Applied Population Pharmacokinetic / Pharmacodynamic Modeling of Antiretroviral and Antimalarial Drug Therapy

Daniel Röshammar

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UNIVERSITY OF GOTHENBURG

Department of Pharmacology
Institute of Neuroscience and Physiology
The Sahlgrenska Academy at University of Gothenburg
Sweden

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The White Rabbit put on his spectacles. 'Where shall I begin, please your Majesty?' he asked.
'Begin at the beginning,' the King said, very gravely, 'and go on till you come to the end: then stop.'

Lewis Carrol

To Schrumfen

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ABSTRACT

HIV/AIDS and malaria are two major global infectious diseases. Although better drugs against these conditions are becoming more available, dosages may not always be optimal with respect to effectiveness, safety, cost or convenience of administration. This thesis aims to quantitate the pharmacological relationship between dosing history, sources of variation between individuals, drug exposure and response to selected antiretroviral and antimalarial regimens.

Pharmacometric, i.e. pharmaco-statistical, models were fitted to observed data from five clinical studies, using the NONMEM software. Several polymorphic genes coding for drug metabolizing enzymes and transporters were found to have impact on the disposition of the non-nucleoside reverse transcriptase inhibitor efavirenz in healthy Ugandan subjects after single dose administration. Moreover, using simulation it was demonstrated that a 200 mg dose reduction in Zimbabwean HIV-patients with genetically decreased metabolic capacity would maintain efavirenz exposure within the therapeutic range during repeated administration. In a typical clinical trial large amounts of drug response data are collected. However, usually only limited amounts of the recorded data are actually used for investigating differences between regimens. Herein, a drug-disease model was developed to describe the time-course of repeatedly measured HIV-RNA levels in Scandinavian patients randomized to one of three commonly prescribed antiretroviral regimens. The initial analysis showed that an efavirenz-containing regimen appeared to be more efficacious compared to two protease inhibitor-containing regimens. Antimalarial artemisinin-based combination therapy bears many resemblances to antiretroviral treatment. The drugs exhibit variable and complex pharmacokinetics and the diseases themselves bring reasonable possibilities for pharmacodynamic assessment. Auto-induction of drug metabolism was described after multiple dosing with artemisinin in Vietnamese patients. The frequency of recrudescent malaria infection was as high as 37% but could not directly be linked to low artemisinin exposure. The elimination half-life of piperaquine, a suitable partner drug for artemisinin-based combination treatment, was estimated to 12 days with large between-subject variability.

The thesis demonstrates the utility of pharmacometric methodology in the analysis of clinical data originating from high-income countries as well as resource-limited settings. Ultimately it can be a tool for decision analysis and policy making.

Keywords: HIV, malaria, pharmacokinetics, pharmacodynamics, pharmacometrics, NONMEM

Farmakokinetisk och farmakodynamisk populationsmodellering av läkemedel för behandling av HIV och malaria

SWEDISH SUMMARY – POPULÄRVETENSKAPLIG SAMMANFATTNING

HIV och malaria är två infektionssjukdomar som orsakar stort individuellt lidande med påtagliga ekonomiska konsekvenser runt om i världen. Den här avhandlingen syftar till att med hjälp av farmakokinetiska och farmakodynamiska modeller beskriva det farmakologiska sambandet mellan dosering, variabilitet mellan individer (pga. vikt, kön, njurfunktion, gener, sjukdom etc.), läkemedelsexponering och effekt av behandling mot dessa tillstånd.

Med farmakokinetik avses vetenskapen om hur läkemedel omsätts (absorberas, fördelas, bryts ner samt utsöndras) i kroppen. Farmakodynamik är det forskningsområde inom vilket sambandet mellan exponering av ett läkemedel och dess farmakologiska effekt/biverkan studeras. Tiden till effektens inträdande, dess grad och duration är direkt eller indirekt relaterad till läkemedelshalten i blodet. Genom att anpassa matematiska och statistiska modeller till experimentellt observerade data kan interaktionen mellan läkemedelskoncentration, effekt och sjukdomsförlopp beskrivas kvantitativt. Sådana, så kallade farmakometriska modeller, kan användas för att optimera nuvarande läkemedelsterapier eller för att avgöra lämpliga doseringsrekommendationer för nya behandlingar.

Betydelsen av genetiska skillnader mellan individer för omsättningen av HIV-läkemedlet efavirenz undersöktes i friska försökspersoner från Uganda. Vidare studerades patienter från Zimbabwe för att avgöra om personer med sämre förmåga att omsätta efavirenz bör administreras en lägre dos för att, med bibehållet behandlingsresultat, minska risken för biverkan. Sambandet mellan antiretroviral läkemedelsterapi och virusnivåer beskrevs efter upprepade mätningar i skandinaviska HIV-patienter som inte tidigare stått under behandling. Därutöver har de farmakokinetiska och farmakodynamiska egenskaperna för de två malarialäkemedlen piperakin och artemisinin beskrivits i två asiatiska populationer.

Både kön och genetik visade sig påverka farmakokinetiken av efavirenz. En dossänkning från 600 till 400 mg dagligen föreslogs vara möjlig i patienter med dålig kapacitet att bryta ner efavirenz. En kombinationsbehandling innehållande efavirenz visade sig vara effektivare än två andra vanligt förekommande behandlingar. Artemisinin gav som väntat inte tillräckligt god effekt när det gavs som monoterapi till vietnamesiska vuxna och barn. Piperakin skulle kunna vara en lämplig partner till artemisinin i kombinationsbehandling mot malaria, men dess långa terminala halveringstid bör tas i beaktande då resistenta parasiter kan uppstå vid otillräckliga läkemedelsnivåer.

Sammanfattningsvis beskriver detta arbete hur farmakometriska modeller är användbara verktyg för att sammanfatta och utvärdera läkemedelsdata som inhämtats såväl i Skandinavien som i världens fattigare länder. Företrädesvis bör denna metodik öka för att på ett kostnadseffektivt tillvägagångssätt erhålla verkningsfullare och säkrare behandlingsalternativ.

Papers Discussed

This thesis is based on the following papers, which will be referred to in the text by Roman numerals.

I. Mukonzo J, Röshammar D, Waako P, Andersson M, Fukasawa T, Milani L, Svensson J-O, Ogwal-Okeng J, Gustafsson LL, Aklillu E (2008) A novel polymorphism in MDR1 gene, CYP2B6*6 and sex predict single dose efavirenz population pharmacokinetics in Ugandans. Submitted

II. Nyakutira C, Röshammar D, Chigutsa E, Chnozi P, Ashton M, Nhachi C, Masimirembwa C (2008) High prevalence of the CYP2B6 516GT(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharm 64: 357-365

III. Röshammar D, Josephson F, Simonsson USH, Ekvall H, Flamholc L, Ormaasen V, Sönnerborg A, Wallmark E, Ashton M, Gisslén M (2009) Assessment of antiretroviral drug efficacy in the NORTHIV study by non-linear mixed effects modeling. Manuscript

IV. Röshammar D, Hai TN, Thanh NV, Huong DX, Huong NV, Thu NB, Ashton M (2007) Modeling of the time-dependent population pharmacokinetics and exposure-response relationship of the antimalarial artemisinin based on sparsely sampled saliva. Manuscript

V. Röshammar D, Hai TN, Friberg Hietala S, Huong NV, Ashton M (2006) Pharmacokinetics of piperaquine after repeated oral administration of CV8 in 12 healthy male subjects. Eur J Clin Pharm 62: 335-41

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List of Abbreviations

ACT	artemisinin-based combination therapy
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
AUC	area under the concentration-time curve
AUC_{dose1}	AUC after the first dose
$AUC_{0-\infty}$	AUC from time zero to infinity
C	drug concentration
CDC	Centers for Disease Control and Prevention
CD4	helper T lymphocyte
CI	confidence interval
CL	clearance
CL_{int}	intrinsic clearance
CV	coefficient of variation
CYP	cytochrome P450
D	duration of zero-order absorption
DNA	deoxyribonucleic acid
E	drug response
EC_{50}	concentration required to achieve 50% of maximal drug response
E_H	first-pass extraction
EM	extensive metabolizer
E_{MAX}	maximal drug response
F	bioavailability
F_H	bioavailability across the liver
FDA	(US) Food and Drug Administration
FO	first-order estimation
FOCE	first-order conditional estimation
fu	fraction unbound drug in plasma
HAART	highly active antiretroviral treatment
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
IC_{50}	concentration required to achieve 50% of maximal inhibition
ICH-GCP	International Conference on Harmonisation – Good Clinical Practise
IIV	interindividual variability
IM	intermediate metabolizer
IOV	interoccasional variability
IP	initial parasitemia
IPRED	individual prediction
ka	first-order absorption rate constant
k_{in}	zero-order production rate constant

k_{out}	first-order removal rate constant
LLOQ	lower limit of quantification
MDR	multi drug resistance
MIT	mean induction time
NIMPE	National Institute of Malariology, Parasitology and Entomology
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
Obs	observed value
OFV	objective function value
P	typical parameter estimate
P_i	individual parameter estimate
PCR	polymerase chain reaction
PCT	parasite clearance time
PD	pharmacodynamics
PI	protease inhibitor
Pgp	p-glycoprotein
PK	pharmacokinetics
PM	poor metabolizer
PRED	population prediction
Q	inter-compartmental clearance
Q_H	hepatic blood-flow
R	viral reproduction ratio
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RSE	relative standard error
SCRIHS	Secretariat Committee for Research Involving Human Subjects
SD	standard deviation
SNP	single nucleotide polymorphism
TB	tuberculosis
V	volume of distribution
V_c	central volume of distribution
V_p	peripheral volume of distribution
VPC	visual predictive check
V_{ss}	volume of distribution at steady-state
WHO	World Health Organization
WMA	World Medical Association
γ	efficacy parameter for PIs
ε	difference between observation and individual prediction (Papers I-II, IV-V)
ε	efficacy parameter for NRTIs and NNRTIs (Paper III)
η	difference between typical and individual parameter estimate
θ	typical parameter value
σ^2	estimable variance of ε
ω^2	estimable variance of η

1 Introduction

1.1 Background

This thesis encompasses clinical pharmacokinetic (PK) and pharmacodynamic (PD) studies of drug regimens for the treatment of HIV/AIDS and malaria. Pharmacokinetics and pharmacodynamics are two connected disciplines of pharmacology dealing with the absorption, metabolism, distribution and elimination of drugs and the relationship between drug exposure and pharmacological response (therapeutic and adverse). The time-course of drug action is governed by the relationship between the drug's pharmacokinetic and pharmacodynamic properties. Consequently, the onset, magnitude and duration of pharmacological response are directly or indirectly related to the drug concentration in the body [1, 2]. Pharmacometrics refers to the development and application of fitting mathematical and statistical models to experimental pharmacokinetic, pharmacodynamic and therapeutic outcome data for descriptive, clarifying, hypothesis generating and predictive purposes [3]. Recently a broader definition of pharmacometrics as a process facilitating translation of complex biological processes to describe interactions between xenobiotics and patients in a quantitative manner was proposed [4]. A pharmacometric model can be used to quantitatively summarize knowledge about the disease, the pharmacological properties of a drug or to provide information for optimized sub-population dosage recommendations.

