Optimization of first-line anti-tuberculosis therapy in patients co-infected with HIV

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Cover photo: Kicukiro TB/HIV center in Kigali, Rwanda. Photo by Jesper Sundell.

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ABSTRACT

Every year over one million people die from tuberculosis. People infected with HIV are significantly more vulnerable to tuberculosis, which is the leading cause of HIV-associated death. The first-line treatment for tuberculosis consists of rifampicin, isoniazid, pyrazinamide and ethambutol. Although the regimen is effective, the risk of treatment failure and acquired toxicity is unacceptably high. Due to the lack of effective alternative therapy against resistant tuberculosis, optimal use of the first-line combination is crucial. The aim of the studies presented within this thesis was to investigate potential for optimization of the first-line tuberculosis therapy in patients co-infected with HIV. A bioanalytical method for quantification of the four first-line antitubercular drugs and their primary metabolites in human plasma was developed and validated. Population pharmacokinetics of the drugs and their metabolites were described using non-linear mixed effects modelling. The effects of genetic polymorphism, concomitant HIV therapy and patient demographics on drug exposure were investigated. Individualized dosing based on patient characteristics to reduce high pharmacokinetic variability was proposed for isoniazid and pyrazinamide. Two drug-drug interactions of potential clinical relevance were described: an effect of HIV therapy on rifampicin pharmacokinetics and an effect of rifampicin on isoniazid pharmacokinetics. In addition, novel effects of polymorphism in cytochrome P450 on the pharmacokinetics of rifampicin and ethambutol were suggested. Lastly, a framework for determination of individual doses based on pathogen susceptibility was developed. Conclusively, new dose regimens for the first-line anti-tuberculosis drugs in patients co-infected with HIV are proposed. Such regimens may reduce the risk of treatment failure, resistance development and toxicity. The drug-drug interactions and pharmacogenetic effects described within this thesis may guide the design of future clinical studies.

Keywords: Tuberculosis, HIV, pharmacokinetics, pharmacogenetics, LC-MS/MS, individualized therapy

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SAMMANFATTNING PÅ SVENSKA

Tuberkulos är den vanligaste dödsorsaken hos personer smittade med HIV. Den nuvarande förstahandsbehandlingen mot tuberkulos består av en kombination av läkemedel innehållande rifampicin, isoniazid, pyrazinamid och etambutol som ges under sex månader. Behandlingen är kliniskt effektiv men resulterar i terapisvikt hos 20 % av behandlade patienter vilket ökar risken för uppkomst av läkemedelsresistens. Kombinationsbehandlingen kan också orsaka allvarliga biverkningar hos patienter så som leverskador. Att optimera behandlingen i syfte att minska risken för terapisvikt, läkemedelsresistens och toxicitet är därför kritiskt. Målet med projekten som är inkluderade i denna avhandling var att utreda hur den nuvarande förstahandsbehandlingen mot tuberkulos kan optimeras hos patienter som är smittade med HIV. En bioanalysmetod för att mäta rifampicin, isoniazid, pyrazinamid, etambutol och deras nedbrytningsprodukter, så kallade metaboliter, i blodprover från patienter utvecklades. Farmakokinetiken (hur läkemedel absorberas, fördelas och försvinner ur kroppen) för de fyra läkemedlen och deras metaboliter definierades med hjälp av matematiska modeller. HIV-behandling, genetiska variationer och andra patientspecifika faktorer testades sedan i modellerna för att bedöma deras eventuella påverkan på läkemedelsexponering. I studierna föreslogs högre doser av etambutol på grund av generellt låg exponering hos patienterna vilket ökar risken för terapisvikt. För isoniazid och pyrazinamid rekommenderades individuell dosering hos olika patienter baserat på patientdemografi och genetik för att på så sätt reducera den höga variabiliteten i läkemedelsexponering av de båda läkemedlen. I studierna identifierades två läkemedelsinteraktioner av potentiell klinisk relevans. HIV-behandling påverkade rifampicins farmakokinetik markant och eliminering av isoniazid ökade över tid på grund av sambehandling med rifampicin. Vidare identifierades påverkan av genetiska skillnader hos läkemedelsmetaboliserande enzymer mellan patienter på tuberkulosläkemedlens farmakokinetik. Slutligen beskrevs ett nytt ramverk för individuell dosering hos patienter baserat på mätning av bakteriers känslighet för läkemedel. Sammanfattningsvis presenteras en optimerad förstahandsbehandling mot tuberkulos för patienter smittade med HIV. Denna optimerade behandling skulle kunna minska risken för terapisvikt, biverkningar och utveckling av läkemedelsresistens. Läkemedelsinteraktionerna och farmakogenetiken som beskrivs i denna avhandling kan vägleda designen av framtida kliniska studier.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Sundell J**, Bienvenu E, Birgersson S, Äbelö A, Ashton M, Hoffmann K-J. Simultaneous quantification of four first line antitubercular drugs and metabolites in human plasma by hydrophilic interaction chromatography and tandem mass spectrometry. *Journal of Chromatography B*. 2019 Jan 15;1105:129-135.

II. **Sundell J**, Bienvenu E, Janzén D, Birgersson S, Äbelö A, Ashton M. Population Pharmacokinetics and Pharmacogenetics of Ethambutol in Adult Patients Coinfected with Tuberculosis and HIV. *Antimicrobial Agents and Chemotherapy*. 2020 Jan 27;64(2):e01583-19.

III. **Sundell J**, Bienvenu E, Birgersson S, Äbelö A, Ashton M. Model-Based Assessment of Variability in Isoniazid Pharmacokinetics and Metabolism in Patients Co-Infected With Tuberculosis and HIV: Implications for a Novel Dosing Strategy. *Clinical Pharmacology and Therapeutics*. 2020 Jul;108(1):73-80.

IV. **Sundell J**, Bienvenu E, Äbelö A, Ashton M. Effect of efavirenz-based antiretroviral therapy on the pharmacokinetics of rifampicin and its primary metabolite in patients co-infected with tuberculosis and HIV. *Journal of Antimicrobial Chemotherapy*. 2021 Jul 31. *Online ahead of print*.

V. **Sundell J**, Wijk M, Bienvenu E, Äbelö A, Hoffmann K-J, Ashton M. Factors Affecting the Pharmacokinetics of Pyrazinamide and Its Metabolites in Patients Coinfected with HIV and Implications for Individualized Dosing. *Antimicrobial Agents and Chemotherapy*. 2021 Jun 17;65(7).

VI. **Sundell J**. Probability functions for susceptibility-guided precision dosing in antitubercular therapy. (*Submitted*)

VII. **Sundell J**, Bienvenu E, Birgersson S, Äbelö A, Ashton M. Time dependent pharmacokinetics and drug-drug interaction between rifampicin and isoniazid during first-line tuberculosis therapy in patients co-infected with HIV. (*Submitted*)

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CONTENT

ABBREVIATIONS

ART	Antiretroviral therapy
AUC	Area under the plasma concentration-time curve
C_{max}	Maximal plasma concentration
CYP	Cytochrome P450
HIV	Human immunodeficiency virus
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MIC	Minimal inhibitory concentration
NAT2	N-acetyltransferase 2
SLCO	Solute carrier anion transporter
SNP	Single nucleotide polymorphism
TB	Tuberculosis
VPC	Visual predictive check
WHO	World Health Organization

DEFINITIONS IN SHORT

Pharmacokinetics	What the body does to the drug including absorption, distribution, metabolism and excretion.
Pharmacodynamics	What the drug does to the body with regard to both desired effects and adverse effects.
Pharmacogenetics	A scientific field relating genetic variation to variability in exposure and/or response to drugs.
Pharmacometrics	A scientific field concerned with application of mathematical models to describe biological systems and pharmacological data.
Population pharmacokinetics	A scientific field aiming to identify and quantify sources of pharmacokinetic variability.

1 INTRODUCTION

"There is no good in anything until it is finished."

Genghis Khan

Every year, around 10 million people develop active tuberculosis (TB). According to the world health organisation (WHO), 1.4 million people died from TB in 2019 (1). Despite an antitubercular regimen, which has evolved since the 1950s, therapy fail to cure up to 20% of patients with drug susceptible TB. The four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) included in the antitubercular cocktail also display a severe toxicity profile.

The current dose regimen is partly designed to avoid the risk of liver toxicity, which has been observed in 4 – 17% of patients on first-line TB therapy (2). There is evidence suggesting that the hepatotoxicity is partly related to metabolic products, which are formed when the drugs are metabolized by the body (3-6). Since there is variability in metabolism between individuals, toxicity caused by metabolites could explain why no direct dose-toxicity relationship has been described for isoniazid or pyrazinamide (7, 8).

The first-line antitubercular therapy is used against susceptible strains of TB, meaning strains that have not developed resistance. When a TB strain is resistant to more than one of the first-line drugs it is referred to as multidrug-resistant. Patients infected with multidrug-resistant TB are treated with second-line therapy. If a TB strain is resistant to several first- and second-line agents, it is referred to as extensively drug-resistant.

Despite available therapy, multidrug-resistant TB and extensively drug-resistant TB are significantly harder to treat than susceptible TB. The duration of regimens are up to a few years and the prognosis for cure is considerably low. Treatment success is only achieved in about 50% of multidrug-resistant and extensively drug-resistant TB cases (9). Many patients on the second-line regimens also live with a reduced quality of life due to the toxicity caused by the antitubercular agents. The risk of drug-induced liver toxicity is additionally higher than during first-line treatment. Therefore, avoiding development of resistance is crucial.

About one third of the global population is estimated to be infected with TB. However, most TB-infected individuals are not affected by the disease since the infection remains inactive. Human immunodeficiency virus (HIV) infection compromises the immune system and therefore significantly increases the risk of developing active TB. In HIV-infected individuals, TB is the most common cause of death.

To decrease the risk of toxicity, treatment failure and development of resistance, the use of the first-line TB agents needs improvement. There is an overall agreement in the scientific community that higher rifampicin doses and genotype-guided dosing of isoniazid is one way forward. Initial clinical trials investigating such a dose optimization have indeed shown promising results in terms of both successful therapy and a reduced risk of toxicity (10-12).

This thesis presents new knowledge on the complexity of first-line TB therapy and offers a starting point for individualized dosing in patients co-infected with HIV. Novel strategies for treatment of TB patients based on drug metabolism, pharmacokinetic variability and bacterial susceptibility are introduced. These concepts and findings can in addition be extended to patients uninfected with HIV.

1.1 TUBERCULOSIS PATHOGENESIS

TB is caused by the bacteria *Mycobacterium tuberculosis*. About half of the individuals who become infected eliminate the bacteria. In the majority of those who do not clear the infection, TB becomes latent, also referred to as inactive TB. In some individuals however, TB becomes active and about 10 million people globally develop active TB each year. Active TB can develop directly (primary infection) or after several years when a latent TB infection progress into an active state (secondary infection). Risk factors for developing active TB include old age, malnutrition, immunosuppressing therapy (e.g. corticosteroids) and immunocompromising diseases such as diabetes or HIV (9, 13).

TB is an airborne disease. Infection is transmitted by inhalation of droplets containing the pathogen and can affect different organs of the body. However, only TB infection in the lungs (pulmonary TB) is transmittable. When the bacteria enters the lung, they are engulfed by macrophages in the alveoli. Unlike many other infectious pathogens, TB can reside inside the macrophages rendering it protected from elimination by the immune system. Gathering of

components of the immune system around the site of pulmonary infection results in formation of granulomas. Such granulomas, also termed tuberculomas, are effective at containing but not eliminating the pathogen (14).

1.2 TUBERCULOSIS AND HIV CO-INFECTION

TB is the most common cause of death in HIV-infected individuals. HIV targets and kills CD4 cells, which play a crucial role in the immune system. As the number of CD4 cells decline, the host becomes increasingly susceptible to other infections. If left untreated, the host eventually progresses to develop acquired immune deficiency syndrome (AIDS). HIV-infected individuals are therefore at a significantly higher risk of developing active TB during a primary infection. The risk of a TB infection progressing from latent to active is also increased. According to the WHO, HIV-infected individuals are 20-fold more likely to develop active TB than individuals uninfected with HIV (9).

