Pharmacokinetic drug-drug interactions in the management of malaria, HIV and tuberculosis

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Abstract

Malaria, Human Immunodeficiency Virus (HIV) and tuberculosis (TB) are global health problems having their worst situation in sub-Saharan Africa. Consequently, concomitant use of antimalarial, antiretroviral and antitubercular drugs may be needed, resulting in a potential risk of drug-drug interactions.

Cytochrome P-450 (CYP) enzyme induction/inhibition may lead to drug-drug interactions and can be detected by probe drugs. An analytical method was developed for the quantitation of mephenytoin, CYP2B6 and CYP2C19 probe, and its metabolites.

Induction/inhibition of principal CYP enzymes by the antimalarials; artemisinin, dihydroartemisinin, arteether, artemether and artesunate, was evaluated using the 4-hour plasma concentration ratios of probe drugs and their metabolites along with modelling the population pharmacokinetics of S-mephenytoin and its metabolites. The extent of change in enzymatic activities was different among the antimalarials, with artemisinin having strongest capacity for induction and inhibition, consequently, the strongest potential risk for drug-drug interactions.

Drug-drug interactions between the antitubercular rifampicin and the antiretrovirals nevirapine and lopinavir were assessed, in TB/HIV patients, by developing population pharmacokinetic models. Rifampicin increased nevirapine oral clearance. Simulations suggested that increasing the nevirapine dose to 300 mg twice daily when co-administered with rifampicin, would result in nevirapine concentrations above subtherapeutic levels, with minimum exposure above the recommended maximum concentration. Lopinavir is co-formulated with ritonavir in the ratio of 4:1. In children, increasing ritonavir dose four times did not completely compensate the enhancement of lopinavir oral clearance caused by rifampicin. However, the predicted lopinavir trough concentration was above the recommended minimum therapeutic concentration.

The work presented in this thesis followed an investigation line though not done for a particular drug. First the CYP enzymes involved in the interaction are identified. Afterwards, the expected drug-drug interaction is investigated where the potentially interacting drugs are concomitantly administered and an adjustment in the dose regimen is proposed that is subsequently evaluated.

Keywords: Pharmacokinetics, Drug-drug interactions, Cytochrome P-450, Artemisinin antimalarials, Nevirapine, Lopinavir, Rifampicin, NONMEM

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urn:nbn:se:uu:diva-8426 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-8426)
To my family

and

to all researchers interested in the field of drug-drug interactions
Papers discussed

The thesis is based on the following papers, which will be referred to, in the text, by the Roman numbers assigned to them below.


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Contents

Introduction ................................................................................................................................. 11
Overview .................................................................................................................................. 11
Pharmacokinetic drug-drug interactions ............................................................................... 11
  Drug-drug interactions associated with CYP enzymes ...................................................... 12
  Drug-drug interactions associated with P-glycoprotein .................................................... 13
  Consequences of drug-drug interactions associated with CYP enzymes and P-gp .............. 14
  Probes used to study CYP enzymes induction and inhibition ............................................. 15
  Enzyme turnover model ......................................................................................................... 16
Selected drugs used in the management of malaria, tuberculosis and HIV infection ............ 17
  Antimalarial drugs: Artemisinin and its Derivatives ............................................................ 17
  Antitubercular drugs: Rifampicin and Isoniazid ................................................................. 18
  Antiretroviral drugs: Nevirapine and Lopinavir/Ritonavir .................................................. 19
  Drug-drug interactions involving antimalarial, antiretroviral and antitubercular drugs: an overview .......................................................... 20
Non-linear Mixed Effect Modelling ......................................................................................... 21
Aims of the thesis ..................................................................................................................... 22
Materials and methods ........................................................................................................... 23
  Experimental procedures ...................................................................................................... 23
    Ethics (Papers II, IV and V) ............................................................................................... 23
    Subjects (Papers II - V) ..................................................................................................... 23
    Study design (Papers II - V) ............................................................................................ 24
Chemical Analysis .................................................................................................................. 25
  Enzyme probes (Papers I and II) ....................................................................................... 26
    Nevirapine and its metabolites 2-, 3-, 8- and 12-hydroxynevirapine (Paper IV) .............. 27
Data analysis ............................................................................................................................. 28
  Noncompartmental data analysis and statistics (Papers II and IV) ..................................... 28
  Modelling (Papers III-V) ..................................................................................................... 28
Results and discussion ............................................................................................................ 31
  Quantification of mephenytoin and its metabolites nirvanol and 4’-hydroxymephenytoin (Paper I) ................................................................. 31
Abbreviations

$A_{e0,m} \quad \text{metabolite urinary recovery}$
$A_{en} \quad \text{amount of enzyme}$
$\text{AGP} \quad \alpha 1$-acid glycoprotein
$\text{AhR} \quad \text{aryl hydrocarbon receptor}$
$\text{AUC} \quad \text{area under the plasma drug concentration-time curve}$
$\text{AUC}_{\text{po}} \quad \text{AUC after oral administration}$
$\text{CAR} \quad \text{constitutive androstane receptor}$
$\text{CL/F} \quad \text{oral clearance}$
$(\text{CL/F})_0 \quad \text{oral clearance at the beginning of the dosing interval}$
$\text{CL}_{u} \quad \text{unbound clearance}$
$\text{CL}_{u,\text{int}} \quad \text{partial intrinsic clearance}$
$\text{C}_{\text{max}} \quad \text{maximum concentration}$
$\text{C}_{\text{min}} \quad \text{minimum concentration}$
$\text{C}_{u} \quad \text{unbound concentration}$
$\text{CYP} \quad \text{cytochrome P-450}$
$\frac{dA_{en}}{dt} \quad \text{rate of change of an enzyme over time}$
$\text{EMs} \quad \text{extensive metabolizers}$
$\text{FO} \quad \text{first-order method}$
$\text{FOCE} \quad \text{first-order conditional estimation method}$
$\text{FOCEI} \quad \text{first-order conditional estimation method with interaction}$
$\text{f}_{u} \quad \text{ratio of unbound and total drug concentration}$
$\text{GR} \quad \text{glucocorticoid receptor}$
$\text{HIV} \quad \text{human immunodeficiency virus}$
$\text{IIV} \quad \text{interindividual variability}$
$\text{IMs} \quad \text{intermediate metabolizers}$
$\text{k}_{a} \quad \text{absorption rate constant}$
$\text{K}_m \quad \text{Michaelis-Menten constant}$
$\text{k}_{\text{out}} \quad \text{elimination rate constant of an enzyme}$
$\text{k}_{r} \quad \text{rate constant of recovery from inhibition}$
$\text{LC/MS/MS} \quad \text{liquid chromatography/tandem mass spectrometry}$
$LLOQ \quad \text{lower limit of quantification}$
$\text{MDR} \quad \text{multidrug resistance}$
$\text{NNRTI} \quad \text{non-nucleoside reverse transcriptase inhibitor}$
$\text{NRTI} \quad \text{nucleoside reverse transcriptase inhibitor}$
$\text{OFV} \quad \text{objective function value}$
$\text{P-gp} \quad \text{P-glycoprotein}$
PI Protease inhibitor
P\textsubscript{i} individual parameter estimate
PMs poor metabolizers
PRED\textsubscript{ij} individual prediction
PXR pregnane X receptor
R\textsubscript{in} production rate of an enzyme
SD standard deviation
TAD time after dose
TB tuberculosis
V/F volume of distribution
V\textsubscript{max} maximum rate of metabolism
WHO world health organization
\(\varepsilon\) difference between individual prediction and observation
\(\eta\) difference between population and individual parameter estimate
\(\theta\) typical predicted value of a parameter
\(\sigma^2\) variance of \(\varepsilon\)
\(\omega^2\) variance of \(\eta\)
Introduction

Overview

*Plasmodium falciparum*, the human immunodeficiency virus (HIV), and *Mycobacterium tuberculosis* are three devastating pathogens in tropical areas [1]. Malaria is a global health problem; each year there are about 500 million cases, mainly in sub-Saharan Africa [2] and Southeast Asia [3]. HIV is also a worldwide health problem. Globally there are about 40 million infected individuals, with an incidence in 2006 of about 4 million new HIV infections, 65% of which occurred in sub-Saharan Africa and 20% in South and Southeast Asia [4, 5]. One eighth of the new HIV infections in the world in 2006 occurred in children younger than 15 years [5]. As with HIV and malaria, the number of tuberculosis (TB) cases worldwide is overwhelming. One third of the world’s population has TB [6]. Southeast Asia is the world's hardest-hit region, with about three million new cases of TB each year. In Sub-Saharan Africa, there are more than 1.5 million cases each year; however, because of the high prevalence of HIV in the region, the number of new TB cases per year is rising rapidly [7]. Due to the geographical overlap of malaria, HIV and TB prevalence, the diseases are likely to co-exist in a great number of individuals. For these individuals, there is an obvious need for concomitant use of antimalarial, antiretroviral and antitubercular drugs. Drug-drug interactions may result from concurrent administration of drugs leading to diminished therapeutic efficacy or increased toxicity from one or more of the administered drugs.

Pharmacokinetic drug-drug interactions

Pharmacokinetic drug-drug interactions occur when one drug alters the absorption, distribution, metabolism or excretion of another drug, leading to alterations in the plasma concentrations of the latter and subsequently at the site of action.

Drug-drug interactions that lead to altered drug absorption can influence the rate and/or the extent of absorption. Interactions affecting absorption can result from the formation of insoluble drug complexes, or from changes to gastric pH or gastrointestinal motility. Displacement of highly protein-bound drugs from their plasma protein binding sites can alter drug distribution.
However, theoretically, this will not affect the average unbound concentration ($C_u$) of drug at steady state for most drugs, with the exception of highly extracted, intravenously administered drugs, which are quite rare [8].

Drug-drug interactions affecting the renal excretion of drugs can arise from alterations to the transporters involved in the efflux of drug molecules into the urine by secretion. Alterations to the pH or flow of urine can also result in drug-drug interactions.

Drug metabolism occurs mainly in the liver, although other sites such as the gastrointestinal tract, kidneys, skin and lungs can be involved. In phase I of drug metabolism, the drug undergoes reactions such as oxidation, reduction or hydrolysis, which introduce a chemically reactive group to the drug molecule or expose such a group. Cytochrome P-450 (CYP) enzymes are the main enzymes responsible for drug metabolism; the CYP1, CYP2 and CYP3 families provide the principal enzymes responsible for the metabolism of 80% of currently known drugs [9]. CYP enzymes are mainly located in the liver but they are also present in other tissues, including the small intestine, which contributes to first-pass metabolism [10-12]. The CYP3A subfamily is the most abundant CYP family in both the liver and the intestine [13, 14]. In phase II of drug metabolism, the reactive groups are conjugated to endogenous molecules by, for example, glucuronidation, glutathione conjugation and sulfation. The products of phase II reactions are more water soluble. Subsequently, the drug is excreted into the bile or urine.

The most clinically important drug-drug interactions result from modification of drug metabolism. Identifying the CYP enzymes that are modified through induction or inhibition by a particular drug helps to predict possible drug-drug interactions. However, understanding of the interactions related to CYP enzymes is complicated by interplay between efflux transporters and the enzymes [15].

Drug-drug interactions associated with CYP enzymes

**Drug-drug interactions due to induction of CYP enzymes**

The up-regulation of CYP enzymes is mainly a consequence of the synthesis of new enzyme molecules [16]. Enhanced synthesis of CYP enzymes occurs upon binding of the inducer to a nuclear receptor [17]. Enzyme induction can also occur as a result of enzyme stabilization, as in the case of CYP2E1 [18-20].

Nuclear receptors regulated by xenobiotics include the aryl hydrocarbon receptor (AhR), the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and the glucocorticoid receptor (GR), which lead to an increase in CYP gene transcription [9, 15, 21]. CAR and PXR are expressed in the liver and the small intestine [22, 23]. AhR regulates CYP1A genes while CYP2 and CYP3 are regulated by CAR and PXR. GR seems to increase the
expression of PXR and CAR. PXR is present in the nucleus while CAR is found in the cytoplasm and translocates to the nucleus upon binding to inducers [21].

