inducer of several phase I and II drug metabolizing enzymes, and transporters [7, 29–31] is postulated to induce *N*-acetyl transferase-1 and 2 enzymes involved in PAS metabolism [7]. Although, non-enzymatic processes of PAS CL contribute little to the overall PAS CL, EFV-mediated induction of transporters in the kidneys and biliary tract could also play a role. PASER administration with an EFV-containing regimen will reduce PAS exposures and upward dose-adjustment of PASER by approximately 40% may be required to mitigate the effect. However, caution should be exercised in the interpretation of this finding since the study was not designed to characterize drug-drug interactions. A prospective study is required to determine the

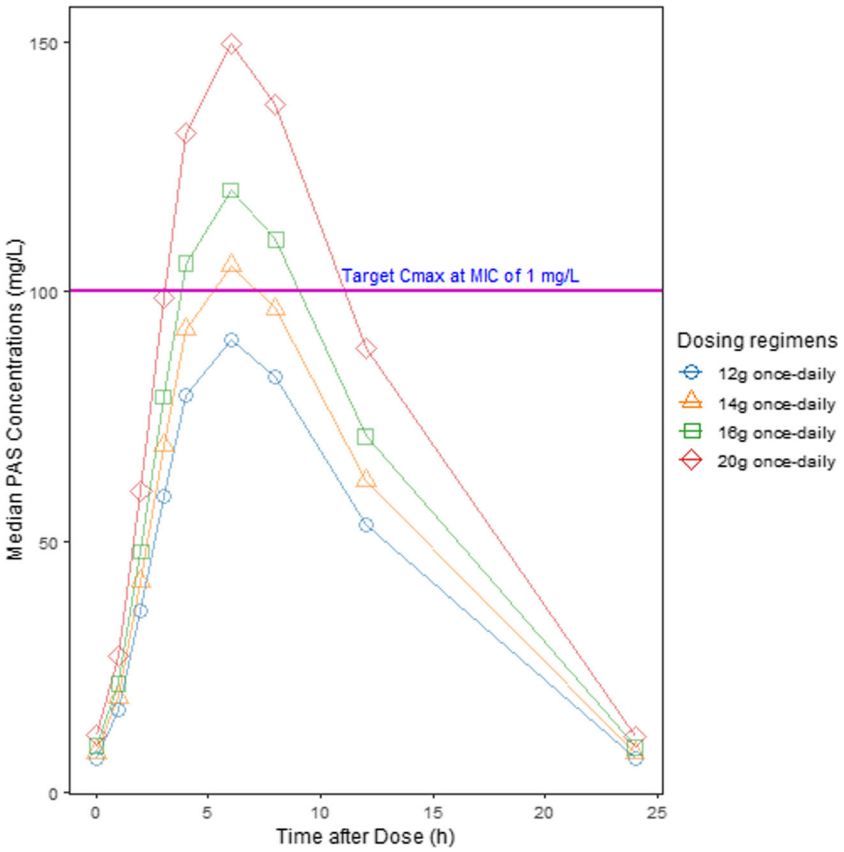

Fig. 4 Simulated median plasma PAS concentrations with novel dosing regimens. PAS is para-aminosalicylic acid, C_{max} is the peak plasma concentration and MIC the minimum inhibitory concentration. The simulation study consists of 1000 virtual patients per dosing regimen

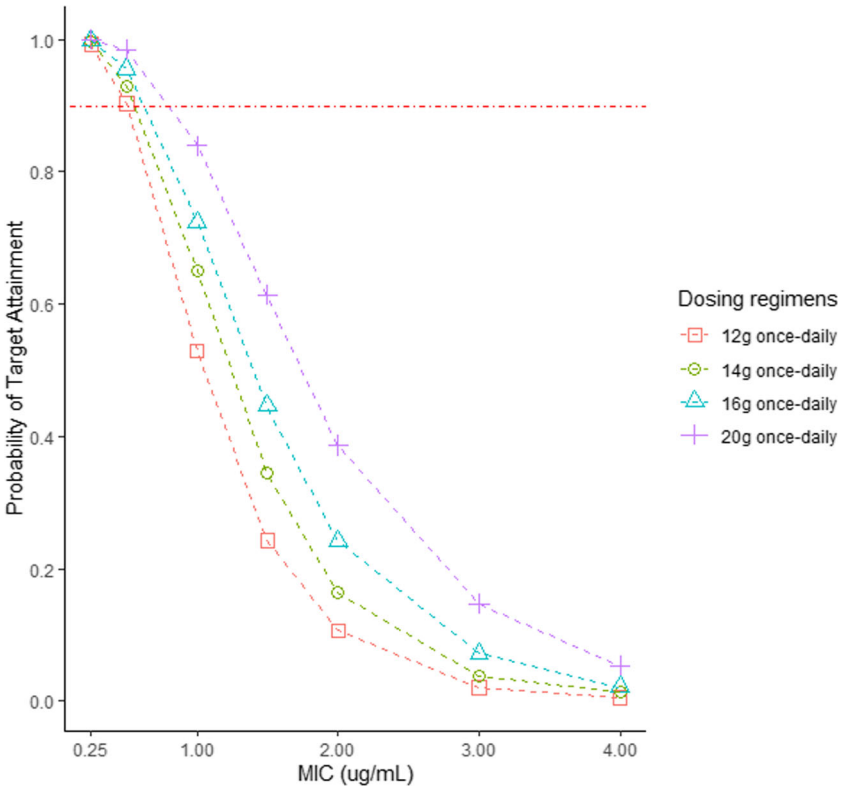

Fig. 5 Probability of target attainment when aiming for peak concentration over minimum inhibitory concentration (MIC) of ≥ 100 with varying MIC. The horizontal, red dashed line corresponds to a probability of target of attainment value of 90% with each dosing regimen. The simulation study consists of 1000 virtual patients per dosing regimen

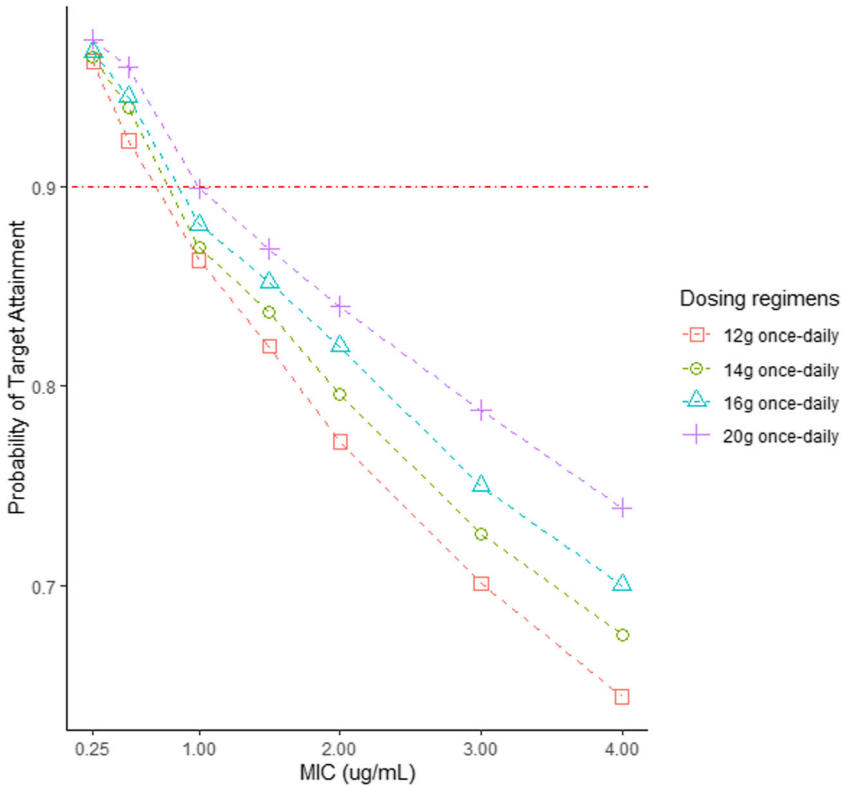

Fig. 6 Probability of target attainment when aiming for trough concentrations ≥ 1 mg/L with varying minimum inhibitory concentration (MIC). The horizontal, red dashed line corresponds to a probability of target of attainment value of 90% with each dosing regimen. The simulation study consists of 1000 virtual patients per dosing regimen

magnitude of EFV-induced PAS CL and to elucidate the pathway(s) involved.

The optimized model was used to predict plasma PAS concentrations following several PASER dosing scenarios not yet investigated in patients. Contrary to the widespread understanding that PAS is essentially a bacteriostatic agent, there is clear evidence that if high enough concentrations are achieved, PAS has bactericidal activity [2–5, 9, 11]. An early bactericidal activity study by Jindani et al. provided evidence in four patients that a 15-g once-daily dose of PAS has bactericidal activity similar to that of rifampicin at 10 mg/kg [11]. The British Medical Research Council studies of the 1940s and 1950s demonstrated the dose-dependent increase in sodium PAS efficacy when used together with streptomycin or isoniazid [2–5]. Efficacy in terms of resistance suppression in the companion drug, streptomycin, increased incrementally across sodium PAS doses of 5 g, 10 g and 20 g per day administered in four divided doses; by the sixth month, streptomycin resistance emerged in 36%, 30% and 7% of patients on 5 g, 10 g and 20 g sodium PAS per day, respectively [2, 3]. A subsequent study showed similar protection of companion drugs between sodium PAS doses of 10 g and 20 g given in two and four divided doses, respectively [4, 5]. It is important to note that regimens that protected companion drugs well were linked by the use of single sodium PAS doses of at least 5 g, suggesting that high peak concentrations are responsible for this effect. These studies did not report the PAS concentrations reached.

Most simulated patients on 14 g, 16 g and 20 g once-daily regimens achieved the C_{max}/MIC target with MICs lower than 1 mg/L. However, this study shows the difficulty in achieving the C_{max}/MIC target when MIC values are at least 1 mg/L. That said, the simulation studies provide evidence of target attainment with novel dosing regimens of PASER. This provides support that high once-daily PASER regimens could achieve the C_{max}/MIC target, while still maintaining PAS concentration of at least 1 mg/L over 24 h (Fig. 6). De Kock et al. concluded that 4 g twice-daily PASER is sufficient to maintain PAS concentrations above MIC of 1 mg/L for 90% of the dosing interval, if %T > MIC is the pharmacodynamic target [7]. De Kock et al. outlined possible advantages related to improved efficacy and ease of programmatic implementation of once-daily doses of PASER. In addition, once-daily dosing will simplify the supervision of drug intake.

Historically, gastrointestinal intolerance to PAS has been linked to the dose, frequency of dosing, formulation type, route of administration and administration with or without food [2, 3, 6, 10, 16, 32, 33]. PAS intolerance was seen in 12%, 15% and 58% of patients who received sodium PAS at 5 g, 10 g and 20 g per day in four divided doses, respectively [2, 3]. Several studies have documented PAS tolerance to be better or at least not worse, when ingested as a single daily dose compared to the same daily dose but divided over several administrations [6, 17, 34, 35]. In the same manner, PASER administration of 8 g once-daily was similarly or better tolerated than 4 g twice-daily [17]. Of interest is that PAS intolerance is low following

intravenous administration, in spite of the very high plasma concentrations reached [12, 32]. This is likely because intravenous PAS administration circumvents the direct gastric irritation effects of PAS and/or metabolites produced while in the gastrointestinal tract. In our study, the mean C_{max} of 197.2 mg/L reached with highest dose of 20 g once-daily is similar to 180.0 mg/L reached with oral PAS-resin complex and much lower than 323.0 mg/L reached with intravenous sodium PAS for infusion (Ferrosan) [33, 35]. Both formulations were well tolerated in spite of the high PAS concentrations reached, an indication that intolerance may not be concentration-related [17, 33, 35]. Hence, we do not anticipate that the suggested dosing regimens will be significantly worse than the current standard of care solely because of increased exposure. However, a prospective study is required to confirm the safety and tolerability of high once-daily PASER regimens.

Our study has several limitations: firstly, the target used in our study is based on extrapolation from historic data and needs to be validated in a prospective study. Secondly, the target attainment rates were obtained by simulations and should be confirmed in real patients. Thirdly, we used protocol time rather than actual time of PK sampling for the analyses. Finally, the population upon which the population PK model was built included only South African patients, and this may limit generalization to other populations.

Conclusion

The target PAS concentrations were achieved in most patients administered 14, 16 and 20 g once-daily PASER. We suggest that a prospective PK study, preferably including assessment of early bactericidal activity, should be performed to confirm our findings and determine the safety and tolerability of the proposed regimens.

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Authors' contributions AAA contributed in developing the research questions, data formatting, designing and execution of the modelling and simulation study, interpreting the results and drafting the manuscript. PA contributed in data formatting, designing of the modelling study and critical review of the manuscript. PRD, AHD and HR contributed in the conceptual design of the study, interpreting the results and critical review of the manuscript. EMS contributed in developing the research questions, designing and supervision of the modelling and simulation study, interpreting the results and critical review of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics declaration Stellenbosch University's Health Research Ethics Committee (S19/01/007) approved this study.

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Probability of target attainment with novel dosing regimens reviving para-aminosalicylic acid in treatment of tuberculosis

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Supplementary materials

Online methods

Online results

Figure S1

Figure S2a

Figure S2b

Figure S3

NONMEM control stream

Online methods

External validation

Twelve adult South African patients diagnosed with drug resistant TB with or without human immunodeficiency virus (HIV) coinfection were enrolled by Liwa et al.[1] In this two-period study, patients were administered oral PASER 4 g twice daily together with acidic beverages for at least four weeks prior to the first PK sampling occasion.[1] The same dosing regimen was continued for at least a week prior to the second PK sampling occasion.[1] PK samples were collected at pre-dose, 2, 3, 4, 5, 6, 8 and 12 h post-dose.[1]

The performance of the model was evaluated through a posterior predictive checks of non-compartmental analysis (NCA) metrics and standard goodness-of-fit plots.[2] It is essential that the model captures NCA metrics well since they are the basis of the target attainment evaluation. The nonlinear mixed effects modeling (NONMEM) software (version 7.4) was used for the implementation of the model.[3] The model was used to simulate concentration-time profiles of each individual 1000 times through Perl-Speaks-NONMEM (PsN, version 4.8.1).[4] R, an open-source statistical software (version 3.5.1)[5] was employed for data management. The *ncappc* package in R[6] was used to perform NCA calculations and simulation-based posterior predictive checks. The NCA metrics of C_{max} and area under the concentration-time curve from time zero to the time of last measured concentration (AUC_{last}, which is the same as AUC 0 to 12 hours) were estimated from both observed and simulated datasets. The population mean of each NCA metric was estimated from a set of observed and simulated data. The distribution of the simulated population means of each NCA metric was graphically compared to the corresponding observed population mean. Diagnostics were based on the observed population mean falling within the 95% nonparametric prediction interval (npi) of the distribution of the simulated population means, suggesting general performance of a population PK model to reproduce drug exposure. Additionally, PsN and Xpose[7] were used to create visual predictive checks (VPCs)[8] and plots of predictions versus observations, and/or residuals. Pirana was used to keep record of model evaluations.[9]

Online results

External validation

Fig. S2 showed a systematic deviation between observed concentrations and population predictions and the posterior predictive checks demonstrated a bias in NCA metrics derived from the model (Fig. 1). The population mean of C_{max} and AUC_{last} of the observed dataset fell outside the 95% npi of the simulated datasets (Fig. 1). These findings lead to the conclusion that the published model needed optimization before it could be used in probability of target attainment simulations.

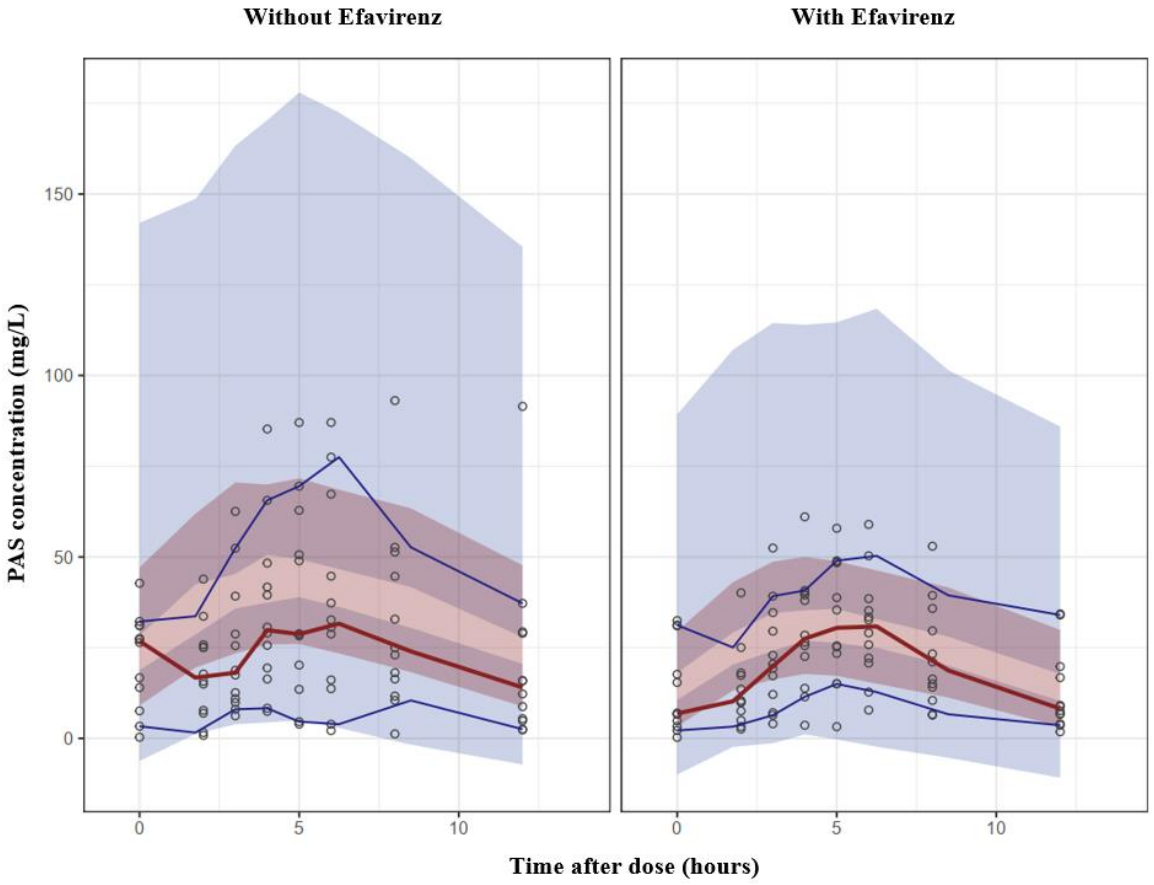

Fig. S1 Visual Predictive Check of the base (published) model[10] on the Liwa et al[1] data stratified by concomitant administration with or without efavirenz. The solid blue lines represent the 97.5th and 2.5th percentiles of the observed para-aminosalicylic acid concentration data (open grey circles), the solid red line connects the median (50th percentile) of the observed data (n=12). The blue shaded areas represent 95% confidence intervals of the 97.5th and 2.5th percentile of the predicted simulated data (n=1000), whereas the red shaded area represents 95% confidence interval of the median (50th percentile) of the predicted simulated data.

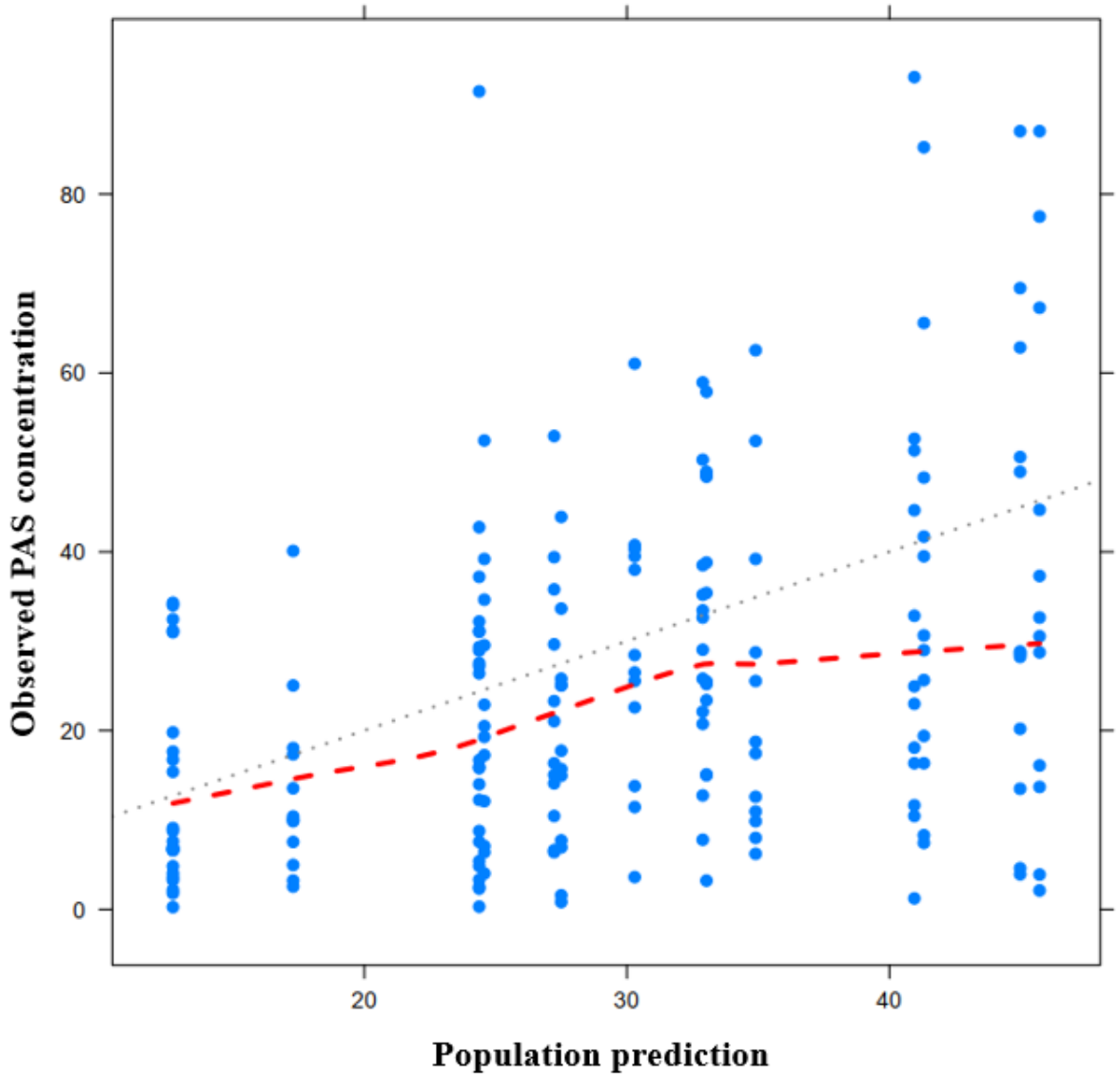

Fig. S2a Basic goodness of fit plot of the base (published) model on the Liwa et al[1] data, showing the observed para-aminosalicylic acid concentrations (mg/L) versus the population predictions (mg/L). The blue dots are the observed concentrations. The grey dashed line is the line of identity (if the predictions were 100% perfect, all the blue dots will fall on this line). The red dashed line is the trend line that shows systematic deviation between observed concentrations (y-axis) and population predicted concentrations (x-axis). The observed and predicted concentrations are from the 12 adult patients in the Liwa et al study.[1]