1.2.1 CLINICAL MANAGEMENT OF TUBERCULOSIS

TB is curable and the first-line TB therapy consists of rifampicin (10 mg/kg), isoniazid (5 mg/kg), pyrazinamide (25 mg/kg) and ethambutol (15 mg/kg) administered once daily for eight weeks followed by 16 weeks of rifampicin and isoniazid alone (15). Streptomycin can be added to the regimen if one of the first-line drugs is contraindicated. Rifampicin and isoniazid exerts the main bactericidal effect whereas pyrazinamide exhibits a sterilizing effect by killing semi-dormant bacteria (16). By adding pyrazinamide to the rifampicin-isoniazid regimen, the duration of therapy was reduced from nine months to six months. The main purpose of ethambutol is to protect against pre-existing mono-resistance to one of the other antitubercular drugs in the cocktail (16).

There are several second-line antitubercular agents, which may be used against multidrug-resistant TB. However, since these agents are less effective than the first-line drugs, the duration of therapies involving second-line agents are commonly 18-24 months. Second-line drugs are also associated with more severe adverse events and the regimens are more expensive.

1.2.2 CLINICAL MANAGEMENT OF HIV

HIV is currently not curable wherefore HIV therapy is lifelong. The goal of HIV therapy is thus to increase quality of life and reduce HIV-associated morbidity and transmission of HIV infection. The drugs used for such purposes are categorized by their mechanism of action, which all reduce viral load by interrupting the HIV life cycle. Both nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) prevents HIV from replicating by blocking reverse transcriptase. Two NRTIs and one NNRTI are recommended as first-line antiretroviral therapy (ART). Until recently, WHO recommended tenofovir and lamivudine (NRTIs) combined with efavirenz as the preferred NNRTI (17). However, updated guidelines recommend dolutegravir to be used as the NNRTI backbone in newly diagnosed HIV patients (18). Efavirenz is currently recommended as an alternative first-line NNRTI.

1.2.3 CLINICAL MANAGEMENT OF TUBERCULOSIS AND HIV CO-INFECTION

In TB/HIV co-infected patients with no prior HIV diagnosis, TB treatment is initiated directly, and ART is started within the first eight weeks of the antitubercular regimen. If patients have a CD4 cell count of less than 50 cells/mm^3, ART should be started within the first weeks of initial TB treatment (15). The rationale for delaying ART in co-infected patients is based on overlapping adverse events, drug-drug interactions, immune reconstitution inflammatory syndrome and avoidance of high pill burden, which may discourage adherence. TB treatment is started directly in co-infected patients with newly diagnosed TB who are already on HIV treatment.

1.3 DRUG SUSCEPTIBILITY OF INFECTIOUS PATHOGENS

The susceptibility to a drug of an infectious strain can be measured by the minimal inhibitory concentration (MIC). The MIC is the lowest concentration of a drug at which there is no visible growth of a pathogen. The cut-off between susceptible and resistant strains is referred to as the susceptibility breakpoint. If the MIC for a pathogen is below the breakpoint, the pathogen is considered

susceptible to the drug. On the other hand, if the MIC is above the breakpoint, the pathogen is considered resistant to the drug.

The susceptibility breakpoints for rifampicin (1 mg/L), isoniazid (0.2 mg/L), pyrazinamide (100 mg/L) and ethambutol (5 mg/L) were introduced in the 1960s (19). However, these breakpoints have recently been challenged and suggested to be lower (19-21). In addition, introduction of an intermediate susceptible MIC range has been proposed (20).

1.4 PHARMACOKINETICS, PHARMACODYNAMICS AND PHARMACOGENETICS

Pharmacokinetics describes the disposition of drugs in the body (i.e. what the body does to the drug) and can be subcategorised into the absorption, distribution, metabolism and excretion of drugs. Primary parameters such as drug clearance and volume of distribution are used as metrics to define the disposition. Secondary parameters including maximal concentration (C_{max}) and area under the plasma concentration-time curve (AUC) are used as measurements of exposure to the drug.

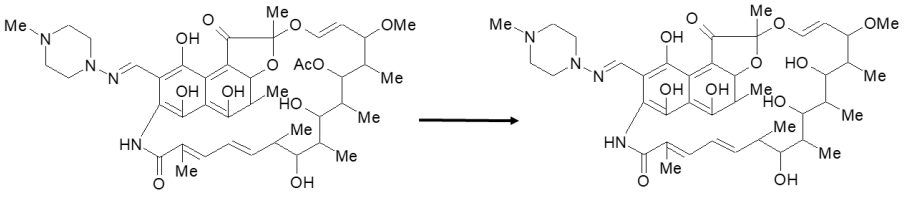
Pharmacodynamics describes both therapeutic and toxic effects following administration of a drug (i.e. what the drug does to the body and/or pathogen). Such effects may be quantified directly or via biomarkers to define a pharmacodynamic response. In drug development, exposure-response relationships are used to determine clinical doses. If an exposure-response relationship has been described for a drug, a therapeutic exposure target associated with a defined effect may be used in individual patients. Such an approach is used in therapeutic drug monitoring where drug exposure is quantified in individual patients for dose adjustments (22, 23).

1.4.1 PHARMACOKINETICS OF THE FIRST-LINE ANTITUBERCULAR DRUGS

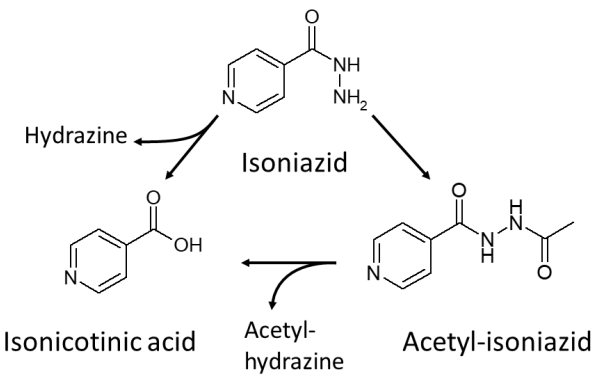
The first-line antitubercular drugs are normally absorbed rapidly with maximal concentrations achieved 1-4 hours post dose. The disposition may be altered by HIV infection since low exposure to the first-line TB drugs has been

reported in patients co-infected with HIV (24, 25). However, there are contradictive results in the literature and another study did not confirm such an effect by HIV-infection (26). Other factors affecting the disposition of the first-line drugs include sex, genetics and diabetes (27-31).

Rifampicin is converted to its major metabolite 25-desacetylrifampicin via esterases (32). The elimination of rifampicin is autoinduced (i.e. repeated administration of rifampicin increases its own elimination over time) via unknown elimination pathways (33). In addition, the bioavailability of rifampicin is both time- and dose-dependent (33, 34).



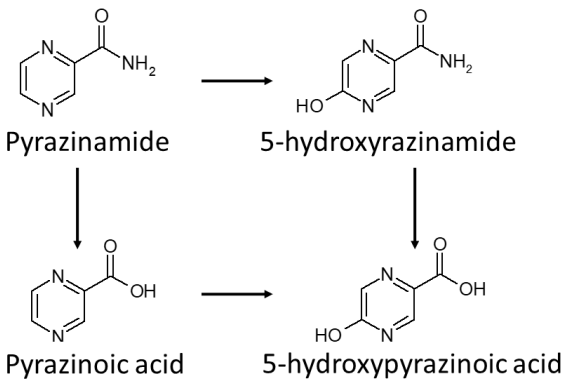
Rifampicin metabolism.



Isoniazid metabolism.

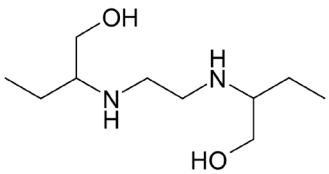
Isoniazid is mainly metabolized via two hepatic pathways. Its two primary metabolites, acetyl-isoniazid and isonicotinic acid, are formed via acetylation by N-acetyltransferase 2 and hydrolysis, respectively (35, 36). Furthermore, hydrazine and acetyl-hydrazine are formed by direct and indirect metabolism of isoniazid (37, 38). These hydrazine metabolites have been suggested to be involved in isoniazid-induced hepatotoxicity (3-5).

Pyrazinamide metabolism results in the formation of pyrazinoic acid and 5-hydroxypyrazinamide (39). Both metabolites are eliminated by further conversion to 5-hydroxypyrazinoic acid or by renal excretion (40). 5-hydroxypyrazinoic acid has been proposed to be responsible for hepatotoxicity caused by pyrazinamide administration (6).



Pyrazinamide metabolism.

Following oral administration, 50 to 70% of an ethambutol dose is excreted via the urine. In addition to renal elimination, approximately 15-25% is converted to inactive metabolites (41, 42). The main enzyme involved in ethambutol metabolism is alcohol dehydrogenase resulting in the formation of a dicarboxylic product via an aldehyde intermediate (42).



Ethambutol.

1.4.2 PHARMACODYNAMICS OF THE FIRST-LINE ANTITUBERCULAR DRUGS

Rifampicin, isoniazid and pyrazinamide are bactericidal whereas ethambutol is bacteriostatic. Rifampicin inhibits RNA polymerase blocking the transcription of bacteria (43). Isoniazid and ethambutol inhibit synthesis of mycolic acid and arabinogalactan, respectively, which are both components of the bacterial cell wall (44, 45). Pyrazinamide acidifies the cytoplasm and inhibits translation (46).

Pharmacokinetic-pharmacodynamic relationships of specific TB drugs are intrinsically challenging to study in a clinical setting due to the administration of multidrug regimens. In addition, the susceptibility of different strains in different patients affect the efficacy of therapy in infectious disease (47). Pre-clinical *in vitro* and animal studies have therefore commonly been used to identify exposure-response relationships. The pharmacodynamic parameters identified in such pre-clinical studies have been demonstrated to correlate well with those estimated in clinical studies (48).

Several studies have investigated exposure to first-line TB drugs in relationship to positive clinical outcome (commonly defined as cure without relapse). In two clinical studies, low exposure to pyrazinamide was associated with poor outcome (25, 49). Low exposure to rifampicin and isoniazid was further associated with an even higher risk of treatment failure and acquired drug resistance in one of the studies (49). Low rifampicin and isoniazid concentrations have also been related to a prolonged effect by the antitubercular regimen (50). A meta-analysis suggested that pharmacokinetic variability of isoniazid resulting in different exposure was associated with poor treatment outcome and acquired drug resistance (51).

A recent clinical study investigated the effect of drug exposure and pathogen susceptibility on pharmacodynamic response, outcome and adverse events (52). The results suggested pyrazinamide and rifampicin AUC/MIC ratios to be predictive of the time course of therapy effect and successful clinical outcome, respectively. Isoniazid and rifampicin AUCs were associated with drug induced liver injury and acute kidney liver injury, respectively.

Another approach for studying exposure-response for multidrug regimens is to compare the pharmacodynamic responses between standard therapy and a regimen where the dose for one of the drugs has been modified. Clinical trials have evaluated the safety and efficacy of high dose rifampicin in comparison to standard rifampicin doses (10, 11). Using a pharmacometric approach,

results suggested that higher doses of rifampicin might be able to reduce the duration of TB therapy (53).

1.4.3 PHARMACOGENETICS

Pharmacogenetics is a field relating genetic variations to variability in the pharmacokinetics and/or pharmacodynamics of drugs (54). Such genetic variations may affect the bioavailability, elimination and distribution of a drug. The studies included in the present thesis focused on variability in genetics (i.e. single nucleotide polymorphism (SNP)) affecting the pharmacokinetics of the first-line TB drugs.

A SNP is defined as a variation at a single nucleotide position in the DNA. If a nucleotide differs from the reference genome on one of the homologous chromosomes, it is referred to as a heterozygous mutation. If the nucleotides differ from a reference genome on both homologous chromosomes, it is referred to as a homozygous mutation. A mutation may affect the transcription or function of a metabolic enzyme or transporter and may therefore influence, for example, the metabolism of endogenous compounds or drugs. Among the first-line antitubercular drugs, impact of SNPs on drug exposure has been described for rifampicin and isoniazid.

SLCO1B1 is a gene coding for the solute carrier organic anion transporter 1B1. The transporter is involved in hepatic cellular uptake of compounds. SNPs in SLCO have been associated with lower rifampicin exposure (29, 55). For isoniazid, the acetylation pathway is polymorphic and an established effector of isoniazid clearance (38). Polymorphism in the responsible enzyme, N-acetyltransferase 2, has been associated with six SNPs, which in combination can be used to classify acetylator status into slow, intermediate or rapid acetylator (56).