HIV/AIDS and malaria are two major global infectious diseases. Although better drugs against these conditions are becoming more available, dosages may not always be optimal with respect to effectiveness, safety, administration convenience or cost. Crucial is that the treatment is effective for delaying resistance development [5, 6]. It is given that the pharmacokinetic and pharmacodynamic properties of antiretroviral and antimalarial agents must be further investigated in order to optimize affordable, effective and safe regimens in the treatment of HIV and malaria. Within this work, principles of pharmacometric methodology were applied to further study the pharmacokinetic and pharmacodynamic properties of selected antiretroviral and antimalarial drugs in one Vietnamese, one Scandinavian and two African populations.

1.2 The diseases

1.2.1 HIV/AIDS

HIV/AIDS is a global health problem. In 2007 an estimated 33.2 million persons were living with the disease, another approximately 2.5 million got infected and 2.1 million died from acquired immunodeficiency syndrome (AIDS) related causes [7]. HIV, human immunodeficiency virus, is a retrovirus belonging to a group of viruses called lentiviruses. The virus attacks immune function cells such as the $CD4^+$ T-lymphocytes (herein referred to as CD4-cells) and macrophages. The virus integrates in the host cell's DNA leading to the production of new virus particles and death of the infected cells [8]. Without treatment, the immune system begins to fail resulting in immunodeficiency and an increased susceptibility to opportunistic infections and cancer. The disease progression is typically characterized by three distinct phases (*Figure 1*).

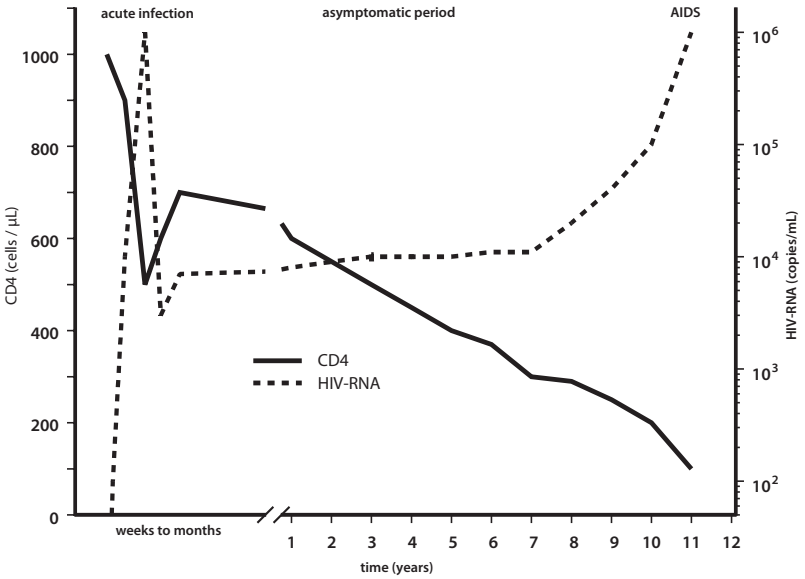

Figure 1. The typical clinical course of HIV disease progression. Adapted from Fauci et al [15] by permission of Annals of Internal Medicine.

After an incubation period lasting a few weeks rapid viral replication occurs. HIV-RNA levels can reach 10^7 copies/mL plasma and CD4-cells decrease substantially from their initial levels [9]. The first clinical manifestations of infection appear within some weeks and include fever, diarrhea, rash and influenza-like symptoms. After the acute HIV-infection there is a reduction in viral levels to a plateau or pseudo stationary state of 10^3-10^5 copies/mL plasma and the CD4-cells partly recover, accompanied by a period of asymptomatic HIV-infection lasting many months to years [10-12]. The asymptomatic period is followed

by a symptomatic infection with continued viral replication and gradual decline in immune response [13]. AIDS is developed when the CD4-cell count falls below 200 cells/μL blood or in the presence of certain AIDS defining conditions [14].

1.2.2 Malaria

An estimated 300 to 500 million cases and 1.5 to 2.7 million deaths occur each year due to malaria [16]. What is more, malaria is directly responsible for around 18% of all child deaths in sub-Saharan Africa [17]. Infection by the *Plasmodium* parasite (*P. falciparum,* which causes the most dangerous infections*, P. vivax, P. ovale or P. malarieae*) is transmitted by mosquitoes. The complex parasite life-cycle is displayed in *Figure 2*.

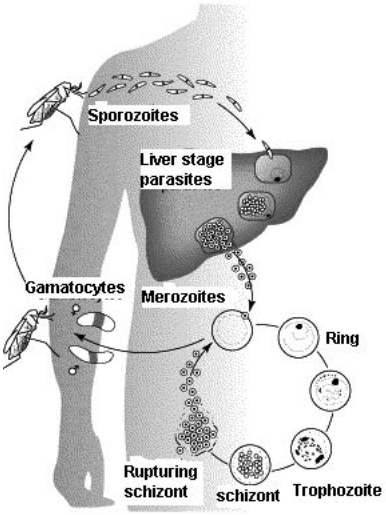

Figure 2. The malaria parasite life cycle in humans [18].

After a bite, sporozoites infect hepatic cells. Subsequently they multiply to merozoites ready to invade erythrocytes. The 48 hour cycle of *P. falciparum* in the red blood cells can be divided into 1-12 intervals to account for the transition between ring, trophozoite and schizont stages. When the schizont ruptures new merozoites are released [19]. Blood stage parasites are responsible for the clinical manifestations of the disease. Symptoms usually occur within weeks after the bite and include fever, headache and nausea for uncomplicated malaria or anemia, organ failure and coma for severe malaria.

1.3 Drug therapy

1.3.1 Antiretroviral drugs

The first antiretroviral compound (zidovudine) was introduced in 1987. Today there exist several classes of antiretroviral drugs. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) incorporate in the viral deoxyribonucleic acid (DNA) during synthesis from viral ribonucleic acid (RNA), resulting in chain termination. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) directly inhibit the activity of the viral enzyme reverse transcriptase. Protease inhibitors (PIs) target the protease enzyme, necessary for post-translational processing of viral proteins. New classes of drugs block the entry of the virus into the cell (fusion inhibitors) or prevent the integration of viral DNA into the host cell's genome (integrase inhibitors), respectively (*Figure 3*).

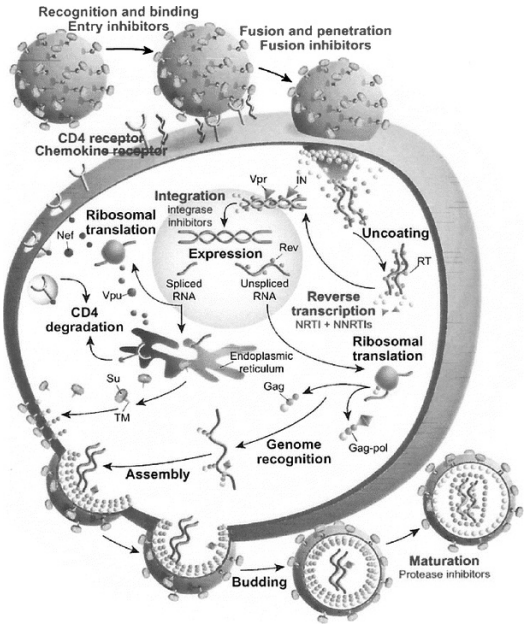

Figure 3. The HIV replication cycle and targets for antiretroviral drugs [20] by permission from Nature Publishing Group.

Since the introduction of highly active antiretroviral therapy (HAART), a combination of at least three drugs from different classes, treatment of HIV-infection has steadily improved. Antiretroviral combination therapy has decreased mortality and morbidity in HIV-disease during the last years [21]. A commonly adopted treatment policy suggests a first-line treatment consisting of a combination of two NRTIs and either a NNRTI or a PI [22].

Treatment initiation in the absence of clinical symptoms is usually guided by the CD4-cell count. In clinical drug trials, the number of HIV-RNA copies, the viral load, is currently used as the primary marker for drug efficacy. The decay in viral levels is such a good measurement of drug efficacy that it has the status of a surrogate endpoint of overall survival and time to clinical events [23]. Long-term antiretroviral combination treatment in HIV-infected patients generally results in a decrease of plasma viral HIV-RNA levels. The first few weeks the decay in viral load is rapid, then the rate of decline tend to slow because of virus reservoirs in latently and long-lived infected cells [24]. It is important to notice that the initial decay in viral load may also be followed by viral rebound which indicates therapeutic failure because of poor adherence to treatment or development of drug resistance.

1.3.1.1 Efavirenz

The efficacy of efavirenz (a NNRTI) based combination regimens has been shown in many clinical trials [25-33]. Despite its long half-life, narrow therapeutic window, large between-subject variability in drug exposure and toxic side effects, efavirenz is preferred to nevirapine due to the more solid efficacy and safety documentation [34]. Efavirenz mid-dosing interval plasma concentrations below 1 mg/L have been associated with treatment failure and may select for viral drug resistance, while concentrations exceeding 4 mg/L increase the risk of adverse neuropsychiatric effects [35]. The pharmacokinetic properties of efavirenz include auto-induction of drug metabolism and a relatively long elimination half-life [36].

1.3.1.2 Lopinavir, atazanavir and ritonavir

Lopinavir, atazanavir and ritonavir are all examples of PIs. Lopinavir is approved for co-formulation with ritonavir and has twice daily dosing. Although it is generally accepted that there exists a relationship between PI exposure and antiviral response [37, 38], no such direct relationship has been observed for lopinavir. Currently applied dosing recommendations may be greater than needed to reach the maximum of the concentration-response curve. Nevertheless a relationship between lopinavir exposure to viral susceptibility and antiviral response has been proven, using logistic regression [39]. Atazanavir is the first and currently the only PI that is registered for once a day administration. A statistically significant relationship between viral reductions and atazanavir trough concentrations divided by the number of protease mutations (associated with reduced atazanavir response) has been observed [40]. Ritonavir is a so called pharmacokinetic 'booster', used because of its capacity to inhibit the drug metabolizing enzyme cytochrome P450 (CYP) 3A4. Given in a low dose, ritonavir reduces the metabolism of lopinavir and atazanavir, which are extensively metabolized by CYP3A4, and thereby also enhancing drug exposure [41].