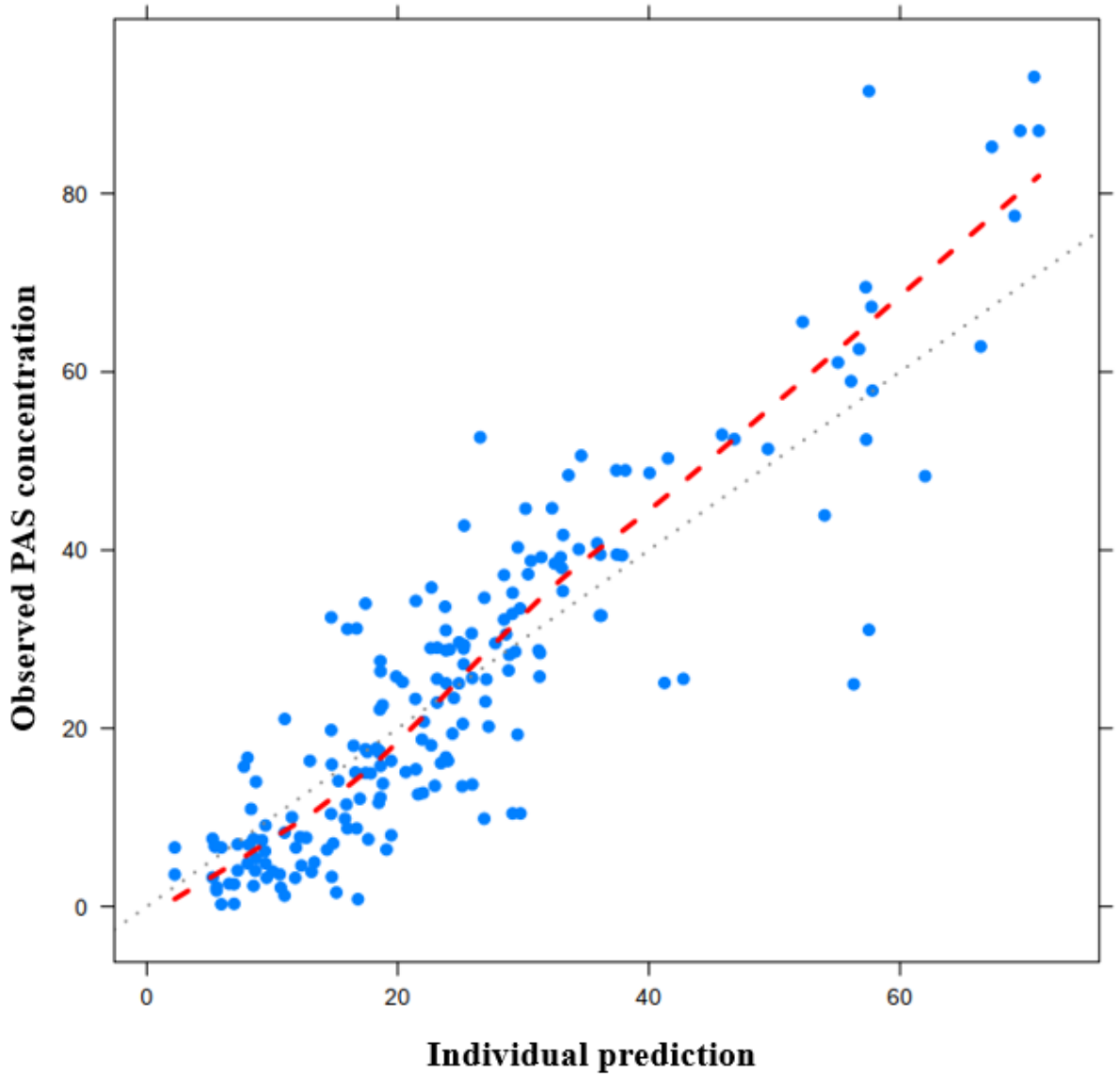

Fig. S2b Basic goodness of fit plot of the base (published) model on the Liwa et al[1] data, showing the observed para-aminosalicylic acid concentrations (mg/L) versus the individual predictions (mg/L). The blue dots are the observed concentrations. The grey dashed line is the line of identity (if the predictions were 100% perfect, all the blue dots will fall on this line). The red dashed line is the trend line that shows systematic deviation between observed concentrations (y-axis) and individual predicted concentrations (x-axis). The observed and predicted concentrations are from the 12 adult patients in the Liwa et al study.[1]

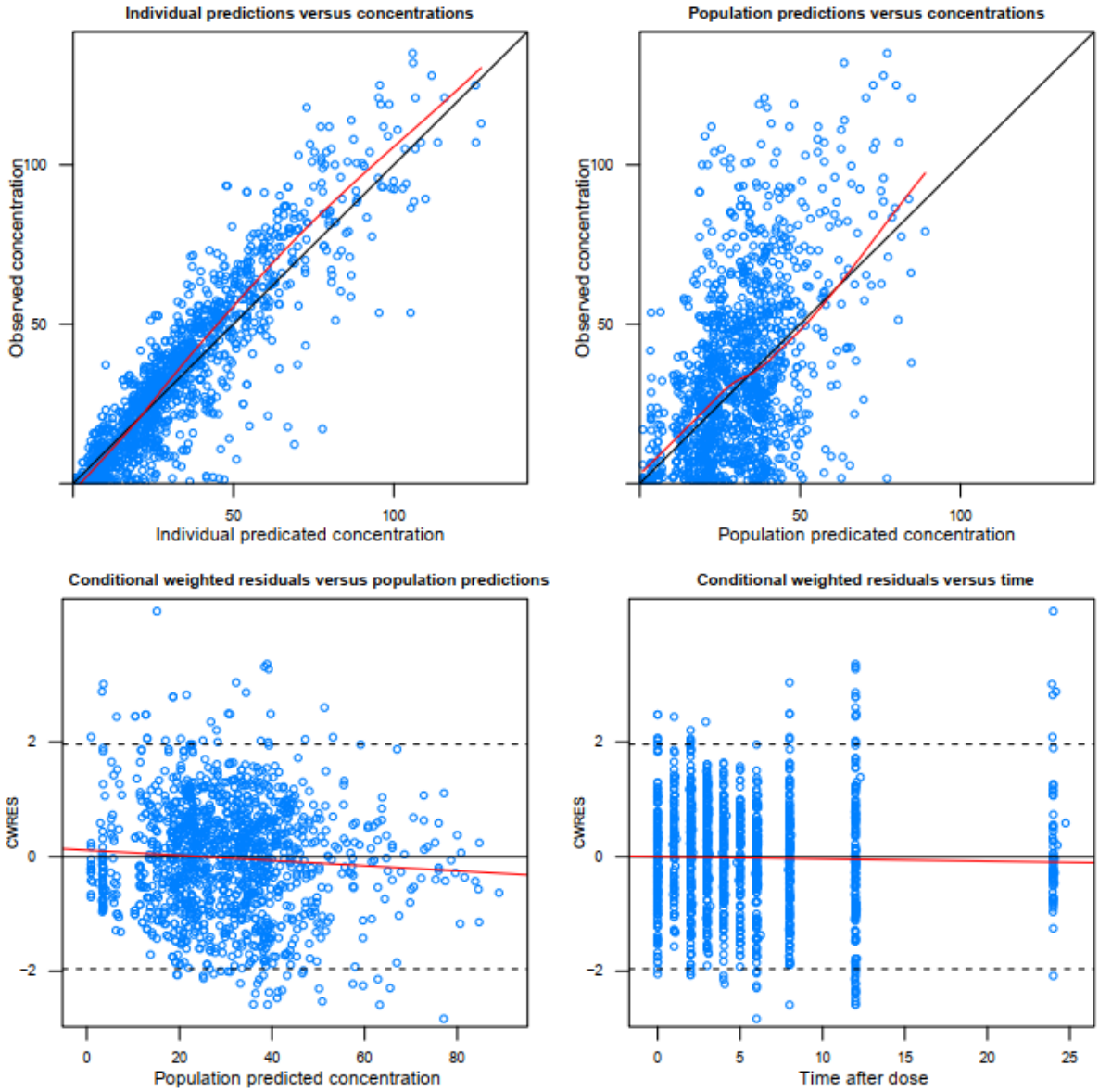

Fig. S3 Basic goodness of fit plots of the final optimized model on the combined data from de Kock et al and Liwa et al studies[1,10] showing the observed concentration (of para-aminosalicylic acid in mg/L) versus the individual predicted concentration in mg/L (upper left) or population predicted concentration in mg/L (upper right). CWRES versus population predicted concentration in mg/L (lower left) or time after dose in hours (lower right). CWRES, conditional weighted residuals. The blue open circles are the observed concentrations. The solid black lines are the lines of identity (top) and zero lines (bottom) if the predictions were 100% perfect, all the blue open circles will fall on these black solid lines. The red solid lines are the trend lines. The horizontal black dashed lines (bottom plots) correspond to +2 and -2 where most of data points should be evenly scattered around the zero line. The observed and predicted concentrations were from the 85 individuals in the de Kock et al and Liwa et al studies.[1,10]

NONMEM control stream

NONMEM code can be found in run001_final_optimized_model.mod.

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```
;; 1. Based on: run#0xx
;; Date of model run#
$PROBLEM PAS Population pharmacokinetic model optimization.
;COMMENTS: Using combined data Liwa and de Kock et al studies.

$INPUT ID TIME DV LDV AMT EVID II SS MDV CMT DOSE OCC WTKG EFV STUD

$DATA dataxx.csv IGNORE=@

$SUBROUTINE ADVAN6 TOL=9

$MODEL
COMP=(DEPOT,DEFDOSE)
COMP=(TRANS2)
COMP=(TRANS3)
COMP=(CENTRAL,DEFOBS)

$PK
Q1 =0
Q2 =0
IF(OCC.EQ.1) Q1 = 1
IF(OCC.EQ.2) Q2 = 1

TVCL=THETA(1)*(1+EFV*THETA(6))                                  ;Apparent
clearance of the typical individual in population PLUS EFV COVARIATE EFFECT ON
CL
TVV=THETA(2)                                                    ;Apparent volume
of distribution of the typical individual inpopulation
TVKTR=THETA(3)                                                  ;KTR is transfer
constant of the transit compartments of the typical individual in the population
TVF1=THETA(7)                                                   ;F1 is the
bioavailability of the typical individual in the population

CL=TVCL*((WTKG/70)**0.75)*EXP(ETA(1)+Q1*ETA(4)+Q2*ETA(5))       ;Individual
apparent clearance, and between-subject variability (BSV) and between-occasion
variability (BOV)
V=TVV*(WTKG/70)*EXP(ETA(2))                                     ;Individual
apparent volume of distribution, and BSV and BOV
KTR=TVKTR*EXP(ETA(3)+ Q1*ETA(6)+Q2*ETA(7))                      ;Individual
transfer rate contant of the transit compartment, and BSV and BOV
F1=TVF1*EXP(ETA(8)) +Q1*ETA(9) + Q2*ETA(10)                     ;Individual
bioavailability, and BSV and BOV

K=CL/V                                                          ;Elimination
rate constant
S2=V

$DES
DADT(1)=-KTR*A(1)                                               ;DEPOT COMP
DADT(2)=KTR*A(1)-KTR*A(2)                                       ;TRANS2 COMP
DADT(3)=KTR*A(2)-KTR*A(3)                                       ;TRANS3 COMP
DADT(4)=KTR*A(3)-K*A(4)                                         ;CENTRAL COMP
```

```
$ERROR                                                          ;Residual error
model
IPRED=A(4)/V
IRES=DV-IPRED
W=SQRT(IPRED**2*SIGMA(1,1) + SIGMA(2,2))                        ;For additive +
proportional error
IWRES=IRES/W
Y=IPRED + IPRED*EPS(1) + EPS(2)

$THETA (0,10.4507)                                              ; 1.CL
$THETA (0,46.2624)                                              ; 2.V
$THETA (0,0.575129)                                             ; 3.KTR
$THETA 0 FIX                                                    ; 4.ADDITIVE
ERROR
$THETA 0 FIX                                                    ; 5.PROPORTIONAL
ERROR
$THETA 0.392677                                                 ; 6.COVARIATE
EFFECT OF EFV ON CL
$THETA 1 FIX                                                    ; 7.F1

$OMEGA 0.0640999                                                ; 1.BSV_CL
$OMEGA 0  FIX                                                   ; 2.BSV_V
$OMEGA 0.136443                                                 ; 3.BSV_KTR
$OMEGA BLOCK(1) 0.0386029                                       ; 4.BOV_CL in
occasion 1
$OMEGA BLOCK(1) SAME                                            ; 5.BOV_CL in
occasion 2
$OMEGA BLOCK(1) 0.167344                                        ; 6.BOV_KTR in
occasion 1
$OMEGA BLOCK(1) SAME                                            ; 7.BOV_KTR in
occasion 2
$OMEGA 0.0791436                                                ; 8.BSV_F1
$OMEGA BLOCK(1) 0.0896854                                       ; 9.BOV_F1 in
occasion 1
$OMEGA BLOCK(1) SAME                                            ;10.BOV_F1 in
occasion 2

$SIGMA 0.100808 28.7776                                         ; Proportional
and additive errors

$ESTIMATION METHOD=1 INTER MAXEVAL=9999 PRINT=1 SORT NSIGDIGITS=3 MSFO=00x.msf
;;standard error estimates calculated

$COVARIANCE
$TABLE ID TIME IPRED IWRES CWRES CWRESI EVID MDV OCC CMT NOPRINT ONEHEADER
FILE=sdtab00x
$TABLE ID CL V KTR ETA(1) ETA(2) ETA(3) ETA(4) ETA(5) ETA(6) ETA(7) ETA(8)
NOPRINT ONEHEADER FILE=patab00x
$TABLE ID EFV WTKG NOPRINT ONEHEADER FILE=cotab00x
;Xpose can read these tables