1.4.4 DRUG-DRUG INTERACTIONS

Drug-drug interactions are defined as one drug affecting the pharmacokinetics and/or pharmacodynamics of another drug when two drugs are co-administered. A pharmacokinetic drug-drug interaction may be clinically relevant if the exposure of the affected drug decreases or increases substantially resulting in a lower or higher efficacy, respectively. Alterations

in the efficacy and/or toxicity profile of a drug may also be the result of a change in the exposure to an active or toxic metabolite. In co-treatment of TB and HIV, there is a significant risk of drug-drug interactions since several drugs are administered simultaneously and some of the included drugs (e.g. rifampicin and efavirenz) are known to affect the disposition of other drugs (57, 58).

A drug may increase the exposure to another drug by inactivation of the metabolic enzyme responsible for metabolism of the victim drug (i.e. inhibition). A drug may also decrease the exposure to another drug by induction of metabolic enzymes resulting in an increased elimination and/or decreased bioavailability of the victim drug. Induction is a mechanism designed to regulate levels of endogenous or exogenous substances in the body. Typically, the inducing drug binds to a transcriptional regulator (e.g. pregnane X receptor) which, in turn activates transcription of drug-metabolizing enzymes. The increased abundance of enzymes results in an increased capacity to eliminate substances. Rifampicin induces several enzymes via the pregnane X receptor pathway, increasing its own elimination (i.e. autoinduction) and the metabolic elimination of other drugs (59).

1.4.5 TOXICITY OF THE FIRST-LINE TUBERCULOSIS DRUGS

The most serious adverse event during first-line antitubercular therapy is hepatotoxicity, which has been reported in 4 – 17% of patients (2). Drug-induced liver injury is associated with rifampicin, isoniazid and pyrazinamide administration. However, no relationship between hepatotoxicity induced by isoniazid or pyrazinamide and dose has been described (7, 8). The lack of relationship to dose is plausibly due to accumulation of metabolites causing the liver toxicity (3-6). With regard to rifampicin, initial studies of higher doses (i.e. up to 35 mg/kg) indicates a safety profile similar to that of standard rifampicin doses (11, 60).

The toxicity of TB therapy and ART is overlapping. HIV infection itself has been reported to increase the risk of developing drug-induced liver injury during TB therapy (61, 62). One study reported higher incidence of serious adverse events in patients co-infected with TB and HIV compared to HIV-uninfected patients (63). However, no difference in drug-induced hepatotoxicity was observed. Nevertheless, concomitant administration of ART and TB therapy has been demonstrated to increase the risk of

hepatotoxicity (64). Additionally, female sex, older age and lower weight have been reported as risk factors for drug-induced liver injury during TB therapy (65-67). Furthermore, although debated, slow acetylator status has been associated with higher incidence of isoniazid-induced liver toxicity (38).

Optic neuropathy is a concern during therapy including ethambutol and a dose-dependency in the ocular toxicity profile of ethambutol has been described (68). The visual loss following therapy containing ethambutol is generally considered reversible with prompt withdrawal of ethambutol. However, irreversible visual loss in TB patients has been observed in a few smaller clinical studies, fuelling a debate on reversibility of reduced vision following ethambutol administration (69, 70).

1.4.6 THERAPEUTIC TARGETS

A therapeutic target is a quantifiable target associated with an adequate efficacy and/or safety of a drug. In infectious diseases, such targets can be based on either drug exposure (e.g. AUC) or drug exposure relative to the susceptibility of the pathogen (e.g. AUC/MIC ratio). The target is typically correlated to a pharmacodynamic response (e.g. an AUC/MIC of 567 for isoniazid is associated with 90% of the maximal kill rate of bacteria). The therapeutic thresholds that have been suggested for the first-line antitubercular drugs in different studies (both exposure-based and AUC/MIC-based) are summarised in **Table 1**.

Table 1. Suggested therapeutic targets of first-line antitubercular drugs.

Drug	C_{max} (mg/L)	AUC (h*mg/L)	AUC/MIC	Reference
Rifampicin	> 8	181 – 214[a]	271, 435	(22, 52, 71-73)
Isoniazid	3 – 6	10.5	567	(22, 74, 75)
Pyrazinamide	20 – 60	363	11.3	(22, 49, 76)
Ethambutol	2 – 6	-	-	(22)

C_{max}; maximal plasma concentration, AUC; area under the plasma concentration-time curve, MIC; minimal inhibitory concentration. [a] Suggested target for therapeutic drug monitoring based on rifampicin doses higher than current standard doses.

1.5 BIOANALYSIS

Bioanalysis generally refers to quantitative concentration measurement of drugs, metabolites or endogenous compounds in biological matrices (e.g. blood or plasma). Bioanalytical methods include two main components: sample preparation and detection of the target compounds. The sample preparation aims to make the samples cleaner (e.g. remove proteins) for improved detection and to avoid compromising the bioanalytical system. Protein precipitation, liquid-liquid extraction or solid phase extraction are commonly used for such a purpose.

Robust and precise bioanalytical methods are crucial in pharmacokinetic/pharmacodynamics studies and drug development. Due to its high specificity and sensitivity, liquid chromatography tandem mass spectrometry (LC-MS/MS) has become the gold standard detection method in bioanalysis. The system combines the separation power of LC and the mass-specific detection by the MS instrument. Selected compound fragmentation in the MS/MS mode adds additional sensitivity to the detection system. Several drugs are administered during TB/HIV co-infection wherefore a specific bioanalytical method is required for quantification of the first-line TB drugs and their metabolites.

LC-MS/MS methods for quantification of first-line antitubercular drugs have been described in the literature (77-86). However, the majority of the methods focus on determination of concentrations of one or a few of the parent drugs. No method for simultaneous quantification of the four first-line TB drugs and their major metabolites has been described.

1.6 PHARMACOMETRICS

Pharmacometrics, the science of quantitative pharmacology, is an inter-disciplinary science, which includes elements from mathematics, statistics, physiology, pharmacology and medicine.

Pharmacometrics can be defined as a field where mathematical models are applied to describe and quantify biological and/or pharmacological processes in a system (e.g. the human body). Application of pharmacometric models is also sometimes referred to as population modelling, modelling and simulation (M&S) or pharmacokinetic/pharmacodynamic (PK/PD) modelling. The models combined with a set of parameters can be used to explore changes in

the system due to certain events (e.g. what happens if the dose is increased) also referred to as simulations. Typically, a pharmacometric model which describes pharmacokinetic data consists of a network of differential equations which describes how drug plasma concentrations (dependent variable) changes over time (independent variable). The plasma concentration may further be linked to pharmacodynamic effects known as the exposure-response relationship.

Population pharmacokinetic studies are concerned with variability in drug exposure and identification and quantification of sources of such a variability. The pharmacometric studies included in this thesis focused on population pharmacokinetics. However, exposure-response relationships and bacterial susceptibility to drugs were taken into account during simulations.

1.6.1 NON-LINEAR MIXED EFFECTS MODELS

In pharmacometric studies, non-linear mixed effects (NLME) models are commonly utilized. Mixed effects models include fixed effects parameters describing a central value of parameters, and random effects describing the variability in the parameters (87).

For continuous data, a dependent variable *y* (e.g. plasma concentration) for the i:th individual and j:th observation can be described as a function (f()) of independent variables *x* (e.g. time and dose) and individual parameters θ (e.g. drug clearance and volume of distribution) according to:

$$y_{i,j} = f\left(x_{i,j}, \theta_i\right) + \varepsilon_{i,j}$$

where ε is the residual error which describes the deviation between the model prediction and observed value.

A NLME model can be categorized into three components: a structural model, a statistical model and a covariate model. A structural model describes the central tendency of the data (i.e. the typical individual). As an example of a structural model, the following equation describes a pharmacokinetic one-compartment model after oral administration:

$$c_{p,t} = \frac{D * \theta_F}{\theta_V}\left(\frac{\theta_{ka}}{\theta_{ka} - \frac{\theta_{CL}}{\theta_V}}\right)\left(e^{-\frac{\theta_{CL}}{\theta_V} * t} - e^{-\theta_{ka} * t}\right)$$

where $c_{p,t}$ is the plasma concentration at time after dose (t), D is the dose, F is the oral bioavailability, V is the volume of distribution, CL is the clearance, and k_a is the absorption rate constant.

The statistical model contains elements describing the variability in the data. Such a variability can further be categorized into inter-individual variability, between occasion variability and residual variability. The inter-individual variability describes differences in individual pharmacokinetic profiles by describing differences in model parameters and is commonly applied in models as an exponential relationship according to:

$$\theta_i = \theta_P * e^{\eta_i}$$

$$\eta_i \sim N(0, \omega^2)$$

where θ_i is the parameter of the i:th individual, θ_P is the population mean of the parameter and η_i is the inter-individual variability. η_i is assumed to be normally distributed with a mean of 0 and variance of ω^2. Due to the exponential relationship of η_i, the parameter becomes log-normally distributed.

Between occasion variability occur within the same individual due to differences in parameters following different dosing occasions. Such a variability may arise from that one individual, for example, had a meal when taking one dose, but was fasting when taking another dose resulting in an altered absorption rate. Between occasion variability may be applied according to:

$$\theta_{i,k} = \theta_P * e^{\eta_i + \kappa_k}$$

$$\kappa_k \sim N(0, \pi^2)$$

where κ_k is the between occasion variability for the k:the occasion. κ is assumed to be normally distributed with a mean of 0 and variance of π^2.

The residual variability is unexplained deviations from the individual predicted profile and is typically applied as an additive, proportional or combined residual error:

$$y_{i,j} = IPRED_{i,j} + \varepsilon_{i,j} \quad \text{Additive error}$$

$$y_{i,j} = IPRED_{i,j} + IPRED * \varepsilon_{i,j} \quad \text{Proportional error}$$

$$y_{i,j} = IPRED_{i,j} + \varepsilon_1 + IPRED * \varepsilon_2 \quad \text{Combined error}$$

where y is the j:th observation for the i:th individual, IPRED is the individual prediction and ε is the difference between the individual observation and individual prediction.

The covariate model accounts for differences in parameters that can be explained by known patient characteristics (e.g. genotype or weight). A covariate is a fixed effects parameter that may partly explain variability in a pharmacokinetic or pharmacodynamic parameter. A covariate effect on a parameter due to a categorical covariate (e.g. genotype) is commonly applied as a difference relative to the most frequent parameter estimate. Alternatively, the parameter can be estimated separately for each subgroup. Continuous covariates such as weight or quantifiable markers of kidney function are typically centred or normalized around the population median with an effect relative to the median covariate value. For certain covariates there are established covariate - parameter relationships such as for weight and drug clearance or volume of distribution (i.e. allometric scaling) (88, 89). Therefore, allometric scaling can be tested in models without estimation of additional parameters.

2 AIM

The overall aim of the work presented within this thesis was to suggest an optimized standard first-line antitubercular regimen in patients co-infected with HIV.

Specific aims were to:

1. Develop a bioanalytical method for simultaneous quantification of first-line TB drugs and their metabolites in plasma.
2. Describe the population pharmacokinetics and metabolism of first-line drugs in patients co-infected with TB and HIV.
3. Describe and quantify potential effects of efavirenz-based ART on the pharmacokinetics of first-line TB drugs and their metabolites.
4. Evaluate patient characteristics and genotypes applicable for individualized dosing.
5. Suggest optimized dosing strategies for the first-line TB drugs in patients co-infected with HIV.

3 PATIENTS AND METHODS

The present thesis is based on material from a clinical study conducted in Rwanda. The study was conducted in agreement with the principals described by the Helsinki Declaration and International Conference on Harmonization guidance for Good Clinical Practice and the study protocol was approved by the National Ethics Committee of the Ministry of Health in Rwanda.

3.1 PATIENTS AND STUDY DESIGN

The clinical study was an observational, open-label study conducted in four clinics. Samples and patient data from 63 patients were used in the studies included in this thesis. Inclusion criteria were: TB diagnosis, new or prior diagnosis of HIV, TB therapy naïve, age of 21 – 65 years, literacy in Kinyarwanda, English or French and a signed consent to participate in the study. Patients were either on concomitant efavirenz-based ART (n = 23) or ART naïve (n = 40). In addition to efavirenz, HIV therapy consisted of lamivudine and zidovudine or tenfovir.