As a consequence of the gene transcription and the following protein synthesis, enzyme induction is a time-dependent process and can take several weeks to occur [24]. In addition, the steady-state level of an enzyme depends on the balance between its rate of synthesis and degradation [25]. Therefore, reaching a new steady-state level upon induction or after withdrawal of the inducer will take time, irrespective of whether the induction was due to synthesis of new enzymes or to enzyme stabilization [26]. The time to reach a new steady state depends on the enzyme half-life, provided that the half-life of the inducer is comparatively less than that of the enzyme [27].

Not all CYP enzymes are inducible; CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, and CYP3A are among the inducible CYP enzymes. The extent of CYP induction depends upon the baseline levels of CYP enzymes, genetic polymorphism in the CYP enzymes or in the nuclear receptors, and environmental factors such as smoking and alcohol consumption [15].

**Drug-drug interactions due to inhibition of CYP enzymes**

Drug-drug interactions due to inhibition, which is usually an immediate process, can be either competitive or noncompetitive. For competitive inhibition, depending on the affinities of the substrate and inhibitor to the enzyme, increasing the concentration of the substrate can lead to recovery from inhibition because the inhibitor would be removed from the enzyme. Recovery from competitive inhibition depends on the half-life of the inhibitor. Non-competitive inhibition occurs when the enzyme can no longer metabolize the substrate because of enzyme inactivation by the inhibitor. Inactivation of the enzyme usually lasts longer with non-competitive than with competitive inhibition, and recovery from non-competitive inhibition, as with induction, is dependent on the half-life of the enzyme if the half-life of the inhibitor is comparatively negligible. Mechanism-based inhibition is a non-competitive process where the metabolite formed by the substrate irreversibly binds to the enzyme responsible for its metabolism or to another enzyme leading to the inactivation of the enzyme [26, 27].

**Drug-drug interactions associated with P-glycoprotein**

P-glycoprotein (P-gp) is a transporter that serves as a defence mechanism against harmful xenobiotics. P-gp is expressed in the intestine, liver, kidney, placenta and blood–brain barrier [28-30]. Drug–drug interactions can arise from induction or inhibition of P-gp because of its broad substrate specificity. PXR ligands increase the expression of P-gp by up-regulating the multidrug resistance 1 (MDR1) gene, which encodes P-gp [21].
Induction or inhibition of intestinal P-gp results in alterations to the rate and extent of absorption of P-gp substrates [31]. P-gp inhibition decreases drug metabolism in the small intestine but enhances drug metabolism in the liver, although the clinical relevance of this discrepancy is not yet confirmed by clinical trials [32]. In the gut, where transporters efflux drug molecules back into the gut lumen and where the drug encounters the transporters before the enzymes, inhibition of P-gp activity results in a decrease in drug metabolism and consequently in drug bioavailability due to saturation of intestinal CYP3A, since P-gp keeps the drug concentration within the linear range of CYP3A capacity. In the liver, the drug has to pass the enzymes before encountering the transporters that will efflux it into the bile. Thus, inhibition of P-gp activity in the liver can result in enhanced drug metabolism since the liver enzymes are not easily saturated because of their greater abundance relative to that in the gut [32]. In addition to absorption, metabolism and excretion into the bile, alterations in P-gp activity can affect drug secretion in the urine as well as distribution to different organs such as the brain [33].

Consequences of drug-drug interactions associated with CYP enzymes and P-gp

Enzyme induction enhances drug metabolism, and may decrease its plasma concentrations, which may be followed by a reduction in drug efficacy, depending on its therapeutic index [24, 34]. Enzyme induction also results in an increase in plasma metabolite concentrations, unless formation of metabolites is the only elimination pathway for the drug. If the metabolite is toxic, enzyme induction leading to higher levels of the metabolite can result in aggravated toxicity. Alternatively, for prodrugs, enhanced drug metabolism will elevate the levels of pharmacologically active metabolite, possibly leading to an enhanced effect.

Enzyme inhibition can decrease drug metabolism and increase plasma drug concentrations, which may lead to increased pharmacological activity and, if the drug has a narrow therapeutic index, to adverse effects. Inhibiting the metabolism of a prodrug would reduce the anticipated pharmacological effect. However, inhibiting the enzyme that is primarily involved in the metabolism of a drug may shift the process to alternative pathways that were otherwise minor or negligible.

Clinically relevant drug–drug interactions involving P-gp have been summarized in a recent review [35]. Nonetheless, induction or inhibition of P-gp is difficult to establish in humans due to the overlap between inhibitors and inducers of P-gp and CYP3A.

Clinically significant drug-drug interactions involve drugs with a narrow therapeutic window, a steep dose-response curve, high first-pass metabolism
or a single route of elimination that can be inhibited. Pharmacokinetic drug-drug interactions can possibly be compensated for by dose adjustment of the target drug [36].

Probes used to study CYP enzymes induction and inhibition

Activities of CYP enzymes and consequently drug-drug interactions occurring due to their inhibition or induction can be studied by using probe drugs. The ideal probe should be selective, so that a quantifiable route of its metabolism is mostly or exclusively mediated by a particular CYP enzyme; it should also be sensitive, well tolerated, minimally invasive, convenient to use, and easy to assay in biological fluids [26, 37]. The probes currently used for measurement of enzymatic activity include: caffeine, a CYP1A2 probe [38-42]; midazolam, a CYP3A probe [39, 42-44]; metoprolol, a CYP2D6 probe [39, 42, 45]; chlorzoxazone, a CYP2E1 probe [38, 39, 42, 46]; mephenytoin, a CYP2C19 probe [38, 39, 42, 47]; and coumarin, a CYP2A6 probe [48]. The advantages and disadvantages of commonly used probes have been reviewed by Streetman and colleagues [49].

The activity of a certain enzyme is determined by the intrinsic clearance of the probe through this enzymatic pathway. The unbound clearance \( CL_u \) relates the rate of metabolism to \( C_u \). \( CL_u \) can be expressed as

\[
CL_u = \frac{V_{\text{max}}}{K_m + C_u}
\]  

where \( V_{\text{max}} \) is the maximum rate of metabolism and \( K_m \) is the Michaelis-Menten constant.

The intrinsic clearance can be viewed as the ratio between \( V_{\text{max}} \) and \( K_m \) [50]. Assuming low renal clearance, intrinsic clearance can be determined by

\[
CL_{u,\text{int}} = \frac{A_{\text{e},m}}{f_u \cdot AUC_{po}}
\]

where \( CL_{u,\text{int}} \) is the partial intrinsic clearance, \( A_{\text{e},m} \) is the urinary recovery of the metabolite of the probe, \( f_u \) is the fraction unbound and \( AUC_{po} \) is the area under the probe plasma concentration time curve after oral administration. Other indices can be used, such as the plasma probe-to-metabolite AUC ratio [51, 52]. Indices based on the ratio of metabolite to parent probe agent are better than those based on either of them alone [51].

The so-called cocktail approach, which involves the concomitant administration of several probes, has been used to estimate the activities of multiple CYP enzymes simultaneously [38, 39, 42, 47, 53]. Limitations of this approach include the risk of interactions between the probes and the necessity for analytical methods that can be used to analyze several drugs and metabo-
lites in the same sample [54, 55]. Since analysis of a probe cocktail can be complicated, time consuming and costly, it is more convenient to measure the ratio between the concentration of the probe and that of its metabolite at a single time point, provided that the ratio is validated as a surrogate for clearance of the probe. This ratio has been validated in the case of caffeine [40, 41], midazolam, [43, 44], metoprolol, [45] and chlorzoxazone [46].

Enzyme turnover model

An enzyme turnover model can be used to describe the time course of induction [56, 57] or inhibition [58] of an enzyme.

\[
\frac{dA_{enz}}{dt} = R_{in} - (k_{out} \cdot A_{enz})
\]  

(3)

where \(dA_{enz}/dt\) is the rate of change of the amount of enzyme over time, \(R_{in}\) is the zero-order enzyme production rate, \(k_{out}\) is the first-order elimination rate constant and \(A_{enz}\) is the amount of enzyme. A model of enzyme induction is obtained by relating \(R_{in}\), through a stimulation function such as the E_{max} model, or \(k_{out}\), through an inhibition function such as the inhibitory E_{max} model, to the plasma concentration of the inducer; however, if the concentration is not available, a step function can be used. A model of enzyme inhibition is achieved by reversing these functions [59]. \(A_{enz}\) affects the elimination of the target drug. At steady state, \(A_{enz} = R_{in}/k_{out}\). \(A_{enz}\) is normalized to unity at baseline by setting \(R_{in}\) to the value of \(k_{out}\) so that changes in the amount of enzyme upon induction or inhibition can be viewed relative to the baseline value.
Selected drugs used in the management of malaria, tuberculosis and HIV infection

Antimalarial drugs: Artemisinin and its Derivatives

Malaria, from the Italian “mala aria” or “bad air”, is an infectious disease that is transmitted by infected female Anopheles mosquitoes. Four species of malaria parasite can infect humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The artemisinin antimalarial drugs are effective for the treatment of *P. falciparum* infection that has developed a widespread resistance to antimalarial drugs [2, 60, 61]. Because recrudescence rates are unacceptably high for the artemisinin antimalarial drugs, their combination with other antimalarial drugs has been recommended [62]. Artemisinin semi-synthetic derivatives have been synthesized to increase solubility and improve antimalarial efficacy. Artemisinin derivatives include artemether, arteether, artesunate and dihydroartemisinin [63].

**Artemisinin**

Artemisinin was isolated in 1971 from sweet wormwood, also known as sweet Annie (*Artemisia annua* L), a Chinese herb that has been used in traditional Chinese medicine for the treatment of fever and malaria [64].

Artemisinin is metabolized by CYP2B6, CYP3A4, and CYP2A6 *in vitro*; CYP2B6 is the primary enzyme involved in the elimination of the drug in human liver microsomes [65]. Four metabolites have been identified in urine after oral administration of artemisinin, namely deoxyartemisinin, deoxydihydroartemisinin, dihydroxydeoxyartemisinin and “crystal 7” [66]. Artemisinin has a strong capacity for autoinduction in humans [67-74]. It also increases the activity of CYP2C19 [73, 75] and CYP2B6 and alters the disposition of R-mephenytoin by an unidentified enzyme [76]. The induction of CYP enzymes by artemisinin seems to be due to activation of the nuclear receptors PXR and/or CAR [77, 78]. Artemisinin inhibits CYP1A2 and CYP2C19 *in vitro* [79]; inhibition of CYP1A2 activity by artemisinin has been confirmed in healthy human subjects [80].

**Dihydroartemisinin**

Dihydroartemisinin is metabolized by glucuronidation [81]. This drug inhibits CYP1A2 and CYP2C19 *in vitro* [79].

**Arteether**

Arteether is the ethyl ether derivative of dihydroartemisinin. *In vitro*, arteether is metabolized to dihydroartemisinin, mainly by CYP3A4 but also by CYP2B6 and CYP3A5 [82].
Artemether
Artemether is the methyl ether derivative of dihydroartemisinin. Artemether is metabolized by CYP1A2, CYP2B6, CYP2C9, and CYP3A4 \textit{in vitro} [63]. In healthy volunteers, intestinal CYP3A4 is involved in the first-pass metabolism of artemether [83], whereas CYP2D6 or CYP2C19 do not seem to contribute to its metabolism [84]. Artemether has shown capacity for autoinduction [85]. Artemether inhibits CYP1A2 in rats [63].

Artesunate
Artesunate is the hemisuccinic ester of dihydroartemisinin. Artesunate appears to act as a prodrug since it is rapidly converted to dihydroartemisinin, probably by non-enzymatic hydrolysis [86]. Dihydroartemisinin is considered to account for the antimalarial effect [66]. Declining plasma artemesunate concentrations during multiple dosing have been reported [87].

Antitubercular drugs: Rifampicin and Isoniazid
TB is an airborne infectious disease, caused by \textit{Mycobacterium tuberculosis}, that is often spread when an infected person coughs or sneezes. The world health organization (WHO) recommends combination therapy for TB, including rifampicin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months [88].