;there must be one empty line after the last command line
```

CHAPTER 4

Drug concentration at the site of disease in children with pulmonary tuberculosis

Re-submitted for peer review and publication in AJRCCM.

Drug concentration at the site of disease in children with pulmonary tuberculosis

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Running Title: Site of disease drug penetration in pediatric TB

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Impact of the research: A better understanding of the penetration of each first-line antituberculosis drug into different parts of affected lymph node tissue and lesions is a necessary first step required to potentially select pediatric doses that achieve appropriate concentrations at these sites, accounting for pediatric disease severity. This has the potential for tuberculosis treatment stratification and for potential future shorter treatment duration. Our approach provides unique and valuable data which will inform pediatric dose optimization and treatment trials in children.

Summary

Rationale: Adequate exposure of antituberculosis drugs at the site of disease is required for sterilisation of tuberculous lesions.

Objective: To characterize, for the first time, the concentrations of first line antituberculosis drugs at the site of disease in children with intrathoracic tuberculosis.

Measurements and Methods: Prospective study in children with severe intrathoracic tuberculosis routinely requiring bronchoscopy or transthoracic surgical lymph node decompression (SD). We aimed to collect a) plasma samples at predose, 2, 4 and 6 hours post-dose, b) site of disease samples at 2, 4, or 6 hours post-dose for the bronchoscopy, and c) samples at 2 hours post-dose for the SD group. Entire plasma pharmacokinetic profiles were reconstructed using a population pharmacokinetic modelling approach. Site of disease data were modelled by an additional compartment for each lesion, and the rate and extent of distribution of each drug from plasma to lesions determined.

Main Results: We enrolled 13 children, median age 8.6 months. Four of seven SD samples were culture positive. Exposures of antituberculosis drugs varied widely amongst compartments and between participants. The penetration coefficients for isoniazid, rifampin and pyrazinamide showed lower penetration in most lymph node areas compared to plasma. Despite similar penetration coefficients compared to adults, overall low plasma exposures led to low site of disease exposures for all drugs except for isoniazid.

Conclusions: Dose optimization of first-line antituberculosis drugs to increase site of disease exposures could facilitate more rapid culture conversion, and potentially allow

for improved treatment strategies and future treatment shortening across the disease spectrum.

Words: 250

Key words: tuberculosis, drugs, pediatric, pharmacokinetics, nodes

Introduction

Only half of the estimated one million incident pediatric tuberculosis (TB) cases globally are treated each year,[1] partly due to the reluctance of clinicians to initiate lengthy treatment regimens without bacteriological confirmation, which is challenging to achieve, especially in young children.[2]

The treatment of paediatric pulmonary TB, including the drug regimen, dosing and duration, is extrapolated from efficacy trials in adults, with limited consideration to differences in the clinical spectrum of disease or the pharmacokinetic (PK) variability observed in children. While many children with pulmonary (intrathoracic) TB have paucibacillary disease limited to the mediastinal lymph nodes, some have extensive disease, including miliary TB, parenchymal pathology or complicated lymph node involvement with breakthrough to endobronchial disease.[3] Even though it is likely that shorter treatment, given in a targeted way, to children with paucibacillary disease would be effective, all children with pulmonary TB are currently treated with the same regimen, regardless of disease spectrum, severity and bacillary burden. The only exception is the addition of ethambutol for children with severe pulmonary disease and in HIV-infected children.[4] Children typically have lower plasma concentrations for the same milligram per kilogram dose compared to adults for most first-line antituberculosis drugs, and particularly for rifampin, a key first-line sterilizing drug.[5,6] Low exposure to rifampin, is associated with worse treatment outcomes in children.[7–11]

To date, attempts to shorten treatment duration in adults with pulmonary TB have resulted in unacceptably high treatment failure and/or recurrence rates, possibly due to sub-optimal drug penetration into the diverse lung lesions.[12–14] Subtherapeutic antituberculosis drug concentrations in lung cavities in adults are associated with acquisition of mycobacterial drug resistance and also predict unfavourable TB treatment

outcomes.[7,15] For the successful treatment of pulmonary TB, drugs need to reach molecular targets at adequate concentrations and for adequate duration, particularly inside the center of complex granulomas where phenotypically drug tolerant quiescent bacteria may remain secluded.[12] Antituberculosis drug dosing is based on target drug concentrations measured in plasma, despite evidence showing a low correlation between drug concentration measured in plasma vs. at sites of disease, where penetration may be both lesion- and drug-specific.[13,16] To date, few studies in humans have evaluated the penetration of drugs into intrathoracic TB lesions. The limited studies to date have been in adults and have focused on lung tissue,[15–17] despite the fact that TB can be thought of as a lymphatic disease.[18,19] Lymph nodes serve as sites of antigen presentation and immune activation during infection, helping to contain the spread of mycobacteria.[19] Lymph nodes also serve as important sites for mycobacterial persistence, with *Mycobacterium tuberculosis (M.tb)* ready to emerge from a non-replicating state if immunological containment fails.[19] Understanding factors that drive drug penetration, particularly of the sterilizing drugs (rifampin and pyrazinamide), into affected lymph nodes, could facilitate the design of more effective and potentially shorter regimens in children across the disease spectrum, but may also have broader implications for adults. We aimed to characterize the concentrations of first-line antituberculosis drugs at the site of disease in children with complicated intrathoracic TB.

Methods

Study design and procedures

From November 2018 through March 2019, we prospectively enrolled children with complicated intrathoracic TB routinely referred to Tygerberg Hospital, Cape Town, South Africa, for the management of severe airway obstruction. Children underwent one of the

following routine procedures to establish airway patency: bronchoscopic decompression or transthoracic surgical lymph node decompression (SD). Children were eligible if they were on a rifampin-containing regimen for at least 10 days. The Health Research Ethics Committee at Stellenbosch University (N18/05/059) approved this study.

On the day of the procedure, antituberculosis treatment was administered after an overnight fast with dosing times planned at 2 hours before SD, or randomized at 2, 4, or 6 hours prior to bronchoscopy. Treatment consisted of weight-banded once-daily doses of isoniazid (10-15 mg/kg), rifampin (10-20 mg/kg) and pyrazinamide (30-40 mg/kg). Ethambutol (15-25 mg/kg) was added to the regimen for children with severe disease or if they were HIV-coinfected using fixed-dose dispersible pediatric combinations (Table E1).[4,20] Venous or arterial blood samples were collected pre-dose, and at approximately 2, 4 and 6 hours post-dose. During the procedure, site of disease samples were collected in theatre. Bronchoalveolar lavage (BAL) samples were taken from children undergoing bronchoscopy and, when available, endobronchial lymph node biopsy specimens were collected for PK analysis and TB microbiology. Lymph node tissue samples were collected during SD for PK, histology and microbiology. Depending on the size and consistency of lymph node fragments, samples were collected in either homogenizing tubes or in Cryomold for the PK analysis. Further details are described in the online supplement.

Analytical methods

PK assays for rifampin, isoniazid, pyrazinamide and ethambutol in plasma, BAL and lymph node tissue were performed using validated methods.[16] Lymph node material obtained through endobronchial biopsy and liquefied lymph nodes from SD were collected in homogenizing tubes and analyzed using previously described validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS)

method.[16,17] Solid SD specimens larger than 5mm were further classified by histology on frozen section into cellular, necrotic, and mixed lesions. For these specimens, drug concentrations were measured from pathologically distinct regions using laser capture microdissection (LCM) combined with LC-MS/MS as previously described.[17]

PK modelling

Published rifampin, isoniazid and pyrazinamide population PK models in children and an adult ethambutol model were optimized, and the final models were fit to the new data.[21,22] All drug PK models included weight based allometric scaling on clearance and volume and the inclusion of postmenstrual maturation for isoniazid and rifampin. Initial estimates for the ethambutol model used allometrically scaled parameters from a validated adult population PK model. The final plasma PK model was linked to each site of disease assessed by modelling an additional compartment. Using an established method,[13] site of disease PK was described by equation 1.

$$\frac{dC_{SOD}}{dt} = k_{pl-SOD} \times \left(R_{SOD-pl} \times \frac{A_{plasma}}{V_{plasma}} - C_{SOD} \right) \quad (1)$$

C_{SOD} represent the drug concentration in lymph nodes or BAL, $k_{pl\text{-}SOD}$ are inter-compartment rate constants for the transfer of drug from the plasma to the site of disease, $R_{SOD\text{-}pl}$ are the penetration coefficients (ratios) between site of disease and plasma, and A_{plasma}/V_{plasma} is the drug concentration in plasma at time *t*. Penetration values equal to one show equal distribution between plasma and tissue, greater than one show accumulation into the tissue, and less than one show lower penetration in the tissue compared to plasma. When the rate parameter could not be estimated reliably, we assumed a fast penetration rate which was more in line with previous models, for example, the slowest penetration half-life previously published in adults occurred within 3 hours,[13] and for rabbits, penetration half-lives were within 90 minutes.[7,23]

To date, studies on outcomes and related target serum concentrations have been limited to the adult population.[8,24,25] Thus, we compared our data to existing adult drug exposures from the similar population using published population PK models.[22,26–28] The plasma and site of disease drug exposures were compared to target minimum inhibitory concentration (MIC), intracellular macrophage IC_{50}[24,29–32] and minimum bactericidal concentration in caseum (caseum MBC)[33] (see online methods).

Results

Participant characteristics

We enrolled thirteen children who collectively underwent 15 procedures including eight bronchoscopies and seven SDs. Baseline clinical and radiological characteristics are shown in Table 1, and individual participant characteristics in Table E2. The median age was 8·6 months (interquartile range [IQR]: 3·5-20·2) with median weight of 8·2 kg (IQR 7·1-10·5). All children had evidence of severe (extensive) intrathoracic disease on chest imaging (Figure E1). The median time on antituberculosis treatment was 64 and 34 days for the bronchoscopy and SD groups, respectively. All cases were bacteriologically confirmed; one child had isoniazid mono-resistant TB and a second had multidrug-resistant TB which was only diagnosed following the procedure.

Sample characteristics

Drug concentrations in BAL could only be determined in 4/8 (50%) of patients due to urea concentrations below the limit of quantification in BAL, a requirement for dilution factor calculation, and were thus excluded from the modeling. Lymph node samples were obtained in 4/8 (50%) of the bronchoscopy cases through endobronchial biopsy, and in all seven (100%) SD cases (Table E2). Histological examination of all SD tissue fragments showed that most specimens were characterized by necrotizing

granulomatous inflammation. None to very little residual normal lymph node tissue was identified in lymph nodes, and only one had preserved architecture displaying reactive follicular hyperplasia. Ziehl-Neelsen staining revealed acid-fast bacilli in four of the seven samples and two had numerous bacilli, which were typically more abundant in necrotic foci. While none of the BAL samples were culture-positive, four of seven SD cases were culture-positive. MICs were obtained for two of the *M.tb* isolates and values were within the range of normal MIC (isoniazid < 0·03 mg/L, rifampin <0·06 mg/L; ethambutol 1 mg/L) (Table E2).

LCM was performed in nine frozen lymph node specimens from four participants allowing the spatial quantification of drugs within the spectrum of different tissue compartments identified (necrotic, cellular, or mixed lymphoid) (Figure 1).

Plasma and tissue PK profiles

The concentrations of rifampin, isoniazid, pyrazinamide and ethambutol were measured in 44 plasma and in 65 BAL and lymph node samples collected at 2 to 8 hours post-dose (Figure 2). There was large variability in the concentrations of all drugs amongst site of disease compartments, between participants, and even within similar compartments in the same participant.

Modeling of plasma PK and tissue distribution

Plasma model and simulations

The final PK estimates are shown in Table 2 and the structural model is shown in Figure E2. The rate and penetration coefficient estimates for each drug are shown in Table 3. Estimates for the plasma PK model were similar to previously published pediatric models (Table E3). Figure 3 shows the simulated concentration-time profiles relative to published concentrations observed in adults.[22,26–28] Mean values of the steady-state $AUC_{0–24}$ in children compared to adults for plasma, cellular and necrotic lesions are shown in Table

4. The values for other compartments where there was no adult reference for comparison (BAL, homogenized lymph node and mixed regions) are shown in Table E4 and Figure E3.

Rifampin

Plasma rifampin exposures were similar to adult reference values ($AUC_{0–24}$ of 41.7 vs. 38.3 mg*h/L).[28] However, only 15% of the 1000 simulated rifampin $AUC_{0–24}$ in plasma were above the proposed adults target of approximately 42 mg*h/L (obtained based on adult rifampin dose of 8-12 mg/kg per day).[34–36] That said, children appeared to have more favourable drug penetration in lymph nodes compared to penetration into lung lesions observed in adults, particularly in cellular regions, where the penetration coefficient was 1·4 (95% CI: 0.66 to 3.54) resulting in an $AUC_{0–24}$ of 55.2 mg*h/L. Mixed tissue, BAL and homogenized lymph node showed similar concentrations compared to plasma (penetration coefficients ranging from 0.9 to 1.0), while cellular and necrotic regions were more diverse (penetration coefficient of 1.4 vs. 0.5). Rifampin concentrations were below the upper end of the MIC range for >50% of the dosing interval in all tissue compartments except the cellular region (Figure 3 and E3). In necrotic areas, exposure was well below the selected efficacy target (caseum minimum bactericidal concentrations [casMBC]).

Isoniazid

Plasma concentrations were similar in children compared to the simulated AUC values for adults ($AUC_{0–24}$ of 25.3 vs. 27.7 mg*h/L). The penetration coefficient was particularly high in necrotic areas (0.84), consistent with adult data. The penetration coefficient was lower in the cellular region of granulomas (0.56), but was twice as high as that observed

in adults. Penetration into BAL was high (2.9). Overall, isoniazid exposure was above MIC for approximately 50% of the dosing interval for all tissue compartments except in necrotic lesions (Figure E3).

Pyrazinamide

Particularly low plasma pyrazinamide exposures were observed compared to adult data ($AUC_{0–24}$ of 248 vs. 466 mg*h/L). Compared to adults, there was a lower ratio in cellular lesions (0.42 vs. 0.70), but a higher ratio in mixed lesions (1.40 vs. 0.61). The penetration into BAL was the highest for pyrazinamide compared to other drugs. Despite the low plasma pyrazinamide concentrations, our simulations showed pyrazinamide exposure was above the "acidic" macrophage MIC for approximately 54% of the dosing interval in cellular compartments, but below adult target values in homogenised lymph node and necrotic lesions.

Ethambutol

The overall plasma ethambutol exposure was low. However, there were high penetrations into all compartments, ranging from 1.1 in necrotic lesions, to 6 in cellular components. The AUC in children was six-fold lower than the simulated adult value of 78.5 mg*h/L, and as a consequence, the AUC was above MIC for only approximately 29% of the dosing interval, using the lowest range of the MIC distribution. In all compartments except for necrotic lesions, ethambutol appeared to accumulate compared to plasma. There are no prior adult data on ethambutol concentrations at pulmonary site of disease to allow for comparison with this pediatric data.

Discussion

In this proof-of-concept study, we report data, for the first time, on first-line antituberculosis drug penetration into the site of disease in children with pulmonary TB. Specifically, we show drug penetration into intrathoracic lymph nodes and BAL in children with severe forms of pulmonary TB. The penetration coefficients for isoniazid, rifampin and pyrazinamide showed lower penetration in most lymph node areas compared to plasma. Conversely, rifampin accumulated in cellular regions, while ethambutol showed a particularly high accumulation into all tissues compared to plasma. Simulations to investigate drug exposures in lymph node lesions in children compared to adult lung lesions showed that overall, penetration into the site of disease was similar in children compared to adults. However, the standard antituberculosis drug dosing resulted in low plasma and consequently, low site of disease exposures for all drugs evaluated except for isoniazid. Because only 15% of simulated plasma rifampin $AUC_{0\text{-}24}$ in children reached the proposed adults target[34–36] and given that rifampin was administered at lower end of the 10-20 mg/kg range, possibly indicate the need for use of higher end of dose range (20 mg/kg). Several ongoing trials are evaluating higher doses of rifampin up to 50 mg/kg in adults and 70 mg/kg in children. Higher doses of key first-line drugs would likely result in higher plasma exposures, and consequently more optimal exposures in other relevant compartments.[37]

The current long 6-month standard duration of antituberculosis treatment is a result of the proportion of the bacilli that remain dormant in a non-replicating state.[33,38] To guide better decision-making for improved antituberculosis regimens, a better understanding of the potency of each drug against bacilli in different lesions is required. Important factors to consider include the location, state (i.e. quiescence and presence of cell wall) and quantity of *M.tb* bacilli during treatment, all of which are still poorly understood. Our results offer

new data on lesion-focused penetration of antituberculosis drugs and lay the foundation for future research addressing important questions on the exposure-relationship in children at the site where disease needs to be effectively treated, across the spectrum of severe and non-severe TB.

Lymph nodes are critical in primary *M.tb* infection in children and adults, and are the primary disease site in children, particularly in the very young, who are at highest risk of severe TB and mortality. However, the bacterial dynamics and the effect of *M.tb* infection on lymph node structure and function remain understudied. In our study, histopathological examination of SD tissue fragments showed predominantly necrotizing granulomatous inflammation, with little residual lymph node tissue. This was probably a reflection of the nature of the procedure (decompression rather than complete lymph node excision) and disease severity, as these lymph nodes were affected to the extent of causing airway compression. The effect of corticosteroid therapy on lymph node histology is uncertain. Acid-fast bacilli were still seen on Ziehl-Neelsen staining in four of seven SD samples after at least four weeks of TB therapy. Although Ziehl-Neelsen staining does not differentiate viable from non-viable organisms, positive culture results in four of the seven (including two that were acid-fast bacilli negative) demonstrated that at least some viable organisms remained in lymph nodes after more than a month of treatment. Bacilli were more numerous in necrotic foci. This is similar to data previously reported in animal models[39] and in adult humans[40] and highlights the need to optimize drug concentrations in this hard-to-reach compartment. Both these findings (extensive destruction of lymph node tissue and viable *M.tb*, particularly in necrotic foci) are consistent with recent macaque studies suggesting that standard antituberculosis treatment regimens provide poor killing of *M.tb* in lymph nodes compared to that in lung granulomas.[19]

The histological spectrum of the tissue samples between adults and children was similar, despite different excision locations. Considering that pediatric dosing is extrapolated from adults, we compared drug exposures in lesions in children to those in adults. The penetration coefficients of necrotizing lymph nodes in children were remarkably similar to what has been described in adult lung granulomas, suggesting that penetration properties could be scaled between children and adults for these lesions, which in turn could inform future regimen and dosing strategies. The vascular supply of these infection sites could possibly be different for cellular lesions, with necrotic foci being avascular. The penetration was highest for the two drugs with limited activity against quiescent mycobacteria: isoniazid and ethambutol. Conversely, the two drugs with sterilizing activity, rifampin and pyrazinamide, had penetration coefficients of 0.55 and 0.40, respectively, with exposures in the caseum below their target concentrations (CasMBC). A recent *ex vivo* model has shown that only rifampin fully sterilizes bacilli in caseum[33] with a CasMBC of 6.5 mg/L. A higher or different dosing schedule could achieve desirable concentrations in children.

All of the first-line drugs other than rifampin accumulated in the epithelial lining fluid of BAL compared to plasma. For isoniazid, the results were consistent with previous reports (2.36-2.57).[41] For rifampin, we observed a 4-fold higher penetration coefficient than in adult reports (0.86 vs. range 0.22-0.34).[42–44] Differences could be due to lysis of the alveolar cells during sample collection and processing, leading to a false increase in the epithelial lining fluid drug concentrations.[45] Indeed, adult studies typically report higher rifampin concentrations in alveolar cells than in epithelial lining fluid. With the exception of ethambutol, the drug concentrations in BAL were above the MIC ranges explored, in line with our microbiological data showing all eight BAL samples that were Xpert MTB/RIF positive but culture negative.

We present, to our knowledge, the first human data on the penetration of ethambutol into pulmonary compartments. Ethambutol is an important component of first-line antituberculosis treatment in adults and in children, despite its poor plasma-based PK/pharmacodynamic (PD) profile. Our results support the hypothesis that the efficacy of ethambutol might be explained by the favourable penetration of this drug into *M.tb* lesions.[17] We observed ethambutol accumulation in all compartments relative to plasma, but particularly in the cellular areas (penetration coefficient 6.17) where ethambutol targets intracellular bacilli.[29] Overall, ethambutol exposures in different compartments were limited by the low plasma concentrations, which were significantly lower than in adults (C_{max} 2.3 vs. 11.3 mg/L) and below the theoretical MIC target. This is consistent with previous pediatric studies which show erratic absorption and low concentrations of ethambutol in children dosed at 10-20mg/kg per day.[46] Given the favourable penetration into lymph nodes, our results suggest that ethambutol should be dosed at 25 mg/kg (i.e. at the higher end of the dosing range of 15-25 mg/kg) to achieve higher site of disease exposure. Such doses are considered safe and are associated with very low risk of ocular toxicity.

Although the nonlinear mixed effects approach utilized in our study is useful to evaluate sparse PK data, further studies are required to characterize the dynamics of diffusion into caseum over time, including better characterization of the rate of movement of drugs into lesions compared to plasma. For all four drugs, the absorption models had to be adapted. This could be due to the different drug formulations used and/or not having enough data available during the absorption phase. Isoniazid had different peripheral volume and intercompartmental clearance estimates, which were not in line with what has been previously reported, but data were collected only up to six hours making this compartment difficult to parameterize. Therefore, isoniazid simulations used previously published PK

parameters with lesion parameters from our model to ensure accurate prediction of terminal concentrations.

Our cohort included young children with severe forms of pulmonary TB undergoing surgery at one single site which may have introduced selection bias. However, this is the most vulnerable population where optimized antituberculosis treatment strategies are most needed. All children were anaesthetized at the time of the procedure, and particularly those from the SD group received a significant number of concomitant drugs and intravenous fluids, all of which could have affected drug disposition. An additional limitation in our simulation study is that total but not unbound plasma concentrations were quantified thus, protein binding in plasma and tissues were not taken into account. However, isoniazid, pyrazinamide and ethambutol are small polar antibiotics that exhibit low protein binding. Importantly, the macrophage IC_{50} and caseum MBC potency assays used to measure drug potency at the site of disease both correct for protein binding, as they are performed in matrices that reproduce *in vivo* drug binding. The classification of the lymph node histology on the fresh frozen tissue selected for PK into cellular, mixed (cellular but necrosis in the background) and necrotic was challenging in some of our study samples due to freezing artefact, absence of clear area demarcation and the fragmented nature of the tissue samples. However, routine histology samples (formalin-fixed paraffin-embedded) were usually available and provided guidance on interpretation in areas that were difficult to classify on frozen section. Finally, our PK-PD simulations in BAL should be interpreted with caution given the limited number of patients in whom BAL urea concentration was successfully measured and the technical caveats such as dwelling time and volume of instilled lavage fluid.

In conclusion, we have shown that measuring the penetration of antituberculosis drugs in intrathoracic lymph node compartments and BAL in young children was feasible and that

the penetration coeficients of first-line antituberculosis drugs into these compartments was similar to adults. Nevertheless, the overall plasma exposures of all the drugs were low, particularly for ethambutol. Similarly, all the first-line drugs had exposure in necrotic tissue at levels lower than target concentrations. Our results support the hypothesis that exposure of first-line antituberculosis drugs is both lesion and drug specific, and indicate that current pediatric dosing guidelines are not likely to result in target exposures at sites of disease. With an improved understanding of site of disease penetration, and using a data-driven modelling approach, it could be possible to model optimized doses to result in increased exposures of drugs at the site of disease. This in turn can lay the ground for a more appropriate dosing in children and consequently improve the efficacy and duration of antituberculosis drugs and regimens at relevant sites of disease across the disease spectrum.

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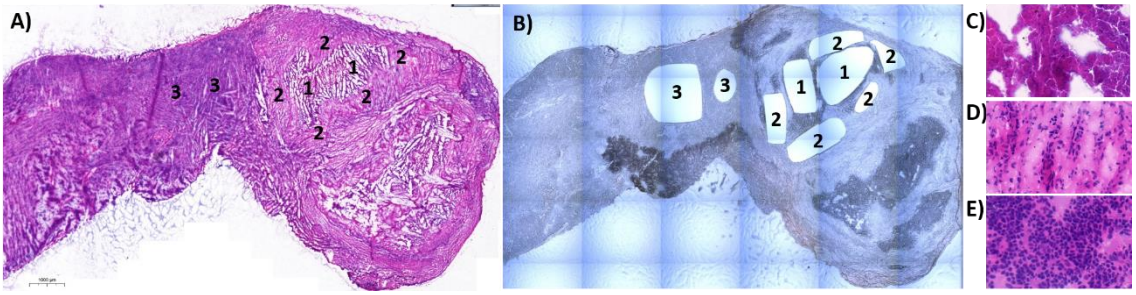
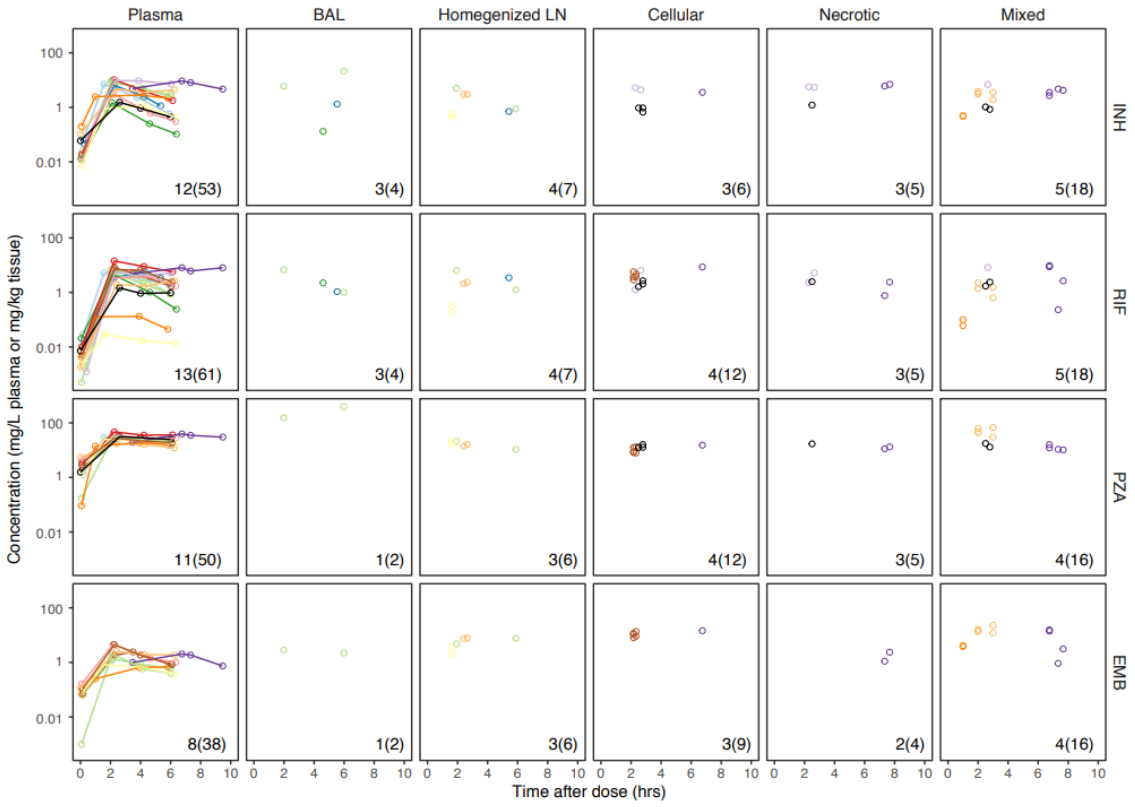
Tables and Figures

Figure 1. LCM in a representative lymph node specimen.



Haematoxylin and eosin stained lymph node (frozen section) containing two lesions (A) and its corresponding serial section taken for laser capture microdissection (B). Regions 1-3 represent the areas dissected for drug quantitation by LC-MS/MS. Example histology of the different areas dissected are shown and correspond to necrotic areas of the lesion (A/B1 and C), the cellular layer of the lesion (A/B2 and D), and a lymphocyte rich region (A/B3 and E).

Figure 2: Raw PK data for each drug in each lesion type.

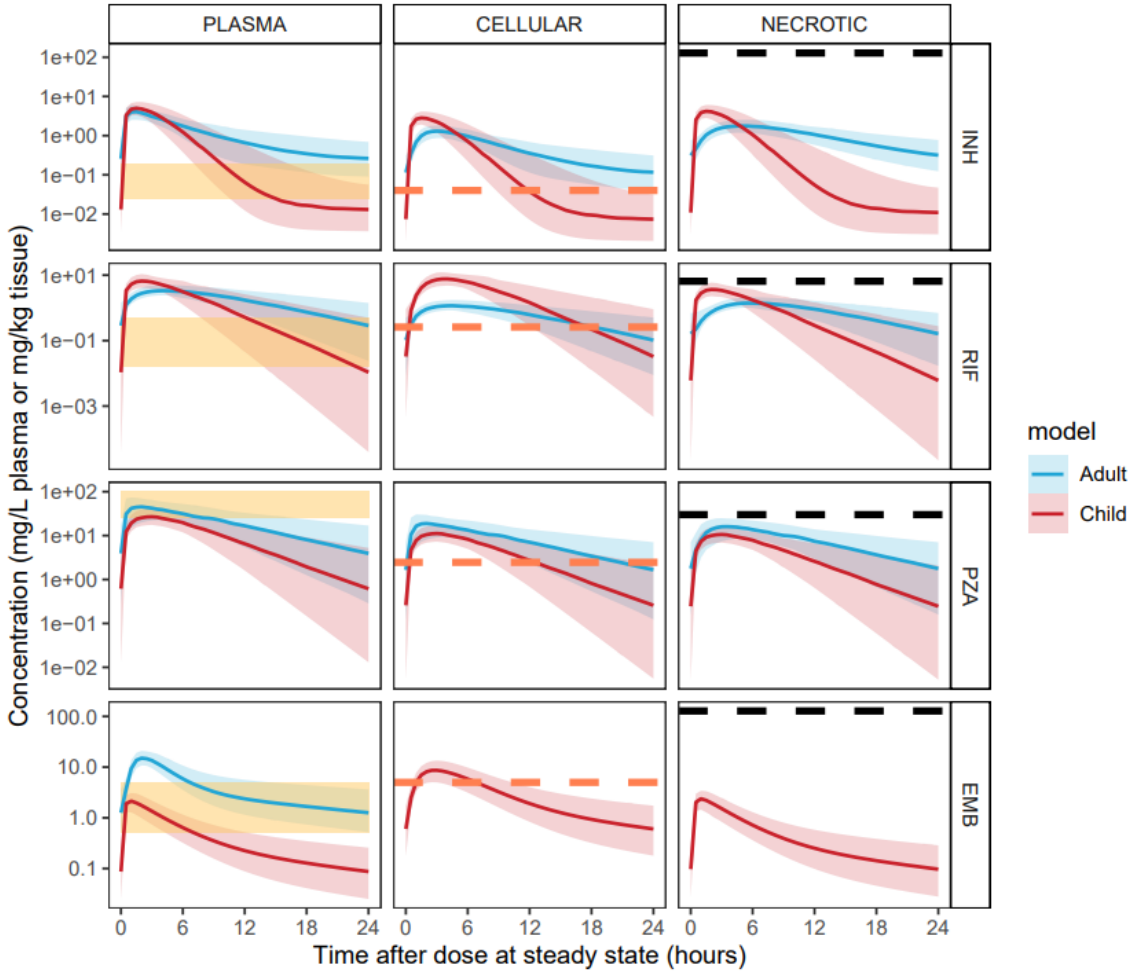


Log-scale Concentration–time profiles are shown for five lesion types and four drugs by respective panel. Plasma concentrations over time for each individual were measured at multiple time points after the time of drug administration and before bronchoscopy or surgical decompression and are shown as individual lines of different colours. Lesion concentrations were measured at a single time point (time of resection) per subject and are represented by circles of different colours that correspond to their individual subject plasma line.

The number of patients (and observations) for each lesion and drug are shown in bottom, right corner of each image.

Abbreviations: BAL, bronchoalveolar lavage; LN, lymph node; INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutol.

Figure 3: Simulated concentration-time profiles of children and adults relative to exposure target.



Simulations for 1000 patients with the same representative characteristics were performed and their steady state concentration-time profiles taken over 24 hr. Red represents an 8.2 kg, 8.6 month child with median and 95% CI. Blue represents simulated 60 kg adult profiles with available parameters from plasma (RIF, INH, PZA and EMB from Smythe et al., Wilkins et al 2011, Wilkins et al 2006, Jönsson et al, respectively) and lesion parameters (from Strydom et al). Dosing for child was H = 120 mg, R = 120 mg, Z = 250 mg, E = 200 mg and adult, H = 300, R = 600, Z = 1600, E = 1100. Yellow bands represent the distribution of PD exposure target selection: wild type MIC for homogenised lymph node; intracellular macrophage IC_{50} for cellular