Patients were initiated on TB therapy according to national guidelines on TB treatment. Rifampicin-based fixed-dose combinations containing 150 mg of rifampicin, 75 mg of isoniazid, 400 mg of pyrazinamide and 275 mg of ethambutol were administered according to the following weight-bands: 20 – 28 kg: 1.5 tablets, 29 – 37 kg: 2 tablets, 38 – 54 kg: 3 tablets, 55 – 70 kg: 4 tablets and >70 kg: 5 tablets. Eight patients were administered streptomycin in addition to the four first-line TB drugs. Blood samples for drug quantification were collected prior to dose and at 1, 2, 3, 4, 6 and 8 hours post dose following the first dose and at steady state of antitubercular therapy for HIV therapy naïve patients (arm A). For patients on HIV therapy, sampling was performed only when TB therapy was initiated (arm B). Sampling has been illustrated in **Figure 1**.

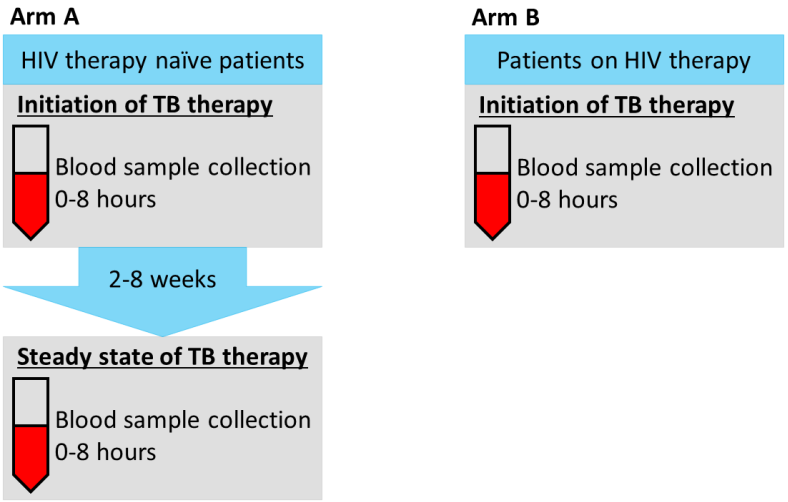


Figure 1. Overview of blood samples collected for pharmacokinetics analysis in the clinical study.

3.2 BIOANALYSIS

Patient plasma concentrations of rifampicin, 25-desacetylrifampicin, isoniazid, isonicotinic acid, acetyl-isoniazid, ethambutol, pyrazinamide and 5-hydroxypyrazinamide were determined by a LC-MS/MS method (**Paper I**). The method was modified to assess high concentrations of pyrazinamide and to quantify pyrazinoic acid and 5-hydroxypyrazinoic acid plasma concentrations (**Paper V**).

3.2.1 INSTRUMENTATION

The LC-system consisted of a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) connected to two PE-200 LC pumps (Perkin Elmer, Waltham, MA, USA). Chromatographic separation was carried out on an Inertsil HILIC column (75mm×2.1 mm, 3 µm, GL Sciences, Tokyo, Japan). Mobile phases consisted of methanol and water containing formic acid as described in **Paper I** and **Paper V**, respectively. Detection of analytes was achieved by multiple reaction monitoring using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems Sciex, Framingham, MA, US) with an electrospray ionization source operating in positive mode. MS configurations were optimized for accurate fragmentation of the molecules in the collision

cell by direct infusion of each substance using a Harvard syringe pump (Harvard Apparatus, Holliston, MA, USA).

3.2.2 SAMPLE PREPARATION

Samples were prepared using a sequential liquid-liquid extraction. Methanol containing the two internal standards phenformin and rifaximin was added to calibrator, QC or plasma sample. Ethyl acetate was added, followed by mixing and centrifugation of the sample. Following removal of the supernatant (sample A), acidified ethyl acetate was added to enhance extraction of isonicotinic acid and 5-hydroxypyrazinamide. The sample was mixed and centrifuged. The supernatant was then removed and combined with sample A and dried under a gentle flow of air at 40^{o} C. The sample was reconstituted with water containing 0.3% formic acid, mixed and centrifuged. The supernatant was injected into the LC-MS/MS system.

The sample preparation method was modified to extract pyrazinoic acid and 5-hydroxypyrazinoic acid (**Paper V**) by adding hydrochloric acid (1 mM) prior to extraction with ethyl acetate. Therefore, acidified ethyl acetate was not required in the extraction steps. Further, the final reconstituted sample was diluted 5-fold with water prior to injection into the LC-MS/MS instrument.

3.2.3 VALIDATION

Method validation was performed with regard to intra- and inter-day accuracy and precision, lower limit of quantification, selectivity, stability, recovery and matrix effect according to FDA recommendations (90).

3.3 GENOTYPING

Genotyping of 20 SNPs of metabolic enzymes and drug transporters (NAT2 282 C>T, 803A>G, 481C>T, 590G>A, 857G>A and 341T>C, CYP2C19*17, *2 and *3, CYP2E1 1053T>G, 1293G>C, 71G>T and 7632T>A, SLCO1B1 463C>A, 388A>G, 11187G>A, rs4149032, 521T>C and 1436G>C, and SLCO1B3 334T>G) was performed for 56 patients. Blood samples for genotyping from seven patients were not available. The genotyping has been described in detail in **Papers II, III, IV and VII**. Genotyping of other CYP

genotypes including CYP1A2 has been described in a previous study by Bienvenu *et al* (91).

Acetylator status was determined by combining N-acetyltransferase 2 genotypes using an accessible web server algorithm (56). Acetyl-isoniazid/isoniazid exposure ratio was utilized to determine acetylator status in patients with missing genotypes (92, 93). The exposure ratios in slow, intermediate and rapid acetylators in the present cohort was compared to reference ratios for the respective phenotypes to ensure adequacy of assigned acetylator statuses. Patients were assigned intermediate (*1/*2 or *2/*17), extensive (*1/*1 or *1/*17) or ultra-rapid (*17/*17) CYP2C19 phenotypes based on combined genotypes (94).

3.4 PHARMACOKINETIC ANALYSIS

3.4.1 SOFTWARE

Non-linear mixed effects modelling of pharmacokinetic data was performed in NONMEM version 7.4 (Icon Development Solutions, Ellicott City, MD, USA) (95). First order conditional estimation with interaction method was used to fit models to plasma concentration-time observations. Perl-Speaks-NONMEM version 4.8.1 (Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden) was used for model automation and interaction with NONMEM. R version 3.4.1 – 4.0.3 was used for model diagnostics (R Foundation for Statistical Computing, Vienna, Austria). Wolfram Mathematica version 11.2 (Champaign, IL) was used for the identifiability analysis performed in **Paper III**. Simulations were performed in NONMEM (**Paper II and Paper III**) and R (**Paper IV – Paper VII**), respectively.

3.4.2 POPULATION PHARMACOKINETIC MODELLING

One- and two-compartment disposition models with first-order absorption and elimination were evaluated to describe the plasma-concentration time observations. In **Paper IV**, a semi-mechanistic model to estimate hepatic elimination was evaluated for rifampicin. Structural models for parent drugs

were evaluated prior to simultaneous modelling of drug and metabolite observations. Discrimination between models was based on reduction in objective function value (OFV). A reduction in OFV of -3.84 and -6.63 is equivalent to a statistically significant model improvement by p = 0.05 and p = 0.01, respectively. Model parameters were evaluated by precision, plausibility and comparison to parameters included in published models on the respective drugs.

Different absorption models including lag time, and transit compartment models with a fixed or estimated number of compartments were tested (96). Inter-individual variability was applied on model parameters as described in the introduction. In **Paper VII**, between occasion variability was evaluated on parameters of the absorption models including the relative bioavailability. Additive, proportional or combined error models were evaluated to describe the residual error. In **Paper II** the dependent variable was log-transformed. Therefore, the residual error was modelled as an additive error, equivalent to a proportional or exponential error on a non-transformed scale. Allometric scaling by body weight on all clearance and volume parameters with a power of 0.75 and 1, respectively was applied in all models. Bioavailability fixed at 1 with an estimated inter-individual variability was included in all models.

Disposition of metabolites were evaluated as one- and two-compartment models. The volume of distribution of metabolites were fixed to previously estimated values to ensure identifiability. In the rifampicin models, the volume of 25-deascetylrifampicin was assumed to be the same as the volume of rifampicin (**Paper IV** and **Paper VII**). In **Paper V**, all elimination of pyrazinamide was assumed to lead to the formation of its primary metabolites. Isoniazid was assumed to be eliminated via two or three pathways out of which two resulted in the formation of its two primary metabolites (**Paper III and Paper VII**). Addition of a third elimination pathway was tested as a fixed fraction of the total isoniazid clearance described in the literature (97). Elimination of rifampicin was described as two separate pathways. One pathway lead to the formation of 25-desacetylrifampicin (**Paper IV and Paper VII**).

Induction of drug clearances (**Paper VII**) were modelled as functions of time according to:

$$CL_t = CL_{FD} * (1 + Induction)$$

where CL_t is the clearance at time t and CL_{FD} is the clearance following first dose. The induction was defined as:

$$Induction = \frac{IND_{max} * time}{IND_{50} + time}$$

IND_{max} is the maximal induction and IND_{50} is the time after first dose required to achieve 50% of the maximal induction during daily administration. The change in relative bioavailability of rifampicin from first dose to steady state (**Paper VII**) was described as:

$$F_t = F_{SS} - (F_{SS} - F_{FD}) * e^{-k_{out}*t}$$

Where F_t, F_{SS} and F_{FD} is the bioavailability at time t, steady state and first dose, respectively, and k_{out} is the induction rate constant.

Visual predictive checks were performed for final models to assess model performance of predicting observations. Non-parametric bootstraps or sample importance resampling were performed to estimate parameter precisions and confidence intervals.

3.4.3 COVARIATE EVALUATION

The effect of patient characteristics, polymorphism in CYP enzymes, NAT2 and SLCO transporters and concomitant HIV therapy was evaluated on pharmacokinetic parameters. Covariate evaluation was performed by forward addition of covariates followed by backward elimination. Covariates were added and retained in the models at significance thresholds of p = 0.05 and p = 0.01, respectively. Continuous covariates were tested as linear relationships on pharmacokinetic parameters centred around the median. If the effects were statistically significant, power and exponential relationships were explored.

During the initial screening of genotype effects on the pharmacokinetic parameters, patients with missing genotypes were assigned to a separate group. For genotypes that had a statistically significant effect, patients with missing genotypes were assigned the most frequent genotype. In **Paper IV**, mixture models estimating the proportion of patients with missing genotypes most likely to be carriers of a specific genotype (e.g. wild-type or heterozygous/homozygous mutation) were evaluated (98). Genotype probability was then checked by evaluating individual parameters computed by the covariate model. Covariates included in final models were evaluated by covariate-parameter relationship plausibility, reduction in inter-individual variability and precision of the covariate effect.

3.4.4 SIMULATIONS

Final models were used to simulate exposure differences comparing the standard first-line regimen to alternative regimens (e.g. changes in dose or dosing interval). Previously described drug exposures associated with adequate clinical outcome were used as target exposures. If targets included both exposure and MIC, distribution of wild-type MICs were taken into consideration.

Stochastic simulations for typical individuals weighing 50 kg (the median weight of the cohort) accounting for inter-individual variability were performed in **Paper II** to **Paper VI**. In **Paper VII**, simulations were performed using mean parameter estimates only to illustrate exposure trends over time.

Rifampicin pharmacokinetics is both dose- and time-dependent. Rifampicin bioavailability increases with higher doses and both bioavailability and clearance is autoinduced (33). Therefore, to simulate exposure following high dose rifampicin (up to 35 mg/kg) in **Paper IV**, estimates from a previously described pharmacokinetic model for rifampicin (33) were used to describe the increase in bioavailability with higher doses according to:

$$F_D = F_{450} * \left(1 + \frac{F_{max} * (Dose - 450)}{FD_{50} + (Dose - 450)}\right)$$

Where F_D is the estimated bioavailability for dose D, F_{450} is the relative bioavailability for a dose of 450 mg (assumed to be 1), F_{max} (0.5) is the maximal increase in bioavailability, and FD_{50} (67 mg) is the increase in dose from 450 mg corresponding to 50% of the maximal increase in bioavailability.