1.3.2 Antimalarial drugs

If diagnosed correctly and treated promptly, malaria is a curable disease. However, the therapeutic arsenal is relatively old and drug resistance among the parasites is an emerging problem. To increase therapeutic success rates and diminish the spread of resistance the World Health Organization (WHO) recommends antimalarial drugs in combinational treatment [42]. In artemisinin-based combination therapy (ACT) one of the artemisinin derivatives is combined with a drug with another mechanism of action and a longer elimination half-life. The aim is to protect the artemisinin drugs from resistance, prevent recrudescence and reduce the duration of treatment [43].

The efficacy of ACT in clinical drug trials is usually determined by the therapeutic outcome such as recrudescence and cure rates evaluated after a 14- or 28-day follow-up period. Although not fully correlating to disease severity and therapeutic outcome, parasitological biomarkers, i.e. changes in the parasite load over time, can also be used to assess treatment response. Conventionally, parasitological recovery from malaria is determined by the absence of parasites in peripheral blood smears. The parasite clearance time (PCT), a commonly used efficacy parameter, can be defined as the time required to reach the first of two negative smears after initiation of treatment [44].

1.3.2.1 Artemisinin

The drug, originally used in the Chinese traditional medicine, is active against all blood-stage parasites. Its structure includes an endoperoxide bridge which has been suggested to be essential for the antiparasitic effect. Treatment with artemisinin results in a rapid parasitological decline with few adverse effects. If artemisinin is used in monotherapy the success of therapy stays low unless administered over seven days. However, when used in combination treatment, the duration of treatment is normally reduced to 3 days [45]. Due to auto-induction of drug metabolism the relative drug exposure of artemisinin is reduced over time during the course of treatment [46]. The original compound artemisinin is currently replaced by its more potent derivatives dihydroartemisinin, artesuante and artemether.

1.3.2.2 Piperaquine

Piperaquine, first synthesized in 1966, has been a suggested partner drug to the artemisinin derivatives because of its large volume of distribution, resulting in a long elimination half-life and post-treatment prophylactic effect. It is likely that piperaquine inhibits the detoxification process of hemoglobin in the parasite food vacuole. The drug, mainly used in Asia, is well tolerated and is manufactured at a low cost. To date, only sparse characterization of the drug's pharmacokinetic properties is available [47].

1.4 Pharmacokinetic and pharmacodynamic non-linear mixed effects models

1.4.1 Regression models

Mathematical and statistical models can be fitted to experimental (preclinical, clinical and literature) data using linear or non-linear regression methods to explain the time-course of drug exposure and response as well as their relationship [48, 49]. Pharmacokinetic/pharmacodynamic software packages, e.g. NONMEM [50], iteratively find the specific estimates of a set of predefined model parameters that give the best prediction of the observed data. The model performance, i.e. how well the model describes the data can be evaluated using various goodness-of-fit metrics, e.g. the objective function value (in the present case equal to $-2 \times$ log-likelihood of the data under the given model) and through the use of diagnostic plots, such as the visual predictive check (VPC) [51]. If an alternative model with additional parameters fits the data better than a reduced nested model, this will be reflected by a drop in the objective function value. A more appropriate model does not necessarily mean a better fit to the data. In general, it is the purpose of the model that determines its appropriateness. In fact, all models are wrong but some may be useful [52]. Ideally a model should be mechanistic in nature. This facilitates extrapolation from the experimental condition under which the model was built. Sometimes the use of an empirical model, lacking any biological interpretation, but still capable of describing the data, is satisfactory for the specific purpose of modeling [53].

1.4.2 The structural model (fixed effects model)

The one-compartment pharmacokinetic model, describing the time-course of drug exposure after an intravenous administration, is one example of a structural model:

$$C = \frac{Dose}{V} \times \exp(-\frac{CL}{V} \times time) \tag{1}$$

C is the drug concentration at any time, Dose is the amount of administered dose, V and CL are pharmacokinetic parameter estimates describing the volume of distribution and elimination clearance, respectively. The E_{MAX}-model is another example of a structural model. It describes the basic shape of a relationship between two variables, e.g. drug concentration and response [54]:

$$E = \frac{(E_{MAX} \times C)}{(EC_{50} + C)} \tag{2}$$

E is the drug response at any drug concentration C. E_{MAX} and EC_{50} are pharmacodynamic parameter estimates describing the maximal response achievable and the drug concentra-

tion producing 50% of the maximal response, respectively.

There are also, mathematically and mechanistically more complex, indirect response models taking temporal delays between drug concentrations and response into account, e.g. because of drug distributional delay to the site of drug action (effect compartment or biophase) or describing the cascade of events that occurs in a biological system due to the pharmacological mechanism of action [55, 56]. Pharmacodynamic models described in this thesis can be classified as irreversible since they aim to describe the inactivation of proliferative cells such as parasites or virus populations.

1.4.3 The individual model (mixed effects model)

Through the advent of the population approach, there is today software not only quantitating pharmacological data on a population level but, importantly, simultaneously also estimating the between- and within-subject variability [57]. Average population PK/PD model parameter values as well as their between- and within-subject variability can be obtained by non-linear mixed effects regression, even when data are sparse (typically 1-3 samples per patient) [58]. In fact, a few samples from many individuals can give as accurate parameter estimation as rich data obtained from a limited number of subjects. Considering a general model the observed value in an individual i at occasion j (Obs_{ij}) can be described by the following equation:

$$Obs_{ij} = Pred_{ij} + \varepsilon_{ij} \quad (3)$$

The individual prediction ($Pred_{ij}$) is a function of this individual's set of specific parameter estimates, sampling-times and other fixed input. Any difference between the individual predictions and the observations, ε (mean 0, variance σ^2), may be due to model misspecification, sampling errors or within-subject variability. A specific parameter estimate for an individual (P_i) may be described as:

$$P_i = P \times \exp(\eta_i) \quad (4)$$

P is the typical parameter estimate in the studied population and η is the log-normally distributed between-subject variability (mean 0, variance ω^2). The differences between observed and predicted data are illustrated in *Figure 4*.

1.4.4 Covariates

Sources of variation among individuals can be manifested both in drug disposition and dynamics. Some of this variability is predictable whereas other is random. One important cause of variability is that of varying expression and function of drug metabolizing enzymes across and within populations [59]. The CYP P450 family, and also drug transport

membrane proteins, exhibit many allelic variants which may encode defective function or no function at all. In addition to genetic factors, other covariate factors such as body weight, age, sex, renal function, drug-drug interactions or concomitant diseases can also influence drug exposure and subsequently treatment outcome. The effects of a continuous covariate, such as body weight on a parameter P may be described as:

$$P_i = P \times [1 + factor \times (\text{body weight}_i - \text{median body weight})] \times \exp(\eta_i) \quad (5)$$

Here, *factor* represents a parameter estimate describing the fractional change in the typical estimate of P with body weight. The effects of a categorical covariate, such as a genetic polymorphism, on a parameter P may rather be described as:

$$P_i = (P_{\text{wild-type}} + P_{\text{heterozygous mutant}} + P_{\text{homozygous mutant}}) \times \exp(\eta_i) \quad (6)$$

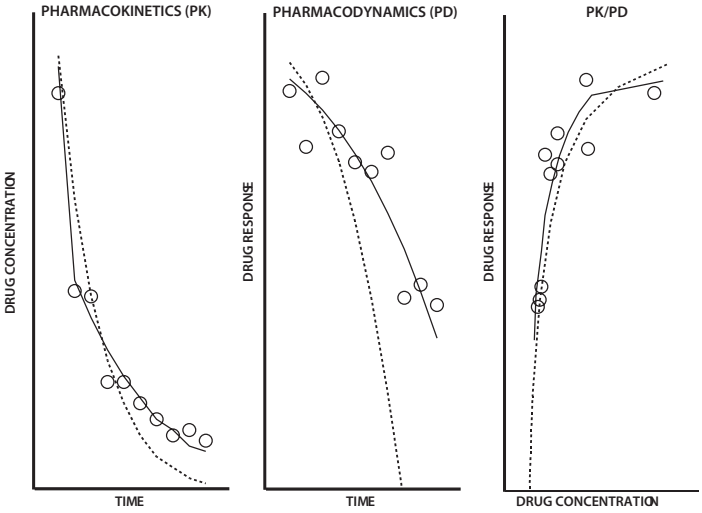

Figure 4. An illustration of a pharmacokinetic, pharmacodynamic and PK/PD model fitted to repeatedly measured data of drug exposure, response and their relationship, respectively. Observed and predicted drug concentrations and response are plotted versus time or concentration, respectively. The open circles represent experimentally observed measurements in a studied individual. The solid lines are the model predictions in the same individual. The model predictions for a typical individual of the population receiving the same drug and dose are seen as dashed lines. The difference between the individual and typical model predictions symbolizes between-subject variability while the difference between observed and individually predicted data may be due to model misspecification, measurement errors or within-subject variability.

1.4.5 Drug-disease models and simulation

Pharmacokinetic and pharmacodynamic models as well as models describing disease-progression [60] can be combined in a drug-disease model describing the links from drug administration to treatment outcome in various patient populations *(Figure 5)* [61].

Ultimately the complete drug-disease model or separate modules of it could be used as a guidance tool. Simulation refers to the use of a model in prediction [62]. Stochastic simulation refers to simulation including the elements of between-subject variability and sometimes also uncertainty. Simulations have successfully been performed to investigate planned study designs [63], for sample size considerations (e.g. how many patients should be recruited to ensure that the population is properly represented) [64, 65], rational dose selection (e.g. which drug or drug combination, dose levels, how often and for how long) [66], adherence issues (e.g. what is the impact of missed doses or discontinuation of therapy) [67], and for optimal sampling strategies (e.g. when it is most informative to draw sparse blood samples for PK or PD analyses) [68].