lesions (orange dashed line) and caseum MBC for the necrotic tissue (black dashed line).

Abbreviations: INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutolM PD, pharmacodynamics; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Table 1. Patient characteristics at time of procedure by study group

	Bronchoscopy (N=8)	Surgical Decompression (N=7)
Male Sex (%)	3/5 (37.5)	4/3 (57.1)
Median age in months (IQR)	17.6 (6.3-41.0)	6.9 (3.4-17.2)
Median weight in kg (IQR)	9.9 (8.2-12.4)	7.1 (4.1-8.3)
Median weight-for-age Z-score (IQR)*	0.1 (-1.3, 0.8)	-1.0 (-4.2, -0.1)
HIV-positive, † N (%)	1 (12.5)	0 (0.0)
Child has current TB source case, N (%)	5 (62.5)	5 (71.4)
TB disease type, N (%)		
PTB only	6 (75.0)	6 (85.7)
PTB and EPTB ‡	2 (25.0)	1 (14.3)
Previous TB episode, N (%)	1 (12.5)	0 (0.0)
Median days on treatment (IQR)	64 (60-73)	34 (28-74)
Regimen, N (%)		
HR§,	2 (25.0)	0 (0.0)
HRZ ll	2 (25.0)	1 (14.3)
HRZE	3 (37.5)	5 (71.4)
RZEL**	1 (12.5)	1 (14.3)
Median dose in mg/kg (IQR)		
Rifampin	12.8 (12.1-16.0)	12.3 (11.1-15.0)
Isoniazid	12.8 (11.4-14.8)	12.2 (11.1-12.7)

Pyrazinamide	28.5 (23.8-30.9)	30.5 (25.3-34.2)
Ethambutol	20.2 (18.6-22.8)	20.8 (20.2-24.1)
Receiving oral steroids, N (%)	6 (75.0)	7 (100.0)
Chest X-ray characteristics, N (%)		
Consolidation	7 (87.5)	4 (57.1)
Collapse	3 (37.5)	1 (14.3)
Cavity	1 (12.5)	0 (0.0)
Paratracheal nodes	3 (37.5)	4 (57.1)
Hilar nodes	6 (75.0)	5 (71.4)
Airway Compression	6 (75.0)	6 (85.7)
Pleural effusion	1 (12.5)	0

Abbreviations: IQR, Inter quartile range; EPTB, extrapulmonary TB; PTB, pulmonary TB; TB, tuberculosis; H, isoniazid; R, rifampicin; Z, pyrazinamide; E, ethambutol; L, levofloxacin; HIV, human immunodeficiency virus.

Footnote: *Anthropometric Z scores were calculated based on WHO growth standards. †HIV infected child on abacavir, lamivudine and Lopinavir/ritonavir; ‡ EPTB included: group 1- disseminated (N=1) and miliary (N=1); group2 -abdominal (N=1); §One case without prior bacteriological confirmation was diagnosed with multi-drug resistant TB detected on the day of the procedure; ll One child received HRZ plus ethionamide for disseminated disease; ** One case of isoniazid mono-resistance diagnosed at the time of TB treatment initiation.

Table 2. Final plasma PK parameters

	Isoniazid	Rifampin	Pyrazinamide	Ethambutol
Tlag (h)	0.4 (1.00)*	0.961 (14.3)	NA	0.493 (13.8)
ka (1/h)	0.654 (15.1)	0.592 (13.3)	0.586 (14.9)	0.324 (7.00)
CL/F (L/h)	7.36 (29.8)	4.64 (8.90)	0.977 (9.90)	15.8 (18.0)
Vc/F (L)	8.72 (36.8)	8.27 (11.2)	5.23 (13.3)	8.59 (5.60)
Q (L/h)	0.0751 (76.2)	NA	NA	7.65 (2.30)
Vp/F (L)	12.1 (59.5)	NA	NA	87.2 (2.30)
TM50 (weeks)	49.0 (FIXED	58.2 (FIXED)		
Hill	2.19 (FIXED)	2.21 (FIXED)		
IIV CL/F	0.817 (41.5)	0.187 (47.9)	0.0538 (59.7)	0.24 (36.8)
IIV Vc/F		0.48 (48.3)	0.0629 (68.2)	

Abbreviations: Tlag, lag in absorption time; Mtt, mean transit time; NA, Not applicable; NN, number of transit compartments; ka, rate of absorption; CL, clearance; Vc, central volume; Q, inter-compartmental clearance; Vp, peripheral volume; F, bioavailability. TM50, post-menstrual age at 50% of adult clearance; Hill, steepness of the maturation function; IIV, inter-individual variability. Footnote: Parameters scaled to 8·6 month, 8·2 kg individual**. Individual clearance and volume values were adjusted according to allometric scaling on weight, CLi = CLstd•(WT/8.2)0.75, V1i = V1std•(WT/8.2)1, Qi = Qstd•(WT/8.2)0.75, V2i = V2std•(WT/8.2)1,**

*Values in parenthesis = percentage relative standard error (RSE).

Table 3. Final site of disease PK parameters according to site of disease

	INH			RIF			PZA			EMB	
Lesion	**Rate**	**Ratio**		**Rate**	**Ratio**		**Rate**	**Ratio**		**Rate**	**Ratio**
		Present study	Adult reference		Present study	Adult reference		Present study	Adult reference		
BAL	20*	2.86 (1.53 – 2.91)		20*	1.13 (0.998 – 1.262)		0.218 (0.124 – 0.314)	20.4 (16 – 25)		20*	1.34 (0.248 – 2.92)
Homogenised lymph node	20*	0.513 (0.28 – 0.75)		20*	1.17 (1.044 – 1.296)		20 *	0.753 (0.63 – 0.88)		20*	3.16 (0.16 – 6.74)
Cellular	20*	0.556 (0.32 – 0.79)	[0.228] (0.223 – 0.233)	0.639 (0.568- 1.008)	1.37 (0.874 – 1.866)	[0.348] (0.122 – 0.574)	20*	0.416 (0.36 – 0.48)	[0.698] (0.597 – 0.799)	0.574 (0.384 – 1.368)	6.17 (0.914 – 14.4)
Necrotic	20*	0.843 (0.80 – 0.88)	[0.824] (0.776 – 1.01)	20*	0.552 (0.477 – 0.641)	[0.443] (0.251 – 0.635)	20*	0.395 (0.30 – 0.50)	[0.394] (0.266 – 0.5219)	20*	1.11 (0.430 – 1.95)
Mixed	2.58 (0.684 – 4.416)	0.486 (0.46 –0.80)		20*	0.873 (0.725 – 1.065)		20*	1.40 (0.91 – 1.9)		20*	5.44 (2.15 – 10.5)

Abbreviations: BAL: bronchoalveolar lavage; NA, Not applicable

The rate (kpl in hr^{-1}, inter-compartmental rate constants for the transfer of drug from the plasma to the lesion) and ratio (Rpl, the penetration coefficients (ratios) between lesion and plasma**.**) for each drug and lesion are shown together with the adult ratio coefficient (Strydom et al)[13] in parenthesis, when available.

The following definitions were used for adult lesion: Cellular (defined as small cellular nodules); necrotic (caseum from closed nodule)

*Values with asterisk were fixed in model to assume an almost instantaneous penetration of the drug.

Values in parenthesis = 95% Confidence interval

Square parenthesis= adult reference.

Table 4: Area under the curve (0-24 hours.) values comparing adults and children exposure

		Area under the curve$_{0\text{-}24}$ (mg*h/L) (95% CI)		
		Plasma	**Cellular**	**Necrotic**
Isoniazid	Adult[23]	27.7 (23.8 - 32)	11.8 (10.1 - 13.6)	23.3 (20 - 26.9)
	Child	25.3 (22.4 - 28.6)	10.8 (9.53 - 12.1)	21.6 (19.1 - 24.3)
Rifampicin	Adult[24]	41.7 (35.8 - 49.4)	14.5 (12.5 - 17.2)	18.5 (15.9 - 21.9)
	Child	38.3 (33.5 - 43.7)	55.2 (48.3 - 63)	21.2 (18.5 - 24.1)
Pyrazinamide	Adult[22]	466 (395 - 539)	194 (165 - 225)	184 (157 - 214)
	Child	248 (213 - 294)	103 (88.6 - 122)	97.9 (84.1 - 116)
Ethambutol	Adult[21]	105 (91.6 - 124)	NA	NA
	Child	12.3 (10.6 - 14.6)	76.8 (66.6 - 91.1)	13.7 (11.9 - 16.3)

Abbreviations: CI, confidence intervals; NA, Not applicable;

Footnote: Median of simulation of 1000 individuals with individual variability is shown with 95% CI as shaded area. Adults received South Africa standard of care doses assuming 60 kg patient (doses were:rifampicin 600 mg; isoniazid 300 mg; pyrazinamide 1600 mg and ethambutol 1100mg). Children were 8.2 kg and received: rifampicin 120 mg, isoniazid: 120 mg, pyrazinamide:250 mg, ethambutol:200 mg

Supplementary appendix

Drug concentration at the site of disease in children with pulmonary tuberculosis

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1. Supplementary Online Methods

Study setting and population

This study was conducted at Tygerberg Hospital, a large academic referral hospital in Cape Town, Western Cape Province, South Africa, with an estimated provincial tuberculosis (TB) incidence of 681 per 100,000 population in 2015.[1] The paediatric pulmonology department at Tygerberg Hospital serves as referral unit for complicated TB cases and manages approximately 400 cases per year with approximately 20% of confirmed TB cases routinely undergoing diagnostic or therapeutic bronchoscopy.[2]

The standard clinical management of children with intrathoracic TB in South Africa, based on current World Health Organization (WHO) and national guidelines, is weight-banded once-daily doses of isoniazid (10-15 mg/kg), rifampin (10-20 mg/kg) and pyrazinamide(30-40 mg/kg). Ethambutol (15-25 mg/kg) is added in children with severe disease or those HIV-coinfected (Table E1).[3,4] Children with severe intrathoracic TB complicated by significant airway obstruction receive prednisone (2 mg/kg daily) and frequently undergo therapeutic interventions to establish airway patency either through bronchoscopy, or surgical relief via surgical transthoracic lymph node decompression (SD), according to local protocols at Tygerberg Hospital.[5–7]

Flexible bronchoscopy is performed under general anaesthesia. Following evaluation of the airways, bronchoalveolar lavage (BAL) is completed with a weight-based volume of 1 mL/kg per aliquot of 0.9% saline. If lymph nodes have ulcerated into the airway, the

BAL is completed in that specific airway. In cases where no ulceration has occurred, the bronchoscope is wedged into the most involved lobe or segment as determined radiologically, and the lavage performed. Two 1-2mL BAL samples are routinely obtained, one for mycobacterial smear microscopy and automated liquid culture, and one for Xpert MTB/RIF-Ultra (Xpert; Cepheid, Sunnyvale, CA, USA). Visible intraluminal tissue is biopsied (endobronchial biopsy) and specimens are analysed microbiologically.[6] Children with life-threatening airway obstruction undergo transthoracic SD, performed according to a standard protocol which involves thoracotomy and lung compression to expose the relevant lymph nodes.[5] Suction or forceps are used to remove the nodal contents, often extracted in a piecemeal manner, which is then analysed histologically and microbiologically.

Study design and clinical procedures

In this study, we excluded children below 3 kg and those with haemoglobin <8g/dL due to safety considerations. Written informed consent was obtained from parents/legal guardians after obtaining approval of the relevant Health Research Ethics Committee.

On the day of the bronchoscopy or surgery, the study team administered antituberculosis treatment, antiretrovirals (ARVs) and prednisone, as relevant, after a standard overnight fast. Antituberculosis drugs consisted of a weight-band fixed-dose combination tablet of rifampin and isoniazid (60:60), 500 mg pyrazinamide and 400 mg ethambutol (Sandoz, Sanofi Aventis and Pharmacodynamics, respectively), administered as crushed tablets, diluted in a maximum 5 mL of water. The exact timing of antituberculosis and ARV drug administration on pharmacokinetic (PK) sampling day and the preceding 2 days was

documented. Participants receiving other concomitant medications were given these on the evening after the procedure, or on the following day.

The study was designed so that different participants contributed samples at different time points to attempt to reconstruct the entire PK profile for BAL and tissue samples. Thus, dosing times were planned at approximately 2 hours before surgical decompression or randomized, at 2, 4, or 6 hours prior to bronchoscopy. The exact time of the blood sample targeted to be coupled to the site-of-disease sample was recorded in relation to the time of bronchoscopy/SD.

Blood samples were collected in ethylenediaminetetraacetic acid-coated tubes, centrifuged, and the plasma frozen at -80℃ within 30 minutes of sampling. An additional blood sample was collected within 4 hours of bronchoscopy to determine the urea concentration. BAL was collected in polypropylene tubes, immediately placed on ice, and frozen at -80℃, within 1 hour of collection.

Lymph node samples were collected, and depending on size and quality were divided for PK analysis, routine histology, and TB microbiology. Small lymph node tissue obtained through endobronchial biopsy at the time of bronchoscopy and liquefied lymph node obtained at the time of SD were collected in pre-weighed homogenizing tubes and frozen at -80℃ within 1 hour of collection until PK analysis was performed. Larger, solid lymph node fragments (>5 mm) from SD were divided into samples for routine histology, TB microbiology and PK analysis. Tissue for routine histology was submitted in a tube containing 10% neutral buffered formalin. Tissue submitted for TB microbiology was collected in a non-additive tube. Tissue submitted for PK analysis was placed in a

cryomold, wrapped with aluminium foil and immediately frozen in liquid nitrogen vapour for at least 5 minutes. All samples were stored within an hour of collection at -80°C until analysis.

Analytical methods

Plasma pharmacokinetics

The concentrations of rifampin, isoniazid, pyrazinamide and ethambutol were quantified in plasma using a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.[8,9] Quality control analysis showed good reproducibility with a coefficient of variation of ≤15% and accuracy, and inter-sample differences of <15%. The lower limit of quantification (LLOQ) in plasma for both isoniazid and ethambutol were 2 ng/mL, 1 ng/mL for rifampicin, and 50 ng/mL for pyrazinamide.

Bronchoalveolar lavage PK

BAL samples were analysed by LC-MS/MS using a validated method.[8,9] The ratio of urea concentration in BAL and serum was determined to calculate the volume of epithelial lining fluid recovered.[10] The LLOQ in BAL for both isoniazid and ethambutol were 2ng/mL, 1ng/mL for rifampicin, and 50 ng/mL for pyrazinamide.

Lymph node PK

All lymph node samples obtained through endobronchial biopsy (bronchoscopy group) and necrotic liquid lymph node contents from SD group were collected in homogenizing tubes, and analyzed using a validated LC-MS/MS method previously described and

labelled as "homogenized LN".[8,9] Larger (>5 mm) and solid tissue specimens obtained from SD were further classified by histology on frozen section and labelled into, cellular, necrotic, and mixed (cellular with necrosis in the background). We measured drug concentrations in tissues using LC-MS/MS with tissue imaging by laser capture microdissection (LCM) as previously described.[8] The LLOQ in tissues was 200 ng/g for rifampin, 50 ng/g for isoniazid, 500 ng/g for pyrazinamide, and 100 ng/g for ethambutol.

Mycobacteriological examination

BAL and tissue samples were sent to on-site nationally accredited routine TB laboratory (National Health Laboratory Services, NHLS) for acid-fast bacilli smear, liquid culture (Mycobacteria Growth Indicator Tube (MGIT), Becton-Dickinson, USA), and drug susceptibility testing using line probe assays (GenoType MTBDR*plus* and MTBDR*sl*; Hain Lifescience, Nehren, Germany) as per standard of care. . We estimated minimum inhibitory concentrations (MIC) to first- and second-line antituberculosis drugs for mycobacterial isolates from lymph node samples by using Sensititre *Mycobacterium tuberculosis* MYCOTB Plate (Thermo Fisher, Waltham, MA, USA).[11]

Clinical data

We collected data on demographic and clinical characteristics, the duration of antituberculosis therapy prior to initiation of the observed study doses, concomitant medications, age, weight and height on the day of the procedure, human immunodeficiency virus (HIV) infection and exposure status, sex, ethnicity, TB disease spectrum and severity, TB microbiology and other routine laboratory data. TB disease severity was classified using modified Wiseman criteria.[12] Chest radiographic data and

chest computed tomography data were collected as standard of care. Chest radiographs (AP and lateral) were reported by a pediatric pulmonologist (PG).

Statistical considerations

Randomization assignment for PK sampling

Assignment to different dosing times was done by simple randomisation with an equal number of participants per group. We prepared a single list of identity numbers with the three dosing times (2, 4 and 6 hours prior to bronchoscopy) permuted randomly across them, and assigned identity numbers consecutively to enrolled participants.

Sample size calculation

Given the lack of any previous pediatric data on which to base calculations, we estimated that a sample size of approximately 15 cases would provide reasonable data for the population PK model and drug penetration ratio estimates, based on two adult studies which included sample sizes of 14, and 15, respectively.[9,13]

Pharmacokinetic modeling

PK modeling

Concentration-time data for each drug in plasma were modelled using a population PK approach to best capture the expected variability between participants. Nonlinear mixed-effects (NLME) models with first-order conditional estimation with interaction as implemented in NONMEM (version 7.4.2; ICON Development Solutions, Ellicott City, MD, United States) were used to estimate PK parameters. Simulations were performed with R software using the mlxR package from Lixoft (version 2019R1; Antony, France: Lixoft SAS, 2019).

Initial estimates used rifampin, isoniazid, and pyrazinamide parameters from existing plasma population models based on data from South African children.[14] In addition, rifampin and isoniazid had postmenstrual age as a covariate on clearance according to Zvada et al.[14] To model ethambutol in children, South African adult PK parameters from Jönsson et al, were scaled allometrically to the weights of the children.[14,15] The parameters from these models were fixed and the model fit to data evaluated and subsequently re-estimated where necessary. Structural model changes were made when model parameters could not be reliably estimated due to the sparse nature of the data. For example, transit compartments were excluded and simplified to a lag in absorption time for isoniazid, rifampin and ethambutol, Figure E2.

The process of model selection and evaluation was guided by physiological plausibility, clinical relevance, basic goodness of fit plots, visual predictive check (VPC) plots, (Figure E4) parsimony, and statistical significance using likelihood ratio test (LRT) for nested models. Assuming the LRT is χ^2 distributed, a p-value of 0.05 was defined statistically significant which equals a reduction in LRT $\geq$ 3.84 for each additional model parameter.

Exposure simulations

Concentration time profiles and derived area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) at steady state were simulated for the average age and weight of children in the cohort (8.6 months, 8.2 kg) for all tissue types using our cohort plasma and tissue estimates. To best compare these outputs to adult exposure from the same population we used published population PK models from South African adults for rifampin, isoniazid, pyrazinamide and ethambutol.[16–19] Cavity from caseum and small

cellular nodule rate and ratio estimates from clinical lesion data,[20] were added to the South African adult plasma models to simulate necrotic and cellular lesion exposures and compared with children. We further compared the simulated time concentration profiles for the four drugs in the different compartments to appropriate inhibitory and bactericidal target concentrations, adjusting the target potency values to reflect drug tolerance of local bacterial populations in each compartment. For plasma, BAL, homogenized LN and mixed tissue, we used published distributions of wild type minimum inhibitory concentration (MIC)[21–24] since these compartments potentially harbor a mix of intracellular, extracellular, replicating and non-replicating bacteria; intracellular macrophage IC_{50}[25] was used in cellular lesions where bacilli reside mostly in macrophages and caseum MBC[26] was used in necrotic tissue or caseum where non replicating persisters reside (Table E4).

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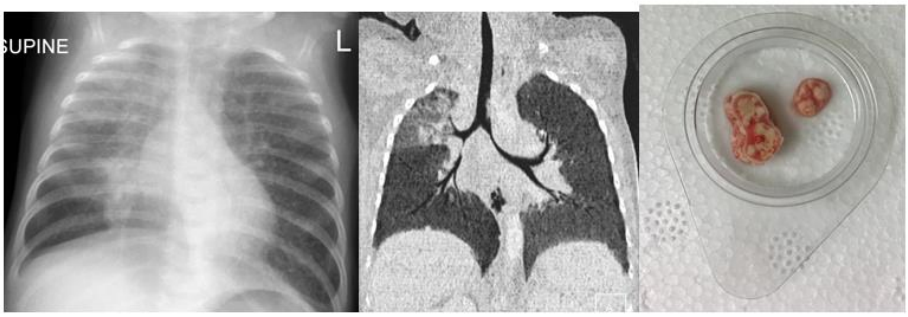
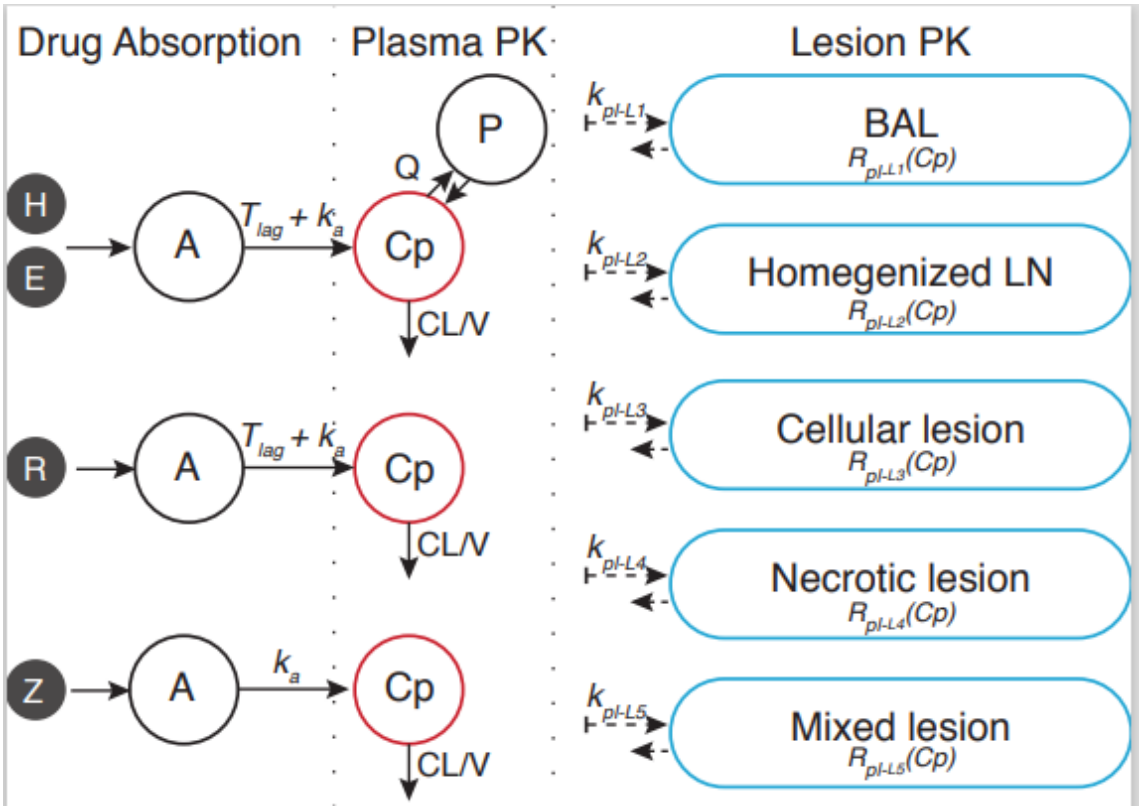
3. Supplementary Figures

Figure E1. Chest X-ray and accompanying CT scan image and lymph node sample



Chest X Ray and CT Scan show bilateral mediastinal necrotic hilar lymph node and compression of left main bronchus and bronchus intermedium (90%) and collapse consolidation of right upper and middle lobe with multiple scattered nodules (endobronchial spread). Resected hilar lymph node frozen with liquid nitrogen and placed on cryomold for pharmacokynetic analysis.

Figure E2: Schematic of structural model

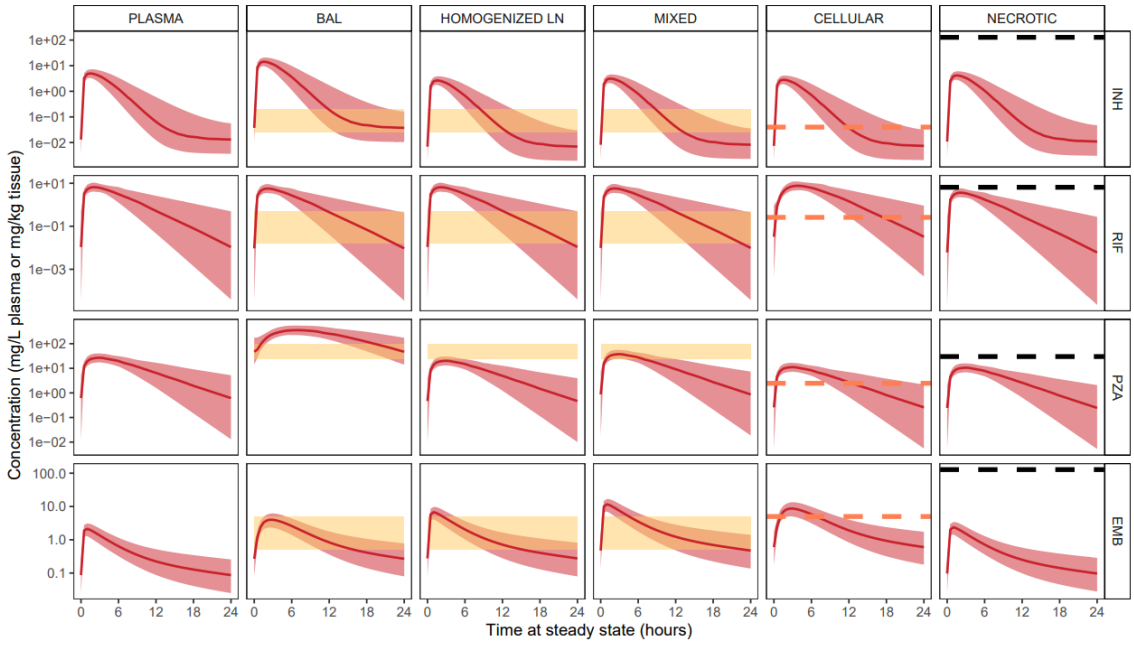


First-line drugs R, H, Z and E with parameters CL, V on the central compartment and ka from an absorption compartment (A). Z required a transit model to capture absorption, and H and E required an additional compartment (P) with Q to model plasma data. Tissue concentration–time profiles were modelled with the addition of 2 parameters to describe the rate of drug absorption into the tissue compartment and the partition coefficient of observed tissue concentration to plasma concentration.

Abbreviations: A, absorption compartment; CL, clearance; Cp, plasma concentration; E, ethambutol; H, isoniazid; ka, rate of absorption; P, peripheral compartment; PK, pharmacokinetic; Z, pyrazinamide; Q, intercompartmental clearance; R, rifampicin; Tlag, lag time before absorption; V, volume; K_{L1-5}, inter-compartmental rate constants

for the transfer of drug from the plasma to the lesions 1-5; R_{L1-5}, the penetration coefficients (ratios) between lesions 1-5 and plasma.

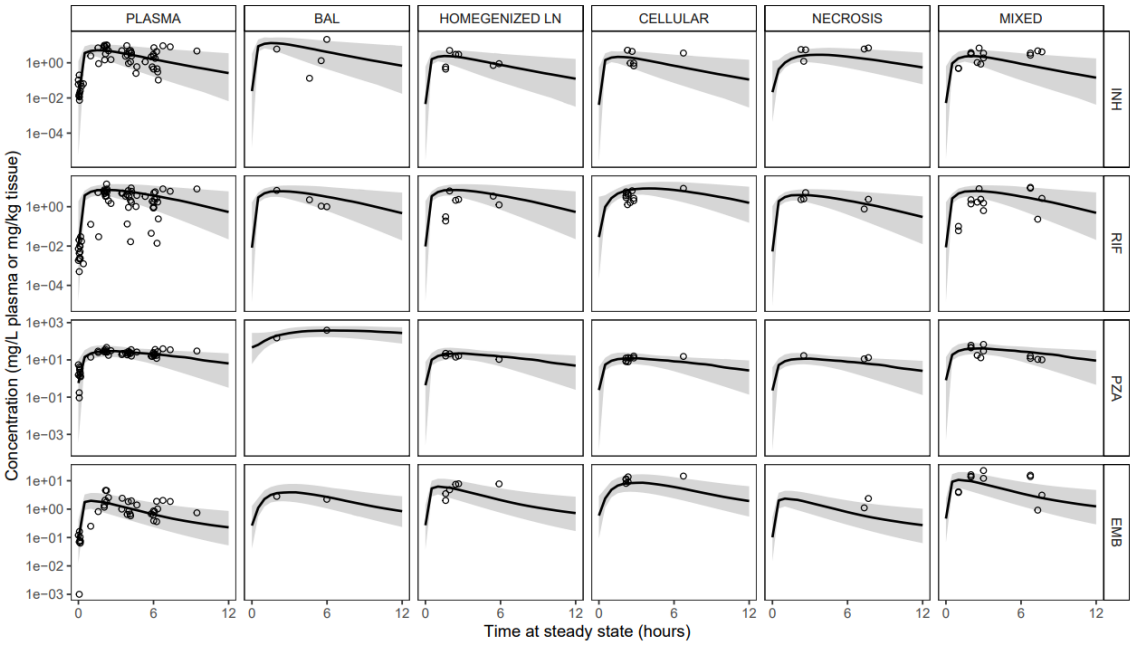
Figure E3: Simulated concentration profiles of children



Simulations for 1000 patients were performed and their steady state concentration time profiles taken over 24 hrs. Red represents an 8·2 kg, 8·6 month child with median and 95 percentile shaded area. Yellow bands represent the distribution of exposure target selection: wild type MIC[21–24] for homogenized LN; intracellular macrophage MIC[21] for cellular (orange) and caseum MBC[26] for the necrotic tissue (black).

Abbreviations: INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutol; PD, pharmacodynamics; LN, lymph node; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Figure E4. Visual predictive check of PK model



Black line is the median prediction from the model, grey ribbons represent the prediction interval for the median (95% CI) and black circles represent original data. INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutol; BAL, bronchoalveolar lavage.

4. Supplementary Tables

Table E1. Weight-band doses used for the treatment of complicated tuberculosis in children <8kg or <30 kg

Body weight	Rifampicin/ Isoniazid 60mg/60mg	Pyrazinamide 150mg tablet or 150 mg/3ml	OR Pyrazinamide (Z) 500 mg	Ethambutol 400mg or 400mg/8ml
2-2.9 kg	1/4 tablet	1.5 ml (75 mg)		1ml (50 mg)
		2.5 ml (125 mg)		
3-3.9 kg	3/4 tablet	or	1/4 tablet (125 mg)	1.5 ml (75 mg)
4-5.9 kg	1 tablet	3 ml (150 mg) or	1/4 tablet (125 mg)	2ml (100mg)
6-7.9 kg	1 + 1/2 tablets		1/2 tablet (250 mg)	3 ml (150 mg)
8-11.9 kg	2 tablets		1/2 tablet (250 mg)	1/2 tablet (200 mg)
12-14.9 kg	3 tablets		1 tablet (500 mg)	3/4 tablet (300mg)
15-19.9 kg	3 + 1/2 tablets		1 tablet (500 mg)	1 tablet (400mg)
			1+1/2 tablet (750	
20-24.9 kg	4 + 1/2 tablets		mg)	1 tablet (400mg)
				1 + 1/2 tablets
25-29.9 kg	5 tablets		2 tablet (1000 mg)	(600mg)

Excluding tuberculosis meningitis and miliary tuberculosis.

Table E2. Individual clinical features of study participants in bronchoscopy (ID 1-8) and surgical decompression (ID 9-15) groups.

ID	Sex	Age	Weight in Kg (WAZ-score)	Days on treatment and regimen	AP	Microbiological characteristics		Site of disease sample	Histology
						baseline	procedure		
1	F	20·2	8·1 (-3·0)	70	HRZ	S	Xpert -; C+	BAL: Xpert +; C -	2h: BAL λ	
2 #	F	6·6	8·21 (0·1)	64	RZEL[a]	N/A	Xpert +; C+	BAL: Xpert +; C -	4h: BAL λ	NA
3 *	F	17·4	10·5 (-0·3)	62	HRZE	S	Not available	BAL: Xpert +; C -	6h: BAL + 1 LN (hiliar)	

4 ł	M	61·8	16·4 (-0·7)	102	HR	F	C+	BAL: Xpert +; C -	6h: BAL + 1 LN (hiliar)	
5*	F	17·8	11·0 (0·1)	76	HRZE	S	Not available	BAL: Xpert +; C -	2h: BAL + 1 LN (hiliar)	
6	M	1·4	7·1 (3·4)	64	HR[b]	S	Not available	GA: Xpert +; C+ BAL: Xpert trace; C -	4h: BAL	
7	F	6·1	9·4 (1·5)	17	HRZE	I	Xpert +; C+	BAL: Xpert +; C -	2h: BAL λ	
8 γ	M	64·5	13·9 (-1·9)	58	HRZ+ Eto	I	C+	BAL: Xpert +; C -	4H BAL λ	
9	M	3·4	5·4 (-1·0)	48	HRZE	S	Xpert +	LN: C +[c]	4 LN (hiliar, subcarinal) 2 ◊	NGI, Langhan's cells, ZN+

10	F	3·5	4·0 (-4·2)	32	HRZE	S	C+	LN: Xpert +; C -	1 LN (hiliar)	NGI, ZN+++
11	M	37·9	7·11 (-5·2)	81	HRZE	S	C+	LN: C +	2 LN (hiliar, subcarinal)	NGI, Langhan's cells, ZN-
12	M	17·2	9·9 (-0·1)	11	HRZE	S	Xpert +; C+	LN: C +	3 LN (paratracheal, hiliar, subcarinal)	NGI, foreign body cells, ZN-
13	M	8·6	7·3 (-0·9)	34	HRZE	S	Xpert +; C+	LN: C -	2 LN (hiliar, subcarinal) 2◊	NGI, foreign body cells, calcification, ZN+++
14 #	F	6·9	8·3 (0·0)	74	RZEL[a]	N/A	Xpert +; C+	LN: C -	2 LN (paratracheal subcarinal)	GI without necrosis, reactive lesion
15	F	3·1	4·1 (-3·7)	28	HRZ	S	C+	LN: C +[d]	2 LN (paratracheal, subcarinal)	NGI, ZN+,

Abbreviations: AP, acetylator phenotype; C+, culture positive for *M. tuberculosis*; E, EMB, Ethambutol; Eto, Ethionamide; F, fast acetylator phenotype; GI, granulomatous inflammation; H, INH, isoniazid; HIV, human immunodeficiency virus; I, intermediate acetylator phenotype; L, levofloxacin; LN, lymph node; MDR-TB, multi-drug resistant tuberculosis; NA Not applicable; NGI, necrotizing granulomatous inflammation; R, RIF, rifampicin; S, slow acetylator phenotype; TB, tuberculosis; Xpert +, Xpert MTB/RIF Ultra positive for *M. tuberculosis;* Z, pyrazinamide; ZN, Ziehl Nielsen.

Footnote: * and # represent same individual; ǂ HIV co-infected on antiretroviral therapy (abacavir, lamivudine and lopinavir/ritonavir).

γ recurrent TB; Age is shown in months; Days on TB treatment represent the total number of days since the initial regimen was started and regimen shows the specific drugs present at the time of the procedure. Acetylator phenotype was inferred from estimated individual clearance values.

All cases were bacteriologically confirmed for drug susceptible *M.tuberculosis* except for: [a] one child with INH mono resistant TB and [b] a child diagnosed with MDR-TB at the time of the procedure who did not have prior bacteriological confirmation. MIC values were obtained for 2 isolates [c,d] and were: INH: < 0·03 mg/L[c] and <0·06 mg/L [d]; RIF <0·06 mg/L; EMB 1 mg/L.

λ BAL samples in which urea dilution factor could not be calculated.

◊ surgical decompression nodes processed as homogeneized LN, either due to liquid nature of the sample or logistic constrains.

Total number of homogeneized LN: 7 (3 bronchoscopy biopsies from ID 3, 4 and 5 plus 4 LN from ID 9 and 13).

Total number of LN for LCM analysis 9 LN from 4 distinct patients (11, 12, 14, 15)

Table E3: Reference table of plasma pharmacokinetic parameters estimates

	INH		RIF		PZA		EMB	
	New	Zvada 2014	New	Zvada 2014	New	Zvada 2014	New	Jönsson 2011*
Tlag (hr)	0·4 (1·00)	NA	0·961 (14·3)	NA	NA	-	0·493 (13·8)	-
Mtt	NA	0·179 (10·9)	NA	1·04 (6·10)	NA	0·10 (17·7)	-	0·78 (7)
NN	NA	4 (FIX)	NA	8·04 (11·9)	NA	3·94 (8·00)	-	1 (FIX)
ka (1/hr)	0·654 (15·1)	2·47 (12·6)	0·592 (13·3)	NA	0·586 (14·9)	4·48 (6·10)	0·324 (7·00)	0·474 (24)
CL/F (L/h)	7·36 (29·8)	6·52 (13·9)**	4·64 (8·90)	5·94 (9·00)	0·977 (9·90)	0·787 (5·60)	15·8 (18·0)	10·5 (3·1)
Vc/F (L)	8·72 (36·8)	7·22 (10·2)	8·27 (11·2)	10·6 (10·2)	5·23 (13·3)	6·32 (2·60)	8·59 (5·60)	13·4 (42)
Q (L/h)	0·0751 (76.2)	1·46 (26·3)	NA	NA	NA	NA	7·65 (2·30)	8·75 (10)
Vp/F (L)	12·1 (59·5)	3·30 (33·9)	NA	NA	NA	NA	87·2 (2·30)	102 (22)

Abbreviations: INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutol**;** Tlag, lag in absorption time; Mtt, mean transit time; NA Not applicable; NN, number of transit compartments; ka, rate of absorption; CL, clearance; Cl_{int}, intermediate acetylator; Vc, central volume; Q, intercompartmental clearance; Vp, peripheral volume; F, bioavailability.

Footnote: Parameters shown represent a child with a median weight of 8.2 kg. RSE (%) values in parenthesis

* Adult CL, Vc, Q and Vp parameters allometrically scaled to median cohort weight

** Cl intermediate acetylator

Table E4: Area under the curve values for matrixes in children

	Area under the curve (mg/L.h)				
	Plasma	BAL	Homogenized LN	Cellular	Necrotic
INH	23·4 (14·5 - 37·8)	67·1 (41·7 - 108)	12·5 (7·75 - 20·1)	13·3 (8·26 - 21·4)	19·6 (12·2 - 31·6)
RIF	38·3 (33·5 - 43·7)	32·9 (28·8 - 37·5)	38·1 (33.3 - 43·4)	55·2 (48·3 - 63)	21·2 (18·5 - 24·1)
PZA	248 (213 - 294)	5057 (4343 - 5995)	187 (160 - 221)	103 (88·6 - 122)	97·9 (84·1 - 116)
EMB	12·3 (10·6 - 14·6)	34·6 (30 - 41·1)	39·1 (33·8 - 46·4)	76·8 (66·6 - 91·1)	13·7 (11·9 - 16·3)

Abbreviations: INH, isoniazid; RIF, rifampicin; PZA, pyrazinamide; EMB, ethambutol; BAL, bronchoalveolar lavage; LN, lymph node.

Footnote: Median area under the curve is shown with 95% confidence interval (CI) in parenthesis, simulated in population of n=1000 with interindividual clearance for isoniazid, rifampicin, pyrazinamide, ethambutol.

Table E5: Exposure target selection (mg/L)

	MIC [21–24]	Macrophage IC_{50} [25]	Caseum MBC[26]
Isoniazid	0·025 - 0·2	0·04	>128
Rifampicin	0·06 - 0.5	0·26	6·5
Pyrazinamide	25 - 100	>2·46	30
Ethambutol	0·5 - 5	>4·09	>128

Abbreviations: MIC, minimum inhibitory concentration (wild type); caseum MBC, caseum minimum bactericidal concentration.

Footnote: Values and distribution of pharmacodynamic exposure target selection selected.

CHAPTER 5

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis

Re-submitted for peer review and publication in Frontiers in Pharmacology

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis

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Figures: 4

Supplementary materials: 2 figures

Abstract

Background

Meropenem is being investigated for repurposing as an anti-tuberculosis drug. This study aimed to develop a meropenem population pharmacokinetics model in patients with pulmonary tuberculosis and identify covariates explaining inter-individual variability.

Methods

Patients were randomized to one of four treatment groups: meropenem 2g thrice daily plus oral rifampicin 20 mg/kg once daily, meropenem 2g thrice daily, meropenem 1g thrice daily, and meropenem 3g once daily. Meropenem was administered by intravenous infusion over 0.5-1h. All patients also received oral amoxicillin/clavulanate together with each meropenem dose, and treatments continued daily for 14 days. Intensive plasma pharmacokinetics sampling over 8h was conducted on 14th day of the study. Nonlinear mixed-effects modelling was used for data analysis. The best model was chosen based on likelihood metrics, goodness-of-fit plots and parsimony. Covariates were tested stepwise.

Results

A total of 404 concentration measurements from 49 patients were included in the analysis. A 2-compartment model parameterized with clearance (CL), inter-compartmental clearance (Q), central (V1) and peripheral (V2) volumes of distribution fitted the data well. Typical values of CL, Q, V1 and V2 were 11.8 L/h, 3.26 L/h, 14.2 L, and 3.12 L, respectively. The relative standard errors of the parameter estimates ranged from 3.8 to 35.4%. The covariate relations included in the final model were creatinine clearance on CL, and allometric scaling with body weight on all disposition parameters. An effect of age on CL as previously reported could not be identified.

Conclusion

A 2-compartment model described meropenem population pharmacokinetics in patients with pulmonary tuberculosis well. Covariates found to improve model fit were creatinine clearance and body weight but not rifampicin treatment. The final model will be used for an integrated pharmacokinetics/pharmacodynamics analysis linking meropenem exposure to early bactericidal activity.

Introduction