3.4.5 PROBABILITY OF TARGET ATTAINMENT ANALYSIS

Probability of target attainment (PTA) analysis was performed by simulating exposure in patients and calculating the probability of achieving a therapeutic target (i.e. AUC/MIC) dependent on MIC and dose. In **Paper VI**, the drug exposure data was derived from simulations with the isoniazid model developed in **Paper III** and a rifampicin model which was developed based on data from doses ranging from 10 mg/kg to 35 mg/kg (33). MIC distributions from patient isolates (21, 47) were used to calculate PTA depending on MIC.

To describe the relationship between two independent variables (MIC and dose) and one dependent variable (PTA) the following equation was developed:

$$PTA = 1 - \frac{MIC^{\lambda}}{MIC^{\lambda} + PMIC_{50}^{\lambda}}$$

where λ is the slope factor and $PMIC_{50}$ is the MIC at which PTA corresponds to 0.5. The therapeutic target is AUC/MIC where AUC is proportional to dose (D), bioavailability (F) and drug clearance (CL) according to:

$$\frac{AUC}{MIC} = \frac{\left(\frac{D * F}{CL}\right)}{MIC}$$

The probability to achieve the therapeutic target will therefore increase with higher doses. Assuming linear pharmacokinetics, a parameter (k) describing the linear relationship between $PMIC_{50}$ and dose can thus be implemented:

$$PMIC_{50} = D * k$$

If individualized doses are used, the relationship between $PMIC_{50}$ and dose can instead be described as a fractional change from the individual dose according to:

$$PMIC_{50} = f_D * k$$

where f_D is 1 if the suggested individualized dose is used and which can be modified based on a quantified MIC.

4 RESULTS AND DISCUSSION

4.1.1 BIOANALYSIS (PAPER I)

Although several methods for quantification of the first-line antitubercular drugs have been described, no single method included quantification of all four first-line drugs and their primary metabolites. A LC-MS/MS method for such a purpose was therefore developed. The method was further modified in **Paper V** to enable quantification of the pyrazinamide metabolites pyrazinoic acid, 5-hydroxypyrazinoic acid and high plasma concentrations of pyrazinamide. During method development, several sample preparation methods and columns were evaluated. Chromatographic conditions with regards to mobile phases were assessed. Further, potentials and settings of the mass spectrometer were optimized for each compound. Phenformin and rifaximin were used as internal standards. Both compounds are inexpensive and commercially available.

In the final method, samples were prepared by a sequential liquid-liquid extraction consisting of two extractions (one under neutral conditions and one under acidic conditions) followed by evaporation of the organic phase and reconstitution of the sample residue. Such a sample preparation is time consuming in comparison to commonly used protein precipitation and may limit the use of the described method for therapeutic drug monitoring. However, the developed sample preparation method enhanced recovery of all compounds and thus optimized quantification of lower concentrations of the investigated drugs and metabolites.

The method was accurate, precise and specific according to standards for bioanalytical methods recommended by the FDA (90). Results from the inter- and intraday accuracy and precisions for all compounds included in both the original method and the modified method are summarized in **Table 2**. Stability testing demonstrated adequate stability of all included compounds during handling, storage and preparation of plasma samples. Moreover, the recovery of the compounds was consistent. Notably, the method was validated over plasma concentration ranges expected in patients although plasma levels of 5-hydroxypyrazinamide were below the lower limit of quantification (**Paper V**). Further, no interference from HIV drugs was observed in the chromatograms when analysing pre-dose samples from patients on HIV therapy. Results from the full validation can be found in **Paper I**. The robustness and specificity of the method demonstrated suitability for drug-drug interaction studies.

Table 2. Intraday and inter-day accuracy and precision of all compounds.

Compound	Nominal concentration (ng/mL)	Intraday (n = 5)		Inter-day (n = 5)	
		Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Rifampicin	200	97.1	10.3	91.5	10.5
	500	99.9	4.8	94.3	8.1
	10,000	98.7	2.5	99.1	5.1
	22,500	95.1	3.4	93.3	6.9
25-desacetyl-rifampicin	40	88.8	6.7	97.7	9.9
	100	102.5	4.6	102.7	8.2
	2,000	103.9	3.3	104.7	4.9
	4,500	97.9	1.7	93.3	5.2
Ethambutol	40	89.4	11.4	100.5	12.6
	100	104.1	6.2	100.2	8.4
	2,000	109.0	2.6	114.9	7.5
	4,500	92.0	2.6	87.1	6.7
Isoniazid	80	105.2	8.8	112.1	9.5
	200	98.9	4.9	92.4	6.3
	4,000	102.2	5.3	110.6	10.0
	9,000	92.7	2.7	94.2	12.8
Acetyl-isoniazid	40	96.6	3.8	102.1	5.8
	100	100.8	3.1	100.0	5.9
	2,000	98.7	1.7	96.3	4.5
	4,500	92.6	3.7	95.0	6.4
Isonicotinic acid	80	100.3	10.4	97.4	14.4
	200	109.9	5.2	106.1	6.8
	4,000	104.7	1.3	104.6	5.2
	9,000	90.7	4.1	88.8	8.8
Pyrazinamide[a]	1,200	104.0	-	110.3	5.0
	5,000	88.9	6.9	89.7	6.2
	50,000	92.6	3.8	92.2	9.9
	90,000	92.5	4.3	94.2	7.9
Pyrazinoic acid[a]	750	106.5	-	109.5	8.4
	3,125	93.2	6.3	90.7	6.2
	31,250	94.5	7.2	95.8	10.0
	56,250	100.8	8.1	101.4	12.0
5-hydroxy-pyrazinoic acid[a]	1,500	94.8	-	94.8	10.4
	3,125	103.7	8.1	103.5	10.8
	31,250	99.3	7.0	99.4	10.2
	56,250	104.0	2.4	96.6	9.2
5-hydroxy-pyrazinamide	60	98.1	8.6	101.3	11.7
	150	104.3	4.1	105.3	9.3
	3,000	94.3	1.4	95.3	5.4
	6,750	90.7	3.2	91.0	9.5

[a]Compounds included in the modified LC-MS/MS method (n = 3 for both inter-day and intraday validation).

4.1.2 GENOTYPES (PAPER II, PAPER III, PAPER IV AND PAPER VII)

Genotyping of patients was performed to investigate pharmacogenetic effects on the first-line TB drugs and their metabolites. N-acetyltransferase 2 and SLCO genotypes were included due to their effects on exposure to isoniazid and rifampicin, respectively (29, 38, 55). CYP2E1 genotypes were included since CYP2E1 has been suggested to be involved in toxicity induced by isoniazid administration (38). Further, patients were genotyped for CYP2C19 since, despite lack of knowledge on the enzymes involved in rifampicin autoinduction, rifampicin is known to induce CYP2C19 (99). All genotypes were in Hardy-Weinberg equilibrium. Genotype frequencies are summarised in **Table 3** and **Table 4**.

Table 3. Genotype frequencies of 56 Rwandan TB/HIV patients.

Enzyme	SNP	Reference SNP	Genotype	No (%)
CYP2C19	806C>T	rs12248560	C/C	33 (59)
			C/T	20 (36)
			T/T	3 (5)
	681G>A	rs4244285	G/G	43 (77)
			G/A	13 (23)
			A/A	0 (0)
	636G>A	rs4986893	G/G	51 (91)
			G/A	5 (9)
			A/A	0 (0)
CYP2E1	1053C>T	rs2031920	C/C	56 (100)
			C/T	0 (0)
			T/T	0 (0)
	1293G>C	rs3813867	G/G	54 (96)
			G/C	2 (4)
			C/C	0 (0)
	71G>T	rs6413420	G/G	56 (100)
			G/T	0 (0)
			T/T	0 (0)
	7632T>A	rs6413432	T/T	52 (93)
			T/A	4 (7)
			A/A	0 (0)

Table 4. Genotype frequencies of 56 Rwandan TB/HIV patients.

Enzyme/ transporter	SNP	Reference SNP	Genotype	No (%)
NAT2	282C>T	rs1041983	C/C	24 (44)
			C/T	26 (48)
			T/T	4 (8)
	341T>C	rs1801280	T/T	16 (28)
			T/C	30 (54)
			C/C	10 (18)
	481C>T	rs1799929	C/C	18 (32)
			C/T	31 (55)
			T/T	7 (13)
	590G>A	rs1799930	G/G	34 (60)
			G/A	21 (38)
			A/A	1 (2)
	803A>G	rs1208	A/A	8 (14)
			A/G	31 (55)
			G/G	17 (31)
	857G>A	rs1799931	G/G	55 (100)
			G/A	0 (0)
			A/A	0 (0)
SLCO1B1	463C>A	rs11045819	C/C	53 (95)
			C/A	3 (5)
			A/A	0 (0)
	388A>G	rs2306283	A/A	3 (5)
			A/G	15 (27)
			G/G	38 (68)
	11187G>A	rs4149015	G/G	48 (86)
			G/A	8 (14)
			A/A	0 (0)
	-	rs4149032	C/C	27 (48)
			C/T	21 (38)
			T/T	8 (14)
	521T>C	rs4149056	T/T	46 (82)
			T/C	10 (18)
			C/C	0 (0)
	1436G>C	rs59502379	G/G	51 (91)
			G/C	5 (9)
			C/C	0 (0)
SLCO1B3	334T>G	rs4149117	T/T	24 (43)
			T/G	19 (34)
			G/G	13 (23)

4.2 POPULATION PHARMACOKINETICS AND PHARMACOGENETICS

4.2.1 ETHAMBUTOL (PAPER II)

Ethambutol plasma concentration-time profiles were described by a one-compartment disposition model. The absorption model consisted of four transit compartments. Inter-individual variability was included on oral clearance, oral volume of distribution, mean transit time and relative bioavailability. Patients with the G/A mutation in CYP1A2 2159 G>A had 45% lower relative bioavailability compared to carriers of the wild-type (G/G) genotype.

The final model was used to simulate ethambutol C_{max} relative to the described target C_{max} of 2 – 6 mg/L following different doses. The simulations indicated that ethambutol doses of 30 mg/kg and 50 mg/kg for G/G and G/A carriers, respectively would result in adequate exposure with regards to the exposure interval suggested to be therapeutic. Observed and simulated C_{max} values are illustrated in **Figure 2**.

The effect of CYP1A2 polymorphism on ethambutol pharmacokinetics was surprising due to the large fraction of ethambutol excreted unchanged via the kidneys (100). Ethambutol is a potent inhibitor of CYP1A2 and CYP2E1 (101). An interaction between ethambutol and CYP1A2 is therefore evident although ethambutol metabolism by CYP1A2 has not been reported.

Mutations in genes coding for enzymes commonly result in a reduced function of the affected enzyme. The finding was therefore further surprising since the bioavailability was lower in patients with the G/A mutation (n = 12). However, carriers of the G/A mutation in CYP1A2 2159 G>A have lower exposure to the CYP1A2 substrate agomelatine (102).

Overall, ethambutol peak concentrations were low in the studied cohort. However, increasing doses may be undesirable due to dose-dependent ocular toxicity of ethambutol (103). Whereas the utility of 30 mg/kg doses may be investigated, 50 mg/kg doses could expose patients to an unacceptably high risk of adverse events. Studies confirming the effect of CYP1A2 polymorphism on ethambutol pharmacokinetics are therefore needed. Of note, sampling up to eight hours post dose limited the development of a potential

two-compartment disposition model (104, 105). However, the model adequately described peak concentrations.