Rifampicin
Rifampicin is metabolised by deacetylation through B-esterases [89]. Rifampicin activates the nuclear receptor PXR [90-92]. This drug is considered to be amongst the most potent inducers of CYP3A4 known. The induction by rifampicin at clinical doses of 450-600 mg is near maximal [15]. Significant CYP3A4 induction was detected 8 hours after a single rifampicin dose [93]. The activity of CYP3A4 during rifampicin administration reached steady state after 4 days and returned to baseline about 2 weeks after termination of rifampicin administration [94]. Rifampicin also induces other CYP enzymes, including CYP2A6, CYP2B6, CYP2C9, phase II enzymes and transporters such as P-gp [33, 92, 95, 96].

Isoniazid
Isoniazid is metabolized by acetylation via N-acetyltransferase [97]. This drug is a mechanism-based inhibitor of a number of CYP enzymes but mainly of CYP3A4 and CYP2C19 [98-101]. It can both inhibit and induce CYP2E1 [102, 103]. Because isoniazid is usually co-administered with rifampicin, the net effect of drug-drug interactions involving isoniazid and rifampicin may be the induction driven by rifampicin.
Antiretroviral drugs: Nevirapine and Lopinavir/Ritonavir

The acquired immunodeficiency syndrome is the advanced stage of infection by HIV, a retrovirus that infects the immune system. HIV can be transmitted through sexual intercourse, blood transfusion, contaminated needles, and from a mother to an infant during pregnancy, delivery and lactation [104]. In resource-limited settings, the WHO recommends a first-line antiretroviral regimen that consists of two nucleoside reverse transcriptase inhibitors (NRTIs), and one non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine or efavirenz. Nevirapine is cheaper and more widely available than efavirenz. Protease inhibitors (PIs) are used in the second-line treatment [105, 106]. In general, NRTIs are renally eliminated whereas NNRTIs and PIs are metabolized by CYP enzymes, mainly CYP3A4. In addition, many NNRTIs and PIs can act as inducers or inhibitors of CYP3A4 [107].

Nevirapine

Nevirapine appears to be predominately metabolized by CYP2B6 and CYP3A4 [108, 109]. Metabolism results in four metabolites: 2-, 3-, 8- and 12-hydroxynevirapine; the main metabolites are 2- and 12-hydroxynevirapine. 2-Hydroxynevirapine and 3-hydroxynevirapine seem to be exclusively formed by CYP3A4 and CYP2B6, respectively, while formation of 8- and 12-hydroxynevirapine involves multiple enzymes. CYP3A4, CYP2B6 and CYP2D6 are involved in 8-hydroxynevirapine formation while 12-hydroxynevirapine is mainly formed via CYP3A4, although other enzymes such as CYP2D6 and CYP2C9 are also involved. The four metabolites are further glucuronidated, with 2-, 3- and 12-hydroxynevirapine glucuronide being the major metabolites in plasma. Nevirapine can induce CYP2B6 and CYP3A [110]. It appears to have a capacity for autoinduction, since its plasma levels decline upon starting administration and reach steady state after about 2 weeks [110, 111]. In addition, nevirapine seems to be a substrate for P-gp [112, 113].

Lopinavir/ritonavir

The PI lopinavir is currently available in coformulation with the PI ritonavir in a ratio of 4:1. Ritonavir is used in a subtherapeutic dose to boost lopinavir plasma levels by inhibiting the activity of CYP3A4, the principle enzyme involved in the metabolism of lopinavir. In addition, ritonavir inhibits P-gp, which is involved in lopinavir transport. Ritonavir can induce metabolic enzymes and thus its own metabolism [114]. Lopinavir plasma levels decrease after starting lopinavir/ritonavir administration and reach steady state in about 2 weeks [114]. Upon administration of radioactive lopinavir in combination with ritonavir, unchanged lopinavir accounted for more than 88% of plasma radioactivity [115]. The lopinavir/ritonavir coformulation
induces CYP1A2, CYP2B6, CYP2C9 and CYP2C19 and inhibits CYP3A [116, 117]. In addition, lopinavir/ritonavir seems to enhance glucuronidation [117].

Drug-drug interactions involving antimalarial, antiretroviral and antitubercular drugs: an overview

Drug-drug interactions are an important concern in the management of patients with HIV because of the large number of antiretroviral drugs and other drugs that are required by these patients for the management of comorbidities and opportunistic infections. Combination therapy has also been introduced in the management of malaria and TB, to overcome drug resistance [118]. Drug-drug interactions can lead to reduced therapeutic efficacy due to diminished plasma concentrations. In general, trough samples of NNRTIs and PIs are used for monitoring virological efficacy [119]. The efficacy of artemisinin has been evaluated based on the relationship between the parasite life-cycle and the duration of maintaining artemisinin concentrations above a minimum inhibitory concentration [120]. The microbial effect of rifampicin is related to its concentration [121].

Rifampicin has been implicated in many clinically significant drug–drug interactions, which have been extensively reviewed [95, 122-128]. Because rifampicin is a potent inducer of CYP enzymes and P-gp, it reduces the plasma concentrations of some coadministered antiretroviral drugs, particularly PIs but also, to a lesser extent, NNRTIs [129]. According to a recent review, however, antiretroviral drugs do not seem to influence rifampicin concentrations [130]. Limited information is available in the literature on drug-drug interactions between the artemisinin antimalarial drugs and other drugs such as antiretrovirals [118, 131, 132] or antitubercular drugs; consequently, the extent of such interactions is not fully known. Because of its wide therapeutic index, it is suggested that the efficacy of artemether is unlikely to be affected by drug interactions [118].
Non-linear Mixed Effect Modelling

Non-linear mixed effect modelling involves the simultaneous analysis of data obtained from all individuals. In the two-stage approach, a model is fitted to individual data separately and the variability between individuals is calculated subsequently. The advantages of non-linear mixed effect modelling include its applicability to sparse data that may have been collected using flexible assessment schedules. Further, the approach allows the possibility to separate residual variability from other sources of variability such as interindividual (IIV) and interoccasion variability. The non-linear mixed effects model includes fixed effect parameters, which are the same for all individuals, and random effect parameters that handle different types of variability: between individuals (IIV), between study occasions (interoccasion variability) and between individual predictions and observations (residual variability).

An individual parameter can be expressed by

\[ P_i = \theta_p \cdot e^{\eta_i} \]  

where \( \eta_i \) is the random effect that describes IIV, which is the difference between the typical predicted value \( (\theta_p) \) and the individual estimate \( (P_i) \). The variable \( \eta_i \) is assumed to be normally distributed with mean 0 and variance \( \sigma^2 \). Many biological parameters are assumed to be log-normally distributed, and IIV is therefore described exponentially.

Variability between individuals can be explained by covariates such as weight, age, gender, bilirubin level, and other drugs taken.

Residual variability may be due to experimental error, dose or sampling time error and model misspecification. Residual error can be investigated using additive, proportional or slope-intercept models. The following equation applies to additive errors:

\[ \text{Observation}_{ij} = PRED_{ij} + \varepsilon_{ij} \]  

where \( \varepsilon_{ij} \) is the random effect that describes the difference between the individual prediction \( (PRED_{ij}) \) and the \( j^{th} \) observation for the \( i^{th} \) individual. The variable \( \varepsilon_{ij} \) is assumed to be normally distributed with mean 0 and variance \( \sigma^2 \).
### Aims of the thesis

The overall aim of the thesis was to investigate possible pharmacokinetic drug-drug interactions between antimalarial, antitubercular and antiretroviral drugs.

The specific aims were to:

- develop and validate a sensitive and specific analytical method for the detection and quantification of mephenytoin and its metabolites nirvanol and 4'-hydroxymephenytoin in human plasma and urine

- investigate the ability of the artemisinin antimalarial drugs to induce and/or inhibit principal CYP enzymes and to compare the potential for drug-drug interactions among the artemisinin drugs to choose a derivative that is suitable for combination therapy from a drug-drug interaction perspective

- develop an integrated pharmacokinetic enzyme turn-over model to describe the pharmacokinetics of the probe drug $S$-mephenytoin and its metabolites $S$-nirvanol and $S$-4'-hydroxymephenytoin in order to investigate the CYP2B6 inductive potential of artemisinin antimalarial drugs and to verify CYP2B6 induction by artemisinin and CYP2C19 induction by artemether and arteether

- describe the population pharmacokinetics of nevirapine in HIV-infected patients taking nevirapine-based antiretroviral therapy in the presence and absence of the antitubercular drug rifampicin

- compare the population pharmacokinetics of lopinavir in TB/HIV co-infected children taking lopinavir/ritonavir in a ratio of 1:1 in the presence of the antitubercular drug rifampicin, with that of lopinavir in HIV-infected children taking lopinavir/ritonavir in a ratio of 4:1.
Materials and methods

Experimental procedures

Ethics (Papers II, IV and V)
The study in paper II was approved by the Ministry of Health Hanoi, Vietnam and by the ethics committee of Göteborg University, Göteborg, Sweden and the Swedish Medical Products Agency, Uppsala, Sweden. The studies in papers IV and V were approved by the Research Ethics Committees of the Universities of Cape Town (papers IV and V), Stellenbosch and the Witwatersrand (paper V), South Africa. The studies were conducted in accordance with the Helsinki Declaration after obtaining informed consent from the participants (papers II and IV), a parent or legal guardian (paper V).

Subjects (Papers II - V)

Artemisinin antimalarial drugs studies (Papers II and III)
Seventy-five healthy Vietnamese volunteers were included in paper II study. Subjects who had received any antimalarial drug within one month or other medication within two weeks of the study and those known to be alcohol abusers were excluded. Thirty-six of the subjects were smokers (all were men) of no more than 10 cigarettes per day. In paper III, data from the aforementioned subjects were included in addition to data obtained from another study (rich data study) of fourteen healthy male Vietnamese volunteers, comprising six CYP2C19 poor metabolizers (PMs) and eight CYP2C19 extensive metabolizers (EMs) [76].

Nevirapine-rifampicin interaction study (Paper IV)
Twenty-seven HIV-infected patients were enrolled during the continuation phase of standard TB treatment. Twenty-six HIV-positive patients who were without TB were enrolled as a control group. Patients with Karnofsky scores below 70, known severe renal, hepatic or gastrointestinal disease including malabsorption or severe diarrhoea, or those receiving other medication that might have affected the plasma concentrations of the drugs in the study were excluded.
Lopinavir/ritonavir-rifampicin interaction study (Paper V)
The study consisted of two parallel treatment groups, TB/HIV and HIV. The TB/HIV group consisted of 15 TB/HIV coinfected children and the HIV group consisted of 15 HIV-positive children without TB.

Study design (Papers II - V)

Artemisinin antimalarial drugs studies (Papers II and III)
In paper II, the volunteers were randomized to receive one of the following five drugs: artemisinin (500 mg), dihydroartemisinin (60 mg), artemether (100 mg), arteether (100 mg) or artesunate (100 mg). Repeated oral doses were administered once daily in the morning for five days (day 1 to day 5). A cocktail containing the probe drugs caffeine (100 mg), coumarin (5 mg), midazolam (7.5 mg), metoprolol (100 mg), mephenytoin (100 mg), and chlorzoxazone (250 mg) was given orally one week before administration of the artemisinin drug (on day -6). Administration of the probe drugs was repeated on day 1 and day 5, one hour after intake of the artemisinin antimalarial drugs. After a wash-out period of five days (day 10), administration of the probe drugs was repeated. Probe drugs were measured in blood samples obtained pre-dose, 4 and 8 hours after the cocktail drugs on days -6, 1, 5 and 10. On these days, total voided urine was also collected for 8 hours after administration of the cocktail. The total weight of each urine sample was recorded. A physical examination was performed on days -11 and 15. Subjects were interviewed about adverse events on days 5 and 15. Henceforth, paper II study will be referred to as “sparse data study” when its data are discussed as part of paper III.