The epidemic rise in multi-drug resistant (MDR) and extensively-drug resistant tuberculosis (XDR-TB) threatens the progress made in reducing morbidity, mortality and efforts in tuberculosis eradication[1]. Medicines included in the current World Health Organization's guideline for MDR- and XDR-TB treatment may be inaccessible in resource-limited settings and many older second-line anti-tuberculosis agents have significant toxicity, some of which could be life threatening and/or irreversible[1]. Drug repurposing or the optimized use of existing drugs or combination of drugs is a cheaper alternative to development of new chemical entities and could accelerate the process of finding good alternative treatments.

Mycobacterium tuberculosis is historically considered resistant to β-lactam antibiotics including carbapenems because of the constitutive production of a broad-spectrum β-lactamase called BlaC[2,3]. The addition of a β-lactamase inhibitor such as clavulanate prevents BlaC-mediated breakdown of β-lactams[3]. Furthermore, meropenem is both a poor substrate and inhibitor of BlaC, thus, administering meropenem together with clavulanate is an attractive combination[4]. Recent evidence from *in vitro* and *in vivo* experiments show that carbapenems including meropenem in combination with amoxicillin/clavulanate have synergistic antimycobacterial activity[2,4]. Similarly, the combination of meropenem with rifampicin shows synergistic activity against not only rifampicin-sensitive *Mycobacterium tuberculosis*, but also against rifampicin-resistant strains *in vitro*[5]. Considering the important role of rifampicin in shortening treatment duration of drug-sensitive pulmonary tuberculosis to 9 months and then to 6 months when combined with pyrazinamide[6,7], any strategy that increases or even restores rifampicin susceptibility could improve treatment options in patients with drug-resistant tuberculosis.

Case reports and observational studies show that regimens containing meropenem, amoxicillin and clavulanate has been safely used in the successful treatment of patients with MDR-/XDR-TB[8–10]. In addition, an influential meta-analysis of individual patient data provided evidence of better treatment outcome in MDR-TB patients receiving regimens containing carbapenems[11]. A limitation of the meta-analysis is the observational nature of the included studies, necessitating the need for robust clinical trials to validate the findings[11]. Diacon and colleagues recently investigated the early bactericidal activity (EBA) of meropenem administered intravenously (IV) at 2g thrice daily together with oral amoxicillin/clavulanate 500mg/125mg as part of NCT02349841[12]. The meropenem arm resulted in mean decline of 14-day EBA, $\log_{10}$ colony-forming units (CFU) per mL of sputum of 0.11 (95% confidence interval [CI], 0.09 to 0.13) versus 0.17 (95% CI, 0.15 to 0.19) obtained following administration of first-line combination of rifampicin, isoniazid, pyrazinamide and ethambutol in the same study[12]. Faropenem, an orally administered carbapenem, failed to demonstrate measurable EBA in the same study (NCT02349841), likely owing to drug concentrations below required levels (unpublished report). Novel oral carbapenems are in development for tuberculosis. It is therefore crucial that pharmacokinetics/pharmacodynamics determinants of efficacy for carbapenems be evaluated. A population pharmacokinetics model of meropenem in patients with pulmonary tuberculosis is the first step in performing an integrated pharmacokinetics/pharmacodynamics analysis linking carbapenem exposure to EBA. This work aimed to develop such a model and identify covariates improving predictive performance within the COMRADE trial (NCT03174184).

Methods

Pharma-Ethics (Ethics reference number: 170516584) and Stellenbosch University Health Research Ethics Committee (Ethics reference number: S19/01/007) approved the clinical study and this analysis, respectively.

Study population and design

COMRADE is a phase 2, open-label randomized clinical trial enrolling South African male and female patients aged 18-65 years with sputum smear-positive pulmonary tuberculosis. The eligibility criteria are detailed in the supplementary materials. Participants with *Mycobacterium tuberculosis* strains without rifampicin-resistance conferring *rpoB* mutations were randomized into one of four study arms receiving daily treatments for 14 days: MACR2X3 received meropenem 2g IV over 0.5h thrice daily and oral rifampicin 20 mg/kg once daily; MAC2X3 received meropenem 2g IV over 0.5h thrice daily; MAC1X3 received meropenem 1g IV over 0.5h thrice daily; and MAC3X1 received meropenem 3g IV over 1h once daily. All participants were administered oral amoxicillin/clavulanate together with each meropenem dose at doses of 500mg/125mg in the thrice daily dose arms and at 875mg/125mg in the once daily dose arm. Intensive pharmacokinetics samples were collected at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8h post-dose at day 14 of treatment. At the end of the study, participants received Directly-Observed Treatment, Short course (DOTS) to treat pulmonary tuberculosis as recommended in the South African National Tuberculosis Treatment Guidelines.

Participants' data recorded included age, sex, race, weight, height, body mass index (BMI), fat-free mass (FFM), serum creatinine, creatinine clearance calculated based on Cockcroft-Gault equation (CLCR), and human immunodeficiency virus (HIV) status.

Bioanalytical method

Plasma meropenem concentrations were measured using a validated Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) at FARMOVS (Pty) Ltd, South Africa (see supplementary materials). The quality control analysis showed acceptable reliability and reproducibility with precision and accuracy ≤15%. The lower limit of quantification (LLOQ) for meropenem was 0.5 mg/L.

Population pharmacokinetics modeling

We used nonlinear mixed-effect modeling and the first-order conditional estimation with interaction (FOCE-I) method in the software NONMEM, version 7.4 for all analyses to describe the population pharmacokinetics of meropenem[13]. The execution of NONMEM control stream was implemented through Perl-speaks-NONMEM (PsN, version 4.9.0)[14,15].

Data formatting

Data assembly, formatting, and visualizations were conducted with R (an open source statistical software, version 3.5.1)[16] and Phoenix® WinNonlin™ (version 8.1)[17].

Structural and stochastic models

One- and 2-compartment models were evaluated for the best model fit to the data. Two levels of variability were evaluated: inter-individual variability (IIV) and residual unexplained variability (representing reporting errors, assay errors, model misspecification). The IIV in pharmacokinetic parameters was assumed to be log-normally distributed. Additive, proportional, and combined error models were explored to characterize the residual unexplained variability.

Covariate model

Age, body weight and CLCR are covariates previously shown to impact meropenem disposition, whereas, rifampicin is a potent inducer of drug metabolizing enzymes and transporters[6,21–25]. For this reason, these covariates were tested first, and those found to impact meropenem disposition were included in the model used as base for further covariate exploration using stepwise covariate model (SCM) building.

Prior to the SCM procedure, the base model with both structural and stochastic components was assessed using stepwise generalized additive modeling (GAM) implemented in Xpose to identify potential candidate Empirical Bayes estimates (EBEs) and covariate relationships[20].

Finally, SCM was implemented through PsN[14,15]. The potential parameter-covariate relationships were tested one at a time, and the likelihood ratio test (LRT) used to discriminate between two nested models at a statistical significance level of 5% and 1% for the forward inclusion and backward elimination procedures, respectively. The investigated covariates' influence on meropenem pharmacokinetics parameters included those of age, height, HIV status, race and sex on clearance from the central compartment (CL) and those of race and sex on central volume of distribution (V1).

Model selection and evaluation

The process of model selection for nested models was based on LRT. Thus, for each additional parameter, a reduction in objective function value (OFV) of ≥3.84 corresponding to a significance level of 5% was considered statistically significant. Akaike information criterion (AIC) was used to choose between non-nested models. In addition to the goodness-of-fit statistics, the process of model selection and evaluation was guided by visual predictive checks (VPCs), prediction- and residual based goodness-of-fit plots, and also biological plausibility, clinical relevance and parsimony[18,19]. The basic goodness-of-fit plots and VPCs were visualized with the Xpose package (version 4) and Pirana[18,20]. Pirana was also used to manage run records.

Model validation through non-parametric bootstrapping was utilized to establish the reliability and stability of the final model[26]. The bootstrapping procedure entails random sampling with replacement of each patient to form a new dataset stratified on study arm to retain proportions of the same sample size as the original dataset. We fitted the final model to each of the 1000 generated bootstrap datasets. The point estimates and their corresponding 95% CI were calculated for the model parameters.

Results

Sixty participants with drug-sensitive pulmonary tuberculosis aged between 20 and 63 years, of whom 75% (45/60) were males, participated in the study. Of the 60 participants, 11 withdrew from the study prior to the intensive pharmacokinetic sampling visit, thus, 49 participants provided plasma samples for analysis. The demographics of the 49 participants are reported in Table 1. Of the 441 concentration observations available, 404 were included in the analysis. Whereas 34 plasma samples were below the quantification limit (BQL), three samples (one at pre-dose and two at 8 hours after dose) were excluded with the motivation that the concentrations were at least 10-fold higher than expected, and their conditional weighted residuals (CWRES) ≥4. Figure 1 displays the individual meropenem concentration-time profiles per study arm.

The meropenem concentration-time data were fitted best with a 2-compartment model (Figure 2). Table 2 provides the estimated typical values of the structural pharmacokinetic parameters with low uncertainty in parameter estimates ranging from 3.8 to 27.5%.

We estimated a relatively low IIV in CL and V1 with coefficient of variation (CV) of 20% and 13.1%, respectively. No significant variability could be detected in inter-compartmental clearance (Q) while for peripheral volume of distribution (V2) the variability between individuals was high (CV of 106%).

Combined additive and proportional error model was used to quantify the residual unexplained variability (Table 2).

The addition of allometric scaling with body weight on disposition parameters, normalized to 70kg with fixed theoretical exponents of 1 for volume of distribution and 0.75 for clearance, resulted in 22.2 points OFV reduction[27]. A further 14.4 points reduction in OFV occurred with the inclusion of size-standardized CLCR normalized to the median population value of 115 mL/min. Conversely, both age and rifampicin had insignificant impact on meropenem CL.

Following the SCM's three forward selection and two backward elimination procedures, none of the other parameter-covariate relationships met the criteria for inclusion in the full final model.

The final model provides good fit to the observed population distribution of concentration-time data (Figure 3), and the observed individual concentration-time profiles (Figure S1). Figures S2a and S2b display plots of residual-based diagnostics, whereas, Figure 4 display the VPCs of the final model.

Model validation through non-parametric bootstrap procedure demonstrates the final model's robustness in describing meropenem pharmacokinetics (Table 2).

Discussion

To our knowledge, we describe for the first time, the population pharmacokinetics of meropenem in patients with tuberculosis. A two-compartment model fit the data best. We estimated with good precision the typical values of CL, Q, V1 and V2. The IIV in V2 was high, but low for CL and V1. The structural, stochastic and covariate parameter estimates of the typical individual obtained from NONMEM analysis fell within 95% CI of the non-parametric bootstrapping procedure, an indication of the model robustness in predicting meropenem concentrations (Table 2). However, the uncertainty in the estimate of IIV on V2 was high (Table 2). The difficulty in estimating the IIV on V2 did not affect the model's purpose of describing meropenem population pharmacokinetics. Further, rifampicin did not affect meropenem CL in the current study. While this is not surprising, because meropenem is predominantly excreted unchanged in urine, rifampicin, a potent inducer of both metabolizing enzymes and transporters, could theoretically increase meropenem CL by inducing renal drug transporters. This provides reassurance that these two drugs can be used together, as needed, without need for dose adjustment to mitigate a drug interaction.

In the current study, meropenem CL of 11.8 L/h to a 70 kg individual with CLCR of 115 mL/min confirms previous reports that renal elimination of meropenem involves both the processes of glomerular filtration and tubular secretion[23,28]. Meropenem is a polar carbapenem that distributes into extracellular fluid, with approximately 70% of a dose excreted unchanged in urine[23,28]. It is biologically plausible to expect meropenem clearance to change with body weight and renal function. To this end, the inclusion of allometric scaling with body weight on disposition parameters resulted in drop in OFV by 22.2 points, and thus, improving the model fit[27]. The allometric scaling with centering at 70 kg was done to allow comparison of the disposition parameters with results of other studies in adults or children. For example, Rapp

and colleagues reported the typical value of clearance of 6.82 L/h normalized to 70 kg adult[23]. Reasons for the lower clearance in this population of critically ill children compared to 11.8 L/h in the current study could include renal impairment and/or maturation in organ function in the very young.

Other investigators sequentially evaluated the covariate effect of body weight on disposition parameters and found significant impact on only V1[21]. This contrast with our approach, and that of others[23], in which body weight was included as the allometric size-descriptor simultaneously on all disposition parameters. This is based on the understanding that volumes increase linearly with body weight (fixed theoretical exponent of 1) while clearance increases following a power function (fixed theoretical exponent of 0.75)[27].

Because of the polar nature of meropenem, it is reasonable to expect better model fit when allometric scaling is with FFM rather than total body weight. On the contrary, the model with FFM resulted in a lesser (20.4 points) OFV reduction, than that with body weight (22.2 points). For this reason and that of parsimony, we chose to keep the model with total body weight. The finding is not entirely surprising given the data: the median weight in the current study population is 52.7 kg (range, 39.3-76.3), and no patient was obese. The model with FFM could be more useful when describing patients with extreme body weights[29].

We found creatinine clearance to account for some variability in meropenem clearance between individuals and to provide an improvement in goodness of fit statistics. The covariate effect co-efficient of size-standardized creatinine clearance on CL is 0.416 (95% CI of 0.171 to 0.661) in the current study and is similar to 0.62 (95% CI of 0.34 to 0.83) reported by Li and

colleagues[21]. Other investigators documented similar results in children[22,23]. The clinical implication of the estimated effect of creatinine clearance on CL is that in a 70 kg patient with severe renal impairment (CLCR of 5-30 mL/min), about 40-70% reduction in meropenem doses would be required. Compared to other studies[21,22], the relatively low IIV in CL and V1 in the current study might be explained by the homogeneous patient population.

Few investigators reported a significant effect of age on meropenem CL, no such effect was seen in the current study[21,22]. It should be noted however, that the age range in our study was 20-63 years, but 18-93 years in the study by Li and colleagues[21], whereas the study by Du and colleagues enrolled children aged 0.08 to 17.3 years[22]. The significant impact of age on drug clearance in children could be explained in part by the effect on drug elimination of size and maturation of organ function[30].

The study has limitations. Firstly, the model was developed in adult tuberculosis patients and cannot be extrapolated to children. Secondly, the sample size might not provide enough power to pick up covariate relations with weak effects or occurring very rarely. However, such covariate effects are of limited clinical importance.

Conclusion

A 2-compartment population pharmacokinetics model described the pharmacokinetics of meropenem well with good precision in parameter estimates. The addition of both allometric scaling with body weight on disposition parameters and creatinine clearance on meropenem clearance increased the model's predictive performance. Rifampicin exposure did not influence meropenem parameters. The model will be used for integrated

pharmacokinetics/pharmacodynamics analysis linking meropenem exposure to early bactericidal activity.

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Table 1 Characteristics of patients who participated in pharmacokinetic sampling

Characteristics	MACR2X3 (n=12)	MAC2X3 (n=13)	MAC1X3 (n=12)	MAC3X1 (n=12)	Overall (n=49)
Age (years)					
Median (Q1, Q3)	32.3 (27.6, 40.2)	36.5 (33.2, 45.4)	40.9 (28.6, 45.8)	34.0 (28.2, 39.1)	36.0 (28.6, 45.4)
Max-min	21.1-58.6	23.1-61.2	20.0-62.7	20.3-55.6	20.0-62.7
Sex					
Female	3 (25.0%)	6 (46.2%)	2 (16.7%)	1 (8.3%)	12 (24.5%)
Race					
Black	2 (16.7 %)	2 (15.4 %)	5 (41.7 %)	7 (58.3 %)	16 (32.7 %)
Coloured	10 (82.3 %)	11 (84.6 %)	7 (58.3 %)	5 (41.7 %)	33 (67.3 %)
HIV status					
Positive	1 (8.3 %)	3 (23.1 %)	3 (25.0 %)	4 (33.3 %)	11 (22.4 %)
Weight (kg)					
Median (Q1, Q3)	52.3 (48.2, 55.9)	50.3 (48.3, 55.5)	55.2 (51.6, 62.1)	49.6 (45.8, 56.8)	52.7 (47.5, 57.1)
Max-min	39.3-62.4	40.3-65.9	45.1-65.5	43.0-76.3	39.3-76.3

Height (m)					
Median (Q1, Q3)	1.65 (1.60, 1.68)	1.62 (1.57, 1.71)	1.73 (1.67, 1.7)	1.66 (1.62, 1.69)	1.66 (1.60, 1.71)
Max-min	1.54-1.76	1.54-1.82	1.58-1.76	1.59-1.73	1.54-1.82
Creatinine clearance					
(mL/min)					
Median (Q1, Q3)	126 (90.3, 145)	109 (83.3, 139)	98.6 (94.1, 129)	112 (99.5, 127)	115 (94.3, 137)
Max-min	76.7-203	57.7-173	61.9-187	93.9-185	57.7-203

MACR2X3, intravenous meropenem 2g 8-hourly plus oral rifampicin 20 mg/kg once daily; MAC2X3, intravenous meropenem 2g 8-hourly; MAC1X3, intravenous meropenem 1g 8-hourly; MAC3X1, intravenous meropenem 3g once-daily; HIV, human immunodeficiency virus; Q1, lower quartile; Q3, upper quartile; Min, minimum; Max, maximum.

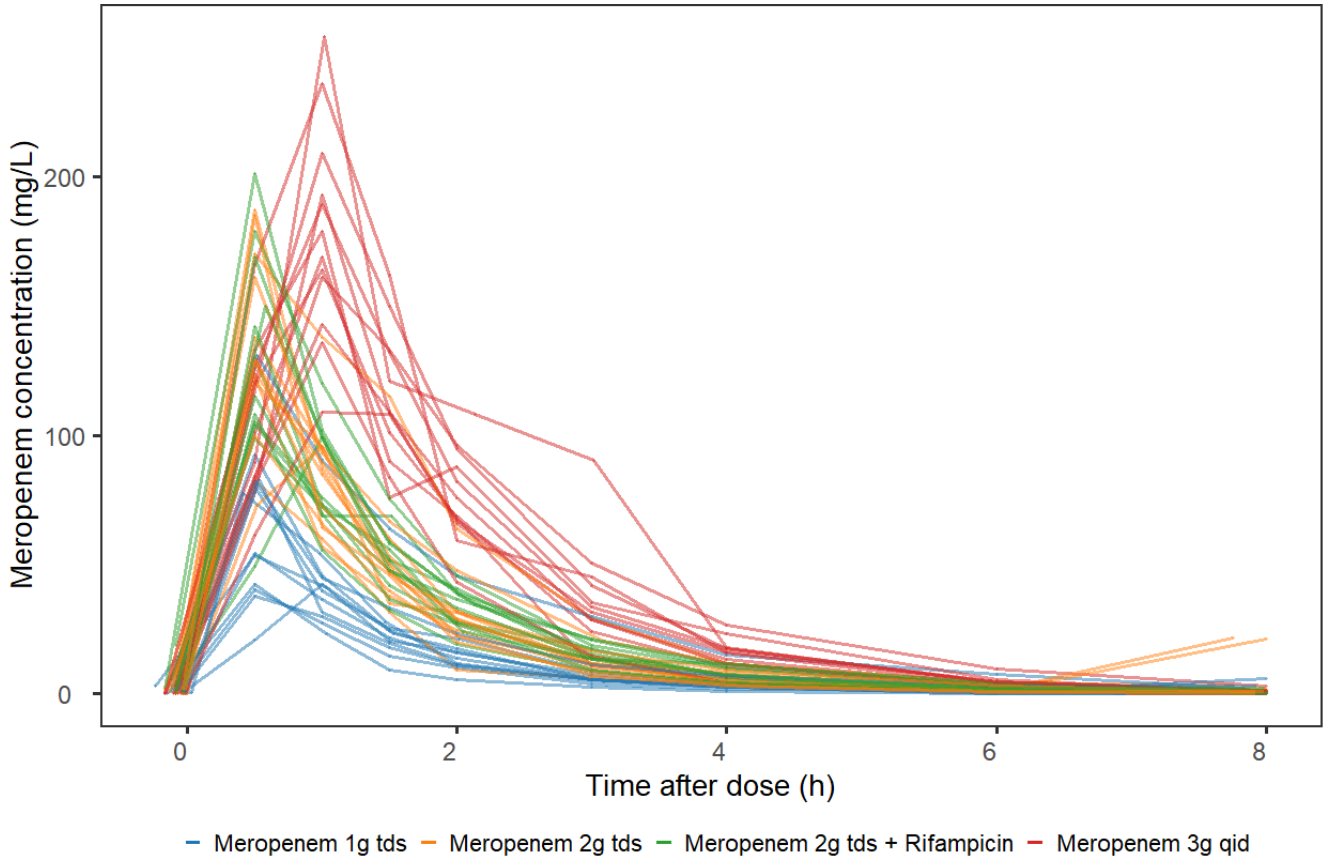
Table 2 Meropenem population pharmacokinetic model parameters.

Parameter	Population estimate (%RSE[a])	Bootstrap median (95% CI)
Structural model parameter		
CL (L/h/70 kg)	11.8 (4.9)	11.9 (10.5 to 12.8)
V1 (L/70 kg)	14.2 (3.8)	14.6 (13.4 to 16.4)
Q (L/h/70 kg)	3.26 (27.5)	3.15 (0.777 to 4.84)
V2 (L/70 kg)	3.12 (10.8)	3.17 (1.54 to 78.4)
Inter-individual variability (IIV) as %CV[b]		
IIV CL	20 (15.5)	19.3 (13.7 to 25.4)
IIV V1	13.1 (35.4)	12.7 (0.131 to 21.2)
IIV V2	106 (30.7)	111 (0.868 to 710)
Residual variability		
Proportional residual error (%)	0.178 (14.8)	0.178 (0.127 to 0.229)
Additive residual error (mg/L)	1.16 (19.6)	1.13 (0.388 to 1.54)
Covariate		
Creatinine clearance on CL	0.416 (30.5)	0.403 (0.203 to 0.704)

[a]Relative standard error (%RSE) was calculated as standard error from the covariance step/population estimate.
[b]Coefficient of variation (%CV) for IIV was calculated as (SQRT(EXP(OMEGA)-1)*100.
Confidence interval (CI), clearance from the central compartment (CL), central volume of distribution (V1), intercompartmental clearance (Q), and peripheral volume of distribution (V2). The bootstrap median and 95% CI were calculated from fitting of the final model to the 1000 bootstrap datasets.

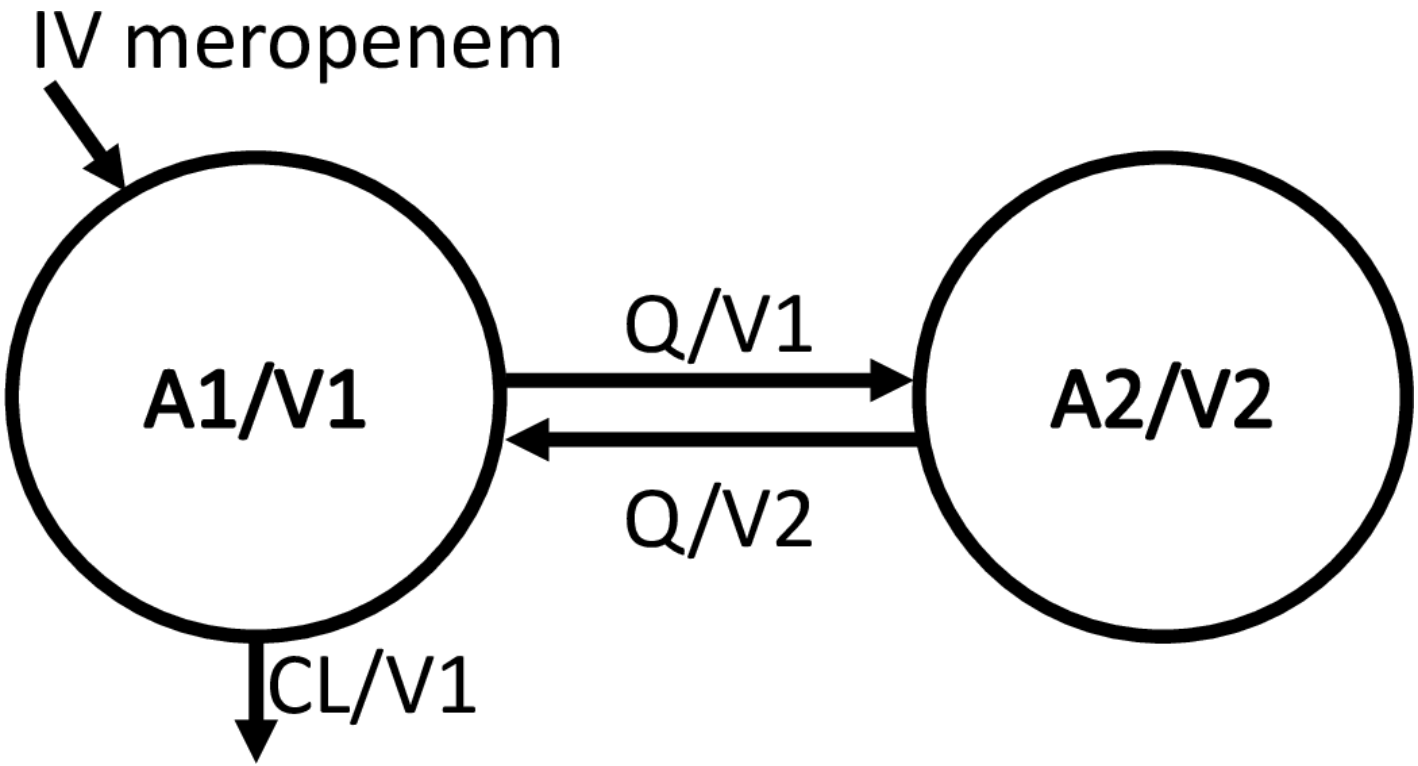
TVCL = THETA(1)*((WTKG/70)**0.75)*((CLCR*70/WTKG)/115)**THETA(7); TVCL is the meropenem clearance in the typical individual.
TVV1 = THETA(2)*WTKG/70 ; TVV1 is the meropenem volume of distribution in the central compartment in the typical individual.
TVQ = THETA(3)*((WTKG/70)**0.75); TVQ is the meropenem inter-compartmental clearance in the typical individual.
TVV2 = THETA(4)*WTKG/70; TVV2 is the meropenem volume of distribution in the peripheral compartment in the typical individual.

Figure 1. Meropenem plasma concentration-time profile stratified by study arm



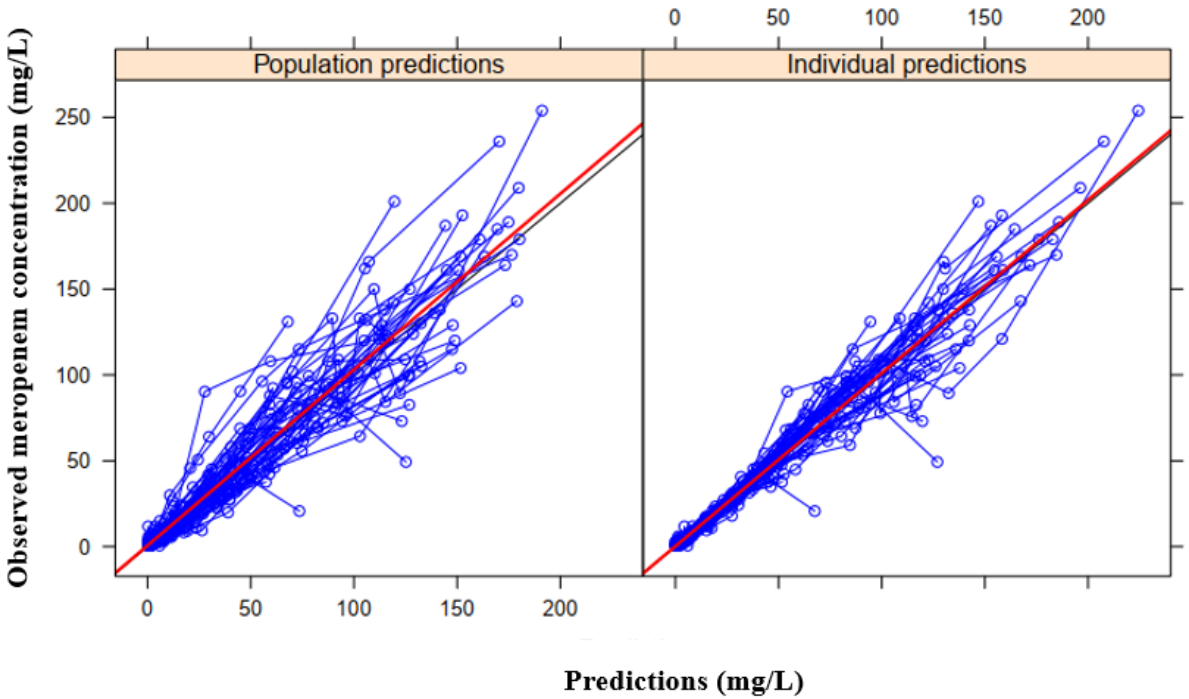
MACR2X3, intravenous meropenem 2g thrice daily plus oral rifampicin 20 mg/kg once daily; MAC2X3, intravenous meropenem 2g thrice daily; MAC1X3, intravenous meropenem 1g thrice daily; MAC3X1, intravenous meropenem 3g once daily.

Figure 2. Structural model schema



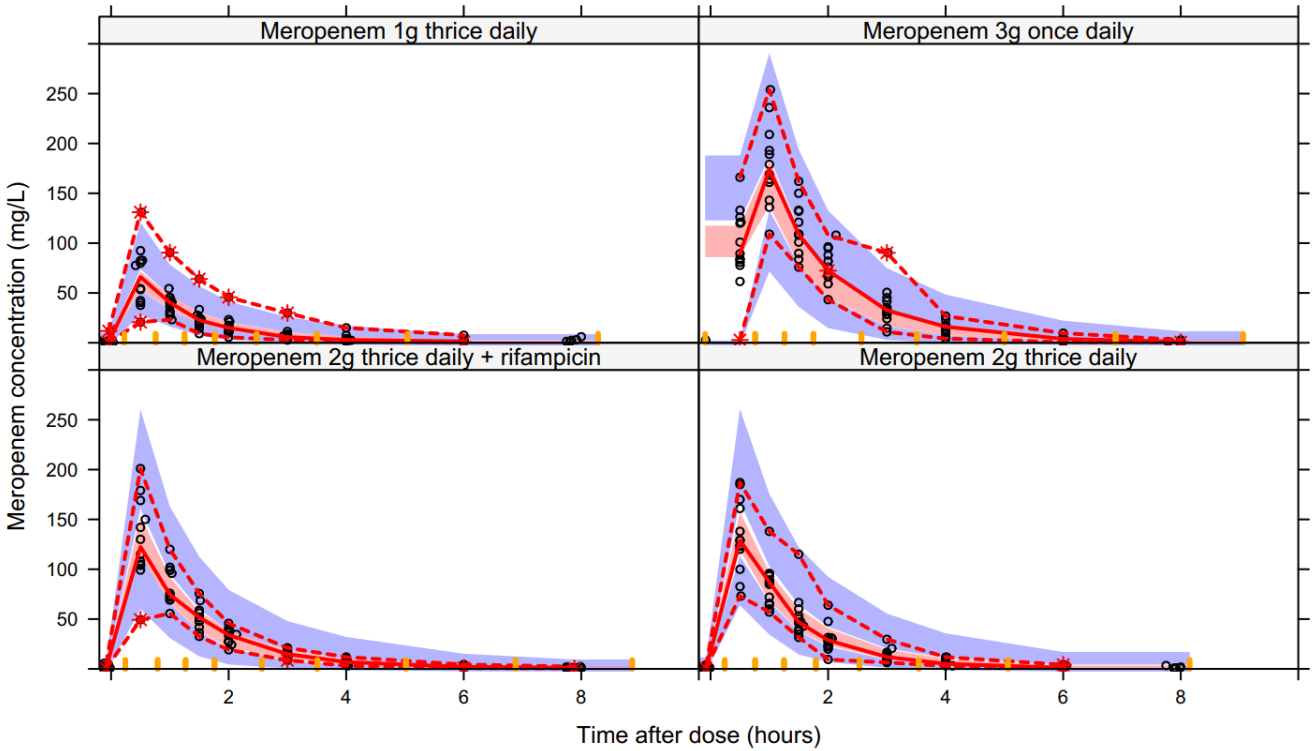
Meropenem amount in the central compartment (A1), central volume of distribution (V1), intercompartmental clearance (Q), meropenem amount in the peripheral compartment (A2), peripheral volume of distribution (V2), total plasma clearance (CL), meropenem concentration in the central compartment (A1/V1), meropenem concentration in the peripheral compartment (A2/V2), elimination rate constant is CL/V1, transfer rate constant from central to peripheral compartment (Q/V1), and transfer rate constant from peripheral to central compartment (Q/V2).

Figure 3. Basic goodness-of-fit plots of the final model



Basic goodness of fit plots of the final model showing the observed meropenem concentration versus the individual predicted concentration (right) or population predicted concentration (left). The observed and predicted concentrations are from the 49 individuals in the study.

Figure 4. Visual Predictive Check of the final model stratified by study arms.



The dashed red lines represent the 97.5th and 2.5th percentiles of the observed meropenem concentration data (open black circles), the solid red line connects the median (50th percentile) of the observed data (n=49). The blue shaded areas represent 95% confidence intervals of the 97.5th and 2.5th percentile of the predicted simulated data (n=1000), whereas the red shaded area represents 95% confidence interval of the median (50th percentile) of the predicted simulated data.

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis

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Supplementary materials:

Methods

Figure S1: Meropenem population pharmacokinetics model fit to individual data

Figure S2a: CWRES versus population prediction

FigureS2b: CWRES versus time after dose

NONMEM control stream of the final model

Methods

Eligibility Criteria

Inclusion criteria for participation in this study

- New or recurrent pulmonary tuberculosis (TB) with one or both of the following:
    - Sputum positive for acid-fast bacilli on direct microscopy of at least grade 1+ (International Union Against Tuberculosis and Lung Disease [IUATLD] scale) on at least one pre-treatment sputum sample.
    - Sputum positive for M. tuberculosis by Xpert MTB/RIF testing, semiquantitative result of "medium" or "high" on at least pre-treatment sputum sample.
- Age ≥18 and ≤65 years at screening.
- Ability and willingness to provide informed consent.
- Body weight 40 kg to 90 kg, inclusive.
- Laboratory values obtained within 30 days prior to or at study screening:
    - Absolute neutrophil count (ANC) ≥750 cells/mm^3.
    - Hemoglobin ≥7.0 g/dL.
    - Platelet count ≥50,000/ mm^3.
    - Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤3 X upper limit of normal (ULN).
    - Serum total bilirubin ≤2.5 X ULN.
    - Serum creatinine <1.5 X ULN.
- Human immune-deficiency virus (HIV) infection must be documented as either absent or present.
- For HIV-positive participants, only: CD4+ cell count of ≥100 cells/mm^3, performed within 30 days prior to or at study screening.
- For females of reproductive potential, negative serum or urine pregnancy test within 7 days prior to study screening. Female participants who are engaging in sexual activity that could lead to pregnancy must agree to use one reliable non-hormonal method of contraception (condoms or an intra-uterine contraceptive device), or another method

(diaphragm or cervical cap) if it is approved by the national regulatory authority and used according to package insert, while receiving study medications.

- Willingness to be hospitalized for a minimum of 16 consecutive days.
- Ability to produce an overnight sputum sample of sufficient quality and quantity. As a guideline, this should be 10 mL or more during a 16-hour collection period.
- Xpert MTB/RIF result performed on sputum within 14 days prior to or at study screening that shows either “Rifampicin resistance detected” or “Rifampicin resistance not detected”.

Exclusion criteria

- Treatment with any drug active against *M. tuberculosis* within the 3 months prior to study screening.
- Breast-feeding.
- Known allergy or sensitivity to any of the study drugs.
- Participants receiving valproate sodium or probenecid.
- Karnofsky score <60 or poor general condition such that, in the opinion of the investigator at screening, any delay in initiation of definitive TB treatment cannot be tolerated.
- Known current neurological TB or seizure disorder.
- Any condition as determined by physical examination, medical history, laboratory data, or chest x-ray which, in the opinion of the investigator, would interfere with safety or endpoint assessments in the study.

Bioanalytical method

Meropenem was quantified by a validated analytical method using Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). The validated method was developed to simultaneously quantify meropenem and ertapenem in human dipotassium ethylenediaminetetraacetic acid (K_2EDTA) plasma over the range of 0.5-256 µg/mL.

The analytes were extracted from the biological matrix (i.e., plasma) using protein precipitation with methanol. This was followed by dilution of the supernatant with a mixture of acetonitrile and formic acid solution.

The liquid chromatographic separation was with Phenomenex® Kinetex XB-C18, 150 x 4.6 mm, 5 µm analytical column. Mobile phases: mobile phase A (formic acid solution) and B (acetonitrile) were delivered using a gradient flow. The autosampler, equipped with a 96-well tray, was used to inject 2 µL of each sample onto the column, at a temperature of approximately 5 ºC. Meropenem retention time was ~1.44 minutes.

Mass spectrometer, Sciex API4000 coupled to Watson LIMS™ software version 7.4.2 and Analyst® software version 1.6.2 was used. Ionization mode was with electrospray Ionization (ESI) in positive mode. Meropenem protonated precursor ion with m/z 384.0 and product ion with m/z 141.0. The internal standard working solution with a concentration of ~12 µg/mL meropenem-d6 in methanol was prepared in a polypropylene container by dissolving the reference substance directly in the methanol. The internal standard working solution was added to each sample (excluding blank samples). Calibration standards (STDs) in human K_2EDTA plasma and quality control samples (QCs) in human K_2EDTA plasma and in stabilized human lithium heparin (LH) plasma, were prepared gravimetrically in human plasma.

The validation process comprised of three accuracy and precision validation runs during which the accuracy and precision of the method was established and evaluated against acceptance criteria as defined by the regulatory guidelines. The meropenem regression model used was: log-log linear calibration curve (log y = a logx + b. The Response Type was peak area ratio.

Absolute recovery (extraction efficiency) for meropenem was 90.4% (mean % coefficient of variation [%CV] = 4.7), whereas, the relative recovery was 0.903 (mean %CV = 4.0). The average Signal-to-Noise Ratio at lower limit of quantification (LLOQ) was 54.4. No inherent carry-over was detected in the blank samples. In addition, no interfering peaks from endogenous and other matrix components were observed at the retention times of meropenem or the internal standard.

Meropenem was accurately quantified in the presence of commonly used over-the-counter drugs (paracetamol, ibuprofen, cyclizine, cetirizine, pseudoephedrine, codeine and diclofenac). A multi-component analysis confirmed that meropenem can be accurately determined in the presence of amoxicillin, clavulanic acid, rifampicin and each other. The variability of the internal standard-normalized matrix factor (IS-MF) was <15% at both low and high analyte concentrations, indicating that the analysis is reproducible in the various matrices.

Dilution integrity: the following dilutions were successfully validated;

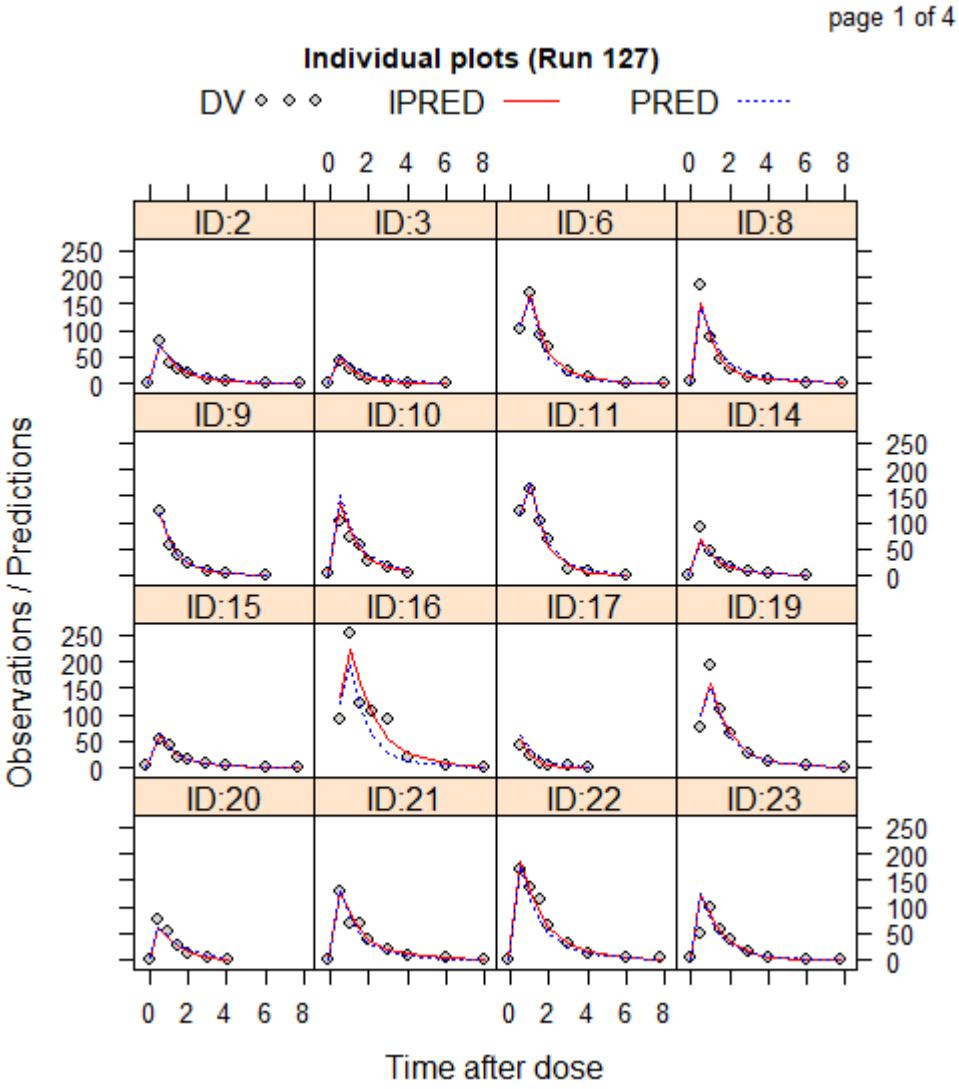
1. 2-fold dilution: K_2EDTA plasma samples diluted with K_2EDTA plasma.
2. 2-fold dilution: stabilized LH plasma samples diluted with stabilized LH plasma.
3. 5-fold dilution: stabilized LH plasma samples diluted with K_2EDTA plasma.
4. 15-fold dilution: K_2EDTA plasma samples diluted with K_2EDTA plasma.

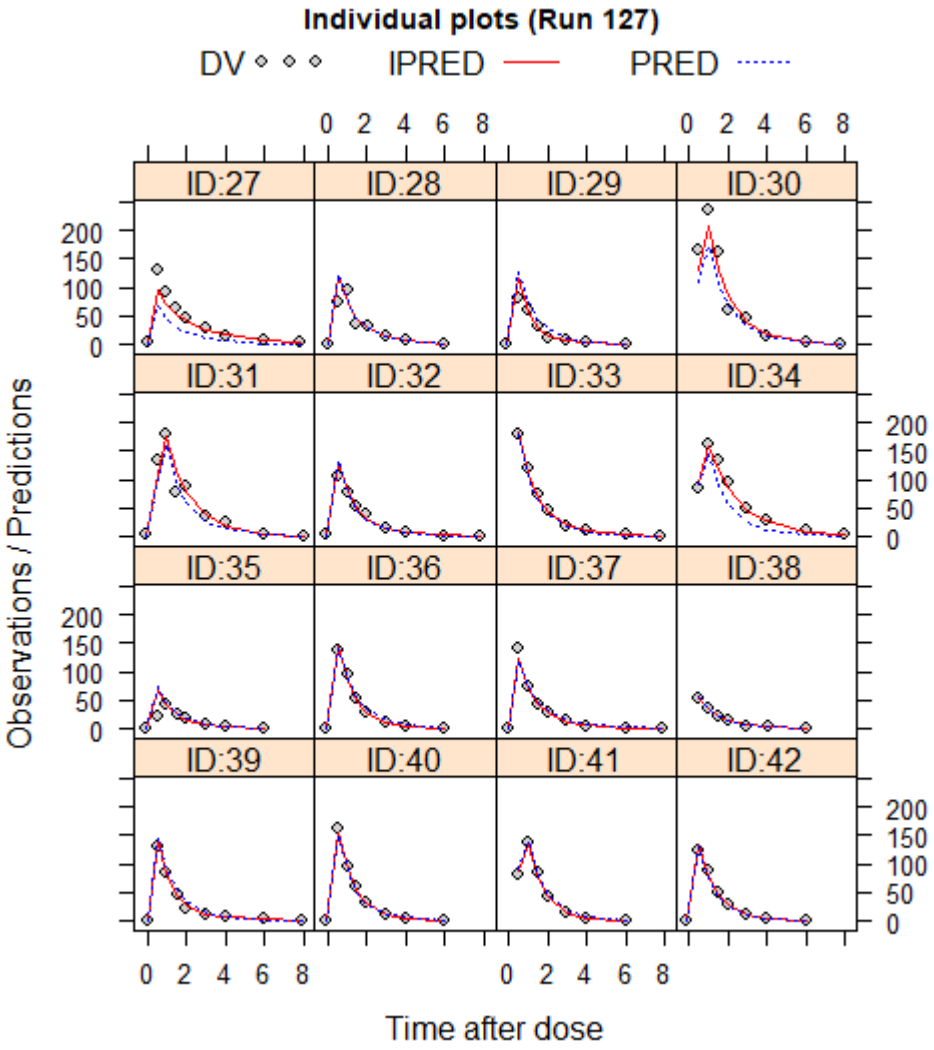
Plasma samples with meropenem concentration above the upper limit of quantification can be analyzed by applying 2-fold, 5-fold and 15-fold dilutions.

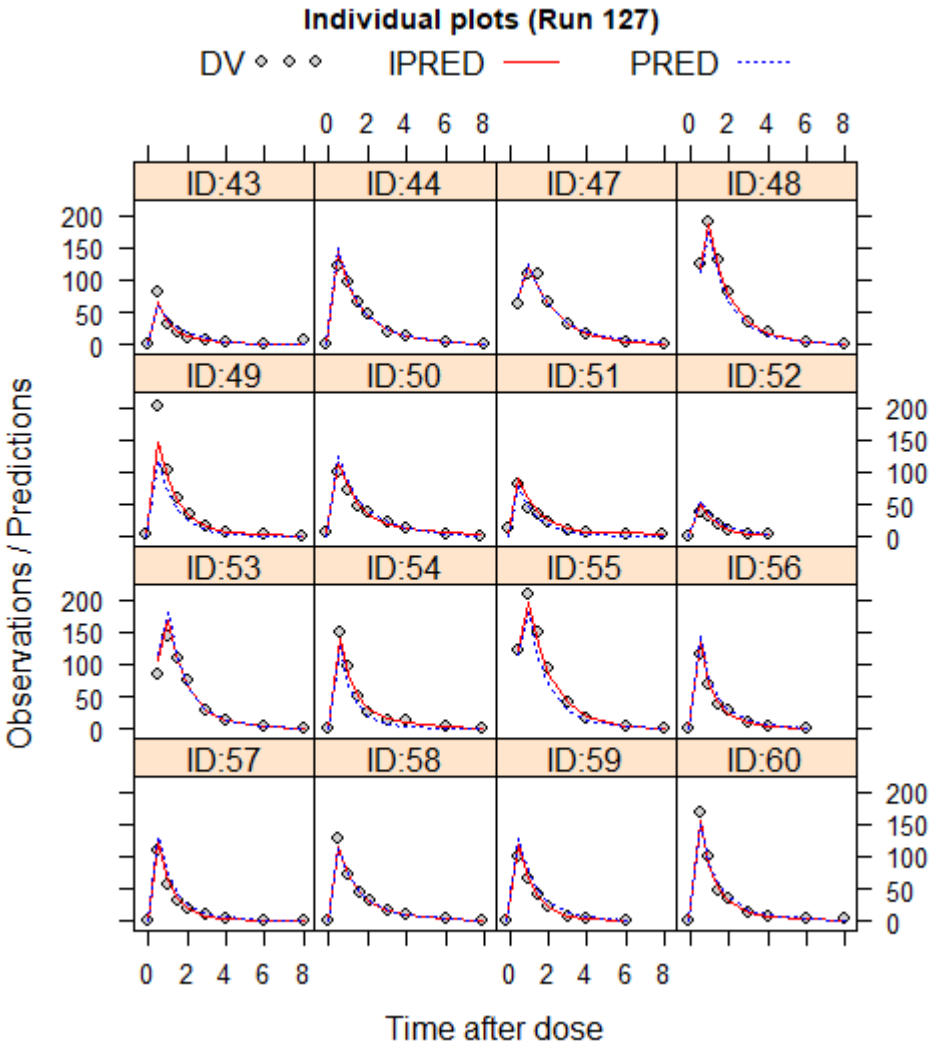
Finally, the 3 consecutive accuracy and precision validation runs met the acceptance criteria:

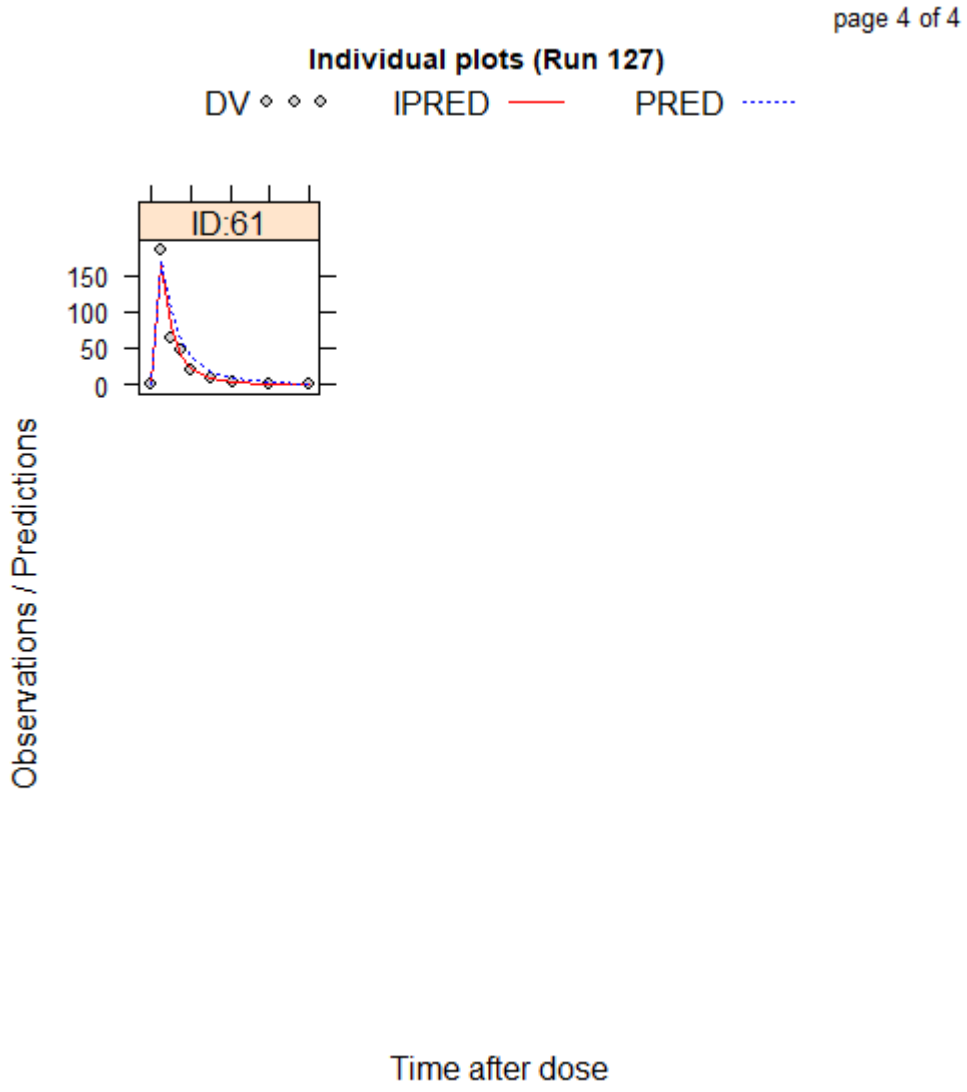
1. The between-run accuracy calculated over all 3 consecutive validation runs (expressed as %Bias) must be within 15% over the range and within 20% of at the LLOQ, and
2. The between-run precision calculated over all 3 consecutive validation runs (expressed as %CV) must be ≤15% (20% at the LLOQ).

Figure S1. Meropenem population pharmacokinetics model fit to individual data



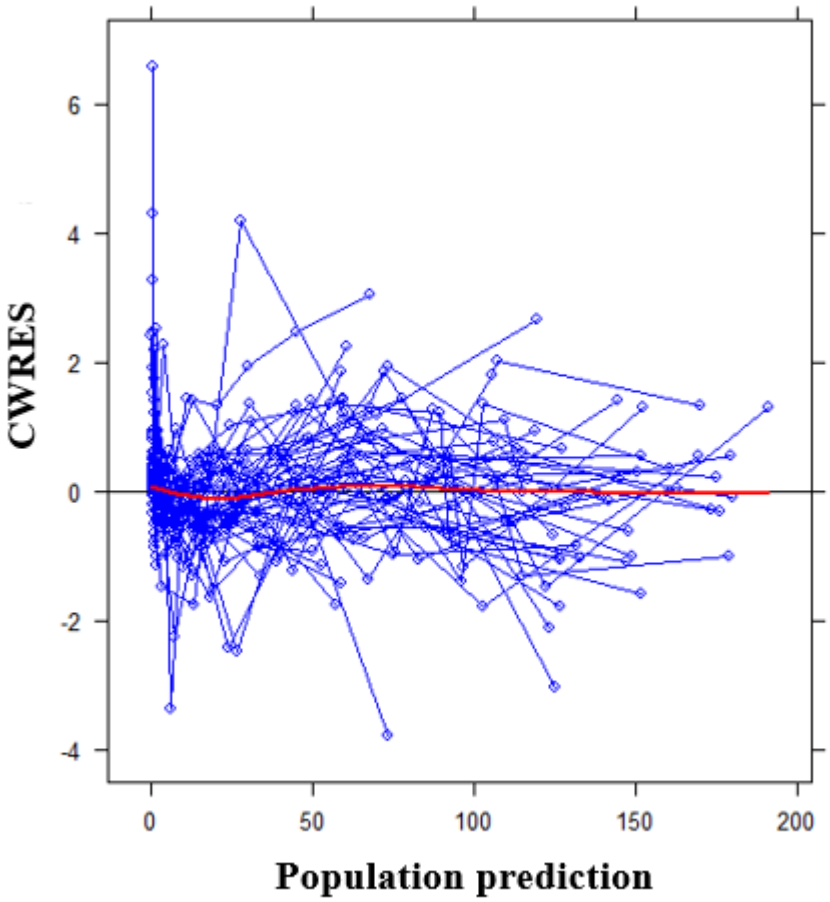






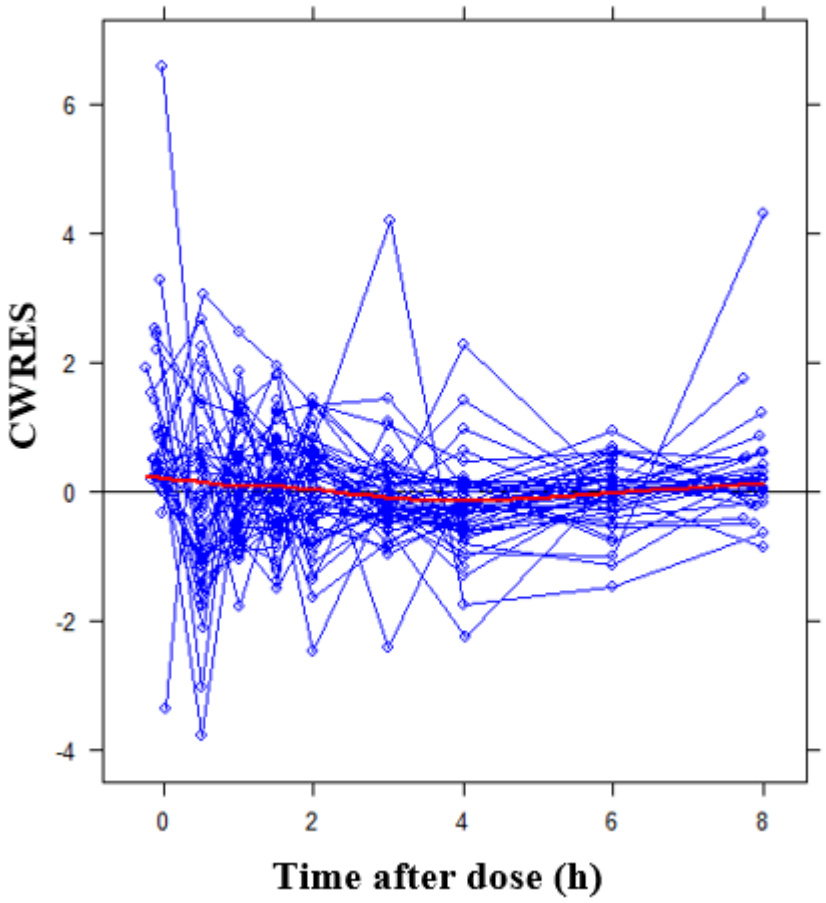
DV, observed meropenem concentration (mg/L); IPRED, individual prediction (mg/L); PRED, population prediction (mg/L).

Figure S2a. CWRES versus population prediction in mg/L



CWRES, conditional weighted residuals. The blue open circles are the observed concentrations. The solid black horizontal line is the zero line. If the predictions were 100% perfect, all the blue open circles will fall on the solid black line. The solid red line is the trend line.

Figure S2b. CWRES versus time after dose



CWRES, conditional weighted residuals. The blue open circles are the observed concentrations. The solid black horizontal line is the zero line. If the predictions were 100% perfect, all the blue open circles will fall on the solid black line. The solid red line is the trend line.

NONMEM control stream of the final model