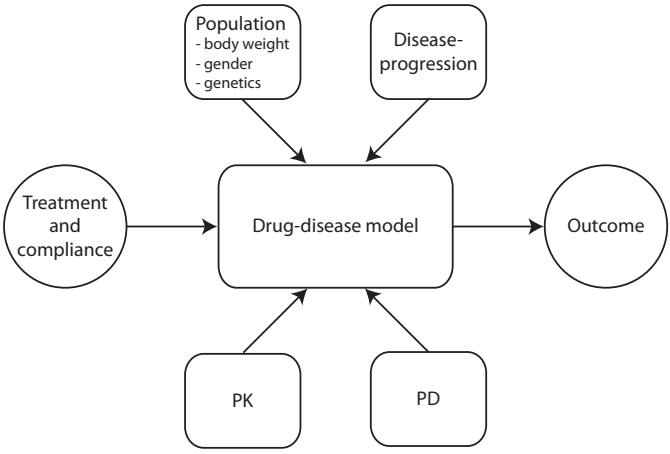

Figure 5. Schematic illustration of a template drug-disease model, describing the links between the study population, dosing, pharmacokinetics, pharmacodynamics, disease-progression and treatment outcome.

2 Aims of the Thesis

The overall aim of this thesis is to highlight the use of pharmacometrics when analyzing data collected in anti-infective drug studies performed in resource-limited settings as well as in high-income countries. In a further perspective the thesis aims at deriving information, facilitating the optimization of existing and novel antiretroviral and antimalarial pharmacotherapy, through applied PK/PD modeling and simulation.

Primary objective

> To mathematically describe the pharmacological relationship between dosing history, between-subject variability, drug exposure, response and/or treatment outcome in various patient populations for selected antiretroviral and antimalarial drugs, using PK and PK/PD models

Secondary objectives

1. To search for covariates (demographic or disease-specific) explaining between-subject variability in the pharmacokinetic and pharmacodynamic parameters

2. To specifically examine the effects of pharmacogenetic polymorphisms in drug metabolizing enzymes and transporters on the pharmacokinetic and pharmacodynamic properties

3. Provide rationale for individualized therapy, i.e. dose adjustments by patient specific features, to target the desired drug exposure and to improve clinical success rates

4. To demonstrate the utility of specific, empirical or mechanistic, PK- and PD-models to estimate and report experimental data from studies performed in various parts of the world

3 Methods

3.1 Clinical investigations

3.1.1 Ethics

Observed data were obtained from five clinical studies. All trials were conducted according to the principles set down by the International Conference on Harmonisation – Good Clinical Practise (ICH-GCP) guidelines, the Declaration of Helsinki, as modified by the 48th World Medical Association (WMA) General Assembly, Somerset West, Republic of South Africa, October 1996 (Paper IV) and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 (Papers I-III and V) and applicable regulations. Participants provided signed informed consent prior to study entry. Ethical approval for the first study (Paper I) was given by the Uganda National Council of Science and Technology. The second study (Paper II) was approved by ethics committees at the Medical Research Council of Zimbabwe and by the Joint Parirenyatwa Hospital and College of Health Science Research, Harare. The third study (Paper III) was approved by an independent ethics committee and the Swedish Medical Products Agency. The forth study (Paper IV) was approved by Vietnamese Ministry of Health and the WHO Secretariat Committee for Research Involving Human Subjects (SCRIHS). The fifth study (Paper V) was approved by the local review board at the National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi and the Vietnamese Ministry of Health.

3.1.2 HIV study designs

3.1.2.1 Paper I

In the first study, performed in Uganda, the objective was to investigate the impact of pharmacogenetics on the population pharmacokinetics of efavirenz. Efavirenz was given as a single dose of 600 mg to healthy subjects (52 males and 69 females, 19-59 years of age). The participants did not use any other medications including herbal preparations one week prior to or during the study period. Blood samples for efavirenz concentration determination were collected from 32 of the participants at 0, 4, 8, 12, 24, 48 and 72 hours after dose. Additional samples were taken at 4 and 24 hours from the 89 remaining subjects. Plasma concentrations of efavirenz were analyzed using a reversed-phase high-performance liquid chromatography (HPLC) method with UV-detection. The lower limit of quantification (LLOQ) was 0.35 μM. The study participants were characterized for 30 single nucleotide polymorphisms (SNPs) in *CYP2B6*, *CYP3A5*, and *MDR-1* genes

by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

3.1.2.2 Paper II

The second study, performed in Zimbabwe, sought to investigate the relationship between efavirenz exposure and the *CYP2B6* 516 G>T (*CYP2B6*6*) genotype in HIV/AIDS patients through pharmacokinetic modeling and simulation. Seventy-four HIV-positive patients (26 males and 48 females, 20-56 years of age) assigned to receive efavirenz (600 mg) once a day, in combination with two NRTIs, were included in the study. Single blood samples were collected at 11-16 hours after reported last dose intake from patients who had been prescribed efavirenz for at least 3 weeks. A HPLC assay with UV-detection was used for the determination of efavirenz steady-state concentrations (LLOQ = 0.47 mg/L). Patients were genotyped for *CYP2B6*6* polymorphism using PCR-RFLP.

3.1.2.3 Paper III

The NORTHIV study was a randomized open-label multi-centre trial performed in Norway and Sweden. The study aimed to compare efficacy, side-effects, and treatment adherence to three commonly prescribed regimens given to antiretroviral naïve HIV-infected patients. The objectives of the present analysis were to describe the time-course of antiretroviral drug exposure, search for covariates influencing drug exposure and evaluate potential differences in drug response between the treatment arms, using pharmacokinetic and pharmacodynamic models. Patients (158 males and 81 females over 16 years of age) were repeatedly followed with respect to HIV-RNA levels, CD4-cell count and drug exposure for up to three years after study initiation. Patients were randomized to one of three study arms; *i.* lopinavir/ritonavir (400/100 mg), co-administrated with two NRTIs twice daily, *ii.* atazanavir (300 mg), co-administrated with ritonavir (100 mg) and two NRTIs once a day, *iii.* efavirenz (600 mg), co-administrated with two NRTIs once a day. Plasma concentrations of lopinavir (LLOQ = 0.25 μM), atazanavir (LLOQ = 0.14 μM), ritonavir (LLOQ = 0.50 μM) and efavirenz (LLOQ = 0.47 μM) at weeks 4, 12, 48 and 144 were analyzed by HPLC. HIV-RNA levels were determined using the Roche Amplicor v1.5 reverse transcriptase PCR assay. CD4-cells were counted using flow cytometry.

3.1.3 Malaria study designs

3.1.3.1 Paper IV

In the fourth study the pharmacokinetics of artemisinin and the relationship between drug exposure and treatment outcome were modeled. In this observational, non-randomized study, 97 Vietnamese patients (67 males and 30 females, 5-88 years of age) were

treated according to then current national recommendations with artemisinin (500 mg) administered orally twice the first day followed by single dose administration for the next four days. Study intervention was limited to saliva samples for pharmacokinetic evaluation being collected at approximately 0, 2, 4 and 6 hours after the first dose and optionally at 4 and 6 hours after any of the following doses. Parasite counts (number of asexual parasites) were determined pre-treatment and every eight hours after initiation of therapy, until three negative smears. Parasite clearance times were defined as the time from the first dose to the first of three negative smears. Patients were followed up on day 21. Cure rate was determined as the proportion of patients with no detectable parasites at this visit. Artemisinin saliva concentrations were determined using HPLC with post column derivatization and UV-detection (LLOQ = 2 μg/L).

3.1.3.2 Paper V

This was a pilot study designed and conducted at a time when there was no prior information available on human piperaquine pharmacokinetics, despite the drug having been used clinically for some time. The principle aim was to obtain basic pharmacokinetic information to enhance future study designs and sampling strategies. Twelve healthy Vietnamese males were administered 1280 mg piperaquine phosphate orally as a single dose on day 1 followed by another 640 mg in the morning on days 2 and 3, respectively. Blood samples for drug concentration measurement were frequently collected after the first and third dose for a total of 29 days. Piperaquine in plasma was quantified by solid phase extraction followed by a HPLC method with UV-detection (LLOQ = 7 nM). The complex drug plasma concentration-time profiles obtained necessitated a modeling approach to characterize the drug's irregular absorption profile and long elimination half-life.

3.2 Model development

3.2.1 Software and model building

Pharmacokinetic and pharmacodynamic models were fitted to data obtained from clinical studies I-V, using the NONMEM version V or VI software [50] under a Compaq Visual Fortran v. 6.6 compiler.

3.2.2 Pharmacokinetic and pharmacodynamic HIV models

In Paper I, a two-compartment pharmacokinetic model with zero-order followed by first-order absorption, which can be interpreted as dissolution rate limited absorption, was fitted to the data (*Figure 6*). Age, body weight, sex, albumin, alanine aminotransferase (ALT), urea, serum creatinin and pharmacogenetic polymorphisms in genes (n=30) coding for metabolizing enzymes and transporters (CYP3A5, CYP2B6 and MDR-1) were

covariates considered for inclusion in the model. Pharmacogenetic covariates were tested on apparent clearance and on the relative bioavailability parameter (F_{rel}).

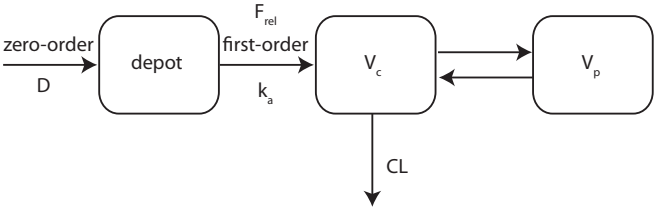

Figure 6. Zero-order followed by first-order sequential absorption two-compartment pharmacokinetic model. The drug is introduced into a depot compartment through zero-order absorption. The drug is further absorbed to the central compartment and distributed to a peripheral compartment. The drug is eliminated from the central compartment. D: duration of zero-order absorption, depot: dose compartment, k_a: first-order absorption rate constant, F_{rel}: relative bioavailability (set to 1 in wild-type metabolizers), V_c: apparent central volume of distribution, V_p: apparent peripheral volume of distribution, CL: apparent clearance.

In Paper II, a linear one-compartment pharmacokinetic model was fitted to the observed steady-state drug concentrations. Pharmacogenetic *CYP2B6*6* polymorphism was introduced as a covariate on apparent clearance (CL/F). Homozygous wild-type metabolizers were assumed to have one typical estimate of clearance. The potential reduction in this parameter was estimated for hetero- and homozygous mutant metabolizers. Body weight, sex and age were other covariates considered for inclusion in the model. Using the final model, it was investigated through simulation whether *a priori* dose reduction would be possible in poor efavirenz metabolizing patients. The size of the dose reduction in steps of 100 mg was plotted against the proportion of patients having sub-optimal concentrations (<1 mg/L) and the proportion of patients with toxic exposure (>4 mg/L). If the proportion of patients with sub-optimal concentration was <5% the dose reduction was considered achievable.