In paper III, additional data were obtained from participants in the rich data study who received single oral doses of 200 mg racemic mephenytoin and 500 mg artemisinin. Twenty-eight days later, artemisinin 250 mg/day was given for 9 days, with a 500 mg dose on day 10. A single mephenytoin 200 mg dose was given on the fourth day of the 10-day artemisinin administration. In addition, to monitor CYP2C9 activity, single doses of tolbutamide 500 mg were given at the first occasion of mephenytoin administration, on the seventh day of the 10-day artemisinin administration as well as 2 and 7 days after the last artemisinin dose. In EMs on the second occasion of mephenytoin administration and in PMs, blood samples for the determination of plasma concentrations of mephenytoin were taken 5 minutes before mephenytoin intake and 10 hours after, and then one sample was taken each morning on days 1, 2, 3, 5, 9 and 13 after mephenytoin administration. In EMs on the first occasion of mephenytoin administration, blood samples were taken 5 minutes before and 1, 2, 3, 4, 5, 6, 7, 8, and 10 hours and 1, 2, 3, 5, 9 and 13 days after mephenytoin administration.
Nevirapine-rifampicin interaction study (Paper IV)
The TB/HIV patients were established on an antiretroviral regimen consisting of two NRTIs plus oral nevirapine 200 mg twice daily and standard recommended weight-based dosages of rifampicin and isoniazid. They were enrolled and admitted for pharmacokinetic blood sampling at the beginning of the study and at least 14 days after completion of the TB treatment. The HIV patients were enrolled and admitted for pharmacokinetic blood sampling after being established on the same antiretroviral regimen (two NRTIs plus oral nevirapine 200 mg twice daily) for at least 3 weeks. Blood samples were taken just before and 0.5, 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 hours after drug administration from 17 TB/HIV patients and 8 HIV patients. Two blood samples, a minimum of 1 hour apart, were obtained on each sampling occasion from the remainder of the patients in both groups.

For the part of the study including intensively sampled TB/HIV coinfected patients (up to 12 hours after nevirapine administration) a noncompartmental analysis of nevirapine and 12-hydroxynevirapine pharmacokinetics has been previously reported [133].

Lopinavir/ritonavir-rifampicin interaction study (Paper V)
The TB/HIV group received lopinavir/ritonavir in a dose ratio of 1:1 with a nominal lopinavir dosage of 230 mg/m² twice daily, in addition to two NRTIs and rifampicin 10 mg/kg/day. Blood samples were taken after the children had been established on the TB/HIV co-treatment for at least 4 weeks. Upon completing the TB treatment, the children received standard doses of lopinavir/ritonavir in a ratio of 4:1 with a nominal lopinavir dosage of 230 mg/m² twice daily, in addition to two NRTIs. After a wash-out period of not less than 4 weeks, they were readmitted for blood sampling but only 10 of the 15 children returned for the follow-up. The HIV group received lopinavir/ritonavir in a ratio of 4:1, with a nominal lopinavir dosage of 230 mg/m² twice daily, in addition to two NRTIs. After a minimum of 4 weeks, the children were admitted for blood sampling. Blood samples were taken just before and 2, 3, 4, 5, 6, 8 and 12 hours after drug administration.

Chemical Analysis
All analyses were conducted using high performance liquid chromatography/mass spectrometry systems. Detailed descriptions of the chemical assays can be found in the respective papers.
Enzyme probes (Papers I and II)

**Mephenytoin, nirvanol and 4’-hydroxymephenytoin (Paper I)**

An enantiospecific liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed to separate and quantify mephenytoin and its metabolites nirvanol and 4’-hydroxymephenytoin in plasma and urine. The LC/MS/MS system included two LC-10ADVP pumps (Shimadzu, Kyoto, Japan), a Triathlon 900 autosampler (Spark Holland, The Netherlands) equipped with a 10 μL loop and an extra valve, a Chiral α1-acid glycoprotein (AGP) column, 150 x 4.0 mm i.d., 5 μm particle size (ChromTech, Hägersten, Sweden) and a 0.5 μm A-431 in-line filter (Upchurch Scientific Inc., WA, USA) before the column. The flow was split to 0.25 mL/min before entering the triple quadrupole mass spectrometer (Quattro Ultima; Micromass, Manchester, UK) in electrospray positive ion mode. The source temperature and desolvation temperature were set to 130 and 400°C, respectively. Nitrogen was used as both cone and desolvation gas, held at 200 and 800 L/h, respectively. The pressure of the collision gas, argon, was 3x10⁻³ Torr. The capillary and cone voltages were 2.7 kV and 35 V, respectively. The precursor-product ion pair was \( m/z \) 219 → 134 for mephenytoin, \( m/z \) 205 → 134 for nirvanol and \( m/z \) 235 → 150 for 4’-hydroxymephenytoin. The corresponding collision energies were 18, 17 and 19 eV. The mobile phase consisted of 2% acetonitrile in 5mM ammonium acetate. Plasma samples were precipitated with acetonitrile while urine samples were diluted with the mobile phase. After urine sample injection, the mobile phase was discarded for the period 0-2.5 min by switching the flow using the extra valve. For evaluation of the intra-day precision and accuracy of the assay, one calibration curve, six replicates of the quality control samples and five or six replicates of the lowest standard concentration were processed each day. The plasma inter-assay precision and accuracy were assessed by analyzing duplicate quality control samples on eleven separate occasions. The urinary inter-assay precision and accuracy were assessed by analyzing six replicate quality control samples on three separate occasions. The precision was calculated as the relative standard deviation (coefficient of variation) and the accuracy was determined by the mean calculated concentration as a percentage of the nominal concentration.

**Caffeine, paraxanthine, chlorzoxazone, 6-hydroxychlorzoxazone, 7-hydroxycoumarin, metoprolol, α-hydroxymetoprolol, midazolam and 1-hydroxymidazolam (Paper II)**

An LC/MS/MS method modified from Scott et al. [134] was used to measure plasma concentrations of caffeine, paraxanthine, chlorzoxazone, 6-hydroxychlorzoxazone, 7-hydroxycoumarin, metoprolol, α-hydroxymetoprolol, midazolam and 1-hydroxymidazolam. The LC/MS/MS system included two LC-10ADVP pumps (Shimadzu, Kyoto, Japan) with a
high-pressure gradient mixer, a Triathlon 900 autosampler (Spark Holland, The Netherlands) equipped with a 10μL loop, a reversed phase column (HyPurity C18, Thermo Hypersil-Keystone, PA, USA), 3 μm particle size, 50 x 4.6 mm protected by a 10 x 4 mm guard column of the same material. The analytes were separated using a gradient with the mobile phase changing from 95% of (0.05% formic acid in water) to 95% of (70% acetonitrile in 0.05% formic acid) during 4.5 min and then 0.5 min later changing back to 95% of (0.05% formic acid in water). The flow from the column was split to 200 μL/min before entering the triple quadrupole mass spectrometer (Quattro Ultima; Micromass, Manchester, UK). Plasma samples, incubated with β-glucuronidase solution, were precipitated with acetonitrile. 7-Hydroxycoumarin concentrations in urine were determined using a modified version of the method used for 7-hydroxyccoumarin in plasma. The mobile phase contained 20% acetonitrile in 0.05% formic acid with no gradient. Urine samples, incubated with β-glucuronidase solution, were diluted with the mobile phase.

Nevirapine and its metabolites 2-, 3-, 8- and 12-hydroxynevirapine (Paper IV)

The method used to measure plasma concentrations of nevirapine and its metabolites 2-, 3-, 8- and 12-hydroxynevirapine was developed based on previously published methods [133, 135].

Nevirapine plasma concentrations were measured by the use of a liquid chromatography/mass spectrometry method. LC was performed with a Waters Alliance 2690 LC system and a 20 x 2.1 mm Hypersil Gold C18 column (Thermo). For detection, a Waters/Micromass ZMD single quadrupole mass spectrometer (Waters, Milford, MA, USA) was used in positive ionization mode for nevirapine at m/z 276.2 and neostigmine, the internal standard, at m/z 223.2. Plasma concentrations of 2-, 3-, 8- and 12-hydroxynevirapine were determined by LC/MS/MS method. The system consisted of an Applied Biosystems (Foster City, CA, USA) API 3200 linear ion trap. LC was performed using an Agilent 1200 series instrument and a column: Gemini C18 3 μm particle size, 2.0 x 50 mm (Phenomonex, Torrence CA, USA). The precursor-product ion pairs were m/z 283.2 → 214.1 for 3-hydroxynevirpine, m/z 283.2 → 160.9 for 2-hydroxynevirapine, m/z 283.2 → 265.2 for 12-hydroxynevirapine, m/z 283.2 → 242.3 for 8-hydroxynevirapine, and m/z 276.3 → 162.3 for physostigmine, the internal standard.
Data analysis

Noncompartmental data analysis and statistics (Papers II and IV)

In paper II, the 4-hour plasma concentration ratios of paraxanthine to caffeine, S-4'-hydroxymephenytoin to S-mephenytoin, α-hydroxymetoprolol to metoprolol, 1-hydroxymidazolam to midazolam and 6-hydroxychlorzoxazone to chlorzoxazone were used to evaluate the activities of CYP1A2, CYP2C19, CYP2D6, CYP3A and CYP2E1, respectively. The total amount of 7-hydroxycoumarin excreted in urine from 0 to 8 hours was used to evaluate CYP2A6 activity. The metrics for the individual enzyme activities were calculated from probe drugs concentrations obtained on days -6, 1, 5 and 10. For comparison of enzyme activities between study days, the following four contrasts were estimated in the statistical evaluation; day 1 vs day -6, day 5 vs day -6, day 5 vs day 1 and day 10 vs day -6. A repeated ANOVA model with Gaussian random effects was applied to the log-transformed (natural base) data. An overall test level of 5% for the multiple (four) tests per treatment group was selected. In accordance with the Bonferroni method for multiple testing, 98.75% confidence intervals are presented and p-values are compared with 0.0125 in the sequel. The Proc Mixed in SAS 8.2 (SAS Company Inc, Cary, USA) software was used for the analysis.

In paper IV, the AUC values of nevirapine metabolites within a specific dosing interval were calculated from plasma concentrations following the morning dose (AUC-day) and the night dose (AUC-night). The AUCs were calculated using linear trapezoids for ascending and log trapezoids for descending intensive data. The paired t-test (S-plus 6.2 for Windows, Insightful Corp., Seattle, WA) was used for comparing AUC-day for TB/HIV patients during and after TB treatment. The comparison for AUC-night was performed in the same manner. A p-value of < 0.05 was considered statistically significant.

Modelling (Papers III-V)

The population pharmacokinetics of S-mephenytoin, S-4'-hydroxymephenytoin and S-nirvanol (paper III), nevirapine (paper IV) and lopinavir (paper V) were described by nonlinear mixed effects modelling using NONMEM version VI software (GloboMax, Hanover, MD, U.S.A). [136]. The typical population pharmacokinetic parameters and IIIV and residual variabilities were estimated using the first-order method (FO), first-order conditional estimation method (FOCE) for S-mephenytoin, S- 4'-hydroxymephenytoin and S-nirvanol (paper III) and the first-order condi-
tional estimation method with interaction (FOCEI) for nevirapine (paper IV) and lopinavir (paper V).

Model selection was based on minus twice the log likelihood of the data (the objective function value; OFV), the standard error of the parameter estimates, graphical analysis using Xpose versions 3.104 and 4 [137] and scientific plausibility. A decrease in the OFV of 6.63 ($p < 0.01$) or 3.84 ($p < 0.05$) between two nested models was regarded as statistically significant for estimations using FO and FOCE, respectively. Covariate models were built by forward inclusion of the covariates (OFV decrease of $> 10.83; p < 0.001$) followed by stepwise deletion (OFV increase of $> 12.12; p < 0.0001$).

Model validation was done by bootstrap resampling [138]. Parameter estimates from the final model were re-estimated for each of the 1000 bootstrap samples. The median and 95% confidence intervals were also compared with the NONMEM estimates. Simulations plus predictive checks [139] were used for model validation; the final model and parameter estimates were used to simulate 1000 datasets based on the original design. Observed concentrations were compared with the simulated median and 95% nonparametric prediction interval.