```
$PROBLEM   Meropenem population pharmacokinetics modeling in patients with pulmonary
           tuberculosis.
$INPUT     ID USUBJID DAT2=DROP TIME TAD TAD2 SAMPT=DROP DURHR
           AMT MDV EVID DV BLQP CMT RATE SS=DROP DOMAIN=DROP
           VISIT EXCL  EXCL2 PAGE CLDV PCSP AGE SEXF RACEB HTM
           WTKG BMI FFM LBW HIVP RIFGPS RIF1 AMXCLV G2ARM DOSE
           CREAT CLCR SDAY SUBJECT=DROP STAD=DROP LASTDOSE=DROP
$DATA      Datasetxx.csv IGNORE=@ IGNORE=(EXCL2.EQ.1)
           IGNORE=(EXCL2.EQ.4) IGNORE=(EXCL2.EQ.5)
$SUBROUTINE ADVAN13 TOL=9
$MODEL     COMP (COMP1) ;Central compartment
           COMP (COMP2) ;Peripheral compartment
$PK
D1=DURHR
TVCL = THETA(1)*((WTKG/70)**0.75)*(((CLCR*70/WTKG)/115)**THETA(7))
     ;TVCL is the clearance of the typical individual in the population
     ;+ allometric scaling with body weight centered at 70 kg
     ;+ Covariate effect of size-standardized creatinine clearance centered at 115 mL/min
TVV1 = THETA(2)*WTKG/70
     ;TVV1 is the central volume of distribution for the typical individual in the population
     ;plus allometric scaling with body weight centered at 70 kg
```