In Paper III, linear one-compartment pharmacokinetic models were fitted to the observed steady state drug concentrations of lopinavir, atazanavir, ritonavir and efavirenz. Age, body weight, sex, ethnicity, CDC-stage, clinical chemistry variables and exposure to ritonavir, which is a potent CYP3A4 inhibitor, were covariates considered for inclusion in the models. A drug-disease model, where the interaction between CD4-cells, virus, actively and latently cells are described through a set of differential equations, was fitted to the repeatedly measured HIV-RNA levels (*Figure 7*).

In brief, the virus infects a pool of CD4-cells which can be either actively or latently infected. Latently infected cells are sooner or later activated and new virions are produced from the actively infected cells. In the presence of drugs the infection rate of CD4-cells is inhibited by drug regimen specific factors ranging from 0 to 100% inhibition. In an extended

analysis it will also be evaluated whether drug response can be modeled as a function of drug concentration. The reproduction ratio (R), a derived system specific parameter for infectious diseases, can here be defined as the expected number of new virions produced from a single virus particle introduced among uninfected cells [69, 70]. If R is greater than 1 or less than 1, the virus population will grow or decline, respectively. In this initial analysis, pharmacodynamic models were fitted to HIV-RNA data only. In an extended analysis, models will be fitted to both HIV-RNA and CD4-cell data. HIV-RNA data below the quantification limit was considered using the F_FLAG functionality in NONMEM [71].

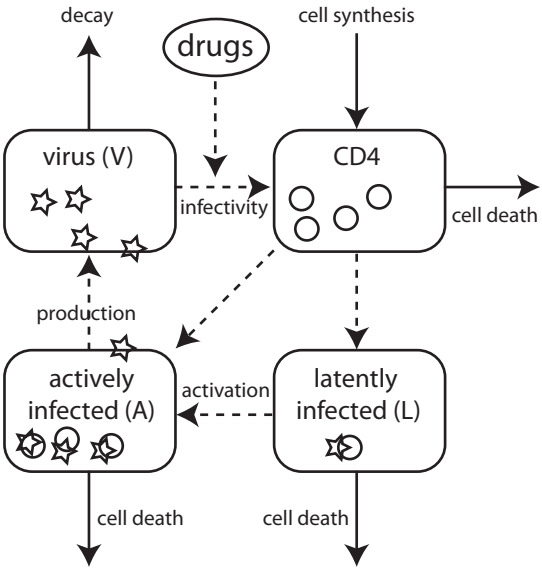

Figure 7. The drug-disease model describing the HIV-infection and action of antiretroviral drugs. The disease model consists of virus, uninfected, actively and latently infected CD4-cell compartments. The virus infects uninfected cells, which either become latently or actively infected. Latently infected cells can become reactivated. Each cell-type has its natural life-span. The interaction between the cells was described by the use of differential equations. The infection rate was assumed to be proportional to the number of uninfected cells and the number of virus particles. The infection rate can be inhibited 0-100% by drug regimen specific factors (ε).

3.2.3 Pharmacokinetic and pharmacodynamic malaria models

To account for auto-induction of artemisinin metabolism, a well-stirred pharmacokinetic model [72] with stimulation of the enzyme precursor production rate by hepatic drug amounts [73] was used in Paper IV (*Figure 8*). The well-stirred model is described by the fraction of unbound drug (fu), liver blood flow (Q_H), and the intrinsic clearance of drug (CL_{int}), which is the enzymatic capacity in the absence of blood-flow limitations and binding of drug to proteins. F_H is the fraction of drug that escapes first-pass metabolism from

the liver compartment. If induction of enzymes occurs, an increment of CL_{int} will be seen, leading to an increase in hepatic CL and/or a decrease in hepatic bioavailability (F_H) depending on the size of the extraction ratio:

$$CL = \frac{Q_H \times fu \times CL_{\text{int}}}{Q_H + fu \times CL_{\text{int}}} \tag{7}$$

$$F_H = \frac{Q_H}{Q_H + fu \times CL_{\text{int}}} \tag{8}$$

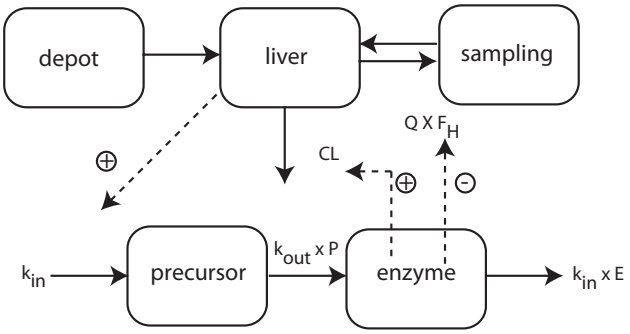

Figure 8. Schematic illustration of the artemisinin auto-induction pharmacokinetic model. Artemisinin is absorbed from a depot compartment into a well-stirred liver compartment and subsequently distributed into the saliva compartment where sampling occurs. The amount of artemisinin in the liver compartment is inducing the enzyme precursor levels. The turnover of liver enzymes is described through an indirect response model with zero-order formation (k_{in}) of the precursor and first-order removal (k_{out}) of enzymes. When enzyme levels are elevated, intrinsic clearance (CL_{int}) is increasing in a corresponding manner, leading to an increase in drug clearance (CL) and/or a decrease in the hepatic bioavailability (F_H).

The relationship between exposure to artemisinin and therapeutic response (PCT and cure) was investigated by pharmacodynamic models, using linear, non-linear and logistic regression methods. The PCT was characterized as a function of the area under the concentration-time curve after the first dose (AUC_{dose1}) by a linear model or a sigmoidal inhibitory E_{MAX}-model with baseline effect. The probability of cure was modeled as a binary logistic function of the cumulative $AUC_{0-\infty}$. PCT normalized for initial parasitemia (IP) was tried as well.

To describe the atypical absorption profile of piperaquine with multiple peaks, a dual absorption pathway was modeled in Paper V, implementing a fast and slow absorption process (Figure 9).

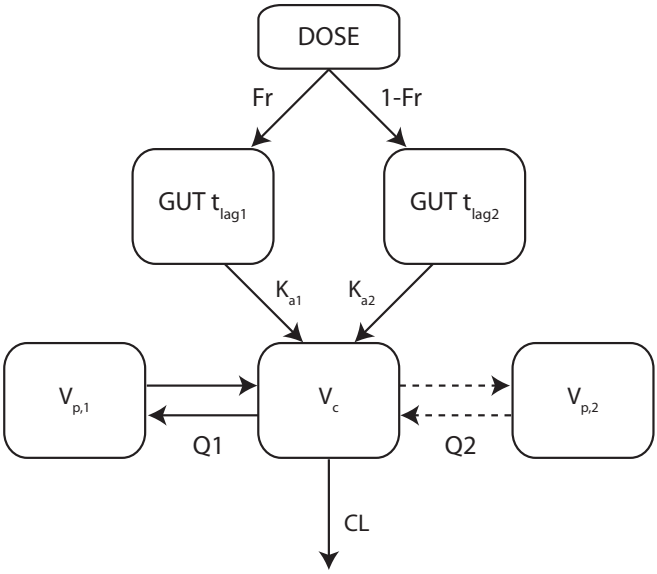

Figure 9. Pharmacokinetic model for piperaquine. Dual pathway absorption was included to account for multiple peaks. The absorption of piperaquine to the central compartment was described by two consecutive first-order processes, separated by a lag-time. The disposition model was composed of either two (solid lines) or three (solid and dashed lines) compartments. V_c: apparent central volume of distribution, Vp: apparent peripheral volume of distribution, CL: oral clearance, Q: apparent inter-compartmental clearance, Fr: fraction of dose being absorbed in the first consecutive absorption step, k_{a1} and $_{ka2}$: first-order absorption rate constants, t_{lag1} and t_{lag2}: absorption lag-times.

4 Results and Discussion

4.1 Efavirenz pharmacokinetics

The use of mixed effects modeling allowed examining the impact of multiple pharmacogenetic and demographic covariates on the single dose efavirenz population pharmacokinetics in healthy subjects. Homozygous carriers of the *CYP2B6*6* and *CYP2B6*11* genotypes were identified to have a 20% reduction in apparent clearance compared to wild-type metabolizers. A novel polymorphism in *MDR-1* (c 4036 A>G) was estimated to increase the relative bioavailability by 25% and the apparent peripheral volume of distribution was two-fold higher in females compared to males (Table 1, *Figures 10 and 11*).

Table 1. Parameter estimates for the pharmacokinetic/pharmacogenetic model (Paper I).

Parameter	Estimate (95% CI)	CV% (95% CI)
CL/F (L/h)	4.00 (3.47, 4.53)	14.0 (2.8, 25.2)
Effect of *CYP2B6*6*	-0.209 (-0.386, -0.032)	
Effect of *CYP2B6*11*	-0.199 (-0.329, -0.0691)	
V_c/F (L)	19.1 (7.46, 30.7)	99.5 (49.4, 132)
V_p/F (L)	155 (131, 179)	27.9 (14.8, 36.7)
Effect of sex	2.08 (1.64, 2.52)	
Q/F (L/h)	13.7 (6.1, 21.3)	32.1 (20.5, 40.5)
ka (h^{-1})	0.146 (0.0558, 0.236)	19.7 (8.6, 30.8)
D (h)	1.07 (0.758, 1.38)	69.7 (15.3, 97.4)
F_{rel}	1 FIX	18.8 (11.9, 23.9)
Effect of MDR-1 (c 4036)	0.257 (0.0873, 0.427)	
σ_{prop} (CV%)	13.9 (9.62, 17.1)	

*CL/F: apparent clearance, Effect of CYP2B6*6 and *11: fractional change in CL/F for poor metabolizers, V_c/F: apparent volume of distribution of the central compartment, V_p/F: apparent volume of distribution of the peripheral compartment, Effect of sex: factor expressing peripheral volume of distribution in females relative to peripheral volume in males, Q/F: inter-compartmental clearance, ka: absorption rate constant, D: duration of zero-order absorption, F_{rel}: relative oral bioavailability, Effect of MDR-1: fractional change in F_{rel} for mutant subjects, σ_{prop}: random residual error, CI: confidence interval, CV: coefficient of variation.*

The results are in agreement with other studies that have previously reported poor efavirenz clearance among carriers of *CYP2B6*6* [74-76]. Although the *CYP2B6*11* mutation seems to significantly affect efavirenz clearance, its clinical role and implications

need to be further investigated after multiple dose administration. P-glycoprotein (Pgp) is coded by the multiple drug resistance gene (*MDR-1*). There are conflicting suggestions on whether efavirenz is a substrate for Pgp and the role of *MDR-1* genetic variation in efavirenz plasma exposure and treatment outcomes is not clearly defined [77-80]. Favorable virological response with *MDR-1* 3435 C>T has been reported [76] but no systematic study has monitored the role of other SNPs in the *MDR-1* gene for treatment outcome.