In paper III, the data were pooled from the sparse and rich data studies [76]. The data were analyzed in steps. The data of the first occasion of mephenytoin administration in both studies were analyzed by initially fitting a submodel to the S-mephenytoin data followed by simultaneously fitting a submodel to S-mephenytoin, S-4’-hydroxymephenytoin and S-nirvanol data using fixed S-mephenytoin pharmacokinetic parameters. Finally, all data were incorporated then simultaneously analyzed to develop the final model. One- and multi-compartment models were fitted to S-mephenytoin, S-4’-hydroxymephenytoin and S-nirvanol data. Due to the reported high oral first-pass effect of S-mephenytoin in EMs [140], first-pass formation of S-4’-hydroxymephenytoin and S-nirvanol was tested by evaluating the need for a hypothetical absorption compartment [141] for each of the substances. Graphical analysis revealed that the formation of S-nirvanol was slow in PMs. The need for up to ten transit compartments between the central compartments of S-mephenytoin and S-nirvanol was tested. Induction of CYP2B6 and CYP2C19 by artemisinin and the inductive potential of dihydroartemisinin, arteether, artemether and artesunate were characterized by an enzyme turn-over model as described by equation 3. Enzyme induction was achieved by multiplying $R_{in}$ by a factor (DRUG) describing induction of the enzyme.

Subjects from the rich data study were screened for CYP2C19 phenotype [76]. In the sparse data study, although the subjects were not phenotyped before the study, those with S-4’-hydroxymephenytoin plasma concentrations below the lower limit of quantification (LLOQ) were judged to be PMs. The $\text{MIXTURE}$ subroutine was used in paper III for assigning all individuals to subpopulations. The agreement between the estimated pheno-
type according to the model and the observed phenotype in the rich data set was noted. The difference in CYP2C19-mediated formation of S-4'-hydroxymephenytoin among the different phenotypes was accounted for in the model by investigating the need for separate fixed effect parameters depending on phenotype.

One- and two-compartment models with linear elimination and first-order absorption were fitted to nevirapine (paper IV) and lopinavir (paper V) data. In paper IV, nevirapine plasma concentrations after twice-daily doses of 300, 400 and 500 mg were simulated in intensively sampled TB/HIV coinfected patients during rifampicin-containing TB treatment based on the individual pharmacokinetic parameters from the final model.
Results and discussion

Quantification of mephenytoin and its metabolites nirvanol and 4’- hydroxymephenytoin (Paper I)

The method showed satisfactory precision, accuracy and reproducibility. The calibration curves in plasma and urine were linear in the concentration ranges tested. The coefficient of correlation was above 0.996 for both plasma and urine curves. LLOQ in plasma and urine was ≤ 3 ng/mL with intra-day CVs of < 12.4% and accuracies ranging between 87.2 and 108.3% in plasma and between 98.9 and 104.8% in urine.

In a previous method [142], a Nucleosil 100 C2 column was coupled in series to the chiral AGP column to retain S- and R-mephenytoin, because of their co-elution on the AGP column. However, the extra column is not needed in this MS method. Because MS/MS detection is more sensitive than UV detection, the samples do not need to be concentrated. This is advantageous for large numbers of study samples (as in paper II), where a simple and less time-consuming sample preparation method was required. Precipitation of plasma proteins with acetonitrile was tested and found to be both simple and rapid.

The selected mobile phase, 2% acetonitrile in 5 mM ammonium acetate with a non-adjusted pH of 6.85, gave good separation between the enantiomers and low ion suppression. A chromatogram of the substances in spiked human plasma sample is shown in Figure 1. Signal suppression, due to endogenous compounds present in the samples, was observed between 5.6 and 6.8 minutes after sample injection. With 3.0-3.5% acetonitrile in the mobile phase, both R-nirvanol and S- and R-mephenytoin peaks were eluted in the critical interval, resulting in decreased responses. A mobile phase containing acetonitrile concentrations <2% resulted in a suppressed S-nirvanol peak. Heating the AGP column to 30°C can be used to shorten retention times without negatively affecting the enantioseparation. However, for maximum separation between S- and R-nirvanol using the chosen mobile phase, and to avoid the signal suppression interval, the column was maintained at room temperature. The retention times were 4.8 and 7.8 minutes for S- and R-nirvanol, respectively.
Figure 1. Chromatogram of a spiked plasma sample containing 120 ng/mL of R-4’-hydroxymephenytoin (1), S-4’-hydroxymephenytoin (2) and S-nirvanol (3) and 350 ng/mL of R-nirvanol (4), S-mephenytoin (5) and R-mephenytoin (6).
Assessment of the effect of artemisinin antimalarial drugs on enzymatic activity

Noncompartmental data assessment (Paper II)

The cocktail procedure was generally well tolerated by all subjects apart from one who discontinued the study because of nausea on day 1.

The effects of the artemisinin antimalarial drugs on the investigated CYP enzymes are summarized in Table 1.

For CYP3A activity, the mean 1-hydroxymidazolam/midazolam concentration ratio at 4 hours increased significantly after five days intake of artemisinin, artemether or dihydroartemisinin (day 5 vs day -6). The increase in the ratio by artemisinin was evident on the first day of its administration (day 1 vs day -6). In the artesunate group, the ratio significantly increased at day 10 compared to day -6. Previous findings revealed no effect of artemisinin on CYP3A4 activity, using omeprazole as a probe and the urinary excretion ratio of endogenous 6β-hydroxycortisol/cortisol as an additional marker for CYP3A4 [73]. Although it is not selective for CYP3A4 versus CYP3A5, midazolam is considered to be the best available probe for assessment of CYP3A4 activity [143]. Furthermore, artemisinin has induced the expression of CYP3A4 in primary human hepatocytes and in a human intestinal cell line [77]. Midazolam was found to interact with chlorzoxazone when the two probes were used in the same cocktail [144]. Chlorzoxazone seems to inhibit the first pass metabolism of midazolam by CYP3A in the gut but not in the liver. If the artemisinin antimalarial drugs induce intestinal CYP3A activity, this might not be reflected in the present study and the increase in the 1-hydroxymidazolam/midazolam concentration ratio would then be due to induction of hepatic CYP3A activity only.

For CYP2C19 activity, the mean 4-hour S-4'-hydroxymephenytoin/S-mephenytoin plasma concentration ratio increased upon multiple administration of artemisinin, arteether (day 5 vs day -6) or artemether (day 5 vs day 1) for 5 days. S-4'-hydroxymephenytoin concentrations were not measurable in 9 subjects who were excluded from the data analysis since they were considered to be CYP2C19 PMs. Artemisinin has previously been reported to induce CYP2C19 [73, 75]. CYP2C19 activity has been evaluated by total urinary recovery of S-4'-hydroxymephenytoin and S/R-mephenytoin ratio in urine [38, 39, 42, 47]. However, since artemisinin increases the oral clearance (CL/F) of both R- and S-mephenytoin [76], their ratio as a metric for CYP2C19 activity would be confounded. R-mephenytoin concentrations were still present in pre-dose samples from days 1, 5 and 10, further confounding the S/R-mephenytoin ratios. Therefore the S-4'-hydroxymephenytoin/S-mephenytoin concentration ratio, although not a prevalidated metric, was considered the best choice to estimate CYP2C19 activ-
ity. Moreover, a metabolite/drug plasma concentration ratio is a more specific metric for enzyme activity than total recovery of a metabolite in urine.

Table 1. Phenotyping metrics in the five different treatment groups (artemisinin (ART), dihydroartemisinin (DHA), arteether (ARE), artemether (ARM) and artesunate (AS)). The presented quotients (mean, 98.75% CI) are based on anti-logarithms of the contrast for the different occasions.

<table>
<thead>
<tr>
<th>Enzyme Phenotyping metric</th>
<th>Quotients of metric for different occasions(^a)</th>
<th>ART</th>
<th>DHA</th>
<th>ARE</th>
<th>ARM</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP1A2</strong> paraxanthine/caffeine 4-hr concentration ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>0.27 (0.18-0.39)(^a)</td>
<td>0.73 (0.59-0.90)(^a)</td>
<td>0.70 (0.55-0.89)(^a)</td>
<td>0.83 (0.69-1.02)</td>
<td>0.87 (0.69-1.09)</td>
<td></td>
</tr>
<tr>
<td>day 5/day -6</td>
<td>0.59 (0.41-0.85)(^a)</td>
<td>0.85 (0.69-1.06)</td>
<td>0.70 (0.55-0.89)(^a)</td>
<td>0.81 (0.67-0.98)(^a)</td>
<td>1.00 (0.80-1.26)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>2.22 (1.54-3.12)(^a)</td>
<td>1.17 (0.95-1.45)</td>
<td>1.00 (0.78-1.27)</td>
<td>0.97 (0.80-1.18)</td>
<td>1.16 (0.92-1.45)</td>
<td></td>
</tr>
<tr>
<td>day 10/day -6</td>
<td>1.26 (0.88-1.81)</td>
<td>0.94 (0.76-1.16)</td>
<td>0.84 (0.66-1.06)</td>
<td>1.06 (0.87-1.30)</td>
<td>1.10 (0.88-1.38)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2A6</strong> 7-OH-coumarin excreted in 0-8 hour urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>0.74 (0.40-1.40)</td>
<td>1.17 (0.73-1.88)</td>
<td>0.81 (0.38-1.71)</td>
<td>1.01 (0.63-1.62)</td>
<td>0.73 (0.38-1.44)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>0.87 (0.48-1.60)</td>
<td>1.34 (0.84-2.14)</td>
<td>0.95 (0.45-2.02)</td>
<td>0.91 (0.57-1.45)</td>
<td>0.60 (0.30-1.17)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.17 (0.62-2.23)</td>
<td>1.15 (0.71-1.85)</td>
<td>1.18 (0.56-2.51)</td>
<td>0.90 (0.56-1.44)</td>
<td>0.81 (0.41-1.61)</td>
<td></td>
</tr>
<tr>
<td>day 10/day -6</td>
<td>0.96 (0.53-1.74)</td>
<td>1.38 (0.87-2.19)</td>
<td>1.17 (0.55-2.47)</td>
<td>1.22 (0.77-1.94)</td>
<td>0.86 (0.44-1.68)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2C19</strong> S-4'-OH-mephenytoin S-mephenytoin 4 hr concentration ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>0.95 (0.83-1.09)</td>
<td>0.97 (0.78-1.21)</td>
<td>0.93 (0.80-1.08)</td>
<td>0.95 (0.79-1.14)</td>
<td>0.91 (0.73-1.14)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.69 (1.47-1.94)(^a)</td>
<td>1.16 (0.93-1.44)</td>
<td>1.33 (1.15-1.55)(^a)</td>
<td>1.20 (1.00-1.44)</td>
<td>1.12 (0.89-1.40)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.77 (1.54-2.04)(^a)</td>
<td>1.19 (0.96-1.49)</td>
<td>1.44 (1.24-1.67)(^a)</td>
<td>1.26 (1.05-1.52)(^a)</td>
<td>1.22 (0.98-1.53)</td>
<td></td>
</tr>
<tr>
<td>day 10/day -6</td>
<td>1.65 (1.44-1.88)(^a)</td>
<td>1.13 (0.91-1.41)</td>
<td>1.26 (1.08-1.46)(^a)</td>
<td>1.14 (0.94-1.38)</td>
<td>1.18 (0.94-1.49)</td>
<td></td>
</tr>
<tr>
<td><strong>CYP2D6</strong> a-OH-metoprolol/metoprolol 4-hr concentration ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>0.82 (0.70-0.96)(^a)</td>
<td>0.83 (0.71-0.96)(^a)</td>
<td>0.89 (0.75-0.95)</td>
<td>0.90 (0.76-1.05)</td>
<td>0.90 (0.79-1.04)</td>
<td></td>
</tr>
<tr>
<td>day 5/day -6</td>
<td>1.10 (0.94-1.29)</td>
<td>1.16 (0.93-1.44)</td>
<td>1.33 (1.15-1.55)(^a)</td>
<td>1.20 (1.00-1.44)</td>
<td>1.12 (0.89-1.40)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.34 (1.14-1.58)(^a)</td>
<td>1.14 (0.99-1.33)</td>
<td>1.15 (0.97-1.37)</td>
<td>1.08 (0.92-1.27)</td>
<td>1.13 (0.99-1.30)</td>
<td></td>
</tr>
<tr>
<td>day 10/day -6</td>
<td>1.15 (0.98-1.34)</td>
<td>0.93 (0.80-1.08)</td>
<td>0.98 (0.83-1.17)</td>
<td>0.92 (0.78-1.09)</td>
<td>1.07 (0.93-1.24)</td>
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</tr>
<tr>
<td><strong>CYP2E1</strong> 6-OH-chlorozoxazone/chlorozoxazone 4-hr concentration ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>0.68 (0.54-0.86)(^a)</td>
<td>0.93 (0.66-1.31)</td>
<td>1.13 (0.84-1.51)</td>
<td>1.06 (0.85-1.33)</td>
<td>0.96 (0.73-1.26)</td>
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</tr>
<tr>
<td>day 5/day -6</td>
<td>0.74 (0.58-0.94)(^a)</td>
<td>1.00 (0.70-1.31)</td>
<td>1.00 (0.74-1.32)</td>
<td>0.99 (0.86-1.35)</td>
<td>1.08 (0.83-1.43)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.08 (0.85-1.38)</td>
<td>1.07 (0.76-1.52)</td>
<td>0.88 (0.66-1.17)</td>
<td>1.02 (0.81-1.28)</td>
<td>1.08 (0.86-1.48)</td>
<td></td>
</tr>
<tr>
<td>day 10/day -6</td>
<td>0.90 (0.71-1.14)</td>
<td>0.83 (0.59-1.17)</td>
<td>0.83 (0.59-1.17)</td>
<td>1.07 (0.85-1.35)</td>
<td>1.03 (0.79-1.36)</td>
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</tr>
<tr>
<td><strong>CYP3A</strong> 1-OH-midazolam/midazolam 4-hr concentration ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1/day -6</td>
<td>1.60 (1.26-2.02)(^a)</td>
<td>1.11 (0.94-1.30)</td>
<td>0.97 (0.79-1.20)</td>
<td>1.22 (0.90-1.65)</td>
<td>1.17 (0.94-1.47)</td>
<td></td>
</tr>
<tr>
<td>day 5/day -6</td>
<td>2.66 (2.10-3.36)(^a)</td>
<td>1.25 (1.06-1.47)(^a)</td>
<td>1.16 (0.94-1.43)</td>
<td>1.54 (1.14-2.09)(^a)</td>
<td>1.25 (1.00-1.56)</td>
<td></td>
</tr>
<tr>
<td>day 5/day 1</td>
<td>1.67 (1.31-2.12)(^a)</td>
<td>1.13 (0.96-1.33)</td>
<td>1.19 (0.97-1.47)</td>
<td>1.27 (0.93-1.72)</td>
<td>1.06 (0.85-1.33)</td>
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<tr>
<td>day 10/day -6</td>
<td>1.25 (0.99-1.58)</td>
<td>1.16 (0.98-1.36)</td>
<td>1.12 (0.90-1.38)</td>
<td>1.15 (0.84-1.57)</td>
<td>1.26 (1.01-1.57)(^a)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) p < 0.0125 (α adjusted for multiple testing)