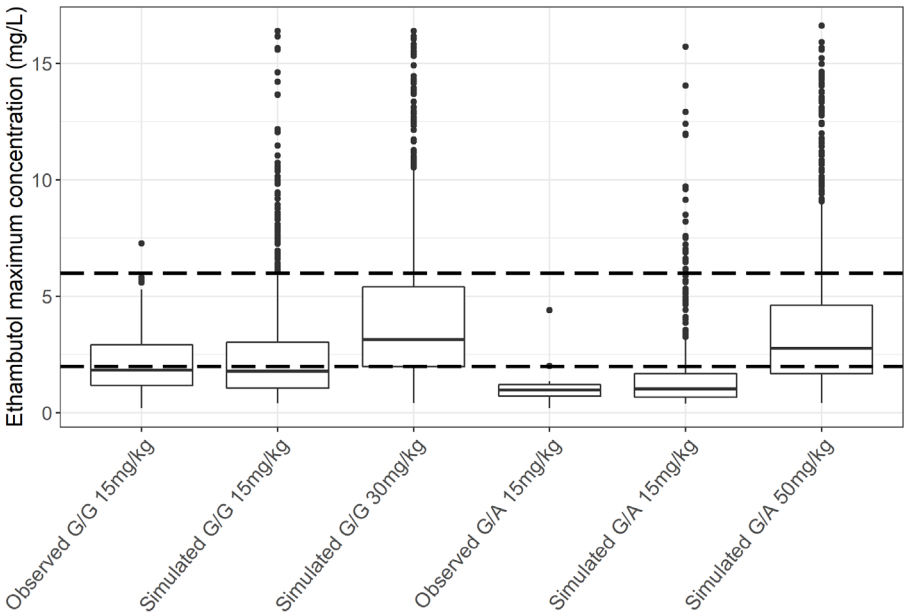


Figure 2. Observed and simulated maximum ethambutol concentration (C_{max}) stratified by dose and CYP1A2 2159G>A genotype. Simulations were performed for individuals weighing 50 kg (n = 200 per group). Dashed lines represent the target C_{max} range.

Model parameters and a VPC can be found in **Paper II**.

4.2.2 ISONIAZID (PAPER III AND PAPER VII)

The structure of the pharmacokinetic model for isoniazid and its metabolites is depicted in **Figure 3**. A two-compartment model described isoniazid disposition adequately. Three pathways were used to characterise isoniazid elimination: formation of acetyl-isoniazid, formation of isonicotinic acid and a third pathway. One-compartment models best described the dispositions of both metabolites.

Isoniazid clearance and fraction of total clearance resulting in the formation of acetyl-isoniazid were estimated based on acetylator status. Total isoniazid oral clearance was 2.3-fold and 1.3-fold higher in patients with rapid and intermediate acetylator status, respectively. Females had a 37% higher relative

bioavailability than males and bioavailability decreased linearly with higher CD4 cell count. Further, clearances of acetyl-isoniazid and isonicotinic acid were 64% and 80% higher in patients on concomitant HIV therapy, respectively.

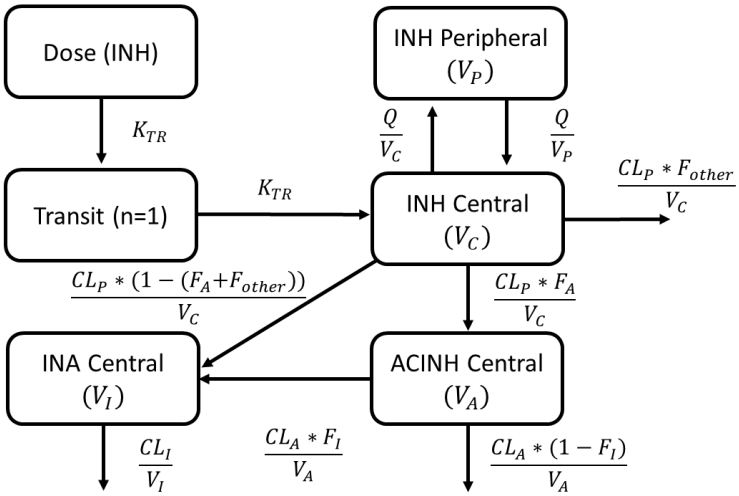


Figure 3. The population pharmacokinetic model for isoniazid and its metabolites developed in Paper III. KTR, transition rate constant; VC, volume of central compartment for isoniazid; VP, volume of peripheral compartment for isoniazid; VA, volume of central compartment for acetyl-isoniazid; VI, volume of central compartment for isonicotinc acid; CLP, clearance for isoniazid; CLA, clearance for acetyl-isoniazid; CLI, clearance for isonicotinc acid; Q, inter-compartmental clearance for isoniazid; FA, fraction of isoniazid metabolized to acetyl-isoniazid; FI, fraction of acetyl-isoniazid metabolized to isonicotinic acid; Fother, fraction of isoniazid eliminated via other routes.

The effect of efavirenz-based ART on the primary metabolites of isoniazid is a novel finding. Since metabolic products cause isoniazid-induced hepatotoxicity (38), alterations in clearances of the primary metabolites may affect the risk of liver injury. However, any such conclusions require further investigation.

Polymorphism in N-acetyltransferase 2 is known to affect isoniazid exposure. Isoniazid dosing guided by acetylator status can reduce risk of hepatotoxicity and improve clinical outcome (12). Further, female sex is a risk factor for development of drug-induced liver injury during first-line TB therapy. Both acetylator status and sex were driving factors of isoniazid exposure in the studied cohort. A dosing algorithm was therefore proposed according to:

$$Dose_i = (Sex_i * 5mg/kg + NAT2_i * 1.5mg/kg) * WT_i$$

where $NAT2_i$ has a value of 0 for slow acetylators, 1 for intermediate acetylators and 3 for rapid acetylators, and Sex_i has value of 1 for males and 0.67 for females and WT_i is the individual weight. Such a dosing strategy may reduce the variability in isoniazid exposure and thus lower the risk of hepatotoxicity, resistance development and treatment failure.

A subset of individuals not yet initiated on HIV therapy (n = 40) was included in the analysis in **Paper VII**. Plasma samples collected at steady state from a proportion of the individuals (n = 26) were analysed and the data was combined with the observations following first dose. A simplified model with no metabolic pathway other than formation of acetyl-isoniazid or isonicotinic acid was used to describe the pharmacokinetics and metabolism of isoniazid following first dose and at steady state. The simpler model was used to increase parameter precisions due to the lower number of individuals included in the analysis. Furthermore, the elimination of isoniazid was estimated as two separate clearance parameters. Isoniazid oral clearance resulting in the formation of isonicotinic acid was 2.3-fold higher at steady state than following first dose. The induction further increased linearly with rifampicin dose. No change in the acetylation pathway of isoniazid or clearance was observed. A statistically significant increase in acetyl-isoniazid clearance could be estimated at steady state. However, the effect was not included in the final model due to poor precision. According to simulations, isoniazid exposure for one dosing interval would be approximately 30% higher when administered alone than during co-administration with standard doses of rifampicin (10 mg/kg) at steady state (**Figure 4**).

Hydrazine products are formed both via direct metabolism of isoniazid and secondarily via metabolism of the isoniazid metabolite acetyl-isoniazid. Induction of isoniazid-mediated formation of hydrazine by co-administration of rifampicin has been proposed (106). The induction was suggested to affect formation of such toxic metabolites by increasing acetyl-isoniazid metabolism. Results from the present cohort indicate an induction of both isoniazid and acetyl-isoniazid clearance. In addition, the induction was dependent on rifampicin dose and may cause an increased risk of toxicity and lower bactericidal effect of isoniazid in regimens containing higher doses of rifampicin. Further studies of the drug-drug interaction are therefore warranted.

Parameters of the final models are summarized in **Paper III** and **Paper VII**, respectively. VPCs are presented in the respective papers.

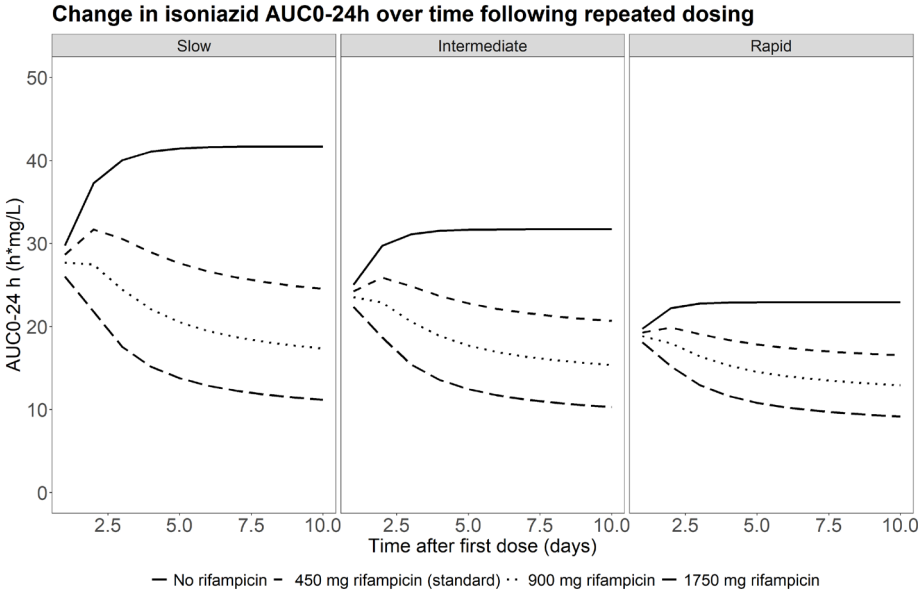


Figure 4. Simulated isoniazid exposure for an individual weighing 50 kg receiving daily isoniazid doses (5 mg/kg) stratified by co-administered rifampicin dose and acetylator status (Paper VII).

4.2.3 RIFAMPICIN (PAPER IV AND PAPER VII)

The dispositions of both rifampicin and 25-desacetylrifampicin were described by one-compartment models. In **Paper IV**, the pharmacokinetics of rifampicin following first dose were investigated. Statistically significant effects of administration of efavirenz-based ART were found on the relative bioavailability (0.6-fold) and oral clearance of rifampicin (1.4-fold). In addition, the oral clearance of 25-desacetylrifampicin was 1.1-fold higher in patients on concomitant ART than patients not yet initiated on HIV therapy. Such effects on rifampicin pharmacokinetics were similar to those of rifampicin autoinduction previously described (33, 34). The time-dependent non-linear pharmacokinetics of rifampicin and its metabolite in HIV therapy naïve patients in the same cohort were therefore investigated in **Paper VII** using a subset of the cohort (n = 40) with the addition of rifampicin steady state data for 26 patients. The changes in oral clearance of rifampicin and 25-desacetylrifampicin due to autoinduction were estimated to be 1.3-fold and 1.1-fold, respectively. Furthermore, the relative bioavailability was estimated to be 0.5-fold at steady state. These findings suggest that both rifampicin and efavirenz-based ART induce rifampicin pharmacokinetics equivalently. The

findings further offer a potential explanation to why the drug-drug interaction has not previously been discovered, since any effect by efavirenz on rifampicin exposure previously has been studied at steady state of antitubercular therapy (107). The initial exposure to rifampicin was lower (0.25-fold) in patients on concomitant ART according to simulations. Such an effect on exposure may promote resistance development due to a reduced early bactericidal activity of rifampicin (108).

Carriers of heterozygous and homozygous mutations in SLCO1B3 334T>G had a lower relative bioavailability (0.7-fold) than carriers of the wild-type variant (**Paper IV**). However, the covariate was excluded from the final model since the difference was not statistically significant. An effect of SLCO1B3 polymorphism on rifampicin pharmacokinetics may be apparent in a study on a larger cohort.

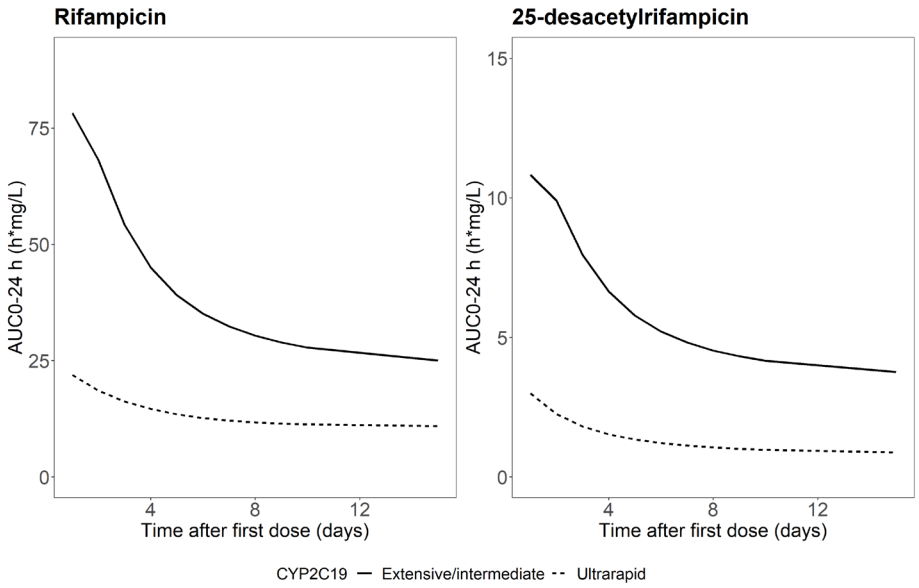


Figure 5. Simulated rifampicin and 25-desacetylrifampicin exposure for a typical individual weighing 50 kg during daily dosing of rifampicin (10 mg/kg) stratified by CYP2C19 phenotype (Paper VII).