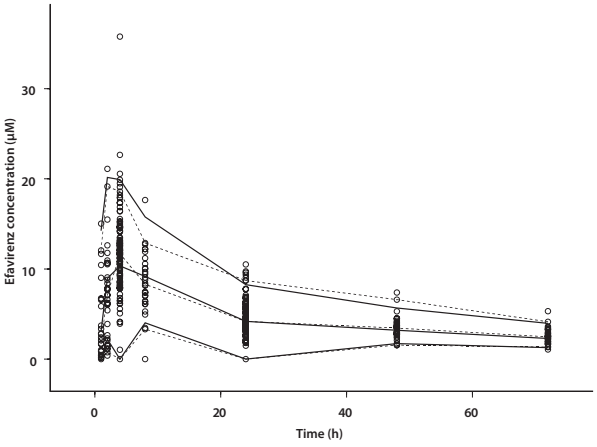

Figure 10. Visual predictive check of how the final efavirenz pharmacokinetic/pharmacogenetic model predicts the observed plasma concentrations (circles). The study was replicated 1000 times. The solid lines constitute a 95% prediction interval around the median predicted efavirenz concentrations. The dashed lines are the corresponding percentiles for the observed concentrations.

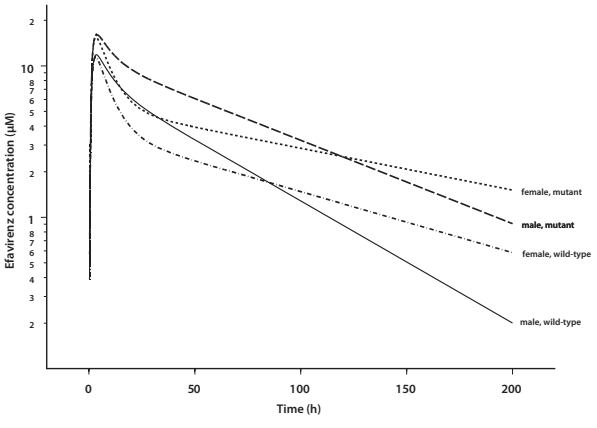

*Figure 11. The simulated concentration time-course after a single dose of 600 mg efavirenz in four typical subjects, based on the final model. Wild-type: CYP2B6 516G/G, CYP2B6*11G/G and MDR-1 c4036A/A (19% of the studied population), homozygous mutant: CYP2B6 516T/T, CYP2B6*11T/T and MDR-1 c4036G/G (3% of the studied population).*

The more pronounced distribution of efavirenz, being a very lipophilic drug (logP = 5.4), to peripheral tissues in women could be due to higher body fat content or due to sex differences in plasma protein binding. However, albumin was not identified to be an important covariate in the present analysis. So far most efavirenz pharmacogenetic studies have focused on a few variant alleles, in particular *CYP2B6*6* and *MDR-1* 3435 C>T, to investigate the potential effect of genetic variation in predicting efavirenz plasma exposure and treatment response. In addition to the previously investigated SNPs, we selected new regulatory and coding SNPs that have not been characterized before but with possible functional effect as predicted by bioinformatics tools. However, this study being a single dose population pharmacokinetic study, the auto-inductive effect of efavirenz following repeated administration could not be considered. There is therefore a need to study the effect of pharmacogenetic polymorphism on efavirenz pharmacokinetics at steady-state, and preferably in a patient population. Anyway, the obtained results indicate which pharmacogenetic polymorphisms that may be therapeutically important to study during chronic administration.

The metabolizing capacity of efavirenz in HIV/AIDS-patients was observed to be decreased in carriers of the *CYP2B6*6* (516 G>T) genotype (*Figure 12*). Approximately 28%, 57% and 15% of the patients were identified to be extensive, intermediate and poor metabolizers of efavirenz, respectively. Typical apparent clearance was estimated to 9.4 (95% CI, 6.2-12.6) L/h in wild-types in contrast to 7.2 (95% CI, 4.3-10.3) and 4.0 (95% CI, 2.0-5.9) L/h in intermediate and poor metabolizers, respectively. Furthermore, the study suggested *a priori* dose reduction from 600 to 400 mg once daily in poor efavirenz metabolizers (*Figure 13*).

Individualization of drug treatment becomes desirable when between-subject variability in PK/PD parameters is extensive and the therapeutic margins narrow [81]. Monitoring of drug concentrations and adjusting the dosage regimen on the basis of these concentrations is a well-known therapeutic intervention defined as therapeutic drug monitoring [82]. An alternative and less costly, conceptual strategy has recently been proposed. The target concentration approach aims at explaining the between-subject variability in concentrations with patient-specific covariate factors such as creatinine clearance, pharmacogenetics, age or body weight and to let the individually predicted PK parameter estimates guide dosing to achieve and maintain optimal drug exposure and the target effect [83]. While therapeutic drug monitoring is an empirical method to predict an optimal concentration, offering no explanation why an individual is outlying, target concentration intervention uses PK/PD models and mechanistic knowledge about the concentration-response relationship. Despite this, in the African context pharmacogenetic testing is also an economical question. Given the large (76%) remaining unexplained between-subject variability in drug clearance, therapeutic drug monitoring may not yet be fully replaced by dose individualization based on pharmacogenetic considerations. In this case *a priori* dose reduction of 200 mg was suggested in poor efavirenz metabolizers. After therapeutic drug monitoring the dose could be further adjusted.

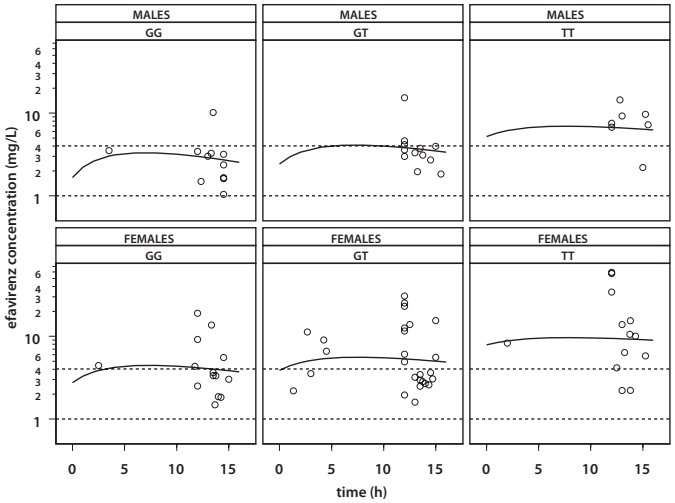

Figure 12. Overall goodness-of-fit plot of the final model. Observed and predicted efavirenz steady-state concentrations are conditioned on sex and genotype. GG: extensive metabolizers, GT: intermediate metabolizers, TT: poor metabolizers. Open circles are the observed concentrations. The solid lines are the model predictions in a typical individual. The dashed horizontal lines show the optimal concentration interval (1–4 mg/L).

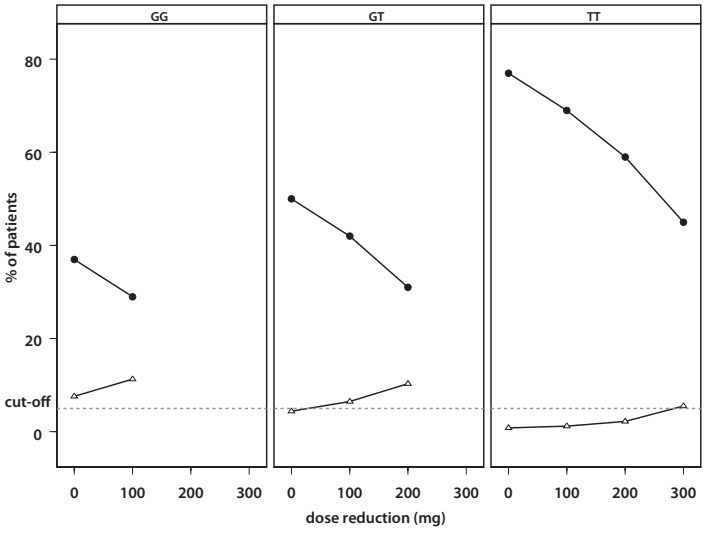

Figure 13. Simulations of efavirenz dose reductions from the standard dosing of 600 mg once a day. The triangles and circles represent the proportion of patients having sub-optimal and toxic efavirenz exposure, respectively. The dashed line symbolizes a cut-off level where 5% of the patients have sub-optimal concentrations. GG: extensive metabolizers, GT: intermediate metabolizers, TT: poor metabolizers.

4.2 Pharmacodynamics of antiretroviral therapy

Lopinavir was identified to give a 2.4-fold (95% CI, 1.7-3.1) increase in clearance of ritonavir. As expected, ritonavir was found to increase the exposure to both lopinavir and atazanavir. The viral load was reasonably well described over time by the pharmacodynamic model (*Figures 14 and 15*). The effectiveness of pharmacological intervention was evaluated by estimating the inhibiting fraction of the *de novo* infection rate and the viral reproduction ratio. Interestingly, in this initial analysis the efavirenz-containing regimen appeared to provide better treatment outcome than two protease inhibitor-containing regimens. Twice daily administration with lopinavir was estimated to be slightly more effective compared to once a day administration with atazanavir, but the differences seemed to be statistically insignificant. In the presence of drugs, the mean viral reproduction ratio was reduced from 3.05 to 1.04, 1.14, and 0.406 for the lopinavir, atazanavir, and efavirenz-containing regimens, respectively.

It should be pointed out that only few studies have previously compared once and twice daily administration [84]. Once a day administration with atazanavir will give rise to more fluctuating drug exposure compared to twice daily administration of lopinavir. However, the risk for viral rebound can potentially be balanced by practical advantages in form of reduced dosing frequency which itself can increase adherence to the prescribed treatment. The available co-formulation of lopinavir/ritonavir also restrains the total daily dose intake.