```
TVQ = THETA(3)*((WTKG/70)**0.75)
      ;TVQ is the intercompartmental clearance of the typical individual in the population
      ;in the population  plus allometric scaling with body weight centered at 70 kg
TVV2 = THETA(4)*WTKG/70
      ;TVV2 is the peripheral volume of distribution for the typical individual in
      ; the population  plus allometric scaling with body weight centered at 70 kg
CL = TVCL*EXP(ETA(1))
      ;Individual clearance, and inter-individual variability (IIV)
V1 = TVV1*EXP(ETA(2))
      ;Individual volume of distribution of the central compartment, and IIV
Q = TVQ*EXP(ETA(3))
      ;Individual intercompartmental clearance, and IIV
V2 = TVV2*EXP(ETA(4))
      ;Individual volume of distribution of the peripheral compartment, and IIV
$DES
CENTRAL_DES = A(1)
PERIPHERAL_DES = A(2)
CC_DES = (CENTRAL_DES/V1)
DADT(1) = (((-(Q)*(A(1)/V1))+(Q*(A(2)/V2))-(CL*(A(1)/V1))))
DADT(2) = ((Q*(A(1)/V1))-(Q*(A(2)/V2)))
```

```
$ERROR                          ;Residual error model
CENTRAL = A(1)                  ;Amount in central compartment
PERIPHERAL = A(2)               ;Amount in peripheral compartment
CC = (CENTRAL/V1)               ;Concentration in the central compartment
IPRED = CC                      ;Individual prediction
IRES = DV - IPRED
W = SQRT((THETA(5)*IPRED)**2 + THETA(6)**2) ;For proportional and additive error
IWRES = IRES/W
Y = IPRED+W*EPS(1)