A statically significant effect of CYP2C19 phenotype on the clearance pathway other than formation of 25-desacetylrifampicin was observed in **Paper VII**. Rifampicin oral clearance was 2-fold higher in ultra-rapid CYP2C19 metabolizers than in extensive/intermediate metabolizers at steady state. Moreover, only accounting for autoinduction in extensive/intermediate metabolizers was stastistically significant compared to accounting for

autoinduction in all patients. CYP2C19 induction is phenotype-dependent and the level of induction is higher in extensive/intermediate metabolizers than in ultra-rapid metabolizers (94). Although rifampicin is a known inducer of CYP2C19 (99), rifampicin has not been reported as a substrate of CYP2C19. The magnitude of the effect should be interpreted with caution due to the wide confidence interval and few individuals with an ultra-rapid phenotype in the studied cohort (n = 2). Further studies of the suggested polymorphic effect on rifampicin pharmacokinetics are therefore required. Simulated typical exposure to rifampicin and 25-desacetylrifampicin from first dose to steady state in TB/HIV patients receiving daily standard doses of rifampicin (10 mg/kg) is illustrated in **Figure 5**.

Parameter estimates and VPCs for the population pharmacokinetic model for rifampicin are presented in **Paper IV** and **Paper VII**.

4.2.4 PYRAZINAMIDE (PAPER V)

The dispositions of pyrazinamide, 5-hydroxypyrazinamide and pyrazinoic acid were described by one-compartment models. Although quantified, the plasma concentrations of 5-hydroxypyrazinoic acid were below the lower limit of quantification in the majority of the samples (94%). 5-hydroxypyranioic acid was therefore excluded from the analysis.

The fraction of total pyrazinamide oral clearance leading to the formation of pyrazinoic acid was estimated to 0.45. Females had a close to 1.5-fold higher relative pyrazinamide bioavailability than males. There was a negative linear relationship between serum creatinine and the oral clearance of pyrazinoic acid: higher serum creatinine levels were associated with lower clearance of pyrazinoic acid. Moreover, the oral volume of distribution of pyrazinamide and both metabolites was lower in patients on concomitant ART.

Simulations with the final model suggested 50 mg/kg and 35 mg/kg doses in males and females, respectively to be adequate with regard to a target AUC of 363 h*mg/L (49) for one dosing interval (**Figure 6**). A PTA analysis further indicated that such doses would increase PTA for a mode MIC of 25 mg/L from 0.73 in females and 0.41 in males to >0.9 in all patients. Since the risk of hepatotoxicity is higher in females (66), dosing by sex is suggested to be clinically evaluated. Simulations further suggested a 40% lower dose in patients with high serum creatinine (> 133 μmol/L) to reduce accumulation of pyrazinoic acid (**Figure 7**). Exposure to pyrazinoic acid has previously been

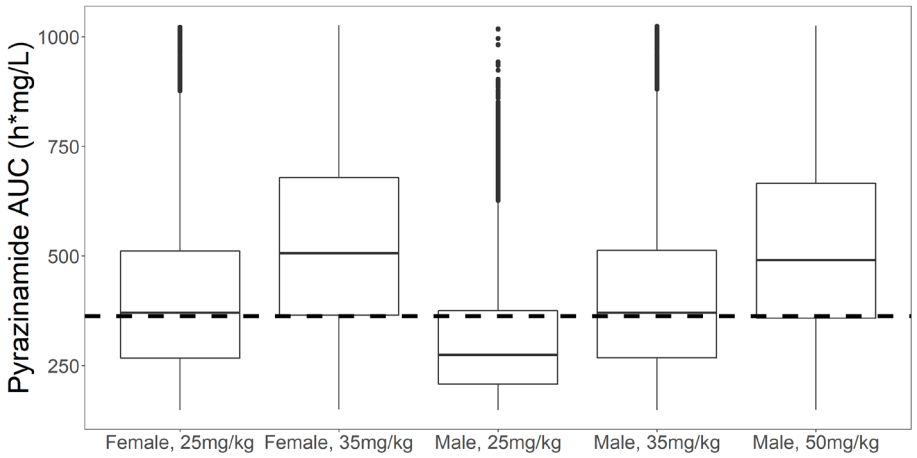


*Figure 6. Simulated pyrazinamide exposure for one dosing interval stratified by dose and sex. Dashed line is the suggested target AUC of 363 h*mg/L.*

observed to be higher in individuals with poor renal function (109, 110). The relationship between serum creatinine and clearance of pyrazinoic acid indicates that serum creatinine may be used as a predictor of exposure to pyrazinoic acid and thus be a useful tool for individualization of pyrazinamide doses.

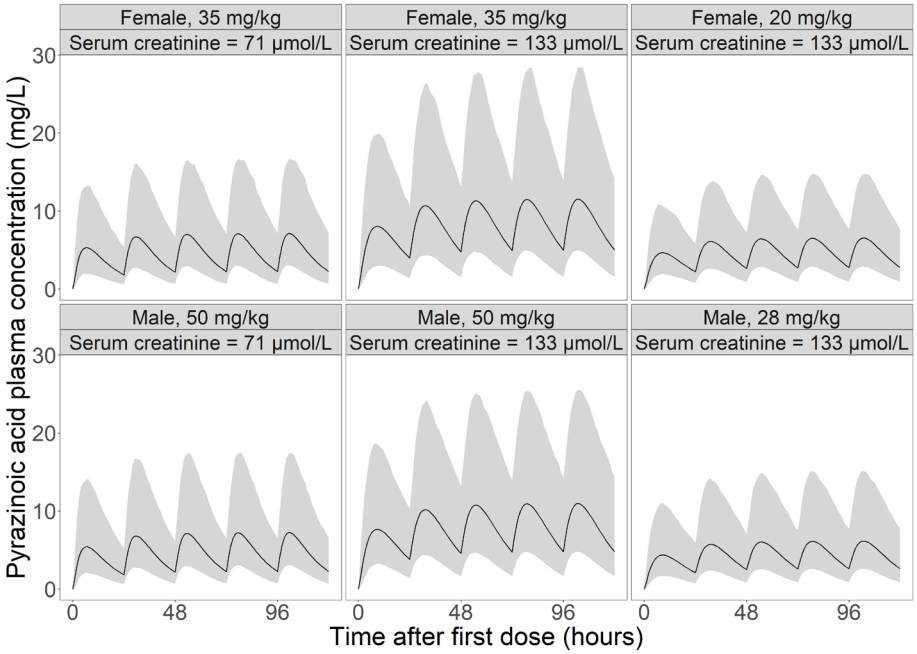


Figure 7. Simulated plasma concentration- time profiles of pyrazinoic acid during daily doses of pyrazinamide stratified by dose, sex and serum creatinine levels.

The effect of ART on the distribution volume of pyrazinamide and both metabolites was limited and may be correlated to an altered clearance. An induction of pyrazinamide clearance by rifampicin has been proposed (111). A similar induction by efavirenz could be plausible since both rifampicin and efavirenz induce a significant amount of enzymes via similar mechanisms. Although the finding is novel, it is unlikely to be of clinical relevance.

Estimated parameters and a VPC of the final pharmacokinetic model of pyrazinamide and its metabolites can be found in **Paper V**.

4.2.5 PROBABILITY OF TARGET ATTAINMENT (PTA) FUNCTIONS (PAPER VI)

Both drug exposure and pathogen susceptibility drive the clinical outcome of the first-line TB drugs (47, 52). Therefore, a model-based approach, which accounts for pathogen susceptibility was developed in this work. The approach was based on functions describing the relationship between dose, MIC and PTA. The functions described the PTA-MIC relationships of rifampicin and isoniazid well, as visualized in **Figure 8**.

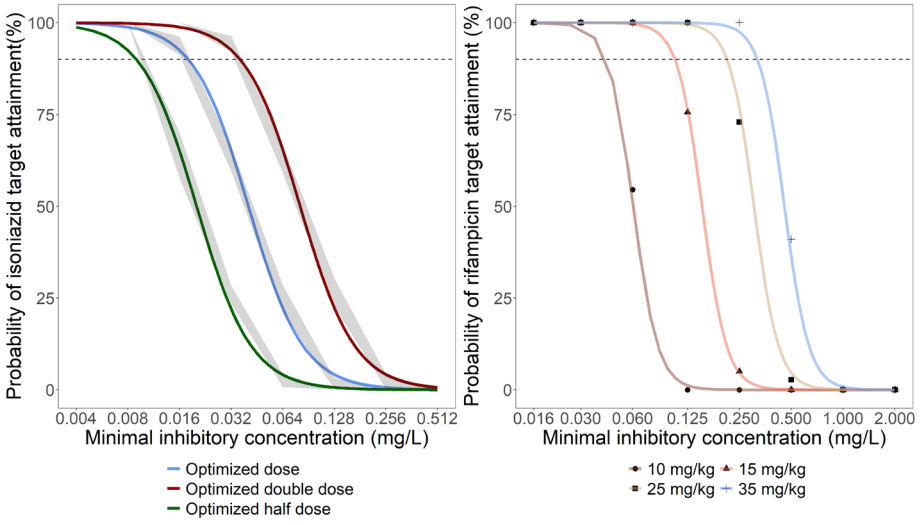


Figure 8. PTA for isoniazid (left) and rifampicin (right). Shaded areas and symbols are the PTA of isoniazid and rifampicin, respectively depending on dose. Coloured lines are predicted PTA for each dose. Dashed line is a PTA of 90%.

Individual MIC-based doses for isoniazid ($D_{i,MIC}$) were calculated with the function according to:

$$D_{i,MIC} = D_i * f_{Di}$$

$$f_{Di} = \left(\frac{\left(\frac{MIC^{\lambda}}{(1 - PTA)} - MIC^{\lambda} \right)^{\frac{1}{\lambda}}}{k} \right)$$

D_i was derived from the dosing algorithm presented in **Paper III**. AUC/MIC ratios following standard isoniazid doses and MIC-guided doses are depicted in **Figure 9**. Whereas standard isoniazid doses would be adequate for MICs up to 0.016, individualized doses could be used for MICs up to 0.064 with regard to the described AUC/MIC threshold. Parameter estimates of the function can be found in **Paper VI**.

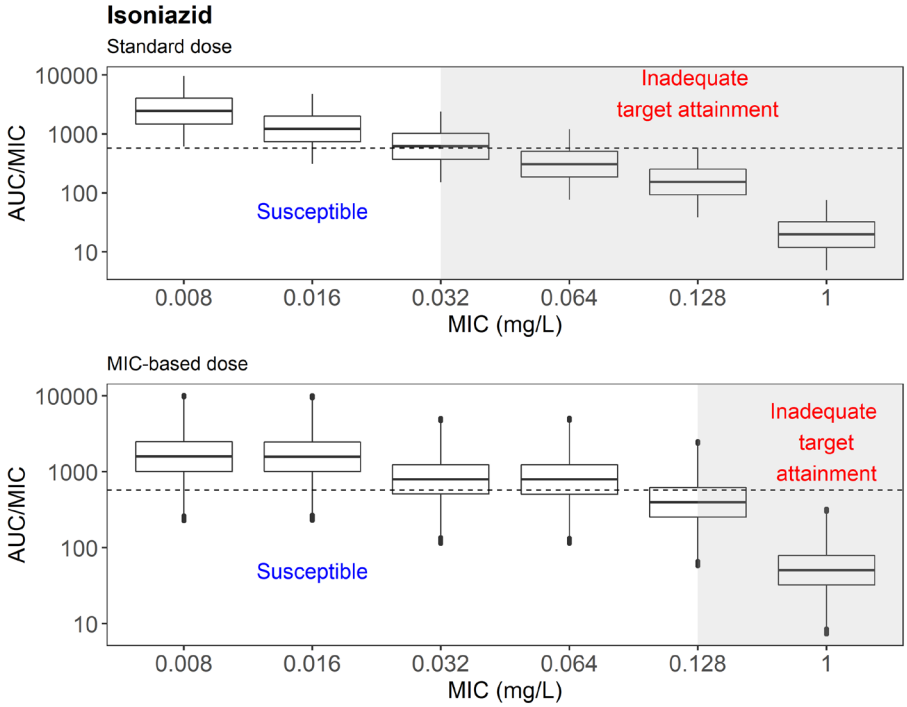


Figure 9. Isoniazid AUC/MIC ratio for standard doses and doses determined with the PTA function. Dashed line is the target AUC/MIC of 567.