There have been previous attempts to model the effects of pharmacotherapy on the pharmacodynamics of HIV-infection [85-92]. Taking HIV-RNA data below the limit of quantification into consideration, we present the application of a drug-disease model for the evaluation of combination therapy. The same model parameters related to the underlying disease were used for all patients. In contrast, treatment specific parameters were assumed to vary between the treatment arms and drugs. The application of drug-disease models is expected to increase in the future drug development process. The United States Food and Drug Administration (FDA) recommends the use of disease-drug-trial models in their FDA Critical Path document as a potentially valuable tool to improve the predictability and productivity of the drug development process for HIV and other therapeutic areas [93].

4.3 Artemisinin exposure-response and piperaquine pharmacokinetics

The saliva concentration-time profiles to artemisinin after the first oral dose could be described by a linear one-compartment model. However, predicted data did not match concentration-time profiles observed at later doses. A refined model, predicting lower artemisinin saliva concentrations over time due to increased first-pass extraction after in-

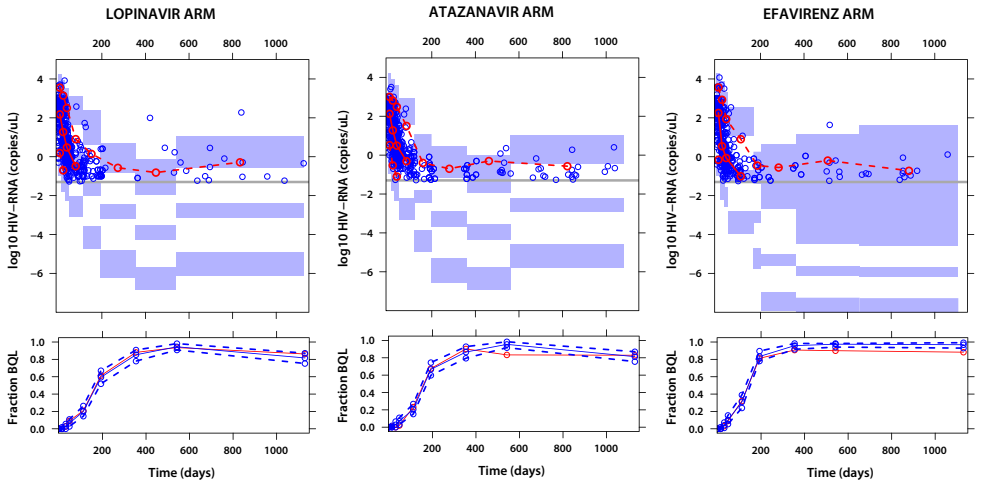

Figure 14. A visual predictive check of how the pharmacodynamic model predicts the observed HIV-RNA data conditioned on treatment group. The study was replicated 1000 times. The red lines are the median and the 2.5th and the 97.5th percentiles of the observed HIV-RNA data which is presented as blue circles. The blue shaded areas are the 95% confidence intervals around the median and the 2.5th and the 97.5th percentiles of model predicted HIV-RNA levels. The grey line symbolizes the limit of HIV-RNA quantification set at 50 copies/mL. Observed data below the quantification limit was omitted from the plot. The lower panels show the predicted and observed fraction of data below the quantification limit.

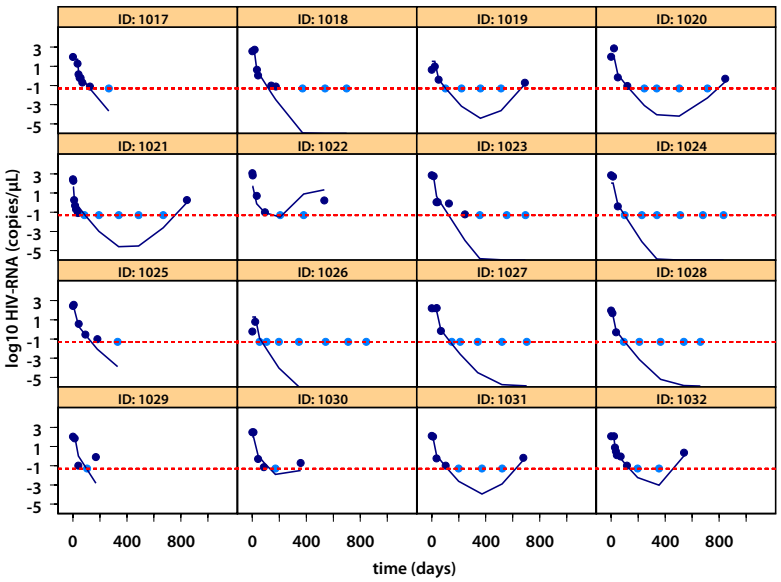

Figure 15. Observations and individually predicted viral levels over time for 16 representative patients. The dark blue circles represent HIV-RNA observations. Light blue circles represent observations below the limit of quantification. The blue lines are the individually predicted HIV-RNA levels. The limit of quantification (50 copies/mL) is symbolized by the red dashed line.

duction of metabolizing enzymes [73], improved fits (*Figure 16*). The enzymatic half-life was estimated to 30.7 (95% CI, 30.4-31.0) hours with a mean induction time (MIT) of 8.21 (95% CI, 8.16-8.26) hours. The observed mean PCT was 40 hours. The observed frequency of recrudescence was high (37%). No direct relationships could be observed between exposure to artemisinin and drug response. Cure rate could not convincingly be related to any measure of exposure, using logistic regression (*Figure 17*).

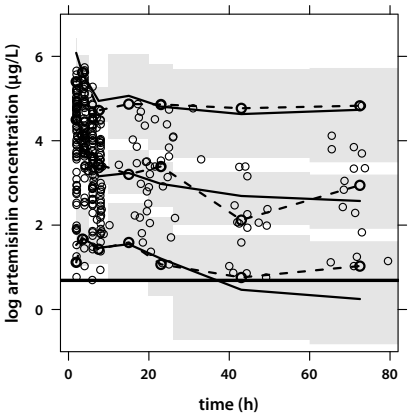

Figure 16. A visual predictive check of how the auto-induction model predicts the observed artemisinin saliva drug concentrations. The study was replicated 1000 times. Observed artemisinin data is presented as circles. The dashed lines represent the median and the 2.5th and 97.5th percentiles of the observed concentrations. The shaded areas represent 95% confidence intervals around the median and the 2.5th and 97.5th percentiles of the predicted concentrations (solid lines). The line at 2 µg/L symbolizes the limit of quantification.

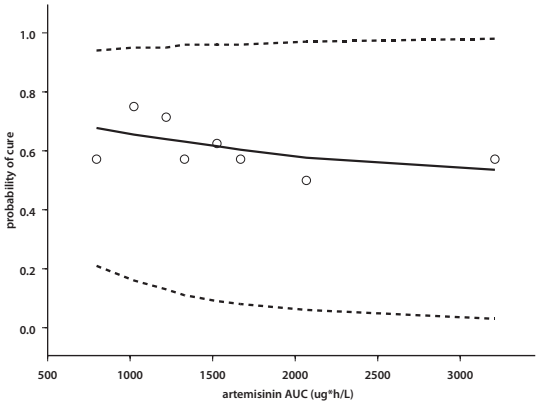

Figure 17. Logistic regression model. Predicted AUC values and observed cure rates are binned in 8 intervals. The solid line represents the probability of cure at varying artemisinin exposure. The dashed lines constitute a 95% confidence interval of the median predicted probability based on parameter estimates from 1000 bootstrap samples.

Similar pharmacokinetic results were reported by Asimus and Gordi after a model-based meta-analysis of six clinical studies involving repeated oral administration of artemisinin to 54 malaria patients and 33 healthy subjects [94]. The present model was fitted to artemisinin concentrations in saliva and not in plasma. Salivary concentrations of artemisinin are well correlated with unbound plasma concentrations [95]. Even though this method has gained little acceptance in clinical practice [96], saliva sampling is a non-invasive method, suitable for sampling in children and for field-studies, facilitating the collection and handling of samples. This study, performed within routine clinical care, illustrates the risk of recrudescence when artemisinin is used in a short course of monotherapy. To prevent the development of resistance and increase efficacy, artemisinin or its derivatives are today therefore mostly used in combination treatment [97].

The dual absorption pathway model gave an adequate fit to piperaquine absorption (*Figure 18*) and facilitated estimation of piperaquine elimination half-life which was 11.7 (95% CI, 8.3-15.7) days with large between-subject variability (5-31 days). Pharmacokinetic parameter estimates are presented in Table 2.

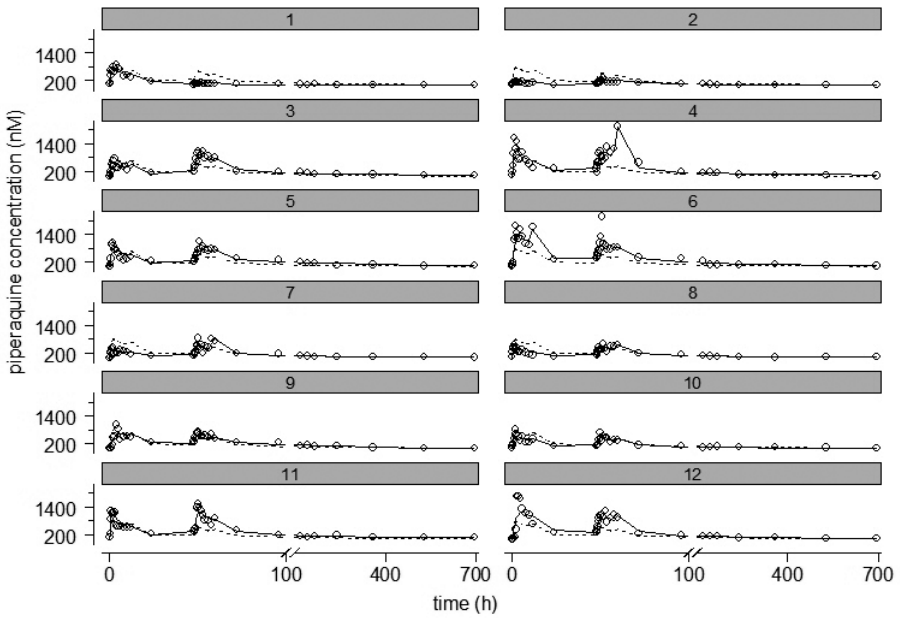

Figure 18. Measured (open circles) and population predicted (dashed lines) or individually predicted (solid lines) piperaquine pharmacokinetic profiles in 12 healthy Vietnamese subjects after repeated oral CV8 administration. Sampling occurred only after the first and last doses. A different time scale was used for the first 100 hours to display the atypical absorption phase.