\(^b\) Quotients > 1 indicate increased enzyme activity, quotients < 1 indicate decreased enzyme activity
For CYP1A2 activity, the mean 4-hour paraxanthine/caffeine plasma concentration ratio was significantly decreased on the first day of administration of artemisinin, dihydroartemisinin and arteether (day 1 vs day -6). However, multiple administration of artemisinin for 5 days elevated the ratio (day 5 vs day 1). Given that residual concentrations of caffeine and paraxanthine were found in the pre-dose samples, the CYP1A2 metric was confounded. It has been suggested that abstinence from caffeine should be extended to 36 hours to avoid measuring paraxanthine remaining in the body [53].

The marker for CYP2D6 activity, α-hydroxymetoprolol/metoprolol concentration ratio at 4 hours, was significantly decreased by artemisinin and dihydroartemisinin (day 1 vs day -6). Artemisinin caused a significant increase in the ratio from day 1 to day 5. Since CYP2D6 is considered to be a non-inducible enzyme, this increase might be explained by induction of an enzyme normally of minor importance for the formation of α-hydroxymetoprolol from metoprolol. Data from two subjects with no detectable α-hydroxymetoprolol concentrations and who were considered to be CYP2D6 PMs were excluded from the analysis.

For CYP2E1 activity, the 6-hydroxyclorzoxazone/chlorzoxazone concentration ratio at 4 hours decreased on the first day of artemisinin administration (day -6 vs day 1) and continued to decrease thereafter (day -6 vs day 5).

For CYP2A6 activity, the 7-hydroxycoumarin amount excreted in 0- to 8-hour urine and plasma concentrations were not affected by administration of any of the studied drugs. Coumarin plasma concentrations were below the detection limit. Coumarin might not be an ideal probe for studying CYP2A6 induction. Coumarin shows extensive first pass metabolism; its systemic availability is only 4% and 7-hydroxycoumarin, its major metabolite, is 95% excreted as the glucuronide in urine within 4 hours [48].

Smoking has been reported to induce CYP1A2 and inhibit CYP2A6 activities [145, 146]. Abstaining from cigarette smoking was not possible in the cultural setting of the present study; consequently, low levels of regular smoking were allowed and monitored by questioning. However, if CYP enzymes activities are induced in smokers, baseline levels could have been increased in these subjects and further induction by the artemisinin antimalarial drugs might not have occurred to the same extent as in non-smokers. The randomization of subjects to each treatment group was not stratified for gender, resulting in a poor match for the number of females in the different groups. However, the inducibility of CYP enzymes in freshly cultured human hepatocytes does not seem to be influenced by gender [147]. Oral contraceptives can alter the base-line levels of enzyme activity, but female participants were not specifically asked about their use of oral contraceptives in the present study.
Model based assessment (Paper III)

An integrated pharmacokinetic enzyme turnover model was developed to describe the concentration-time data of \( S\)-mephenytoin and its metabolites \( S\)-nirvanol and \( S\)-4´-hydroxymephenytoin, and the induction of the formation of \( S\)-4´-hydroxymephenytoin and \( S\)-nirvanol by the antimalarial drugs (Figure 2).

**Figure 2.** Integrated pharmacokinetic – enzyme turn-over model of \( S\)-mephenytoin, and its metabolites \( S\)-nirvanol and \( S\)-4´-hydroxymephenytoin pharmacokinetics in CYP2C19 EMs, IMs and PMs as well as enzyme turn-over model for the induction of \( S\)-nirvanol and \( S\)-4´-hydroxymephenytoin formation. \( F_M \) is the \( S\)-mephenytoin bioavailability. \( k_a \) is the \( S\)-mephenytoin absorption rate constant. \( \text{CL}_{\text{OH}-f} \) and \( \text{CL}_{\text{OH}-e} \) are the formation and elimination clearances of \( S\)-4´-hydroxymephenytoin, respectively. \( k_{N-f1} \) and \( k_{N-f2} \) are the formation rate constant of \( S\)-nirvanol in CYP2C19 EMs/IMs and PMs, respectively. \( \text{CL}_{N-e} \) is the elimination clearances of \( S\)-nirvanol. \( V_{M-c}, V_{OH-c} \) and \( V_{N-c} \) are the volumes of the central compartments of \( S\)-mephenytoin, \( S\)-4´-hydroxymephenytoin and \( S\)-nirvanol, respectively. \( V_{M-p1}/V_{M-p2}, V_{OH-p} \) and \( V_{N-p} \) are the volumes of the peripheral compartments of \( S\)-mephenytoin, \( S\)-4´-hydroxymephenytoin and \( S\)-nirvanol, respectively. \( Q_{M1}/Q_{M2}, Q_{OH} \) and \( Q_N \) are the intercompartmental clearance of \( S\)-mephenytoin, \( S\)-4´-hydroxymephenytoin and \( S\)-nirvanol, respectively. \( k_{enz(n)} \) is the production rate and rate constant for degradation of enzymes.
The S-mephenytoin data were analyzed using a three-compartment model with first-order absorption and two elimination pathways which describe the formation of S-nirvanol and S-4′-hydroxymephenytoin. The S-4′-hydroxymephenytoin data and S-nirvanol data were analyzed using two-compartment models. Two transit compartments before the central S-nirvanol compartment accounted for its slow appearance in PMs. The transit compartments may explain the probable S-nirvanol enterohepatic recirculation, since biliary output has been reported in dogs [148]. S-mephenytoin and S-nirvanol are hydroxylated by the same enzyme [149]. The lack of a requirement for transit compartments in CYP2C19 EMs and intermediate metabolizers (IMs) may be due to the formation of hydroxylated S-nirvanol since enterohepatic recirculation may be influenced by metabolite formation as the metabolite may escape into urine or faeces [150].

Three phenotypes have been reported for CYP2C19: EMs, IMs and PMs [151]. S-mephenytoin, S-4′-hydroxymephenytoin and S-nirvanol data, of the first occasion of mephenytoin administration in the rich and sparse data studies, were used to estimate 3 subpopulations using the $MIXTURE subroutine by evaluating the pharmacokinetic parameters that were different among the three subpopulations. The percentages of subjects estimated to be EMs, IMs and PMs were about 60, 23 and 17%, respectively, which is consistent with literature reports of the distribution in Oriental populations [15]. All individuals phenotyped as PMs in the rich data study and judged to be PMs in the sparse data study were estimated as PMs by $MIXTURE. The phenotyping only distinguished between PMs and EMs. Of the eight subjects phenotyped as EMs in the rich data study, two were estimated to be IMs. The overlap of the distribution of the phenotyping metrics between subjects who are homozygous for the wild type allele of CYP2C19 and those who are heterozygous for $CYP2C19m1$ may explain this [152]. The individual estimated phenotypes were used as a covariate in the final submodel and subsequently in the simultaneous fit of all data to re-evaluate the pharmacokinetic parameters that were different among the three subpopulations. In the final model, the parameters that differed among the three subpopulations were the formation clearance and the central volume of S-4′-hydroxymephenytoin. Final population parameter estimates are presented in Table 2.