Rifampicin PTA functions were determined at steady state due to time-dependent pharmacokinetics. Rifampicin MIC-based doses were determined according to:

$$D_{i,MIC} = \frac{\left(\left(\frac{MIC^{\gamma}}{1-PTA}\right) - MIC^{\gamma}\right)^{\frac{1}{\gamma}} - m}{k}$$

where m is an intercept parameter in the linear relationship between dose and $PMIC_{50}$. Simulated rifampicin AUC/MIC ratios following standard doses and MIC-based doses are visualized in **Figure 10**. Parameter estimates of the function can be found in **Paper VI**. The simulations indicated that standard rifampicin doses (10 mg/kg) would be adequate for MICs up to 0.06 mg/L.

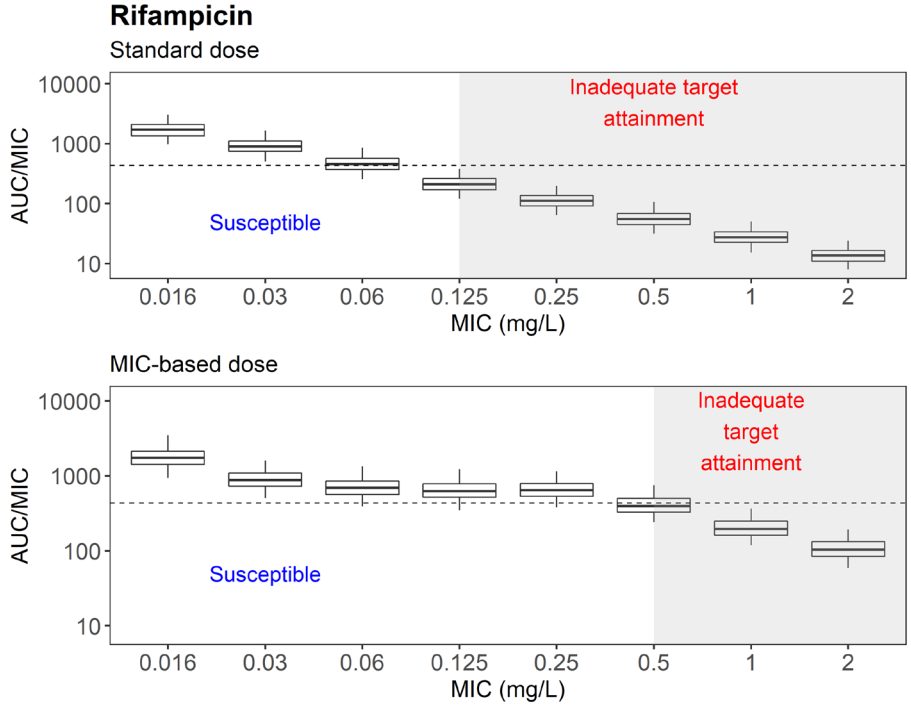


Figure 10. Rifampicin AUC/MIC ratio for standard doses and doses determined with the PTA function. Dashed line is the target AUC/MIC of 435.

The WHO has recommended MIC determination in each patient as part of the Stop TB strategy (112). The described PTA functions may be used for susceptibility-based dose predictions at an individual level or at a population

level utilizing knowledge on local MIC distributions. The functions may further be used in conjunction with bacterial genotypes representative of specific MICs or MIC ranges (113). Determination of strain genotypes could be clinically advantageous since MIC determination for TB is time consuming.

Recently, a study demonstrated a correlation between rifampicin and isoniazid MICs and risk of treatment failure (47). PTA functions offers an approach for MIC-guided dosing. The described functions could be incorporated into a mobile application or website for easy access and utility by clinicians and other health care workers. Implementation of the approach is limited by poor precision in MIC measurement and would thus benefit from standardization of MIC assays (114).

5 DISCUSSION AND PERSPECTIVE

The current standard regimen is effective against active TB in 80% of patients in program settings (9). Development of resistant strains has added further complexity to the clinical management of TB and even untreatable cases have been reported (115). Although non-compliance fuels the emergence of resistance, inadequate drug exposure has been demonstrated to develop resistant strains both *in vitro* and in humans (116, 117). The toxicity profile of the antitubercular regimen also complicates clinical management since it may discourage affected patients from drug intake and may cause serious harm and even death. The risk of treatment failure, resistance development and toxicity emphasize the need for an optimized regimen, especially in vulnerable populations such as HIV-infected individuals.

In this thesis, factors affecting the pharmacokinetics of the four first-line drugs in HIV co-infected patients were investigated. A bioanalytical method suitable for studies of pharmacogenetic effects on metabolism and drug-drug interactions was developed. An updated regimen is proposed, guided by model-based quantification of covariate effects and prior knowledge on clinically relevant exposures. Two drug-drug interactions with potential clinical relevance are described: 1) the effect of concomitant efavirenz-based ART on rifampicin pharmacokinetics and 2) the effect of rifampicin administration on isoniazid pharmacokinetics. The former may affect the early bactericidal activity of rifampicin, whereas the latter may increase risk of isoniazid-induced hepatotoxicity. Moreover, a model-based strategy for susceptibility-guided dosing was developed.

Simulations with the isoniazid and pyrazinamide models suggested dosing regimens based on acetylator status and sex for isoniazid, and serum creatinine levels and sex for pyrazinamide. Higher than the current standard doses of isoniazid (5 mg/kg) have been suggested to be effective even against strains with isoniazid resistance (118). Standard doses of pyrazinamide (25 mg/kg) are lower than in some of the studies leading to the re-introduction of pyrazinamide in the first-line antitubercular cocktail (119, 120). The use of personalized dosing as suggested within this thesis would require lower doses than current standard doses in females with slow acetylator status and high levels of serum creatinine, and higher doses in males with rapid acetylator status and normal serum creatinine levels. Both female sex and slow acetylator status are risk factors for hepatotoxicity and lower doses in such patients could reduce the risk of liver injury (38, 66).

Recent research on optimization of the first-line TB regimen has focused on higher rifampicin doses (10, 11). The two drug-drug interactions of potential clinical relevance described within this thesis suggest that additional studies are required. Firstly, the effect on hydrolysis of isoniazid may potentiate toxicity. Since the data suggested a dose-dependent effect, a study designed to investigate the interaction at different rifampicin doses is warranted. Such a study should include quantification of biomarkers of liver-injury, concentrations of isonicotinic acid and acetyl-isoniazid and possibly genotyping for determination of acetylator status. Secondly, the effect of efavirenz-based ART on rifampicin exposure promotes the use of dolutegravir as a first-line agent for HIV, especially in high endemic areas of TB. The drug-drug interaction may even encourage exchange from efavirenz to dolutegravir in TB/HIV co-infected patients prior to initiation of rifampicin-based TB therapy.

The observed effect of CYP1A2 on ethambutol pharmacokinetic is novel and requires confirmation, especially with regard to the prior knowledge on ethambutol pharmacokinetics. The ethambutol doses required to achieve therapeutic exposure in CYP1A2 2159 G/A carriers as suggested by the presented simulations may increase the risk of developing ocular toxicity (103). Such a risk could discourage the use of higher ethambutol doses. However, an optimized regimen including individualized doses of isoniazid and pyrazinamide, and higher doses of rifampicin, may make ethambutol redundant as part of the antitubercular regimen.

The current first-line TB therapy lasts for six months. A well-known clinical challenge with the standard TB therapy is the long treatment duration since it promotes patient non-compliance. The number of therapeutic failures could be reduced by adequate compliance and drug exposure. High dose rifampicin regimens have been suggested to possibly be able to reduce treatment duration due to a faster killing of bacteria (53). Individualized therapy may reduce the length of therapy and therefore reduce the risk of non-compliance. A clinical study comparing standard therapy to an individualized regimen as suggested within this thesis may be the next step towards a safer and possibly shorter regimen. Further, although this study focused on HIV co-infected individuals, the strategy can be applied on a general TB patient population but may then require adaptation.

Characteristics of both the patient and the bacteria can determine therapy outcome (12, 47). The work in this thesis has demonstrated a novel approach on how combined knowledge on bacterial drug susceptibility and patient factors may be applied for precision dosing in each individual. The poor

precision in MIC assays may provide challenges to the described approach, wherefore local MIC distributions or genotyping of bacteria could be used as an alternative. Genotyping of patients and/or TB strains is in addition limited to the laboratory resources available in any region. Individualized dosing may therefore be restricted to hard-to-treat patients and especially susceptible individuals. However, the lack of effective alternative regimens and the risk of further resistance development strongly encourages optimal use of the drugs available in all patients where such a strategy is feasible.

Although TB is curable, it remains one of the most common causes of death caused by a disease world-wide (1). Development of novel alternative regimens and optimal use of the first-line regimen is thus crucial. Implementation of individualized therapy requires extensive training of experts within the field such as clinical pharmacologists. Modern technology including mobile applications and websites could supply user-friendly tools for clinical implementation. Mobile applications also allow for a continuous update mechanism where data from new clinical studies is added to suggest improvements of the dosing algorithms, which in turn could be evaluated in new clinical trials.

This thesis presents a starting point for an optimized antitubercular regimen in TB/HIV co-infected patients based on an updated scientific rationale. Individualized dosing will likely reduce the variability in exposure to the TB drugs and their metabolites and may in turn lower the risk of treatment failure, resistance development and toxicity. Precision dosing modified by bacterial susceptibility may further improve treatment outcomes. The future of medicine lies within discarding the one-size-fits-all approach in favour of personalized medicine.

6 CONCLUSION

The pharmacokinetics and pharmacogenetics of the four first-line antitubercular drugs in patients co-infected with TB and HIV have been investigated in this thesis. Population pharmacokinetic models were developed and used to identify patients at risk of sub-therapeutic drug exposure. New dosing regimens based on patient characteristics are proposed. Furthermore, a novel model-based dosing strategy accounting for pathogen susceptibility is presented. The conclusions that can be drawn from this thesis are as follows:

- A bioanalytical method for quantification of the four first-line antitubercular drugs and their primary metabolites was developed and validated. The method is suitable for drug-drug interaction studies.
- Ethambutol exposure was overall low in the present cohort. An increase in dose is proposed. CYP1A2 polymorphism was suggested to affect ethambutol pharmacokinetics. The finding requires further studies.
- A novel dosing algorithm based on acetylator status, sex and weight is described for isoniazid to reduce the high pharmacokinetic variability.
- A previously unknown effect of efavirenz-based ART on rifampicin pharmacokinetics was described. The drug-drug interaction affects rifampicin exposure equivalently to rifampicin autoinduction and may affect the early bactericidal activity of rifampicin.
- An effect of CYP2C19 phenotype on rifampicin pharmacokinetics was suggested by the data. The finding requires further studies.
- An effect of rifampicin co-administration on hydrolysis of isoniazid was described. The drug-drug interaction may potentiate isoniazid-related hepatotoxicity and requires further studies with regard to ongoing clinical trials investigating high dose rifampicin.
- Dosing based on sex, serum creatinine and weight is proposed for pyrazinamide to reduce the variability in drug and metabolite exposure.
- Functions describing the relationship between dose, MIC and PTA are presented. The functions may be used as a tool for individualization of therapy guided by bacterial susceptibility.

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APPENDIX