The absorption profiles affected how different disposition models with first-order absorption fitted to the data. It was therefore necessary to allow for some flexibility in the model to account for the multiple peaks in order to obtain better fits to the data during the terminal phase. A reliable estimate of the terminal half-life is of particular value in the case of piperaquine since it determines the duration of effects after treatment (and as such may influence the choice of duration of follow-up in clinical trials) and has bearing on the risk for resistance development [98]. A more recent study, with sampling up to 63 days after drug administration, has suggested an even longer piperaquine elimination half-life of 28 days [99]. What now is more relevant to understand, is the relationship between piperaquine exposure and response, which includes *in vivo* estimates of minimum inhibitory concentrations (MIC) and values of effective concentrations (IC_{50}) for parasitocidal activity.

Table 2. Pharmacokinetic parameter estimates (Paper V).

Parameter	Estimate (RSE %)	IIV (†) or IOV (‡) (CV% (RSE %))
CL/F (L/h)	56.4 (24.8)	111 (69) ‡
V_c/F (L)	82.1 (24.5)	84 (51) ‡
Q/F (L/h)	43.9 (20.4)	59 (68) †
V_p1/F (L)	5920 (21.3)	
V_{ss}/F (L/h)	6002	
k_{a1} (h^{-1})	0.09 (5.8)	
k_{a2} (h^{-1})	0.72 (13.9)	
Fr (%)	91 (2.5)	164 (70) ‡
t_{lag1} (h)	0.42 (3.5)	
t_{lag2} (h)	9.9 (0.5)	
σ_1 (%)	0.34 (13.2)	
σ_2 (nM)	29.4 (11.7)	

*F: oral bioavailability, CL: clearance, V_c: central volume of distribution, Q: inter-compartmental clearance, V_p: peripheral volume of distribution, k_a: first-order absorption rate constant, Fr: fraction of dose being absorbed, t_{lag}: absorption lag-time, σ_1: proportional residual variability, σ_2: additive residual variability, RSE: relative standard error ((SE/mean)*100%), CV: coefficient of variation, IIV: inter-individual variability, IOV: inter-occasional variability.*

5 General Discussion

Treating HIV and malaria infections is challenging for prescribing physicians. This project aims at the identification of better treatment guidelines, taking into account demographic variables and the between-subject variability generated by other covariates such as drug metabolizing enzymes and transporters. Individualization of pharmacotherapy increases the probability that the patient receives the right dose the first time with the expected outcome of improving treatment success rates, decreasing the incidence of drug-induced toxicity and potentially attenuating resistance development.

In developing economies efavirenz is more expensive than nevirapine but is nonetheless finding increasing use and is usually the preferred drug in patients undergoing HIV and tuberculosis (TB) co-treatment [100]. It has been debated whether there is an association between efavirenz concentrations and efficacy or toxicity. Sound understanding of clinical pharmacology and evidence of poor virological response in patients with low efavirenz concentrations in plasma [35, 101, 102] as well as higher toxicity in patients exposed to higher concentrations of efavirenz [35, 103] favor such relationships.

In this thesis several single nucleotide polymorphisms in genes coding for drug metabolizing enzymes and transporters were observed to have effect on the single dose pharmacokinetics of efavirenz in healthy Ugandan subjects. Since efavirenz is known to induce its own metabolism [36], extrapolation to long-term treatment cannot directly be made from these results. After repeated administration however, Zimbabwean patients carrying the *CYP2B6*6* genotype were identified to have a 57% reduction in apparent clearance and a dose reduction from 600 to 400 mg once a day was suggested in this sub-population. Using a quantitative drug-disease model, an efavirenz-containing regimen appeared to provide slightly better treatment outcome compared to two PI-containing regimens in the initial analysis of data originating from a treatment naïve Scandinavian population.

Artemisinin-based combination therapy bears many resemblances to HAART. The drugs exhibit variable and complex pharmacokinetics with potential for drug-drug interactions [104]. The diseases themselves bring reasonable possibilities to measure disease markers (e.g. pathogen load/burden) for pharmacodynamic assessment. Moreover, HIV/AIDS and malaria are two major global infective diseases and in large parts of the world cause co-infections. Herein, no direct relationships could be observed between exposure to artemisinin and parasite clearance times or cure rates. However, exposure to artemisinin was decreased at each repeatedly administrated new dose due to auto-induction of drug metabolism and the rate of recrudescent infection was as high as 37% when artemisinin was used in a short-course of monotherapy. This strongly supports the use of combination therapy. The partnering drug should provide a long-acting cover and there should not be

any pharmacokinetically mediated drug-drug interactions with the artemisinin derivative [43]. Piperaquine was demonstrated to have an erratic oral absorption profile with a long, but a highly variable, terminal elimination half-life. Combining artemisinin with a drug with a longer half-life will increase success rates but caution must be taken since prolonged sub-optimal concentrations of the treatment may lead to the development of parasitological resistance.

Few attempts have previously been made to address dose optimization of HIV and malaria medication. Particularly in the case of malaria, the development of curative drugs and dosage regimens have been driven by empirical trial and error rather than by a sound understanding of the interplay between dosage, pharmacokinetics, pharmacodynamics, and efficacy/safety. However, during the last decades, the science of quantitative pharmacology and PK/PD modeling has evolved [48, 49, 105] and has also been employed in the field of HIV [90, 106]. There are also some examples in the literature of models relating malaria parasite killing rates to drug exposure [107-110]. Nevertheless, these reports are to our knowledge in minority compared to the evolution of modeling in other therapeutic areas. Barret has suggested that modeling and simulation may be used to identify and promote the most beneficial drug therapy in a target patient population and to quantitate sources of variability for improved decision making in the development of antiretroviral regimens [111]. McKenzie gives an excellent review about how mathematical modeling, complementing and extending the scope of classical pharmacokinetic and pharmacodynamic modeling, can reduce the burden of malaria [112].

In the optimization of antiretroviral and antimalarial pharmacotherapy, PK/PD modeling and clinical trial simulation, here referred to as pharmacometric methodology, should be integrated key components. The utility of pharmacometric modeling and simulation is becoming more and more recognized. The objective of pharmacometrics is to transform data into knowledge for effective and safe pharmacotherapy [113] through a learn-confirm process [114]. Similarly, a model may fill the gap between data collection and comprehension [115]. Pharmacometric methodology has had impact on the registration of new drugs [116] and continues to advance in the academic setting [117]. Pharmacometrics can ultimately be a useful tool for decision analysis and policy making in settings where small economical resources are available. However it requires considerable competence and there are few trained users [118].

Efforts to understand the pharmacokinetics and pharmacodynamics of antiretroviral and antimalarial drugs have within this work been extensively based on modeling and simulation activities. Pharmacokinetic and pharmacodynamic models were used to describe and report data originating from resource-limited settings (Papers I-II, IV-V) as well as from high-income countries (Paper III). It was shown that models are valuable tools to describe pharmacokinetic characteristics of drugs (Papers I-V), to identify important covariates (Papers I-III), to suggest new dosing strategies (Paper II) and eventually to describe pharmacodynamic relationships (Papers III and IV).

One advantage of the population based non-linear effects modeling approach, requiring to be emphasized, is that sparse and rich data can be combined in one analysis, sometimes even from different studies [119]. Furthermore, the population method has been shown to generate more accurate parameter estimates compared to the standard two-stage method which provides biased estimates of variability [120]. The replacement of raw data by models facilitates the communication of observed experimental data which often contains an infinite amount of information [121]. When all, and not only fragmentary data, jointly are combined in a model a more robust description is offered [122]. A model may also be used for simulation to gain mechanistic understanding of the system under investigation. Model-based simulations represent powerful tools which may accelerate drug policy decision-making as well as prospective confirmatory drug trials [62, 123]. The purpose of such simulations is not only to alleviate the need for additional clinical studies but also to optimize their design. Anticipated results from simulation should be confirmed in prospective directed studies. Nevertheless, forecasting treatment outcome can generate insight saving time and resources.

Some shortcomings of the efavirenz studies (Papers I-III) are that solid relationships between efavirenz exposure and the risk of having adverse central nervous system effects still have not been documented. Although already extensively studied, the benefit to toxicity ratio of efavirenz needs to be further studied in association with polymorphisms in genes coding for drug metabolizing enzymes and transporters.

Since parasite clearance time was used in Paper IV and no data was available regarding parasite densities over time, logistic regression was used to allow for dichotomized data. If parasite density data would have been available rather than derived parasite clearance times, the pharmacodynamic response could have been characterized over time. Instead there was substantial loss of pharmacodynamic information. Thus it is important to state the purpose of the modeling before the data analysis and possibly already before the conduct of the study (i.e. to enable data collection of needed variables).

Recent advances in handling data below quantification limits will reduce bias in parameter estimates. This has been shown by Beal [124] and more recently by Ahn *et al* [71] when analyzing pharmacokinetic data. Bergstrand and Karlsson have applied the methodology also on pharmacodynamic data [125]. From a retrospective point of view the method could with advantage have been used to handle pharmacokinetic data below the quantification limit in Paper IV. Instead samples below the quantification limit were excluded from the analysis, resulting in discharge of information. In Paper III, the method was used to handle pharmacodynamic HIV-RNA data below the quantification limit of 50 copies/mL in an informative fashion. Instead of just assuming that the HIV-RNA observation is somewhere between 0 and 50 copies/mL, NONMEM is specifying a probability that the observation indeed is below the quantification limit and the model may be used to predict if a patient is stable in treatment or if viral levels are close to rebound.

6 Conclusions

To summarize, in the present work the population pharmacokinetics of efavirenz in healthy subjects and patients of African and European origin were described. Moreover, the relationship between antiretroviral treatments and reductions in viral load over time was modeled. The elimination half-life of piperaquine was quantitated and the exposure-response relationship of artemisinin was assessed in Vietnamese populations. Notably, pharmacological HIV and malaria data originating both from patients resident in low income and industrialized countries were included. Altogether, this research has contributed with knowledge regarding the pharmacokinetic and pharmacodynamic characteristics of drugs indicated for the treatment of HIV and malaria. It remains to be seen whether antiretroviral and antimalarial therapy can be further optimized based on information derived within the frames of this thesis.

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