Induction of the formation of S-4′-hydroxymephenytoin and S-nirvanol was modeled with two enzyme turn-over models. $A_{en1}$ in the first enzyme model (CYP2C19) influenced the formation clearance of S-4′-hydroxymephenytoin and $A_{en2}$ in the other enzyme model (CYP2B6) influenced the formation clearance of S-nirvanol.
Table 2. Final pharmacokinetic parameter estimates of S-mephenytoin and its metabolites S-nirvanol and S-4′-hydroxymephenytoin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extensive metabolizers</th>
<th>Intermediate metabolizers</th>
<th>Poor metabolizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>0.758 fixed*</td>
<td>1 fixed</td>
<td></td>
</tr>
<tr>
<td>kₐ (day⁻¹)</td>
<td>13.8 fixed*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL_OH-f (L/day)</td>
<td>1190</td>
<td>221</td>
<td>-</td>
</tr>
<tr>
<td>CL_OH-e (L/day)</td>
<td>571</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>k_N-f₁ (day⁻¹)</td>
<td>1.38</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>k_N-f₂ (day⁻¹)</td>
<td>-</td>
<td>0.822</td>
<td>10.3</td>
</tr>
<tr>
<td>CL_N-e (L/day)</td>
<td>27.4</td>
<td>13.8</td>
<td></td>
</tr>
<tr>
<td>V_M-e (L)</td>
<td>44.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_M-p₁ (L)</td>
<td>4.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_M-p₂ (L)</td>
<td>41.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_OH-e (L)</td>
<td>22.2</td>
<td>12.6</td>
<td>-</td>
</tr>
<tr>
<td>V_OH-p (L)</td>
<td>2240</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>V_N-e (L)</td>
<td>16.9 fixed*</td>
<td>2.5 fixed*</td>
<td></td>
</tr>
<tr>
<td>V_N-p (L)</td>
<td>65.5</td>
<td>110</td>
<td>65.5</td>
</tr>
<tr>
<td>Q_M₁ (L/day)</td>
<td>6.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q_M₂ (L/day)</td>
<td>230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q_OH (L/day)</td>
<td>219 fixed*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q_N (L/day)</td>
<td>1010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV FM</td>
<td>0.11</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>IIV CL_OH-f</td>
<td>0.47</td>
<td>1.03</td>
<td>-</td>
</tr>
<tr>
<td>IIV CL_OH-e</td>
<td>0.48</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV k_N-f₁</td>
<td>0.35</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIV k_N-f₂</td>
<td>-</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>IIV CL_N-e</td>
<td>-</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>IIV V_M-p₁</td>
<td>3.2</td>
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<td></td>
</tr>
<tr>
<td>IIV V_N-p</td>
<td>0.29</td>
<td>0.27</td>
<td>0.29</td>
</tr>
<tr>
<td>IIV Q_OH</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FM is the S-mephenytoin relative bioavailability. kₐ is the S-mephenytoin absorption rate constant. CL_OH-f and CL_OH-e are the formation and elimination clearances of S-4′-hydroxymephenytoin, respectively. k_N-f₁ and k_N-f₂ are the formation rate constant of S-nirvanol in CYP2C19 EMs/IMs and PMs, respectively. CL_N-e is the elimination clearances of S-nirvanol. V_M-e, V_OH-e and V_N-e are the volumes of the central compartments of S-mephenytoin, S-4′-hydroxymephenytoin and S-nirvanol, respectively. V_M-p₁ / V_M-p₂, V_OH-p and V_N-p are the volumes of the peripheral compartments of S-mephenytoin, S-4′-hydroxymephenytoin and S-nirvanol, respectively. Q_M₁ / Q_M₂, Q_OH and Q_N are the intercompartmental clearance of S-mephenytoin, S-4′-hydroxymephenytoin and S-nirvanol, respectively. IIV is interindividual variability. * fixed from an earlier run.
The rate constant for production and degradation of CYP2B6 could not be estimated and was fixed to 0.177 day$^{-1}$ [153]. The induction factor (DRUG) was estimated as 0.867, 0.797, 0.615, 0.761, 0.199 and 0.169 for artemisinin in the rich data study, artemisinin in the sparse data study, arteether, artemether, dihydroartemisinin and artesunate, respectively. Artemisinin has induced the expression of CYP2B6 in vitro [77]. The different DRUG values for the two studies, in case of artemisinin, may have been due to differences in enzyme levels at the end of artemisinin treatment. The enzyme turnover rate constant was 0.177 day$^{-1}$ corresponding to a half-life of about 4 days. The duration of treatment was 10 days in the rich data study (2.5 half-lives) and 5 days in the sparse data study (1.25 half-lives). Thus, the duration of treatment was not enough in either study for the enzyme level to reach its new steady-state level. The enzyme level reached in the rich data study seems to be higher than that reached in the sparse data study.

The rate constant for production and degradation of CYP2C19 could not be estimated and was fixed to 0.1386 day$^{-1}$, since this gave a lower OFV than other investigated values (0.693, 0.355, 0.231 and 0.173 day$^{-1}$). DRUG was estimated in the sparse data study as 0.512, 0.148 and 0.249 for artemisinin, arteether and artemether, respectively. This confirms the induction of CYP2C19 determined by the non-validated metric, the 4-hour $S$-4' hydroxymephenytoin/$S$-mephenytoin plasma concentration ratio, in paper II.
Interactions between antiretroviral drugs and rifampicin

Nevirapine-rifampicin interaction (Paper IV)

Nevirapine population pharmacokinetics in TB/HIV- and HIV-patients was best described by a one-compartment model with first-order absorption and elimination. The estimates from the final population pharmacokinetic model of CL/F, volume of distribution (V/F) and absorption rate constant (k_a) are consistent with previous reports in other populations [154-156]. Patient characteristics and nevirapine parameter estimates are summarized in Table 3 and Table 4, respectively.

Nevirapine concentrations below 3 mg/L increase the risk of virological failure 5-fold [157] and dose adjustment has been suggested if nevirapine minimum concentration (C_{min}) is lower than 3 mg/L or higher than 12 mg/L [119, 158]. Nevirapine concentrations after 200 mg twice daily of nevirapine taken with rifampicin can be lower than the recommended 3 mg/L (Figure 3). The observed plasma concentrations were always below 3 mg/L in two patients while 11 patients had at least one concentration below 3 mg/L. On average, rifampicin reduced nevirapine C_{min} by 39%. Simulations of different doses of nevirapine revealed that increasing the dosage to 300 mg twice daily resulted in fewer patients exposed to subtherapeutic levels, with minimum exposure levels above the recommended threshold for dose reduction of 12 mg/L (Figure 4a). At nevirapine dosages of 400 mg and 500 mg twice daily, few or no patients, respectively, will be exposed to subtherapeutic levels but this is achieved at a risk of reaching high plasma levels in some patients (Figures 4b and c). From a pharmacokinetic point of view, increasing the dosage to 300 mg twice daily seems to be a satisfactory approach that needs to be studied in patients with respect to nevirapine efficacy and toxicity.

Rifampicin induced the metabolism of nevirapine, seen as an increase in its CL/F by 37.4%, which is in agreement with previous reports that rifampicin alters the pharmacokinetics of nevirapine [111, 159-162]. This may be due to induction of CYP3A4 and CYP2B6 by rifampicin. The absorption rate of nevirapine after the morning dose was almost 6-fold slower when the drug was co-administered with rifampicin. Rifampicin has been shown to decrease the rate of absorption of cyclosporine [31]. Further, in the presence of P-gp inhibitors, the k_a of [^{14}C]bepotastine in the small intestine has been shown to increase [163]. Nevirapine may be a substrate for P-gp [112, 113]. The change in the absorption rate of nevirapine may thus have occurred since rifampicin induces intestinal P-gp [33].
Table 3. Characteristics of South African patient population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients number</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (male/female)</td>
<td>53 (12/40)</td>
<td></td>
</tr>
<tr>
<td>TB/HIV coinfected patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensive</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>sparse</td>
<td>10</td>
<td></td>
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<tr>
<td>HIV infected patients without tuberculosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensive</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>sparse</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Continuous covariates</strong></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>51</td>
<td>34.6 (21.1-58.4)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>52</td>
<td>67.6 (42.5-105)</td>
</tr>
<tr>
<td>Albumin g/L</td>
<td>25</td>
<td>41.0 (24.0-60.0)</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>25</td>
<td>25.0 (10.0-127)</td>
</tr>
<tr>
<td>Total bilirubin μmol/mL</td>
<td>25</td>
<td>5.00 (2.00-17.0)</td>
</tr>
<tr>
<td>Hemoglobin g/dl</td>
<td>25</td>
<td>12.4 (6.00-17.1)</td>
</tr>
<tr>
<td><strong>Categorical covariates</strong></td>
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<tr>
<td>(TB/HIV patients during TB treatment/ TB/HIV patients after TB treatment / HIV patients)</td>
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</tr>
<tr>
<td>WHO disease stage</td>
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<tr>
<td>2</td>
<td>0/0/2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13/8/15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14/14/9</td>
<td></td>
</tr>
<tr>
<td>Concomitant medications</td>
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</tr>
<tr>
<td>Stavudine</td>
<td>16/13/7</td>
<td></td>
</tr>
<tr>
<td>Zidovudine</td>
<td>0/2/1</td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>16/14/8</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>13/10/4</td>
<td></td>
</tr>
<tr>
<td>Vitamin B complex</td>
<td>5/3/2</td>
<td></td>
</tr>
<tr>
<td>Multivitamins</td>
<td>3/0/1</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>5/1/0</td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>2/0/0</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5/0/1</td>
<td></td>
</tr>
</tbody>
</table>
In order to describe the circadian rhythmicity in the observed concentration-time data, covariates for day and night were used. Day was defined as the time between sunrise and sunset while night was the time between sunset and sunrise. The study was conducted over 11 months, with the time of sunrise ranging from 5:29 am to 7:52 am and sunset ranging from 5:48 pm to 7:41 pm. The shortest day was 9 h 56 min long and the longest day was 14 h 9 min long. Three parameters differed between night and day: CL/F, \( k_a \) and absorption lag time. Nevirapine \( k_a \) at night was coded as the sum of the elimination rate constant and an additional estimate to avoid flip-flop. CL/F and the absorption lag-time were higher during the night than the day, whereas \( k_a \) was lower during the night. The circadian rhythm has previously been reported as a small trend toward a higher AUC, due to a higher maximum concentration (C\(_{max}\)) that is reached earlier, and a lower CL/F after the morning dose [164]. Nevirapine is lipophilic [165] and it is known that some lipophilic drugs show an earlier and higher C\(_{max}\) after the morning dose compared to when the drug is given in the evening. Rapid absorption by passive diffusion of drugs given in the morning may be due to faster gastric emptying and higher perfusion of the gastrointestinal tract [166]. Nevirapine is metabolized by CYP3A4, which is found in the intestine. The rapid absorption in the morning may decrease the time of contact between nevirapine and the metabolizing enzymes, thus resulting in decreased first-pass metabolism and increased bioavailability of nevirapine, seen as a lower CL/F in the morning than in the evening. However, this increased bioavailability was not seen in V/F. The model predicted a higher absorption rate, in the presence of rifampicin, after the evening dose than after the morning dose. A possible hypothesis is that isoniazid might act as P-gp inhibitor [98]. The delayed absorption of isoniazid [167] might result in increased nevirapine absorption since isoniazid was given once daily with the morning dose of nevirapine and was not given at night.

Albumin was found to be a predictor of variability in CL/F, which increased by 2.84% with every 1 g/L increase in albumin relative to the population median of 41 g/L. HIV patients suffer from altered metabolism, specific disturbances in protein turnover and weight loss [168]. Consequently, weight and albumin levels seem to reflect metabolic disturbance; a positive correlation between albumin and weight has been found in HIV patients [169]. An increase in clearance with weight has been reported [154, 170] but including weight in the model did not explain the variability in CL/F, either as the only covariate for CL/F or in combination with other covariates. Weight was therefore not included in the final model as a predictor of variability in CL/F.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>NONMEM results</th>
<th>Bootstrap results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F day (L/h)</td>
<td>2.76</td>
<td>0.057</td>
</tr>
<tr>
<td>CL/F night (L/h)</td>
<td>3.78</td>
<td>0.11</td>
</tr>
<tr>
<td>Increase in CL/F with rifampicin (%)</td>
<td>37.4</td>
<td>0.26</td>
</tr>
<tr>
<td>V/F (l)</td>
<td>137</td>
<td>0.072</td>
</tr>
<tr>
<td>kₐ day (h⁻¹)</td>
<td>3.57</td>
<td>0.61</td>
</tr>
<tr>
<td>kₐ night (h⁻¹)</td>
<td>k+0.24</td>
<td>0.39</td>
</tr>
<tr>
<td>kₐ day with rifampicin (h⁻¹)</td>
<td>0.613</td>
<td>0.42</td>
</tr>
<tr>
<td>kₐ night with rifampicin (h⁻¹)</td>
<td>k+0.813</td>
<td>0.55</td>
</tr>
<tr>
<td>Absorption lag time at night (h)</td>
<td>0.73</td>
<td>0.0016</td>
</tr>
<tr>
<td>Effect of age on CL/F (θ&lt;sub&gt;CL/F,AGE&lt;/sub&gt;)</td>
<td>0.0156</td>
<td>0.67</td>
</tr>
<tr>
<td>Effect of albumin on CL/F (θ&lt;sub&gt;CL/F,ALB&lt;/sub&gt;)</td>
<td>0.0284</td>
<td>0.18</td>
</tr>
<tr>
<td>Effect of Vitamin B complex on CL/F (θ&lt;sub&gt;CL/F,BCOM&lt;/sub&gt;)</td>
<td>0.118</td>
<td>0.93</td>
</tr>
<tr>
<td>Effect of body weight on V/F (θ&lt;sub&gt;V/F,WT&lt;/sub&gt;)</td>
<td>0.0142</td>
<td>0.3</td>
</tr>
<tr>
<td>IIV in CL/F (TB/HIV group without rifampicin) and in CL/F day (HIV group)</td>
<td>0.307</td>
<td>0.27</td>
</tr>
<tr>
<td>IIV in CL/F (TB/HIV group with rifampicin)</td>
<td>0.314</td>
<td>0.24</td>
</tr>
<tr>
<td>IIV in CL/F night (HIV group)</td>
<td>0.14</td>
<td>2.3</td>
</tr>
<tr>
<td>IIV in kₐ day</td>
<td>0.71</td>
<td>0.85</td>
</tr>
<tr>
<td>IIV in kₐ night (TB/HIV group)</td>
<td>2.0</td>
<td>0.53</td>
</tr>
<tr>
<td>IIV in kₐ night (HIV group)</td>
<td>1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Proportional residual variability</td>
<td>0.128</td>
<td>0.095</td>
</tr>
</tbody>
</table>