$THETA
(0.0,11.8026)                   ; 1. TVCL
(0.0,14.2024)                   ; 2. TVV1
(0.0,3.26491)                   ; 3. TVQ
(0.0,3.11527)                   ; 4. TVV2
(0,0.178409)                    ; 5. Proportional error
(0,1.15559)                     ; 6. Additive error
0.415838                        ; 7. Covariate effect of CLCR
```

```
$OMEGA

0.0392918                                   ; 1. IIV_CL

0.0170819                                   ; 2. IIV_V1

0 FIX                                       ; 3. IIV_Q

0.753935                                    ; 4. IIV_V2


$SIGMA 1.0 FIX


$ESTIMATION     METHOD=COND INTER NSIG=3 SIGL=9 MAXEVALS=9999
                PRINT=10 NOABORT MSFO=xx.msf


$COVARIANCE


$TABLE      ID USUBJID TAD MDV EVID AMT RATE AGE WTKG CLCR HTM BMI
            FFM LBW RACEB SEXF HIVP G2ARM DOSE BLQP PRED IPRED RES
            IRES IWRES WRES CWRES CIPRED CIRES CIWRES Y DV NOAPPEND
            NOPRINT ONEHEADER FILE=sdtabxx
$TABLE      ID CL V1 Q V2 ETA(1) ETA(2) ETA(3) ETA(4) NOAPPEND NOPRINT
            ONEHEADER FILE=patabxx
$TABLE      ID AGE WTKG FFM LBW CLCR HTM BMI NOAPPEND NOPRINT
            ONEHEADER FILE=cotabxx
```

```
$TABLE      ID RACEB SEXF HIVP G2ARM RIF1 NOAPPEND NOPRINT
            ONEHEADER FILE=catabxx
```

SUMMARY OF FINDINGS

CHAPTER 1

Clinical pharmacokinetics and pharmacodynamics of rifampicin in human tuberculosis

Clin Pharmacokinet. 2019;58(9):1103-1129. doi:10.1007/s40262-019-00764-2.

Key findings

This chapter reviewed rifampicin data spanning five decades since its introduction into TB treatment. Rifampicin was crucial in shortening treatment to six months when combined with pyrazinamide in the first 2 months. AUC_{0-24} (area under the concentration-time curve [AUC] from time 0 to 24 hours)/MIC (minimum inhibitory concentration) is the PK/PD parameter best linked to rifampicin's bactericidal activity.

Rifampicin exposure is complicated by its nonlinear PK, the auto-induction of its own metabolism, and considerable inter- and intra-individual variability that could be reduced by administration under fasting conditions. In addition, therapeutic drug monitoring integrating Bayesian priors could account for variability and allow dose individualisation, and attainment of PK/PD targets faster. Several factors including malnutrition, HIV infection, diabetes mellitus, dose size, pharmacogenetic polymorphisms, hepatic cirrhosis, and substandard medicinal products can alter rifampicin exposure and/or efficacy.

The clinical use of rifampicin at 600 mg (10-12 mg/kg) once daily dosing was selected partly because of cost and toxicity concerns at the time of its introduction. Recent studies suggest higher rifampicin

doses can be used to optimise TB treatment. For example, a clear exposure-response relationship has been defined to treat TB meningitis.

Conclusion

Understanding factors influencing rifampicin disposition are key to its optimal use. Available evidence suggest current rifampicin dosing of 8-12 mg/kg is at the lower end of the dose-exposure-response curve, and that higher doses should be considered. It is reassuring that daily rifampicin doses up to 35 mg/kg for 12 weeks were safe and well tolerated.

Limitations

Firstly, PK data interpretations were complicated by use of different laboratory analytical methods in the published studies. Secondly, while some papers report results as rifampicin AUC (from time 0 to t) or C_{max}, others provide only serum concentrations at certain timepoints after dosing. Thirdly, none of the EBA studies reported rifampicin MICs needed to calculate AUC/MIC. Finally, the included studies have heterogenous designs, outcome measures, and do not take into account variability in drug formulations.

Future research

More studies are needed to confirm whether doses higher than the current standard of care could translate to treatment shortening and/or stratification.

Daily rifampicin at dose of at least 35 mg/kg should be investigated in novel regimens with new or repurposed drugs to optimise TB treatment in terms of shortening treatment duration, improved cure, and relapse rates. The PK/PD determinants of efficacy, and the role of therapeutic drug monitoring should be prospectively investigated. In addition, future studies in patients with TB meningitis should confirm effect on mortality of high rifampicin exposures and further investigate the optimal route of administrations.

CHAPTER 2

The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance

Br J Clin Pharmacol. May 2020; 86(11):2123-2132. doi:10.1111/bcp.14395.

Key findings

The most important finding of this chapter is unravelling the clinical equipoise regarding the historical view of PAS as an exclusively bacteriostatic agent. Available evidence suggests PAS C_{max}, AUC, and, by extension C_{max}/MIC, and/or AUC/MIC rather than %T>MIC are the most important determinants of efficacy and suppression of resistance development in companion drugs.

PAS at high single-daily dosages that achieve high blood concentrations has a hitherto unappreciated bactericidal effect, similar to that of 10 mg/kg rifampicin, and this is not currently exploited. In addition, the size of individual PAS doses administered at each dosing interval rather than total daily dose is important in protecting companion drugs from resistance emergence. Assuming a typical PAS MIC of 1 mg/L, a C_{max}/MIC of ≥100 may be required to replicate the findings seen during the series of clinical trials conducted in 1950s by the British MRC. The contemporary dosing of the widely available PASER formulation makes it highly unlikely to reach C_{max} of ≥100 mg/L. PASER and indeed any formulation of PAS have not been re-assessed by modern methodologies of TB drug development, optimisation, and repurposing.

Gastrointestinal intolerance to PAS is dependent on PAS formulation, and route of administration, which could be avoided if the gastrointestinal tract is bypassed as seen with intravenous administration, which is not a feasible option. Gastrointestinal intolerance seems to not be related to how high the blood PAS concentration is, which is re-assuring.

Conclusion

Substantial evidence are available to question the traditional role of PAS as an exclusively bacteriostatic agent. We are at present not using PASER optimally, necessitating a need for re-evaluation of PAS PK/PD.

Limitations

This chapter has several limitations, firstly, the pivotal British MRC studies did not include quantification of PAS in the serum/plasma. Therefore, extrapolating from a later study that used similar PAS dose and formulation as did the British MRC studies, a C_{max} of 100 mg/L was proposed to have been reached. Secondly, only four patients were included in the PAS EBA study that found PAS to have bactericidal activity similar to that of rifampicin at 10 mg/kg. Finally, PAS MICs were not reported, and no prospective PK/PD study of PAS using PASER formulation was available.

Future research

Future PAS studies should focus on exploring the relationship between PAS dose, PK and pharmacogenetics and the efficacy and tolerability of PAS in different formulations. PAS EBA study should investigate high once-daily PASER dosing regimens, and the exposure determinants of efficacy should be confirmed.

CHAPTER 3

Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens

Eur J Clin Pharmacol. 2020; 76(11):1557-1565. doi:10.1007/s00228-020-02943-8.

Key findings

This chapter presents results of a modelling and simulation study of PASER. Four novel PASER dosing regimens not previously investigated were evaluated.

We found a previously published PAS population pharmacokinetics model needed optimisation prior to being used for target attainment simulations.[43] The model was refined using combined data from two studies in adults.[43,44] The addition of variability in bioavailability and allometric scaling with total body weight on disposition parameters improved the final model fit to the combined data, resulting in a 90.1 points reduction of goodness-of-fit statistic (objective function value, OFV) compared to the published model.[43]

The simulation study in 1000 virtual patients each administered single once-daily 12 g, 14 g, 16 g and 20 g PASER regimens showed the expected dose-dependent increase in exposure. The proposed target for bactericidal activity C_{max}/MIC of ≥100 was reached in 53%, 65%, 72% and 84% of patients administered 12, 14, 16 and 20 g once-daily PASER, respectively, assuming typical PAS MIC of 1 mg/L. Irrespective of the dosing regimen, PAS concentrations remained above 1 mg/L (proposed target for bacteriostatic activity) for >98% of the dosing interval.

Conclusion

Target PAS concentrations were achieved in most patients administered 14, 16 and 20 g once-daily PASER. When PAS MIC is < 1 mg/L compared with ≥ 1 mg/L, the potential for attaining the

proposed target with high once-daily PASER is increased, while ensuring PAS concentrations remain above the target for its traditional bacteriostatic effect.

Limitations

Firstly, the target used in the study is based on extrapolation from historic data and needs to be evaluated in a prospective study. Secondly, target attainment rates were obtained from simulations in virtual patients. Thirdly, protocol time rather than actual time of PK sampling were used for the analyses. Finally, the population upon which the model was built included only 85 South African pulmonary TB patients, and this may limit generalization to other populations.

Future research

A prospective pharmacokinetic study, preferably with assessment of early bactericidal activity and individual MICs, should be performed to confirm our predictions and determine the safety and tolerability of the proposed regimens.

CHAPTER 4

Drug concentration at the site of disease in children with pulmonary tuberculosis

Key findings

This chapter reports on the results of a prospective pilot study investigating the concentrations of first-line antituberculosis drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) at the site of disease in children with severe intrathoracic tuberculosis requiring bronchoscopy or trans-thoracic

surgical lymph node decompression (SD group). A population PK model was therefore developed to reconstruct the full plasma concentration-time profile of the relevant drug in each patient. Furthermore, an additional compartment was modelled to represent each site of disease, and then linked to the plasma PK models

Compared to historical data, the plasma exposure of the first-line drugs were uniformly low except isoniazid. We found the site of disease penetration of antituberculosis drugs to be drug- and lesion-specific. The penetration coefficients of rifampicin, isoniazid and pyrazinamide into most lymph node compartments are <1, an indication of less penetration into tissues compared to plasma. Whereas those for ethambutol are >1 indicating accumulation in the different lymph node compartments. Of note, the key sterilizing drugs rifampicin and pyrazinamide, well known for their antimycobacterial activity against both non-replicating and slowly replicating bacilli, have low penetration into necrotic tissues with a penetration coefficient of approximately 0.5. In addition, rifampicin and pyrazinamide exposures in necrotic tissues were lower than in plasma. Besides, while rifampicin exposure in cellular compartment was higher than in plasma, that of pyrazinamide was lower than in plasma. Thus, creating a potential scenario for monotherapy against "persisters" *M. tuberculosis* population and higher risk of resistance development.

Isoniazid has a low penetration coefficient of 0.56 in cellular regions of granuloma albeit twice that in adults. It is within this compartment that isoniazid is thought to exert its bactericidal activity against rapidly dividing bacilli within the first few days of treatment. Except for BAL, the overall simulated isoniazid exposure in all sites of disease compartments were lower than in plasma.

Furthermore, the penetration coefficients of the first-line antituberculosis drugs in several lymph node compartments were similar to those in adults in several lung tissue compartments. To our knowledge

this is the first human data on ethambutol at sites of disease, and thus, there are no adult data to compare.

Conclusion

Except for isoniazid, plasma exposures of all first-line antituberculosis drugs were low compared to historical data. The proportional distribution to site of disease compartments of rifampicin, isoniazid and pyrazinamide in children are comparable to or higher than in adults for most compartments. Optimising dosing to increase site of disease exposures in children could improve tuberculosis treatment outcomes. Increased knowledge on the PK and PD of first-line drugs may allow treatment stratification or shortening depending on disease severity.

Limitations

This study had several limitations, firstly, selection bias might have been introduced because our study included young children with severe forms of pulmonary TB routinely undergoing surgery at a single site. Secondly, all children were anaesthetized at the time of the procedure, and particularly those in SD group received large number of concomitant drugs and intravenous fluids, all of which could have affected antituberculosis drug disposition. Thirdly, total plasma concentrations of the antituberculosis drugs were measured, and thus, protein binding was not accounted for. Fourthly, the presence of freezing artefacts, absence of clear areas of demarcation and fragmented nature of the tissue samples made classification of the lymph node histology challenging in some of our samples. Fifthly, the VPCs provided do not fully allow evaluation of the variability components since we do not have enough observations to do full confidence interval VPCs. Finally, the precision of the estimated distribution coefficient is generally low due small sample size, and this is particularly true

for BAL whose PK-PD simulations should be interpreted with caution given the limited number of patients in whom BAL urea concentration was successfully measured.

Future research

More prospective studies with larger sample size and varying site of disease sampling timepoints are needed to better characterize the rate and extent of drug transfer between plasma and tissue compartments. The studies should evaluate higher rifampin doses as well as higher end of the dosing range for other first-line antituberculosis agents to ultimately optimise their exposures at the site of disease. In addition, the studies should preferably be powered to investigate relationship between drug exposures in plasma, site of disease, and important clinical outcomes such as cure rate, relapse rate, treatment failure, and mortality.

A more sensitive method of quantifying urea in bronchoalveolar lavage fluid is urgently required to allow for drug measurement in the alveolar fluid.

CHAPTER 5

The population pharmacokinetics of meropenem in adult patients with rifampicin-sensitive pulmonary tuberculosis

Key findings

This chapter presents the results of the first meropenem population pharmacokinetics modelling analysis in pulmonary tuberculosis patients. The data for this chapter was obtained from a prospective phase 2a clinical trial.

A 2-compartment model with first-order elimination process adequately described the observed meropenem concentration-time data. The model was parameterised with clearance (CL) from the central compartment, intercompartmental CL (Q), central volume of distribution (V1) and peripheral volume of distribution (V2). We found low uncertainty in parameter estimates with relative standard errors ranging from 3.8% to 35.4%. Similarly, we found low combined additive and proportional errors of 1.16 mg/L and 0.178%, respectively.

Size-standardised creatinine clearance on CL and allometric scaling with total body weight on all disposition parameters were the only covariates found to have significant impact meropenem disposition and were included in the final model. On the contrary, age, HIV coinfection, and concomitant rifampicin administration did not significantly influence meropenem clearance.

Conclusion

The developed model could adequately describe meropenem population pharmacokinetics in patients with pulmonary tuberculosis. Covariates found to improve model fit were creatinine clearance and body weight but not concomitant rifampicin treatment or age.

Limitations

A major limitation of this analysis is the high uncertainty in the parameter estimates of the peripheral volume of distribution represented by the wide 95% confidence interval of the 1000 bootstrap estimates.

Future research

Future work should focus on an integrated pharmacokinetics-pharmacodynamics analysis linking meropenem exposure to EBA. The PK-PD indices linked to meropenem EBA should be determined.

Appendix A

RESEARCH OUTPUTS OF THE DISSERTATION

The first three manuscripts have been published in international peer reviewed journals. Parts of the results of chapter three, four and five were presented at one local and three international conferences in 2019. Chapter five has been accepted for presentation at the World Conferenceon Pharmacometrics 2022, and the abstract published in CPT: pharmacometrics and system pharmacology supplement. The manuscripts from chapters four and five have been submitted to international peer reviewed journals for publication. A revised version of the manuscript forchapter four has been resubmitted to AJRCCM in response to reviewers' comments.

Published manuscripts and abstracts:

1. Abulfathi AA, Decloedt EH, Svensson EM, Diacon AH, Donald P, Reuter H. Clinical Pharmacokinetics and Pharmacodynamics of Rifampicin in Human Tuberculosis. *Clin Pharmacokinet*. 2019;58(9):1103-1129. doi:10.1007/s40262-019-00764-2.

2. Abulfathi AA, Donald PR, Adams K, Svensson EM, Diacon AH, Reuter H. The pharmacokinetics of para-aminosalicylic acid and its relationship to efficacy and intolerance. *Br J Clin Pharmacol*. May 2020; 86(11):2123-2132.doi:10.1111/bcp.14395.
3. Abulfathi AA, Assawasuwannakit P, Donald PR, Diacon AH, Reuter H, Svensson EM. Probability of mycobactericidal activity of para-aminosalicylic acid with novel dosing regimens.

   Eur J Clin Pharmacol. 2020; 76(11):1557-1565. doi:10.1007/s00228-020-02943-8.

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