RSE= relative standard error. CI= confidence interval. CL/F= oral clearance. V/F= volume of distribution. kₐ= absorption rate constant. k= elimination rate constant which is equal to the oral clearance divided by the volume of distribution. IIV= interindividual variability.

\[
(CL/F)_{pop} = (CL/F).\{1+\theta_{CL,F,AGE}(AGE−346)}\cdot\{1+\theta_{CL,F,ALB}(ALB−410)}\cdot\{1+\theta_{CL,F,BCOM}\}
\]

\[
(V/F)_{pop} = (V/F).\{1+\theta_{V,F,WT}(WT−67.6)}\]
Of the four analyzed nevirapine metabolites (2-, 3-, 8- and 12-hydroxynevirapine), only 12-hydroxynevirapine was found in concentrations above the LLOQ of 0.025 mg/L. Data for 12-hydroxynevirapine were only available from 16 intensively sampled TB/HIV patients during and after TB treatment. Several attempts were made to extend the parent compound pharmacokinetic model to include the metabolite. However, the concentrations were not well predicted. Noncompartmentally derived AUC-day, median (interquartile range), for TB/HIV patients during TB treatment was 2.9 (2.2-3.2) h·mg/L and for TB/HIV patients after TB treatment 2.8 (2.6-4.0) h·mg/L. AUC-night, median (interquartile range), for TB/HIV patients during and after TB treatment was 2.3 (1.7-3.6) and 2.7 (2.1-3.7) h·mg/L, respectively. There were no statistically significant differences between the two occasions (during and after TB treatment) for AUC-day or AUC-night. This may be attributed to differences in the expression of enzymatic activity among patients. Since 12-hydroxynevirapine is metabolized by several enzymes (mainly CYP3A4, but also CYP2D6 and CYP2C9 [42]), net enzymatic activity in each patient and hence the effect of rifampicin on the formation pathway of 12-hydroxynevirapine might explain the lack of a trend.

Figure 3. Individual predicted nevirapine concentrations versus time in TB/HIV co-infected patients during the continuation phase of standard tuberculosis treatment. The lower dashed horizontal line is the cut-off value of 11.3 μmol/L (3 mg/L) below which dose adjustment has been suggested and the upper dashed horizontal line is the cut-off value of 45.1 μmol/L (12 mg/L) above which dose adjustment has been suggested.
Figure 4. Individual predicted nevirapine concentrations versus time in TB/HIV co-infected patients, during the continuation phase of standard tuberculosis treatment simulated, based on the individual pharmacokinetic parameters from the final model, with a dose of 300 mg twice daily (a), 400 mg twice daily (b) and 500 mg twice daily (c). The lower dashed horizontal line is the cut-off value of 11.3 μmol/L (3 mg/L) below which dose adjustment has been suggested and the upper dashed horizontal line is the cut-off value of 45.1 μmol/L (12 mg/L) above which dose adjustment has been suggested.
Figure 4. (Continued).
Lopinavir/ritonavir-rifampicin interaction (Paper V)

Lopinavir showed time-dependent pharmacokinetics where its CL/F increased linearly during the 12-hour dosing interval, probably due to recovery from inhibition by ritonavir. The final model for lopinavir CL/F was:

\[
(CL/F) = (CL/F)_0 (1 + [k_r \cdot TAD])
\]

where \((CL/F)_0\) is lopinavir CL/F at the beginning of the dosing interval, \(k_r\) is the rate constant of recovery from inhibition and TAD is the time after dose in hours.

Lopinavir \((CL/F)_0\) was 1.26, 1.17 and 0.895 L h\(^{-1}\) in the TB/HIV group during TB treatment, the TB/HIV group after TB treatment and the HIV group, respectively. Recovery from ritonavir inhibition of lopinavir metabolism was linear with a \(k_r\) of 0.0582 h\(^{-1}\) in the TB/HIV group during TB treatment and 0.0648 h\(^{-1}\) in the TB/HIV group after TB treatment and in the HIV group. This leads to a gradual increase in CL/F during the dosing interval, reaching 2.14, 2.08 and 1.59 L h\(^{-1}\) at the end of the 12-hour dosing interval in the TB/HIV group during TB treatment, the TB/HIV group after TB treatment and the HIV group, respectively. Therefore, increasing the dose of ritonavir to achieve a lopinavir/ritonavir ratio of 1:1 was not able to completely counteract the induction of lopinavir metabolism by rifampicin since lopinavir CL/F in the TB/HIV group during TB treatment was higher than in the HIV group. The lower CL/F in the TB/HIV group after TB treatment versus during TB treatment was probably due to recovery from the induction effect of rifampicin on lopinavir metabolism.

Lopinavir/ritonavir (1:1) counteracts the effect of rifampicin on lopinavir pharmacokinetics in healthy adults, since this regimen results in similar maximum concentrations and AUC values to those obtained with the standard lopinavir/ritonavir (4:1) regimen without rifampicin [171]. The higher hepatic enzymatic activity in children aged 1-4 years than in adults [172] may explain the inability of the higher ritonavir dose to completely counteract rifampicin induction of lopinavir CL/F in this study. Though full counteraction of rifampicin induction of lopinavir metabolism is the ultimate goal, the clinically relevant criterion for monitoring lopinavir virological efficacy is the trough sample at 12 hours [119]. Lopinavir concentrations predicted at 11.9 hours after dose administration were above the recommended minimum therapeutic concentration (1 mg/L) [119] for all individuals.

Other approaches may compensate for the effect of rifampicin on lopinavir CL/F, although these need to be studied in the target patient population. For example, giving even larger doses of ritonavir may in theory fully compensate for the effect of rifampicin on lopinavir CL/F. However, when
ritonavir was taken in therapeutic doses as an HIV PI, it induced CYP3A4 [173-175]. In vitro, ritonavir activates PXR though it decreases microsomal CYP3A4 activity by irreversible enzyme inhibition [176]. Another alternative might be to give larger doses of lopinavir/ritonavir (in a ratio of 4:1) with rifampicin. In HIV-infected children receiving standard doses of lopinavir/ritonavir (in a ratio of 4:1), 7 of 23 children, mainly younger than 2 years, had C_{min} below 1 mg/L. When the dose of lopinavir/ritonavir (4:1) was increased by 33%, the C_{min} exceeded 1 mg/L [177]. In HIV-infected children, a 30% higher dose of lopinavir/ritonavir (4:1) in the presence of efavirenz seems to completely compensate for the enzyme-inducing effect of efavirenz, although 3 of the 15 studied patients had C_{min} below 1 mg/L [178]. In healthy adults, administering twice the dose of lopinavir/ritonavir (4:1) in the presence of rifampicin resulted in a 57% lower C_{min} compared to that obtained with a normal lopinavir/ritonavir dose in the absence of rifampicin [171].

Assuming mechanism-based inhibition and that the half-life of the inhibitor is negligible in comparison to the turnover half-life of the enzyme, the rate constant for recovery from inhibition corresponds to the rate constant of enzyme turnover [27]. The values of k_r correspond to longer half-life values than the reported values for ritonavir [179, 180], indicating that the enzyme inhibition by ritonavir is mechanism-based and not competitive. The lower k_r in the TB/HIV group during TB treatment than that after TB treatment and in the HIV group may have occurred because patients with TB/HIV co-infection have lower protein anabolism than patients with HIV infection alone [181]. Recovery from ritonavir inhibition of CYP3A4 in adults is nearly complete after 3 days of discontinuation [182]. After 3 days of discontinuation of ritonavir, recovery from ritonavir inhibition in this study was 98% in the TB/HIV group during TB treatment, 99% in the TB/HIV group after TB treatment and 99% in the HIV group.

A previous attempt to link lopinavir population pharmacokinetics with ritonavir exposure resulted in a time-independent inverse relationship between lopinavir CL/F and exposure to ritonavir [183]. However, since ritonavir plasma concentrations were not available from our study, there was no relationship between the pharmacokinetics of lopinavir and of ritonavir in the model.

Lopinavir V/F was 20.9 L in the TB/HIV group and 10.3 L in the HIV group. Lopinavir is 98-99% bound to plasma proteins, mainly alpha-1-acid glycoprotein and albumin [184]. The larger V/F in the TB/HIV group might be attributed to a lower level of albumin in the TB/HIV group compared with that in the HIV group [181, 185]. The larger V/F in the TB/HIV group may have been caused by reduced lopinavir bioavailability compared with that in the HIV group, since the same trend was found in CL/F.
Conclusions

Induction/inhibition of several principal CYP enzymes by the antimalarial drugs artemisinin, dihydroartemisinin, arteether, artemether and artesunate was evaluated using appropriate probe drugs, in healthy subjects. This was done by evaluating the 4-hour plasma concentration ratios of the metabolite to the probe for several probes given simultaneously in a cocktail (paper II) and by modelling the population pharmacokinetics of the probe drug S-mephenytoin and its metabolites S-nirvanol and S-4’-hydroxymephenytoin (paper III). CYP3A activity was induced after multiple-administration of artemisinin, dihydroartemisinin and artemether whereas CYP2C19 activity was induced after artemisinin, arteether and arteether intake. CYP1A2 was inhibited by artemisinin, dihydroartemisinin and arteether from the first day of drug intake and was induced by multiple administration of artemisinin. The CYP2D6 metric decreased on the first day of co-administration with artemisinin and dihydroartemisinin and was later increased following five days of artemisinin intake. CYP2B6 was induced by all the studied artemisinin derivatives. Artemisinin appeared to be associated with the strongest capacity for induction and inhibition in therapeutic doses. The metabolic changes may be clinically significant for some individuals and warrant consideration in combination treatment to avoid or reduce the risk of drug-drug interactions, especially those involving drugs with narrow therapeutic windows.

In TB/HIV co-infected South African patients, administration of rifampicin concomitantly with the antiretroviral drugs nevirapine to adults (paper IV) or lopinavir/ritonavir to children (paper V) resulted in a higher CL/F of nevirapine and lopinavir than occurred in respective patients infected only by HIV. The higher CL/F of lopinavir in the presence of rifampicin was observed despite the receipt of an extra ritonavir dose by TB/HIV co-infected children (lopinavir/ritonavir 1:1). In case of nevirapine, rifampicin co-administration resulted in potentially sub-therapeutic levels of nevirapine in some patients. Simulations of different doses of nevirapine revealed that increasing the dose of nevirapine to 300 mg twice daily elevated nevirapine concentrations above subtherapeutic levels in most patients, with minimum exposure above the recommended maximum concentration. For lopinavir, the predicted trough concentration was above the recommended minimum therapeutic concentration in the TB/HIV co-infected children.
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References


[129] CDC. Table 1. Recommendations for coadministering protease inhibitors and non-nucleoside reverse transcriptase inhibitors with RIFAMPIN 2007 [cited 16 January 2008]; Available from: http://www.cdc.gov/tb/TB_HIV_Drugs/Table1.htm


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